RANGE-WIDE ANALYSIS OF THE SPATIAL DISTRIBUTION AND GENETIC DIVERSITY OF *DELONIX S.L.* (LEGUMINOSAE) IN MADAGASCAR: ENHANCING HERBARIUM-BASED CONSERVATION ASSESSMENTS

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Range-wide analysis of the spatial distribution and genetic diversity of *Delonix s.l.* (Leguminosae) in Madagascar: enhancing herbarium-based conservation assessments

Malin C. Rivers
August 2011

A thesis submitted for the degree of Doctor of Philosophy
University of St Andrews

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I was admitted as a research student in January 2007 and as a candidate for the degree of Doctor of Philosophy in January 2007; the higher study for which this is a record was carried out in the University of St Andrews between 2007 and 2011.

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To my grandparents for their wisdom and inspiration

hazo tokana tsy mba ala
[a single tree is never a forest]

Malagasy proverb

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Abstract

Despite their ecological and economic importance, the majority of plant species and their conservation status are poorly known. Only 4% of plants have been assessed globally and listed on the IUCN Red List of Threatened Species; and without plant conservation assessments, many plant species will not feature in conservation planning.

Herbarium collection information can significantly increase the number of plant conservation assessments. Thus, the aims of this thesis were: (1) to investigate how the quality of herbarium-based conservation assessments can be optimised; (2) to assess the extent to which herbarium-based conservation assessments reflect the reality on the ground; and (3) to scientifically validate genetic and spatial underpinning of IUCN criteria.

Preliminary range-based assessments of the Leguminosae of Madagascar achieved a result consistent with the final conservation rating for over 95% of species when using up to fifteen herbarium specimens. Bioclimatic modelling of range shifts based on future climate change predicted that, in the worst case scenario, up to one third of endemic Leguminosae in Madagascar will be threatened with extinction over the next 100 years. An analysis of the population structure of species of *Delonix s.l.* (Leguminosae) showed that combining spatial analysis with population genetic data provides a more complete picture of landscape-level population dynamics and the impacts on conservation status.

Moreover, range-wide genetic analysis of AFLP markers for four species of *Delonix* demonstrated a genetic basis for IUCN categories distinguishing between threatened and non-threatened species. Although genetic data are currently not often incorporated in conservation assessments, they are crucial in making accurate management decisions and creating effective action plans for conservation. Only by using all available scientific resources can informed conservation decisions be made and the survival of plants and their associated ecosystems be ensured.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF BOXES

LIST OF SUPPLEMENTARY MATERIAL

Chapter 1

Introduction

1.1. Background

1.1.1. Plant biodiversity for future wellbeing

Never before has the human population been larger nor the mean standard of living higher (UNDP 2010) – two trends that come at an increased cost to our planet and its biodiversity. Plants, with their photosynthetic capacity, are the basis of human life and civilisation. Throughout history, plant diversity has provided people with food, shelter, fibre and medicines; and it has maintained vital ecosystem services. Over time, however, civilisation and population growth have imposed a cost, and today many plant species are faced with extinction. Concomitantly, many vital ecosystem services are becoming degraded. The world's plant scientists are faced with a severe challenge to reduce the rate of plant extinctions. If current rates of extinction are allowed to continue, the resulting loss of plant diversity may have catastrophic consequences for human well-being (Corvalan *et al.* 2005). If plant diversity can be conserved and used sustainably, however, the potential benefits are immense.

1.1.2. Policy background

In 1992, the need for conservation of natural resources was brought to the world's attention at the Earth Summit in Rio de Janeiro through the establishment of the Convention on Biological Diversity (CBD) (UN 1992). This treaty called for the conservation and sustainable use of biological resources, and the fair and equitable sharing of benefits deriving from that use. The importance of plant diversity was highlighted ten years later in the Global Strategy for Plant Conservation (GSPC) (UNEP 2002). The GSPC recognised the need to increase knowledge of plant diversity. One of the 16 targets (Target 2) set for 2010 was "*a preliminary assessment of the conservation status of all known plant species, at national, regional and international* *levels*". This target, however, has yet to be met; in October 2010 an updated strategy with revised targets for 2020 was adopted by the Parties to the CBD. Target 2 for 2011-2020 now reads: "*an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action*" (Convention on Biological Diversity 2010).

1.1.3. Conservation assessments for conservation decisions

Conservation assessments of plants are currently lagging behind mammals, birds and amphibians, all of which have been fully assessed (IUCN 2010). Conversely, plants have an order of magnitude more species than all of these groups combined, and although some plant species may have had their conservation status assessed on a national level, or for a Flora treatment, only 4% of plants have been assessed globally by current criteria and listed on the IUCN Red List of Threatened Species (IUCN 2010). Without conservation assessments many plant species will not feature in conservation planning. Hence, there is an urgent need to increase our knowledge of the conservation status of plant diversity. Only by this means can we make informed conservation decisions and ensure the survival of plants and their associated ecosystems.

1.2. IUCN Red List of Threatened Species

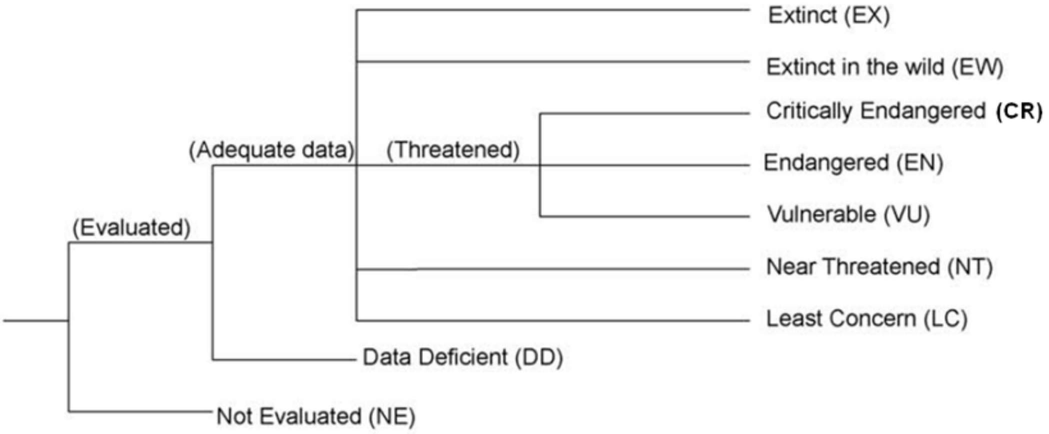
1.2.1. Historical background

The International Union for Conservation of Nature and Natural Resources (IUCN) has produced Red Lists since the 1960s (IUCN 1963). The first Red List assessments were largely based on expert opinion without following a strict protocol; but during the early 1990s a new system was developed that incorporated quantitative criteria, fixed categories, and a unified system to allow comparisons across taxon groups (Mace *et al.* 1992; Mace & Lande 1991; Mace & Lande 1994). New criteria (version 2.4) were published in 1994 (IUCN 1994). In 2001, further changes were made to the criteria, and an updated version of the categories and criteria (version 3.1) was published (IUCN

2001). Since then the wording of the categories and criteria has remained the same, but developments in the interpretation and application of the criteria are published in the continually updated guidelines (version 8.0) (IUCN Standards and Petitions Working Group 2010).

The IUCN Red List aims to identify and document the species most at risk of extinction and, therefore, most in need of conservation attention. It predicts the probability of extinction within a specific time period and aids, but does not directly set, conservation priorities (Lamoreux *et al.* 2003; Mace *et al.* 2008; Rodrigues *et al.* 2006). The IUCN Red List of Threatened Species is today one of the most comprehensive and widely recognised systems used to assess taxa from a wide range of organisms (Hoffmann *et al.* 2008; Lamoreux *et al.* 2003; Rodrigues *et al.* 2006).

Figure 1.1. Structure of the categories in IUCN Red List of Threatened Species, version 3.1 (IUCN 2001)



1.2.2. Categories

The Red List has nine different categories: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Data Deficient (DD) and Not Evaluated (NE) (Figure 1.1 and Table 1.1). CR, EN and VU are the three threatened categories. Taxa that do not qualify for a threatened category, but are close to qualifying for or are likely to qualify for a threatened category in the near future, can be assigned to the category NT. LC is used for species that are assessed but are not considered threatened including widespread species and rare but stable species. The use of the category DD is discouraged, but may be assigned to poorly known taxa.

Table 1.1. Definition of the categories in the IUCN Red List of Threatened Species, version 3.1 (IUCN 2001)

EXTINCT (EX)	no reasonable doubt that the last individual has died
EXTINCT IN THE WILD (EW)	only found in cultivation, in captivity or as a naturalized population(s) outside the past range
CRITICALLY ENDANGERED (CR)	facing an **extremely high** risk of extinction in the wild
ENDANGERED (EN)	facing a **very high** risk of extinction in the wild
VULNERABLE (VU)	facing a **high** risk of extinction in the wild
NEAR THREATENED (NT)	close to qualifying for or likely to qualify for a threatened category in the near future
LEAST CONCERN (LC)	not qualifying for threatened or near threatened categories.
DATA DEFICIENT (DD)	inadequate information to make a direct, or indirect, assessment of its risk of extinction
NOT EVALUATED (NE)	not yet been assessed against the criteria

1.2.3. Criteria

In order to assess whether a species belongs to a threatened category (CR, EN, VU) the species should be evaluated in relation to five criteria: A) Population reduction; B) Geographic range; C) Small population size and decline; D) Very small or restricted population; and E) Quantitative analysis. The criteria are based on a set of thresholds and subcriteria. Extensive guidelines are available to facilitate the process for the conservation assessors (IUCN Standards and Petitions Working Group 2010). In practice, most assessments are based on a combination of population size and range size, either measured directly or inferred. Assessors are encouraged to evaluate taxa using all five criteria, but a taxon only needs to fulfil one of the five criteria to qualify for a threatened category. When several criteria are met resulting in different status assessments, the precautionary principle is applied and the most threatened category should be assigned (IUCN 2001). The criteria can be applied to any taxonomic level at or below the species level; however, the Red List requires an assessment of the full species before an assessment of infraspecific rank can be carried out (IUCN Standards and Petitions Working Group 2010). It is recommended that species on the list are re-evaluated at least once every 10 years (IUCN 2009).

1.2.4. Plants on the IUCN Red List

The *1997 IUCN Red List of Threatened Plants* (Walter & Gillett 1998) included Red List assessments for nearly 34,000 species of plants using the pre-1994 classification system. This was followed by *The World List of Threatened Trees* where 10,091 tree species were assessed using the 1994 IUCN system (v. 2.4) (Oldfield *et al.* 1998). The most recent IUCN Red List (IUCN 2010) records all the species that have been assessed using the 1994 or 2001 criteria. It lists 12,854 species, representing about 4% of the world's plants (Table 1.2). Some groups of plants are particularly under-represented (e.g. bryophytes and pteridophytes); while the gymnosperms are nearly completely assessed (Donaldson 2003; Farjon *et al.* 2006; Farjon & Page 1999). However, only 5,738 species (<2% of the world's plants) actually have current conservation assessments, i.e. done within the last 10 years.

Table 1.2. Numbers of plant species (excluding algae) on the IUCN Red List of Threatened Species (adapted from Schatz (2009) and updated from IUCN (2010), Paton *et al.* (2008) and The Plant List (2010)).

Taxon	Accepted number of species (estimated number of species)	Species on IUCN Red List 2010.4	% accepted number of species on IUCN Red List
Bryophytes	15,344 (20,000)	101	0.7%
Pteridophytes	9,294 (13,000)	243	2.6%
Gymnosperms	1,088	926	85.1%
Angiosperms	273,174 (352,000)	11,584	4.2%
TOTAL	298,900 (386,000)	12,854	4.3%

1.3. Herbarium-based conservation assessments

Despite the policy call nearly a decade ago by the GSPC for conservation assessments of all plant species, thus far only one in 25 has been assessed globally using internationally accepted criteria. One solution to improve this number is to base assessments on information found in herbaria around the world.

1.3.1. Herbarium information

Herbarium and other museum collections provide an important source of data for many of the world's most poorly known species (Brummitt *et al.* 2008; Callmander *et al.* 2005). Historically, herbarium specimens have been collected mainly for taxonomic purposes or for species' horticultural potential. However, the wealth of information associated with herbarium specimens has proven to be very useful in the field of conservation assessments (Nic Lughadha *et al.* 2005; Schatz 2009). Herbarium specimens may not be sampled at random (regarding localities, climate, density, detectability) (Loiselle *et al.* 2008; Parnell *et al.* 2003; Sheth *et al.* 2008). Nonetheless, for many plant species, especially those from the tropics, herbarium specimens are the best source of information available, especially when compiled from multiple relevant herbaria (Schatz 2000b).

As it is difficult to obtain population estimates from herbarium specimens, herbarium-based assessments are often heavily reliant on measures of geographic range. Geographic range is a part of the IUCN Red List assessment process, since species with small or fragmented distributions are more likely to be threatened than species with widespread, continuous distributions. Herbarium-based conservation assessments can be prepared in a two-stage process (Brummitt *et al.* 2008): the first stage is a range-based preliminary assessment and the second stage is a desktop assessment.

1.3.2. Preliminary assessments

Preliminary assessments are based on estimates of geographic range. The range parameters within the IUCN criteria lend themselves to calculation within a geographical information system (GIS). By collating specimen localities for a species and plotting them on a map, it is possible to calculate values for area-based measures such as extent of occurrence (EOO) and area of occupancy (AOO) (Brummitt *et al.* 2008; Willis *et al.* 2003). By comparing these measures against the thresholds set under IUCN Criterion B, a preliminary category of threat is obtained (see Chapter 2). These GIS assessments are described as preliminary, since Criterion B also requires at least two out of three subcriteria (severe fragmentation or number of locations; continuing decline; extreme fluctuation) to be met in order for a species to be classified as threatened (Box 1.1). Preliminary assessments are used by the Royal Botanic Gardens, Kew (Davis *et al.* 2006; Rico Arce & Bachman 2006) and Missouri Botanical Garden (Callmander *et al.* 2007; Good *et al.* 2006; Randrianasolo *et al.* 2002) and collaborating institutions. Preliminary assessments of the endemic legumes of Madagascar reveal that nearly half (49%) qualify for a threatened category (or DD). Furthermore, a quarter of all endemic species qualify for the two most threatened categories, Critically Endangered and Endangered, indicating a very high risk of extinction of legumes in Madagascar. Preliminary assessments for the endemic legumes of Madagascar and for *Delonix s.l.* are found in Appendix 1.

Box 1.1. The range thresholds and subcriteria that need to be met for a species to qualify as threatened under Criterion B

	Critically Endangered	Endangered	Vulnerable
Extent of occurrence	< 100 km^2	< 5,000 km^2	< 20,000 km^2
Area of occupancy	< 10 km^2	< 500 km^2	< 2,000 km^2
and at least two of the following subcriteria:			
(a) Severely fragmented, OR number of locations	=1	≤ 5	≤ 10
(b) Continuing decline in any of the following: (i) extent of occurrence; (ii) area of occupancy; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals.			
(c) Extreme fluctuations in any of the following: (i) extent of occurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals.			

The only previous test of the accuracy of preliminary (range-based) conservation assessments compared to full assessments (accepted by and listed on the IUCN Red List) was carried out using the cypress family (Cupressaceae) (Brummitt *et al.* 2008). The IUCN Conifer Specialist Group has produced full conservation assessments for all cypress species, excluding those with taxonomic uncertainties (Farjon *et al.* 2006). Comparing these “traditional” expert assessments with range-based preliminary assessments, preliminary assessments accurately discriminated threatened and non-threatened taxa, as determined by full assessments, for nearly three-quarters (74%) of the species (Brummitt *et al.* 2008).

1.3.3. Desktop Assessment

The second part of herbarium-based conservation assessments is the desktop assessment. Desktop assessments were developed in order to complete the preliminary assessments and elevate them to full assessments, through evaluation of the subcriteria under Criterion B, as well as using other criteria where possible. In the desktop assessment, as much relevant information as possible about a species is gathered by examining information from specimen labels, searching the literature, incorporating GIS analyses and (where data permit) statistical models, as well as contacting experts (Brummitt *et al.* 2008).

The literature search and specimen labels highlight information regarding species and habitat characteristics that could be of importance. Important information includes life history, habitat requirements, pollination requirements, seed dispersal, population sizes, fire regimes, and logging intensity. GIS analyses of range have already proven valuable for preliminary assessments, but a more detailed GIS analysis could be useful in filling the gaps where experts, specimen labels, or literature cannot provide enough information (Brummitt *et al.* 2008). GIS analysis can be used to estimate the degree of fragmentation, number of locations, continuing decline or extreme fluctuations in range, habitat, or number of locations or subpopulations, and so provide the data for a full assessment under Criterion B (Box 1.1.) (Chapter 3 and Rivers *et al.* 2010). Among other things, GIS analysis can assess collection effort (to determine if the apparent absence of a species is due to true absence or merely to low collection effort), bioclimatic modelling of the species' distribution (to identify new areas with potentially suitable habitat) and predictive mapping of climate change impacts (to assess the status of the habitat in future climate scenarios) (Chapter 4). Feedback and comments are sought from taxonomic and/or regional specialists and their opinions are incorporated in the desktop assessment.

Herbarium-based assessments (preliminary and desktop) use verifiable and repeatable methods, and data sources are referenced, meaning that they can be independently verified and supported. In other words, the herbarium-based assessment is a detailed assessment that stops short of gathering new data from the field. The recent Sampled Red List Index (SRLI) project used herbarium-based conservation assessments (as described above) to assess the conservation status of 7,000 plant species drawn at random from the five major groups of plants (RBG Kew 2010). The SRLI for plants is the first unbiased estimate of the conservation status of the world's plants; it showed that about one fifth of species are considered threatened (RBG Kew 2010).

1.3.4. Ground truthing herbarium-based conservation assessments

Herbarium-based conservation assessments are carried out using existing data in herbaria, in combination with information from the literature, GIS analyses and specialist opinions, to provide a detailed assessment of the plant taxon. Ideally, these assessments ought to be "ground truthed" in the field to confirm findings. There have been few opportunities to evaluate full herbarium-based assessments (preliminary and desktop), and this thesis performs a much called for comparison of the different types of assessments (preliminary, desktop and field-based) and evaluates the strengths and weaknesses of herbarium-based assessments (see Chapter 8 and Appendices 1-4).

1.4. Use of genetic data in conservation assessments

The IUCN recognizes the need to conserve biodiversity at three levels – genetic diversity, species diversity and ecosystem diversity (McNeely *et al.* 1990). To effectively conserve biodiversity, it is essential to take into account the genetic diversity within species to ensure long term survival (Frankel 1974). Understanding the patterns and extent of genetic divergence among populations is crucial in order to identify populations to be conserved and to develop effective conservation plans for a species. The maintenance of such genetic diversity is important to ensure there is enough genetic diversity for present survival, future adaptation to our changing environment and, in the long term, to allow for evolutionary development (Allendorf & Luikart 2007; Frankham *et al.* 2010).

Plant conservation genetics is an important tool to guide conservation and restoration efforts and to minimise extinction risk (Kramer & Havens 2009). Genetic diversity studies are sometimes carried out on species that have already been listed as threatened, as it is important to know the genetic history and structure of these species to be able to effectively assign conservation strategies and management plans (for example Smith & Waldren 2010). However, genetic information is rarely incorporated into the IUCN conservation assessment process, and genetic diversity is not even mentioned in the IUCN conservation assessment guidelines (IUCN Standards and Petitions Working Group 2010). Moreover, many recent genetic diversity studies of endangered plants do not provide conservation assessments of the species analysed (such as Andrianoelina *et al.* 2006), despite these studies containing the information necessary to do so.

Before species go extinct, populations are lost; and before populations disappear, genetic diversity is lost (Spielman *et al.* 2004). It is important, therefore, that genetic diversity considerations are incorporated in conservation assessments. Conservation priorities and plans can then ensure that enough genetic diversity is retained to maintain the species today and ensure its evolutionary potential in the future.

Therefore, in addition to ground truthing herbarium-based conservation assessments in the field, there is also a call for assessing species with regard to their genetic diversity and structure (Chapters 5 and 6). This is important not only to more accurately assess a species' conservation status, but also because knowledge of the genetic history of a species is crucial in designing effective conservation action plans.

1.5. Choice of study area

Plant diversity is distributed unevenly across the globe (Barthlott *et al.* 1996). The tropics contain the highest species diversity, and these areas are also the most threatened and in need of conservation action (Myers *et al.* 2000). Based on the number of endemic species and loss of natural vegetation, Madagascar has been classified as one of the world's biodiversity "hotspots" (Brummitt & Nic Lughadha 2003; Mittermeier *et al.* 2005; Myers *et al.* 2000). Species endemism in Madagascar reaches over 80% among several plant groups (Dransfield & Beentje 1995; Du Puy *et al.* 2002; Hermans *et al.* 2007; Schatz 2000a) and many invertebrate and vertebrate groups (Goodman & Benstead 2005). This species richness is in part due to the age and movement of the island. Madagascar separated from the rest of Africa approximately 165 million years ago (Rabinowitz *et al.* 1983), and since then the Malagasy flora has evolved predominantly in isolation. Phytogeographic connections, however, have been maintained with both the African and the Indo-australo-malesian floras (Schatz 1996).

Madagascar has a population of nearly 20 million people, of whom two-thirds live below the poverty line (World Bank 2010). Much of the natural vegetation in Madagascar has been cleared for slash and burn agriculture, and many plants are used for food, fuel and construction material. The consequence is a rapid rate of deforestation and degradation of natural vegetation. Only about 18% of the natural vegetation still exists (Moat & Smith 2007) and much of this area is in need of conservation action.

Madagascar was also chosen as the study area as it has several large plant groups that are taxonomically well documented (e.g. Leguminosae, Rubiaceae, Orchidaceae, Arecaceae); there is good background information on floristic distributions available, such as the recent vegetation atlas (Moat & Smith 2007); and there is a good infrastructure for fieldwork across the country.

1.6. Choice of taxonomic study group

The choice of taxa for this study was based on a wide range of considerations: the study group needed to be taxonomically well defined, have a well documented collection record, be endemic to the study area, have varied distribution across habitats and across threat categories, and should ideally be representative of other species in Madagascar. For the field element of this study, it is beneficial if the species can be easily detected, recognised and identified in the field.

1.6.1. Importance of Legumes

The target taxa are chosen from the Leguminosae family. Leguminosae is an ideal family to use due to global distribution and representativeness (Schatz 2009). Nic Lughadha *et al*. (2005) showed that on a generic level, the pattern of diversity in the Leguminosae family correlates best with global patterns of angiosperm diversity. It is also the world's third largest angiosperm family, encompassing approximately 19,327 species in 727 genera (Lewis *et al.* 2006). It occurs in a wide range of habits, has a cosmopolitan distribution and is well represented in tropical, temperate, dry and wet habitats. The Leguminosae is a taxonomically well-studied group, and the last decade has seen advances in systematics that have resulted in a new understanding of the patterns of relationship within the family (for example Bruneau *et al.* 2001; Haston *et al.* 2005; Lewis *et al.* 2006). The Malagasy legumes were revised in the publication *Leguminosae of Madagascar* (Du Puy *et al.* 2002); the family has 459 species and 33 genera endemic to Madagascar (Du Puy *et al.* 2002). For this study, whenever possible, analyses were carried out on all endemic legumes of Madagascar. For other analyses, where a smaller number of species needed to be selected, *Delonix* Raf. was the chosen genus.

1.6.2. **Delonix (sensu lato)**

The genus *Delonix* contains eleven species, of which two are native to Africa (one extending to Arabia and India); the other nine are endemic to Madagascar. The most well-known species is *Delonix regia,* which is widely cultivated within Madagascar and throughout the tropics. Recent molecular and morphological studies (Banks 1997; Bruneau *et al.* 2001; Du Puy *et al.* 1995; Du Puy *et al.* 2002; Haston *et al.* 2005) have shown that two monotypic genera, *Colvillea* Bojer ex Hook. and *Lemuropisum* H.Perrier*,* are very closely related to *Delonix*. These genera are therefore also included in the analyses presented here, to provide a broader view of the genus (*Delonix sensu lato*) (Table 1.3). Further phylogenetic studies of the group are currently being carried out by Dr. A. Bruneau at the University of Montreal.

Table 1.3. Taxa included within *Delonix s.l.* in this study

Taxon name	**Distribution**
Delonix baccal (Chiov.) Baker f.	East Africa
Delonix boiviniana (Baill.) Capuron	Madagascar
Delonix brachycarpa (R.Vig.) Capuron	Madagascar
Delonix decaryi (R.Vig.) Capuron	Madagascar
Delonix elata (L.) Gamble	East Africa, Arabia, India
Delonix floribunda (Baill.) Capuron	Madagascar
Delonix leucantha (R. Vig.) Du Puy, Phillipson & R.Rabev.	Madagascar
Delonix pumila Du Puy, Phillipson & R.Rabev.	Madagascar
Delonix regia (Bojer ex Hook.) Raf.	Madagascar
Delonix tomentosa (R.Vig.) Capuron	Madagascar
Delonix velutina Capuron	Madagascar
Colvillea racemosa Bojer ex Hook.	Madagascar
Lemuropisum edule H.Perrier	Madagascar

Figure 1.2. The frequency distribution of the extent of occurrence (km^2) for Leguminosae in Madagascar, (red bars include one or more species of *Delonix s.l.)*

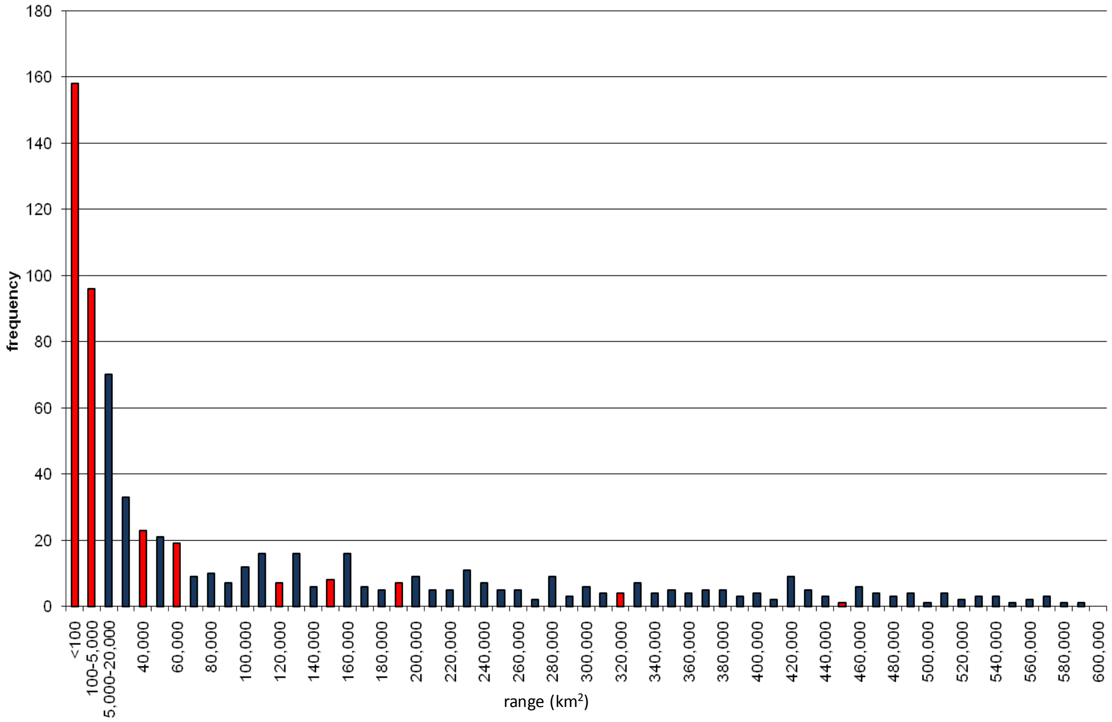
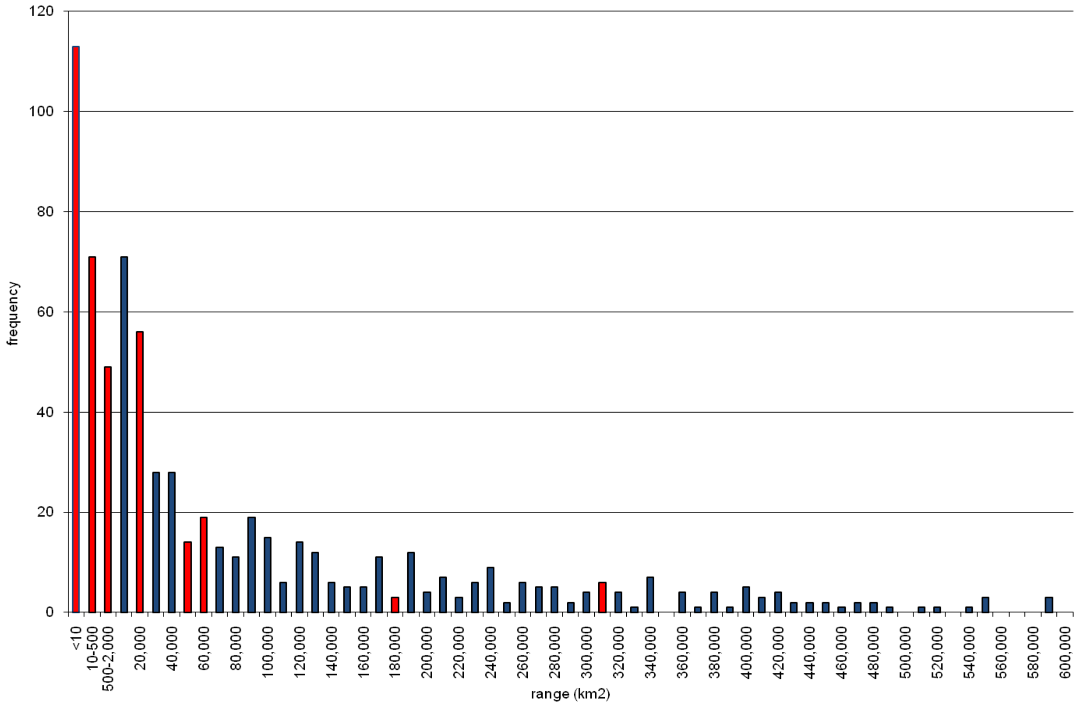


Figure 1.3. The frequency distribution of area of occupancy (km^2) (using the method outlined in Willis *et al.* (2003)) for Leguminosae in Madagascar, (red bars include one ore more species of *Delonix s.l.*)



Analysis of range size was used to test whether *Delonix s.l.* is representative (with regards to range-estimates) of the legumes of Madagascar as a whole. The frequency distributions of the EOO and AOO for all legumes in Madagascar follow a negative exponential function (Figure 1.2 and Figure 1.3). Both range analyses show that the majority of species have small ranges and only a few species have large ranges. The red bars indicate a range interval with at least one species of *Delonix s.l.* Species of *Delonix s.l.* are represented across the whole range of legumes.

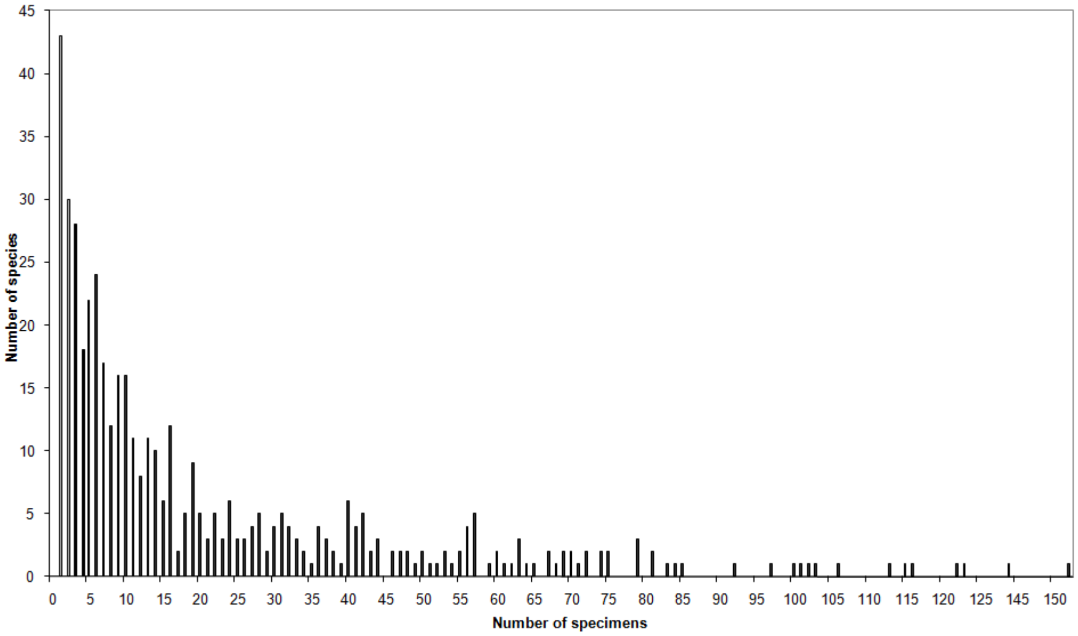
1.6.3. Dataset

In the initial phase of this PhD project, a database containing label data from herbarium specimens of legumes in Madagascar was assembled from more than 17,500 specimens covering 730 species; of these, 87% (15,434) of specimens have been georeferenced (Table 1.4). This gives an average of over 20 georeferenced specimens per species; however, the majority of species of Malagasy Leguminosae are known from very few georeferenced herbarium specimens (Figure 1.4). For example, 9% of endemic legumes in Madagascar are known only from a single specimen; 31% are known from five or fewer specimens; and less than half (42%) of species have 15 or more specimens. This pattern is common in other tropical regions and plant families as well (Haripersaud *et al.* 2010 and Chapter 2). It is unlikely to be due to lack of collection effort, but instead reflects of the narrow distribution and general rarity of these species. The error radius for each georeferenced point was also estimated and recorded.

Table 1.4. Number of species and specimens in the database of Madagascar Leguminosae

	All species	**Endemic species**
Number of species	730	459
Number of specimens	17,742	11,759
Georeferenced specimens	15,434	10,369

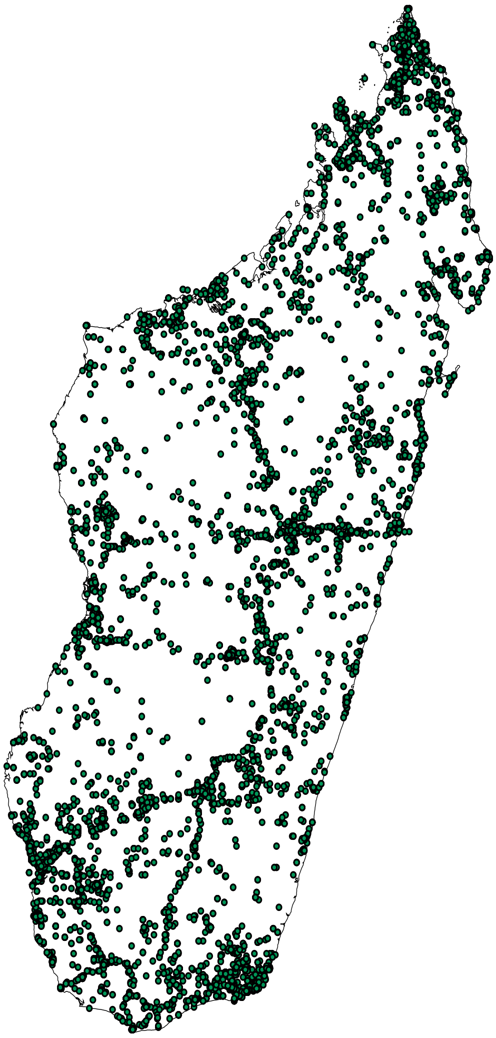
Figure 1.4. The frequency distribution of the number of geographically distinct collections for the endemic species of Leguminosae from Madagascar (n= 459).



The use of herbarium data for conservation assessments relies on having correctly identified and accurately georeferenced collections of maximum coverage. The collections in the legume database used in this thesis are based on material that was reviewed by Du Puy *et al.* (2002) for the monograph on all species of Leguminosae in Madagascar, and therefore the taxonomy and identification is considered to be current. The georeferencing of specimens was carried out on specimens from Paris (P), Missouri (MO) and Kew (K). Cross-checking of the specimens (14%) found at each of the three locations (i.e. databased independently at each of the three herbaria) showed high consistency of the georeferencing among the three institutions. Maximum coverage is difficult to ensure among herbarium collections. Herbarium data, both in numbers of species and distribution of collections, are sometimes clustered around roads and settlements (Figure 1.5). However, specimens are also collected when found at the extremes of species' ranges or at new localities. Importantly, a lack of collections does not always mean a lack of species occurrence. Assuming maximum coverage, and working with the herbarium specimens available, it is estimated that by combining the data from P, MO, and K the majority of all collections available are covered. As an example, an extensive search was carried out for *Delonix s.l.* (including 14 herbaria: B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG (acronyms following Thiers 2009)) and results show that the collections from P, MO and K cover 94% of collections (Figure 1.6), leaving only 6% of specimens found in herbaria elsewhere. If this relationship is true for the family Leguminosae as a whole, then the current dataset of Malagasy legumes is an almost complete set of available specimens.

Figure 1.5. Maps of Leguminosae in Madagascar showing a) the distribution of all available georeferenced herbarium specimens (P, MO, K) b) the distribution of species diversity c) the distribution of collection density. The grid size for b) and c) is 0.5 x 0.5 degree (approximately 50 x 50 km)

a)



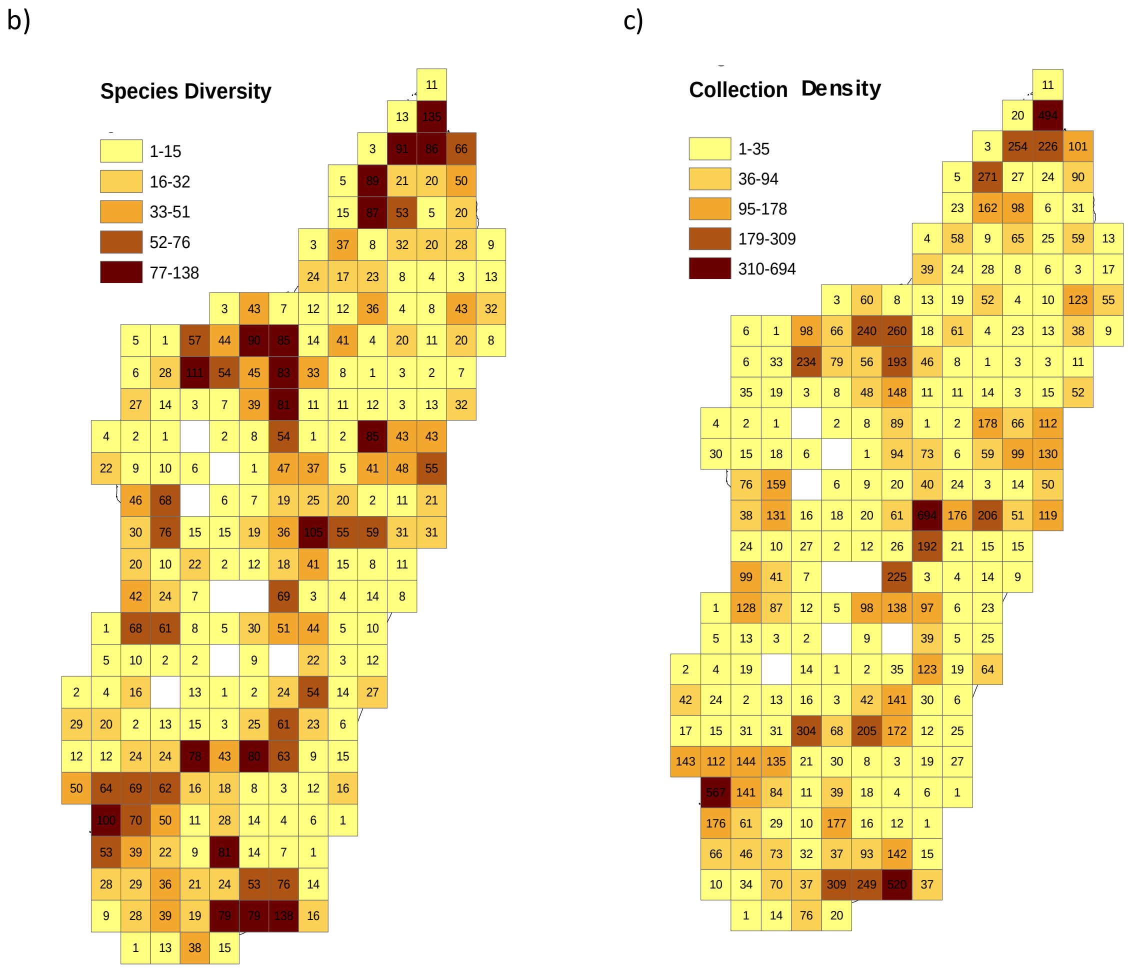
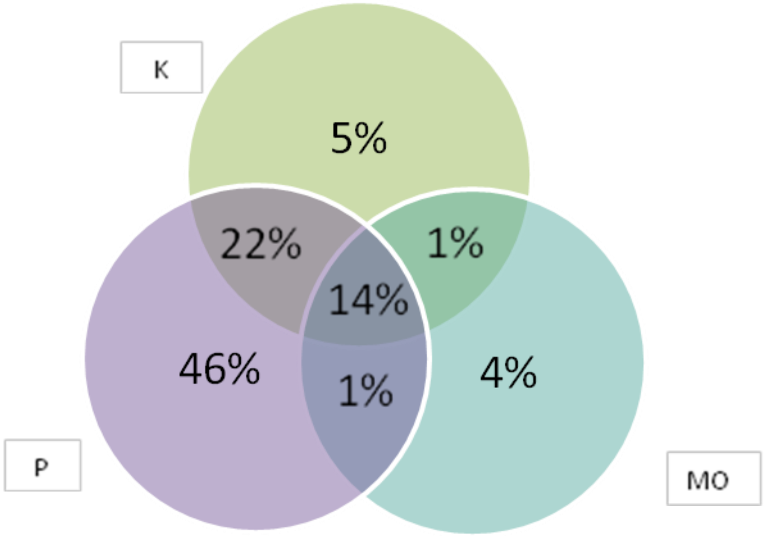


Figure 1.6. *Delonix s.l.* specimens in each of the three main herbaria holding Malagasy material, Kew (K), Paris (P) and Missouri (MO) (6% of specimens are found in other herbaria).



1.7. Thesis structure

This thesis addresses the call at a policy level for more conservation assessments of plants by exploring the potential application of scientific tools to herbarium collection data. The scientific tools applied in this thesis are GIS analysis and the incorporation of genetic diversity information to produce scientifically supported conservation assessments. The three main aims of this thesis are: (1) to investigate how the quality of herbarium-based conservation assessments can be optimised; (2) to assess the extent to which herbarium-based conservation assessments reflect the reality on the ground; and (3) to scientifically validate the genetic and spatial underpinning of the IUCN Criteria.

The thesis is divided into eight chapters including this first chapter, which provides an introduction and background information on the need for, and challenges of, plant conservation assessments. Chapters 2 to 4 address the first research question – optimizing the quality of herbarium-based conservation assessments. Chapter 2 assesses the number of herbarium specimens needed for preliminary range-based assessments. Chapter 3 compares different ways of assessing subpopulation structure using herbarium collection data. Chapter 4 uses further GIS analyses such as species distribution modelling to predict the impact of climate change on threat status. Chapters 5 and 6 address the second research question – assessing the extent to which herbarium-based conservation assessments are true to the reality on the ground. They incorporate genetic data to form a picture of the true level of threat. Chapter 5 presents the general descriptive genetics of *Delonix s.l.* Chapter 6 compares genetic population structure with the spatial population structure. Chapter 7 addresses the third aim – to scientifically validate the genetic and spatial underpinning of the IUCN criteria. Here range-reduction is modelled in four species to assess the genetic basis of the range thresholds found in the criteria of the IUCN Red List. Finally, the conclusion (Chapter 8) compares herbarium-based assessments with the reality on the ground for *Delonix s.l.* and draws on information from the six chapters to offer recommendations for herbarium-based conservation assessments.

Chapter 2

How many herbarium specimens are needed to detect threatened species?[1]

2.1. Summary

The distribution, ecology and conservation status of the majority of plant species are poorly known. One of the challenges ahead is to address this knowledge gap and give more emphasis to this important group of species which represents a critical component of earth's biodiversity. Full conservation assessments require expert knowledge of the group concerned; but, for the majority of plant species, especially those from the tropics, the only source of knowledge is assimilated from specimens housed within herbaria. Digitisation projects are underway to render information from this important global biodiversity resource more accessible; the next step is to bring together and utilise these data to make better informed conservation decisions. One crucial question is: how many herbarium specimens are needed to detect threatened species? Such information would inform and help to prioritise digitisation efforts. Using 11,461 herbarium records we assessed the conservation status of 661 endemic species of Leguminosae and Orchidaceae from Madagascar on the basis of range estimates, following the IUCN criteria. By capturing 15 georeferenced specimens per species we achieved a result consistent with a final conservation rating (based on all known locality records) for more than 95% of species, none of the threatened species were misclassified and less than 6% of species would receive unnecessary conservation support as a result of being falsely identified as threatened. This approach can therefore help to achieve progress towards the Global Strategy for Plant Conservation

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[1] This chapter was submitted as Rivers, M.C., Taylor, L., Brummitt, N.A., Meagher, T.R., Roberts, D.L. & Nic Lughadha, E. How many specimens are needed to detect threatened species? Biological Conservation. December 2010. [MR designed the data analysis protocol and analysed the legume dataset. The orchid dataset was analysed following the same protocol by LT. MR compiled the results and wrote the chapter.]

target of a conservation assessment for each plant species, while reducing digitisation effort by up to half.

2.2. Introduction

It is clear we are entering a time of immense environmental change. For us to gauge more accurately the impact of humans on biodiversity and associated ecosystem services, knowledge of species' conservation status is critical (Rockström *et al.* 2009). In the past, biodiversity conservation has been primarily focused on megafauna, and especially vertebrates such as mammals and birds. A current goal is to address this imbalance and give more emphasis to non-vertebrate species such as plants, fungi and invertebrates, which represent the majority of earth's biodiversity (Stuart *et al.* 2010).

2.2.1. Conservation assessments

In 2002, the Global Strategy for Plant Conservation (GSPC) called for a preliminary conservation assessment for all known plant species by 2010 (Target 2, UNEP 2002). Future targets for GSPC for 2020 are currently under review, but an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action remains a priority. A full conservation assessment of all known plant species to a consistent international standard, such as the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (www.redlist.org; IUCN 2010), is the longer-term aim to facilitate conservation action; however, it is not realistic to assess all species by this method in the near future (UNEP 2010). Major constraints on achieving this target include: lack of funding for field work and data compilation, and lack of expertise for assessment activities leading to incomplete and scattered outputs. To date, only 4% (12,854) of the world's plants species have been fully assessed and included on the IUCN Red List (IUCN 2010).

Full conservation assessments require extensive knowledge of the ecology and range of the taxa concerned, whereas for the majority of plant species, especially those from the tropics, this information is poorly known, and for many the main source of information are herbarium specimens housed within natural history collections. Georeferenced herbarium specimens can be used to model features such as species range (Willis *et al.* 2003) and population structure (Chapter 3 and Rivers *et al.* 2010), and such information can form a basis for preliminary conservation assessments using IUCN criteria (Brummitt *et al.* 2008). However, most plant species are represented by a small number of specimens in the world's herbaria, while few species are well collected (Figure 2.1). Herbarium information is therefore very sparse for the majority of species. However, achieving a full conservation assessment for such species would require considerable time and financial investment in field-based investigation. Until such time as full conservation assessments can be undertaken, preliminary herbarium-based conservation assessments are the only realistic option for many of these species. Therefore, it is critical to have a realistic assessment of the minimum number of herbarium collection records that can provide a viable preliminary conservation assessment. These preliminary conservation assessments should be evidence-based using all readily available information, which means that they can be repeated, independently verified or corrected and potentially upgraded when more information becomes available.

2.2.2. IUCN Red List

The IUCN Red List is widely recognised as the international standard for assessing conservation status, comparable across taxonomic groups, and used extensively in setting conservation priorities by policy makers and other stakeholders (Mace *et al.* 2008). It aims to be applicable to a wide range of taxa, using quantitative criteria and pre-defined thresholds to assess extinction risk. Each taxon is assessed using five criteria: population reduction (A), geographical range (B), small population size and decline (C), very small or restricted population (D) and quantitative analysis (E) (IUCN 2001). A taxon is assigned a conservation category on the basis of one or more of these five criteria that are designed to reflect varying degrees of threat of extinction. There are three threatened categories: Critically Endangered, Endangered and Vulnerable.

The category Near Threatened is applied to taxa that do not qualify as threatened now, but may be close to qualifying as threatened. Least Concern is applied to taxa that do not meet the criteria for threatened or Near Threatened and are not currently regarded as being at risk of extinction. For the purposes of this study, the categories Near Threatened and Least Concern are grouped as "not threatened". The other four categories are Extinct, Extinct in the Wild, Data Deficient and Not Evaluated, which are self-explanatory.

The IUCN Red List states that "*the absence of high quality data should not deter attempts at applying the criteria […]. The Red List criteria should be applied to a taxon based on the available evidence concerning its numbers, trend and distribution*" (IUCN 2001). Although the criteria are highly quantitative and defined, projections, assumptions and inferences can be used in order to place a taxon in the appropriate category (IUCN Standards and Petitions Working Group 2010). Species' range size can be measured using automated GIS techniques on a set of herbarium specimen data, and evaluated against thresholds set for different IUCN Categories (Willis *et al.* 2003), giving a preliminary assessment of threat status based on Criterion B that is objective, repeatable and independent of any projections, assumptions or inferences on the part of the assessor, provided that the set of herbarium specimens gives an accurate picture of the true range of that species. Over time, as more specimens are collected and knowledge improves, the known range of a species may increase, which might (or might not) result in a less threatened conservation rating. The degree to which species conservation assessments are dependent on the number of specimens collected per species is thus of crucial importance. The objective of this study is to establish how many specimens are needed for robust range-based preliminary assessments based on herbarium collections.

2.3. Methods

2.3.1. Study area and species information

Madagascar, the focal region for this study, is one of the world's biodiversity hotspots, based on the number of endemic species and loss of natural vegetation (Brummitt & Nic Lughadha 2003; Mittermeier *et al.* 2005; Myers *et al.* 2000). Species endemism in Madagascar reaches over 80% among many animal groups (Goodman & Benstead 2005) as well as several plant groups, such as legumes (Du Puy *et al.* 2002) and orchids (Hermans *et al.* 2007). However, Madagascar has a rapidly increasing human population and its unique biodiversity is under severe threat from habitat destruction and over-exploitation. A recent vegetation mapping project estimates that only 18% of primary vegetation still exists (Moat & Smith 2007), and many species are in dire need of effective conservation action. Currently, only 386 (3%) of the approximately 12,000 species of vascular plants in Madagascar (Schatz 2000a) are listed on the IUCN Red List (IUCN 2010). There is clearly a need for more conservation assessments of plants in Madagascar; these could be rapid preliminary assessments initially, to identify and prioritise threatened species for a full assessment and conservation action.

The Leguminosae (legume) family is the world's third largest angiosperm family and is well represented throughout tropical, temperate, dry and wet habitats (Lewis *et al.* 2006). The Orchidaceae (orchids) is the largest angiosperm family and occurs in all terrestrial habitats with the exception of the poles and extremely dry deserts, but reaching their zenith in the tropics (Roberts & Dixon 2008). The legumes and orchids are both highly species-rich and have been shown to provide effective representation of global patterns of angiosperm diversity (Nic Lughadha *et al.* 2005). Within Madagascar, both legumes and orchids are found in all habitats, but whereas legumes show greater diversity in the drier, western half of the island, orchid diversity is concentrated in the wetter, eastern half of the island. The two families together were, therefore, chosen as the focal sample for species to use for this study, as the results are likely to be indicative of Malagasy plant species in general. The taxonomy of the species used follows two recently published monographs for the two families in

Madagascar (Du Puy *et al.* 2002; Hermans *et al.* 2007). There are approximately 1,300 species of endemic legumes and orchids in Madagascar. Specimen locality data used in this study were collated from 21 herbaria (AMES, B, BM, BR, C, E, G, HBG, HEID, K, MO, NY, P, PRE, S, TAN, TEF, UPS, W, WAG and ZSS). It was not possible to obtain georeferenced specimen localities (a precise latitude and longitude) for all species, but for legumes our georeferenced dataset encompassed 82% of all legume specimens. Thus, our initial georeferenced dataset comprised 12,435 specimen records covering 1,052 endemic species of legumes and orchids (Figure 2.1, Table 2.1). A minimum of three specimens with distinct, georeferenced localities are needed to calculate the two range estimates: extent of occurrence and area of occupancy (see below) (Willis *et al.* 2003). Thus, species with just one or two collection localities were necessarily excluded from this study, despite the fact that these species are probably the most threatened. Following the restriction of this study to those species with three distinct data points, our final data set included 661 species represented by 11,461 specimens (Table 2.1).

2.3.2. Preliminary Conservation Assessment - IUCN Criterion B

Preliminary conservation assessments were based on each species' geographical distribution using part of IUCN Red List Criterion B (Willis *et al.* 2003). Criterion B, originally developed for plants (Mace *et al.* 2008), allows a species to qualify as threatened when its geographic range is restricted. This is useful, as for many plant species population size (needed in Criteria A, C and D) is not known. Under Criterion B, species must also meet at least two of the following conditions: severe fragmentation or existence at few locations; continuing decline; and/or extreme fluctuations (IUCN 2001). This information is not known for many of our species so the assessments performed here are based purely on range size and should therefore be considered preliminary. Range size, according to IUCN, is measured as extent of occurrence (EOO, the smallest polygon in which no internal angle exceeds 180^{o} and contains all sites of occurrence), and as area of occupancy (AOO, the area occupied by a taxon, excluding cases of vagrancy, at a scale appropriate to the taxon) (IUCN 2001). These two measurements represent different aspects of geographical range size: EOO provides information on overall geographical spread while AOO provides information on the area of suitable habitat (Gaston & Fuller 2009).

Figure 2.1. The distribution of species and their conservation status according to the number of specimens available for endemic a) legumes and b) orchids from Madagascar.

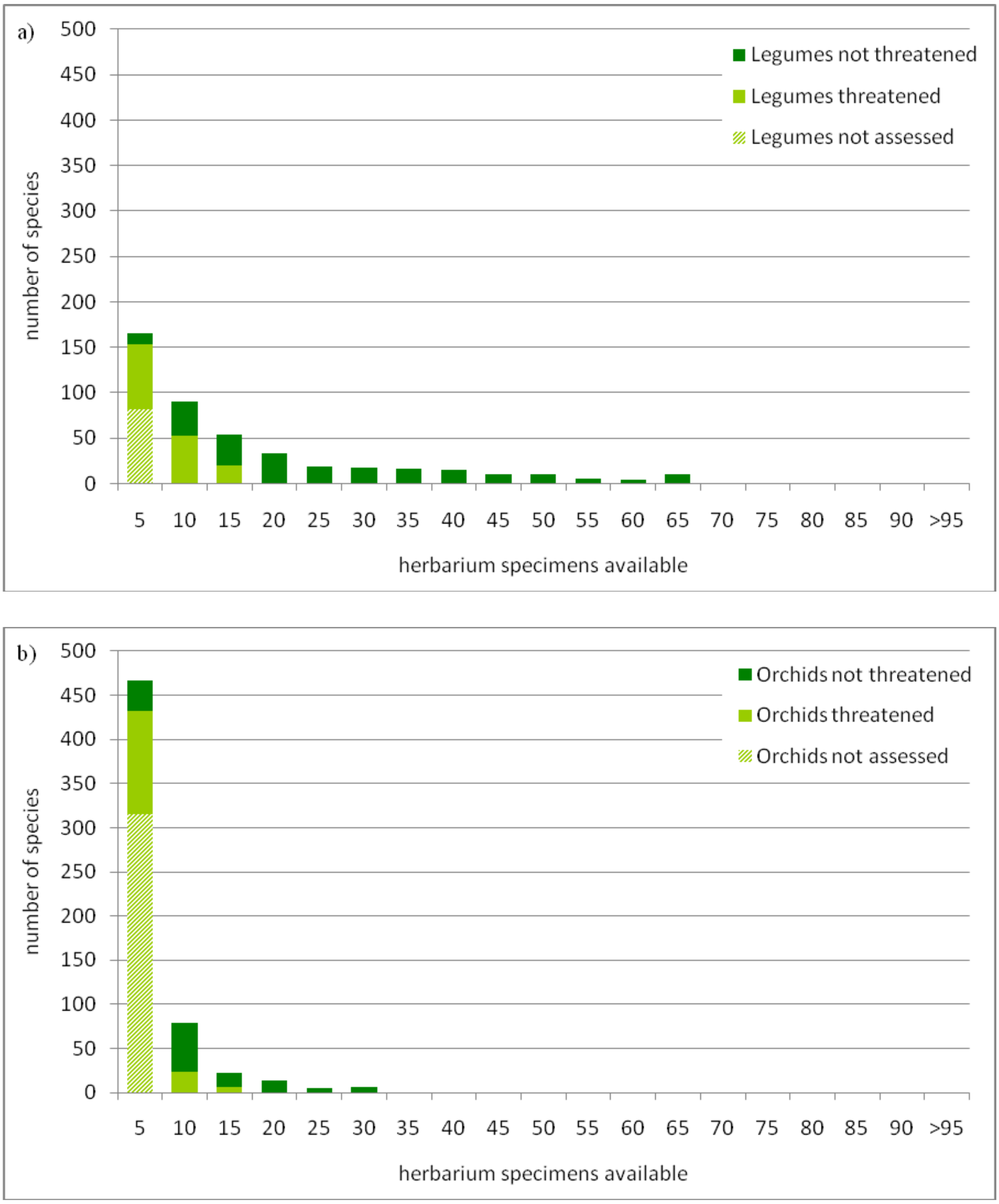


Table 2.1. Number of endemic species and specimens of legumes and orchids in Madagascar.

	Legumes	**Orchids**	**Total**
Endemic species in Madagascar	459 species	~ 850 species	~ 1309 species
Endemic species in initial data set	453 (9,756 specimens)	599 (2,679 specimens)	1,052 (12,435 specimens)
Endemic species with 3 (or more) specimens from distinct collection localities	377 (9,201 specimens)	284 (2,260 specimens)	661 (11,461 specimens)

This study calculated EOO and AOO in ArcView 3.2 using the Conservation Assessment Tools (CAT) extension, developed at the Royal Botanic Gardens, Kew and downloadable from http://www.kew.org/gis/projects/cats (Moat 2007). EOO was calculated by the area of the minimum convex polygon encompassing all the points. AOO was calculated by summing the area of the occupied cells in a grid system, using a cell size (side length) equal to 1/10th the maximum distance between the most distant pair of points (Willis *et al.* 2003). This factor of ten reflects the relationship between EOO and AOO in the IUCN criteria (Table 2.2); it allows the size of the grid to be adjusted to the geographical range of the species. The IUCN states that the appropriate scale to measure AOO will depend on the taxon in question (IUCN Standards and Petitions Working Group 2010). The recommended grid size of 2 x 2 km for AOO was not used as it would classify all 661 species as threatened. To reach an AOO size of > 2,000 km^2, and thus a non-threatened conservation rating, each species would need to be represented by at least 500 collections, each representing a distinct locality more than 2 km apart. The minimum number of localities required to provide a calculation of AOO and EOO are two and three, respectively.

Based on the values for EOO and AOO a preliminary IUCN rating was assigned according to the thresholds set by IUCN (Table 2.2). Species with an EOO smaller than

20,000 km^2 or an AOO smaller than 2,000 km^2 fell into one of the three threatened categories (Critically Endangered, Endangered, and Vulnerable), while species with an EOO larger than 20,000 km^2 or an AOO larger than 2,000 km^2 were considered not threatened. Where the results suggested by EOO differ from those indicated by AOO, a species was assigned the higher category of threat, as recommended by IUCN, according to the precautionary principle (PP). The precautionary principle is the idea that the most threatened category should be assigned when conservation assessment parameters result in different conservation categories.

Table 2.2. Thresholds for geographical range, Criterion B (IUCN 2001).

	THREATENED			**NOT THREATENED**
	Critically Endangered	**Endangered**	**Vulnerable**	
EOO	< 100km^2	< 5,000km^2	< 20,000km^2	> 20,000km^2
AOO	< 10km^2	< 500km^2	< 2,000km^2	> 2,000km^2

For each species all available specimens were used to calculate the EOO and AOO and infer an IUCN rating; in the present study, this is considered the final conservation rating. In order to determine whether a smaller number of collection localities would provide a reasonable preliminary conservation assessment, EOO and AOO were calculated using subsets of specimens. These were taken in chronological order of collection date for 3, 4, 5, 6, etc. specimens, until no further specimens were available. At each step the IUCN status according to EOO, AOO and PP was calculated. The number of specimens needed for a rating the same as the final conservation rating to be obtained was then calculated (see example in Table 2.3).

For species receiving a preliminary assessment as threatened, there is a possibility that some species may have been incompletely sampled and may not have reached their final rating with the specimens available. To avoid this we initially looked only at species that were considered not threatened, as these would not change their rating with the addition of more specimens. We also used Pearson's correlation coefficient (*r*) to check for correlation between the number of specimens available and number of specimens needed to reach the final conservation rating, in order to ensure that the results were not dependent on the number of available specimens. A limitation to this approach is that these preliminary conservation assessments are based only on IUCN Red List Criterion B; species not qualifying for a threatened category based on geographical range may still be threatened based on one of the other categories. However, the information required for assessment using the other four criteria is not available for many of the species used in this study.

Table 2.3. Conservation status for *Delonix floribunda* (Baill.) Capuron using increasing number of specimens up to a total of 50 specimens. The final preliminary conservation rating was reached after only three (AOO) or six (EOO, PP) specimens (marked in bold).

No. specimens used	EOO	AOO	Precautionary Principle
3	Threatened (Vulnerable)	**Not threatened**	Threatened (Vulnerable)
4	Threatened (Vulnerable)	Not threatened	Threatened (Vulnerable)
5	Threatened (Vulnerable)	Not threatened	Threatened (Vulnerable)
6	**Not threatened**	Not threatened	**Not threatened**
7-49	Not threatened	Not threatened	Not threatened
50 (all specimens)	**Not threatened**	**Not threatened**	**Not threatened**

2.4. Results

2.4.1. Threatened or not threatened

The number of specimens needed for an accurate conservation assessment of species not under threat is seen in Figure 2.2. Using only ten specimens per species, the preliminary conservation status of not threatened legumes can be estimated with 87% accuracy, relative to the complete dataset; while orchids have an accuracy of 98%. Using fifteen specimens the threat status of legumes has 96% accuracy, whilst all of the not threatened orchids are correctly identified. As we can be >95% certain to correctly identify a not threatened legume and orchid species after fifteen or ten specimens respectively, we can also have confidence that a species that is given a threatened preliminary conservation rating after fifteen or ten specimens is likely to indeed be threatened and not simply under-represented in collections. Beyond seven specimens, there is no correlation between specimen availability and the number of specimens needed for the final rating for orchids. No correlation is seen between specimens available and specimens needed for the final rating for legumes.

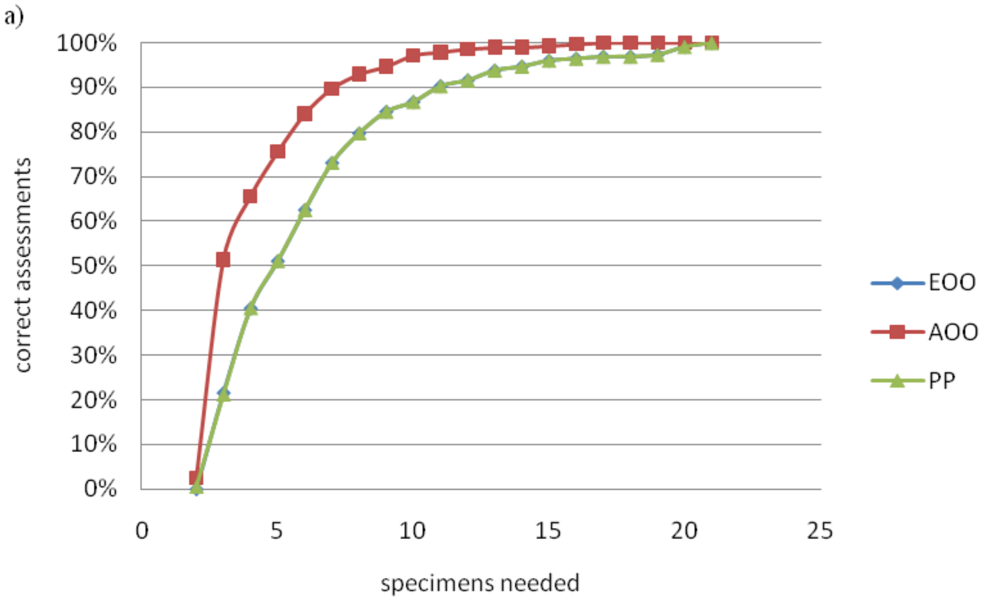
2.4.2. Conservation status

We then extended the analysis to the entire dataset and assessed the number of specimens needed to establish more precise IUCN conservation ratings (i.e. Critically Endangered, Endangered, Vulnerable or Least Concern) for each of the 661 species. With ten specimens there is 90% accuracy for the legumes and 99% accuracy for orchids. With fifteen specimens the accuracies are 97% and 100%, respectively (Figure 2.3). There is no significant correlation between number of specimens available and the number of specimens needed for a robust conservation assessment beyond four specimens for legumes and beyond eight specimens for orchids (Figure 2.3). Overall, fifteen specimens are needed to achieve over 95% accuracy in conservation rating as well as ensure that there is no correlation between number of specimens and rating.

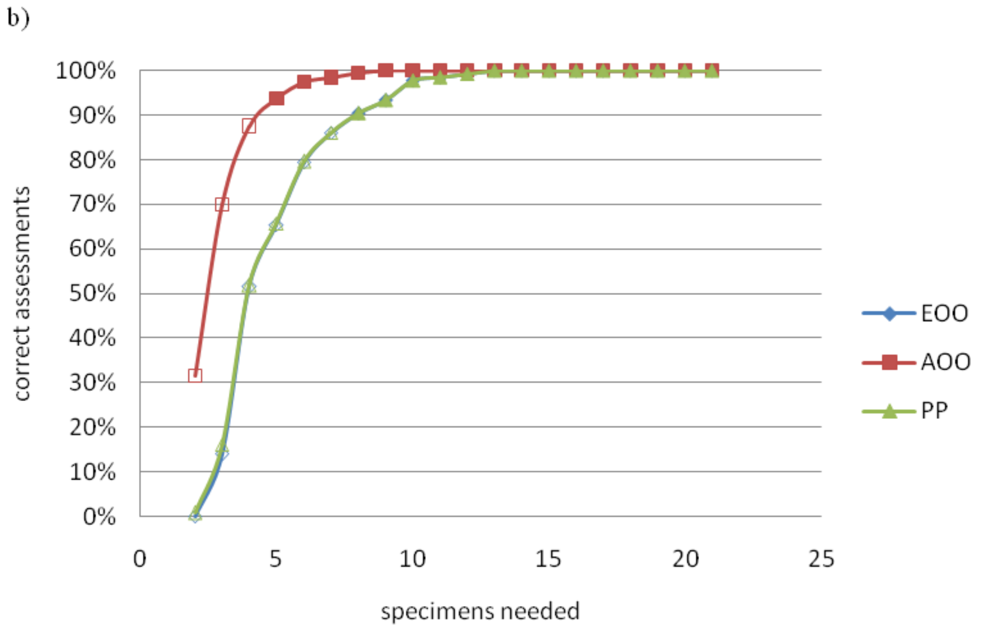
2.4.3. Distribution of threatened species across total specimen numbers

As seen in Figure 2.1, the majority of species are represented by few specimens; and as expected with range-based conservation assessments, the majority of threatened species found are species with fewer available specimens. Some threatened species, however, have more than 40 specimens; similarly some of the not threatened species have as few as three specimens. The non-assessed species are those with fewer than three specimens. Although not included in this study, these species are likely to have very small ranges and therefore belong in the threatened group. The response of legume species in the threatened categories, show that the species classified as CR and EN reach their final rating quickly, while the species classified as VU, also require 15 specimens for 95% accuracy (Supplementary Figure 2.A).

Figure 2.2. The percentage of not threatened species that have reached their final conservation assessment at different numbers of specimens used for a) legumes and b) orchids (open symbols and * represent significant correlation between specimens available and needed for the final rating).



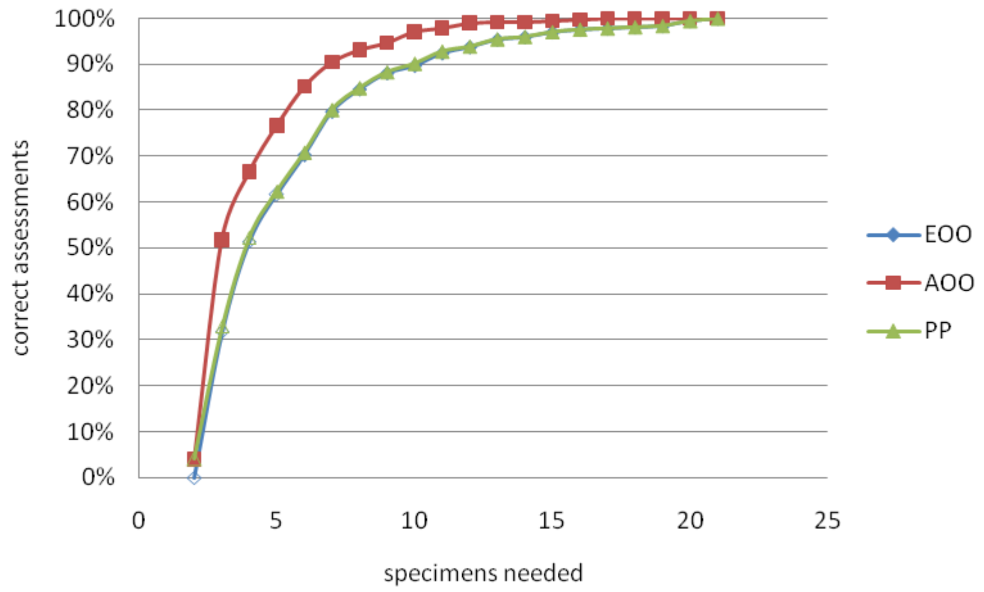
	3	5	10	15	20	21	
EOO	21.6	51.1	86.8	96.0	99.1	100	n = 227
AOO	51.4	75.5	97.2	99.3	100	100	n = 282
PP	23.5	52.6	87.0	96.1	99.1	100	n = 230



	3	5	10	15	20	21	
EOO	14*	65.4*	97.8	100	100	100	n = 136
AOO	69.9*	93.8	100	100	100	100	n = 193
PP	16.1*	65.7*	97.8	100	100	100	n = 137

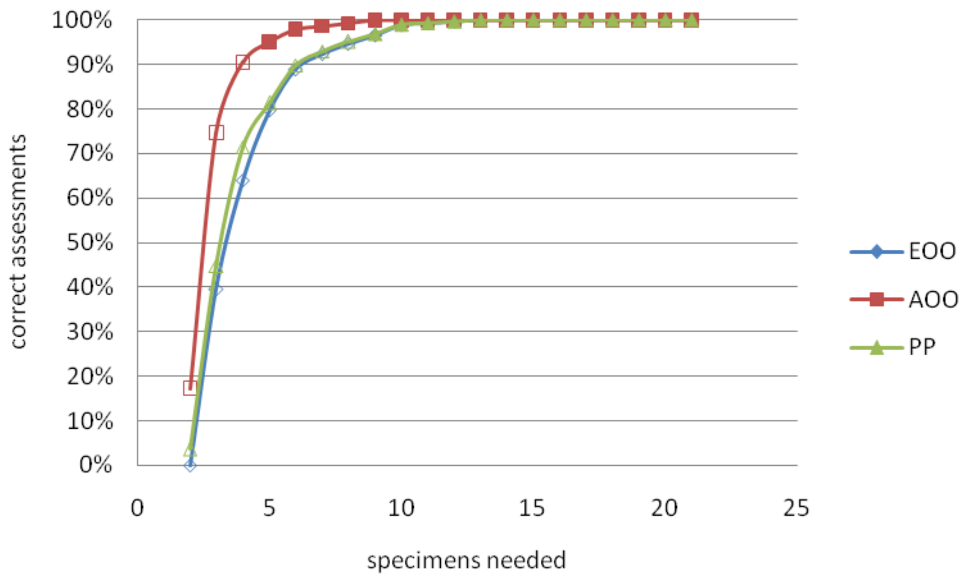
Figure 2.3. The percentage of all species that have reached their final conservation assessment at different numbers of specimens used for a) legumes and b) orchids (open symbols and * represent significant correlation between specimens available and needed for the final rating).

a)



	3	5	10	15	20	21	
EOO	31.9*	61.6*	89.7	97	99.5	100	n = 370
AOO	51.7	76.7	97.1	99.5	100	100	n = 377
PP	32.6*	62.1	89.9	96.8	99.2	100	n = 377

b)

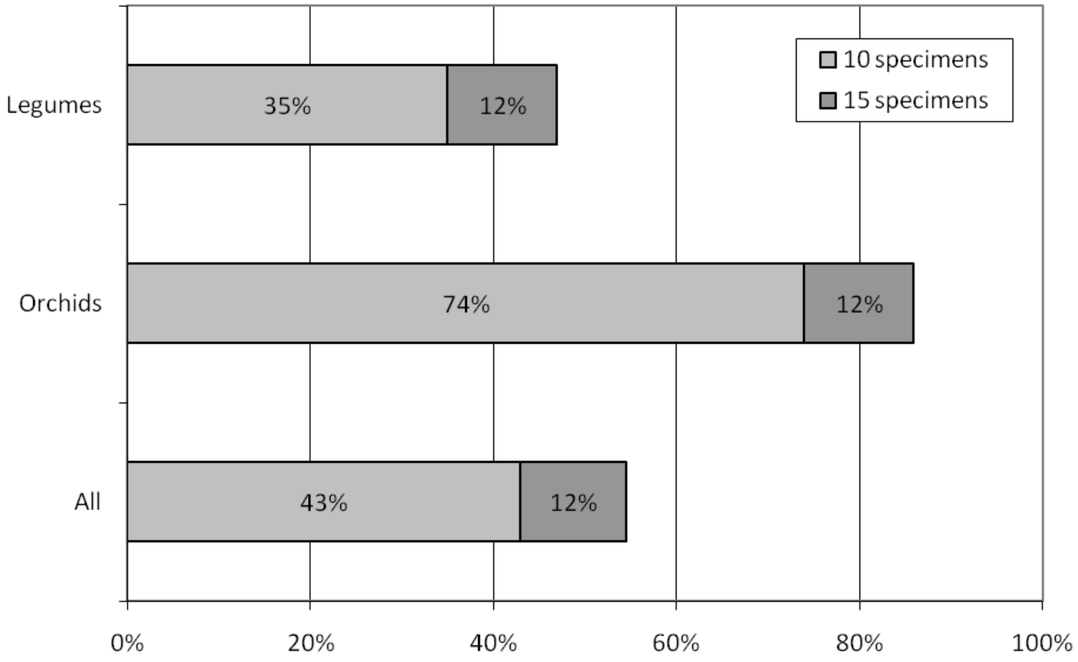


	3	5	10	15	20	21	
EOO	39.5*	79.7*	98.9	100	100	100	n = 260
AOO	74.7*	95.1	100	100	100	100	n = 284
PP	44.7*	81.3*	98.9	100	100	100	n = 284

2.4.4. Digitisation effort

Just fifteen specimens of each species are sufficient to allow robust preliminary conservation assessments to be carried out. Since some species are represented by more than 15 specimens in our herbaria there is significant scope to focus digitisation efforts in order to maximise their conservation relevance. For instance, in this study the conservation status for legumes could be obtained using just half the available digital data (Figure 2.4). In contrast, the majority (86%) of the orchid collections would need to be databased if the threshold was set at 15 specimens.

Figure 2.4. Proportion of herbarium collections needed to be digitised for robust conservation assessments of legume and orchid species. ("All" includes legume and orchid data)



2.4.5. Conservation effort

If conservation decisions were based on the results of this analysis and all species threatened according to our analysis were conserved, then no threatened species would be missed (false negatives). Wasted effort is the number of species that will get conservation support as a result of being falsely identified as threatened (false positives). For legumes and orchids combined, between 3-10% of conservation action would be considered "wasted effort". When ten specimens are used 2% of the not threatened orchid species are misclassified as threatened. With fifteen specimens there is no wasted effort for orchids; for legumes, fifteen specimens lead to 6% wasted effort. No threatened species, however, would be overlooked (Table 2.4).

Table 2.4. The percentage of "missed threatened species" and "wasted effort" for ten and fifteen specimens used in preliminary conservation assessments.

	Legumes (10 / 15 specimens)	Orchids (10 / 15 specimens)	All (10 / 15 specimens)
"Missed threatened species": percentage of threatened species incorrectly classified as not threatened	0% / 0%	0% / 0%	0% / 0%
"Wasted effort": percentage of species that are incorrectly classified as threatened (should be classified as not threatened)	17% / 6%	2% / 0%	10% / 3%

To investigate the effect of collecting effort over time, these analyses were repeated for legumes with specimens in reverse chronological order. The order in which specimens were included had no impact on the eventual conservation assessment. Using the most recent or alternatively the oldest collections first produced the same result (results not shown). However, using the more recent collections may be preferable as georeferencing is often more accurate and the persistence of the species at the collecting locality more likely.

2.5. Discussion

The number of herbarium specimens needed for confident (95% confidence) conservation ratings for both orchids and legumes is 15 specimens. First, we assessed the not threatened species, in order to ensure the species had sufficient sampling and had reached their final preliminary rating. However, as we can be confident that a not threatened species is identified after fifteen specimens, we also have confidence in a threatened status rating at this stage. Similarly, when looking at all the species and at their specific conservation rating the same recommendation of number of specimens remains.

These results should be seen as a conservative estimate as only species endemic to Madagascar were included in the analysis. If more widespread and cosmopolitan species were included, it is likely that a larger proportion of species would reach the geographical thresholds using fewer specimens. Also, more than 600 of the most poorly represented species of legumes and orchids (those with one or two specimens) were excluded from this study.

2.5.1. EOO vs. AOO

The precautionary principle results are mainly driven by the EOO results. This is due to the fact that more species are considered not threatened based on the AOO rating than the EOO rating (282 vs. 227 species for the legumes and 193 vs. 136 species for the orchids). This might be because species distributed linearly over a large distance will have a higher AOO but still have a low EOO. In a few cases, however, the AOO rating is threatened (three legumes and one orchid) while the EOO is considered not threatened. This occurs when species are widespread, but only occur in patchy locations within the range, and emphasizes the need for using both EOO and AOO as one range measure alone does not identify all the species under threat.

2.5.2. Differences between the two datasets

The two datasets complement each other in that the orchids are predominantly from the eastern tropical wet forest, while the legumes are found in all vegetation types of Madagascar, but with most species and collections predominantly found in the western dry forest and the south western dry spiny forest. Legumes have 40% of species with more than 15 specimens, while only 10% of the orchids are represented by more than 15 specimens. Orchids also have smaller ranges than legumes. Nonetheless, preliminary analysis shows that the fact that orchids needed fewer specimens to determine whether a species was threatened or not threatened (ten specimens) compared to the legumes is not due to orchids being represented by fewer specimens generally, nor that they are often of smaller ranges, but due to their patchier distribution. However, further analysis is needed to investigate this. The patchy nature of orchids is probably due to the fact that most orchid populations, particularly in the tropics, are small and clustered in space, which may reflect the distribution and the availability of their associated mycorrhizae (Tremblay *et al.* 2005). Low reproductive success and this island effect result in a small effective population size. Subsequent genetic drift and diversification result in a high speciation rate and small species population sizes (Roberts & Dixon 2008; Tremblay *et al.* 2005). In contrast, legumes are less dependent on mycorrhizal interactions and are therefore less patchy in occurrence. Also, many woody legumes are often the dominant species in a vegetation type, and are sometimes considered “landscape engineers” in the habitats where they are found.

2.5.3. Practicalities

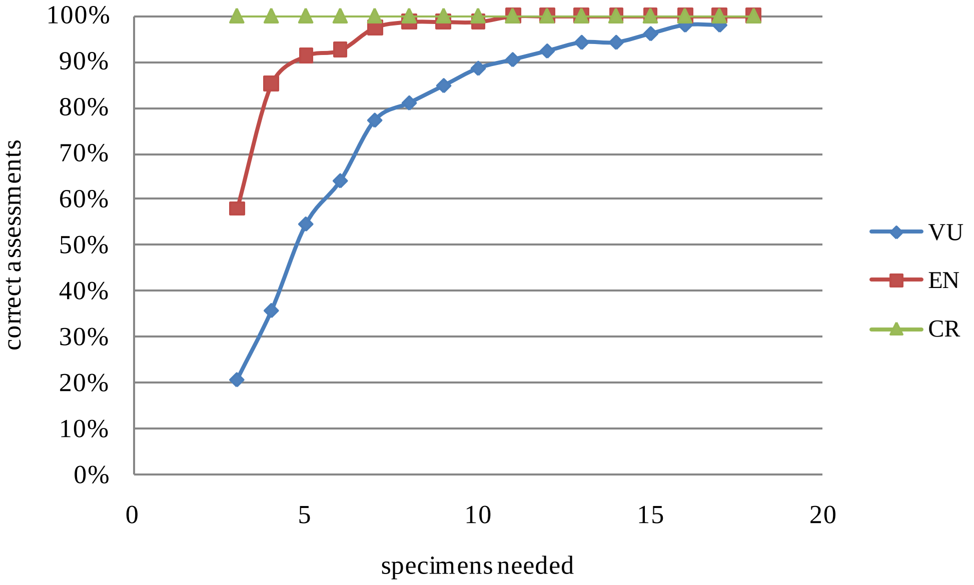
Even though most species are represented by fewer than 15 specimens (Figure 2.1), we would like to emphasise that it is still worthwhile to carry out preliminary (and full) assessments of these species. Conservation assessments should be carried out on species with fewer than 15 specimens using all available information, as advocated by IUCN. Although this study shows that conservation assessments based on geographic range have high confidence (95%) when based on 15 specimens, 70% of all species had reached their final rating at five specimens, and for some species the final rating was reached with as few as three records. Clearly an assessment can be made with a very low number of specimens, and although more data would be desirable it is important to allow assessments based on a low number of specimens when these represent all available information for a species. A more detailed analysis of the orchid data indicates that, where low specimen numbers suggest small range sizes for particular species, this is not an artefact of collection effort (Taylor unpublished results).

We are aware of few herbarium specimen digitisation projects where a primary aim is to support evaluations of conservation status (e.g. the Millennium Seed Bank Enhancement Project (Lindsay 2009) and the Sampled Red List Index for Plants (RBG Kew 2010)). However, our results show that such endeavours have the potential to be very cost-effective, mobilising the most conservation-relevant data from herbaria and underpinning evidence-based preliminary conservation assessments of thousands of species for a fraction of the cost-per-species of alternative approaches such as expert workshops (D. Zappi, pers. comm.). The facts that the material of greatest conservation interest is dispersed in multiple herbaria and that material of one species may be stored in more than one location within each herbarium present a practical challenge which would need to be overcome through careful project management. Our results suggest that the additional costs incurred through selection of material for data capture could result in a far greater return-on-investment in terms of conservation-relevant information.

2.6. Conclusion

Many digitisation projects are underway in herbaria around the world, increasing the availability of georeferenced collection data for plants. The critical next step is to integrate and analyse these data to make better informed conservation decisions. Despite the fact that the majority of the world’s plants have poorly known distributions represented by few specimens, we can still make robust preliminary conservation assessments. This study will help to focus priorities and make digitisation efforts more efficient, and in this way improve the progress towards the GSPC target of a preliminary conservation assessment for every plant species.

Supplementary Figure 2.A. The percentage of legume species that have reached their final conservation assessment at different numbers of specimens used, divided according to threat category (VU – vulnerable, EN – endangered, CR – critically endangered).



	3	5	10	15	20	
VU	20.8	54.7	88.7	96.2	100	n = 53
EN	58	91.4	98.8	100	100	n = 81
CR	100	100	100	100	100	n = 17

Chapter 3

Subpopulations, locations and fragmentation: applying IUCN Red List criteria to herbarium specimen data[2]

3.1. Summary

Despite the ecological and economic importance of plants, the majority of plant species and their conservation status are still poorly known. Based on the limited knowledge we have of many plant species, especially those in the tropics, the use of GIS techniques can give us estimates of the degree of population subdivision to be used in conservation assessments of extinction risk. This paper evaluates how best to use the IUCN Red List Categories and Criteria to produce effective and consistent estimates of subpopulation structure based on specimen data available in the herbaria around the world. We assessed population structure through GIS-based analysis of the geographic distribution of collections, using herbarium specimen data for eleven species of *Delonix sensu lato*. We used four methods: grid adjacency, circular buffer, Rapoport's mean propinquity and alpha hull, to quantify population structure according to the terms used in the IUCN Red List: numbers of subpopulations and locations, and degree of fragmentation. Based on our findings, we recommend using the circular buffer method, as it is not dependent on collection density and allows points to be added, subtracted and/or moved without altering the buffer placement. The ideal radius of the buffer is debatable; however when dispersal characteristics of the species are unknown then a sliding scale, such as the 1/10th maximum inter-point distance, is the preferred choice, as it is species-specific and not sensitive to collection density. Such quantitative measures of population structure provide a rigorous means

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[2] This chapter has been published as: Rivers, M.C., Bachman, S.P, Meagher, T.R., Nic Lughadha, E. & Brummitt, N.A. (2010) Subpopulations, locations and fragmentation: applying IUCN Red List criteria to herbarium specimen data. Biodiversity and Conservation. 19:2071-2085.

of applying IUCN criteria to a wide range of plant species that hitherto were inaccessible to IUCN classification.

3.2. Introduction

Plant diversity is essential for our food security, medicines and ecosystem services, as well as having cultural and aesthetic value. Despite the importance of plants, our current knowledge of their diversity and conservation status is limited and patchy. To date only approximately 4% of plant species have so far had their conservation status assessed by current international criteria and appear on the IUCN Red List (IUCN 2010). To be able to make well informed conservation decisions, there is an urgent need to increase the knowledge of plant diversity and to assess the conservation status of many more plant species worldwide. In 1992, the need for conservation of natural resources was brought to the world's attention at the Earth Summit in Rio de Janeiro, through the establishment of the UN Convention on Biological Diversity. It called for the conservation of biological resources, their sustainable use and the fair and equitable sharing of benefits. The importance of assessing plant diversity specifically was highlighted ten years later by the Global Strategy for Plant Conservation (GSPC). The GSPC recognises the need for plant conservation assessments and calls for the "*preliminary assessment of the conservation status of all known plant species, at national, regional and international levels*" (UNEP 2002).

There is a general, although not comprehensive, understanding of why species go extinct (e.g. range collapse, reduction in number of individuals, severe fluctuation in numbers and/or range), even though factors causing threats to those species will clearly differ. These known characteristics also form the basis for measuring extinction risk according to the IUCN Red List Categories and Criteria version 3.1 (IUCN 2001), hereafter referred to as the IUCN Red List. The IUCN Red List aims to give a warning sign that a species is at risk of going extinct and to give a chance to implement appropriate conservation actions. However, the best methods to measure these early warning signs, especially in poorly-known groups like plants, are not fully developed. Some attention has been given to comparing different range measures (Burgman & Fox 2003; Callmander *et al.* 2007; Gaston & Fuller 2009; Hernandez *et al.* 2006; Willis *et al.* 2003), however, there is no consensus on how best to measure population structure. This paper addresses the immediate need to develop a consensus on the techniques used to measure subpopulation structure against the criteria of the IUCN Red List as well as contributing to broader fields; describing and measuring population structure is highly relevant not only to conservation assessments and conservation biology, but also to population biology more generally, and central to ecology and evolutionary biology (e.g. Waples & Gaggiotti 2006).

Box 3.1. Use of terms relating to population structure used by the IUCN Categories and Criteria v 3.1 (IUCN, 2001).

Subpopulation - Subpopulations are defined as groups geographically or otherwise distinct in the population between which there is little demographic or genetic exchange (typically one successful migrant individual or gamete per year or less).

Location - The term 'location' defines a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present. The size of the location depends on the area covered by the threatening event and may include part of one or many subpopulations. Where a taxon is affected by more than one threatening event, location should be defined by considering the most serious plausible threat.

Severely fragmented - The term 'severely fragmented' refers to the situation in which increased extinction risk to the taxon results from the fact that most of its individuals are found in small and relatively isolated subpopulations (in certain circumstances this may be inferred from habitat information). These small subpopulations may go extinct, with a reduced probability of recolonization.

The IUCN Red List (IUCN 2001) recognises the role population structure plays in extinction risk; and three terms of population subdivision – subpopulations, locations and fragmentation – are included in its categories and criteria (Box 3.1). Ideally, assigning divisions of population structure requires good knowledge of a species' biology, including information on distribution, ecology, reproductive isolation, the degree of genetic exchange and dispersal ability. For most plant species, there is little or no information available from field studies that can be used to determine population structure in such detail. The paucity of such population level data represents a severe constraint to the production of conservation assessments for plants. Where field observations to underpin conservation assessments are lacking, georeferenced herbarium specimens can play an important role (Willis *et al.* 2003). GIS techniques are already being used to assess geographical range using such information; however, they can also be employed for analysing population structure, on the underlying assumption that increased geographical distance between collections means increased genetic separation of subpopulations. GIS analyses are objective and repeatable; data from new collections can be added or old records from now-extinct subpopulations can be omitted, and the data reanalysed.

In order for a species to be listed in one of the Threatened categories of the IUCN Red List (Vulnerable, Endangered, or Critically Endangered), it needs to fulfil at least one of five criteria (A-E; IUCN, 2001). References to the terms of population subdivision (subpopulation, location and severe fragmentation) are found in three criteria, namely Criterion B (geographic range), Criterion C (small and declining population size) and Criterion D (very small or restricted populations). Even when limited data are available, assessors are still encouraged by IUCN to assign a category based on the available data (IUCN Standards and Petitions Working Group 2008). Box 3.2 gives further details on the incorporation of the terms of population subdivision in the IUCN Red List.

The key objective of this paper is to evaluate different ways to effectively and consistently estimate population structure, using the IUCN definitions and criteria, through GIS-based analysis of data available for every species in the herbaria around the world. We use a near-complete set of herbarium specimen data available for eleven species of *Delonix sensu lato* from Madagascar to address population structure according to the terms of the IUCN Red List: subpopulations, locations and fragmentation. Due to equivocal phylogenetic interpretations (Du Puy *et al.* 1995; Haston *et al.* 2005), the related monotypic genera *Colvillea* and *Lemuropisum* are also included in this study as part of *Delonix s.l.*

Box 3.2. Detailed description of the incorporation of population subdivision in the IUCN Categories and Criteria v 3.1

Measures of *subpopulations* are used in Criterion B and Criterion C. In Criterion B, the term subpopulation is included in two subcriteria: continuing decline in the number of subpopulations (subcriterion b) and extreme fluctuation in the number of subpopulations (subcriterion c). Species that fulfil Criteria B and show a decline or extreme fluctuation in subpopulation numbers are listed as *B1/B2b(iv)* or as *B1/B2c(iii)*, respectively. In Criterion C, species that have 90-100% of individuals in a single subpopulation can be listed as threatened under subcriterion *C2a(ii)*. Criterion C requires detailed information on the number of mature individuals which is often not available for plant species. However, species with a single subpopulation can be listed under *C2a(ii)*, as this subpopulation would automatically have 100% of the individuals (subject to the rest of Criterion C being met).

The term *location* is found in Criterion B and Criterion D. In Criterion B the number of locations is included in all three subcriteria. In subcriterion a, a species is considered Threatened if it exists in 10 locations or fewer. A species is also considered Threatened if it shows continuing decline in the number of locations (subcriterion b) and/or extreme fluctuation in the number of subpopulations (subcriterion c). If all subcriteria are fulfilled then species can be listed as *B1/ B2ab(iv)c(iii)*. In Criterion D a species with five (or fewer) locations qualifies as Vulnerable, due to its severely restricted area, and can be listed as *VU D2* (subject to the species being prone to the effects of human activities or stochastic events within a very short time period).

Severely fragmented species are referred to in Criterion B, subcriterion a, where a statement of whether or not the species is severely fragmented is required. Species considered severely fragmented are listed with the code *B1a/B2a*. A taxon is considered severely fragmented if most (>50%) of its total area of occupancy is in habitat patches that are (1) smaller than would be required to support a viable population, and (2) separated from other habitat patches by a large distance.

3.3. Methods

Delonix s.l., a genus from the family Leguminosae, is the taxonomic focus of this study. Leguminosae is the world's third largest angiosperm family and has been identified as a family that can be used as a proxy for evaluating global patterns of angiosperm diversity (Nic Lughadha *et al.* 2005). The taxonomy of *Delonix s.l.* follows Du Puy *et al.* (2002) and recognises 11 species endemic to Madagascar: *Delonix boiviniana* (Baill.) Capuron, *D. brachycarpa* (R.Vig.) Capuron, *D. decaryi* (R.Vig.) Capuron, *D. floribunda* (Baill.) Capuron, *D. leucantha* (R.Vig.) Du Puy, Phillipson & R.Rabev., *D. pumila* Du Puy, Phillipson & R.Rabev., *D. regia* (Bojer ex Hook) Raf., *D. tomentosa* (R.Vig.) Capuron, *D. velutina* Capuron and the monotypic genera *Colvillea racemosa* Bojer and *Lemuropisum edule* H.Perrier. A further two species of *Delonix* found outside

Madagascar are not included in this study. *Delonix s.l.* includes both widespread species and narrow endemics (e.g., *D. tomentosa* is known only from a single locality). Georeferenced specimen data used in this study are primarily from herbaria at the Royal Botanic Gardens, Kew (hereafter RBG Kew), the Museum National d'Histoire Naturelle, Paris, and the Missouri Botanical Garden, with additional specimen data from another eleven herbaria. Estimates show that collections of *Delonix s.l.* from RBG Kew, Paris, and Missouri encompass 94% of all available *Delonix s.l.* collections from Madagascar (M. Rivers unpublished data); duplicate specimens of the same collection from different herbaria were identified and excluded from our analyses so that each collection would be used only once. In total 324 herbarium collections from 14 herbaria (B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG; Thiers, 2009) were used across the 11 species (Table 3.1).

Table 3.1. Summary table of the number of herbarium specimens and georeferenced collections consulted from 14 herbaria: B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG. See Index Herbariorum for full information (Thiers 2009).

	Total number of herbarium specimens	**Uniquely georeferenced collection localities**
Colvillea racemosa	60	43
Delonix boiviniana	93	82
Delonix brachycarpa	9	9
Delonix decaryi	61	38
Delonix floribunda	72	51
Delonix leucantha	19	19
Delonix pumila	28	24
Delonix regia	39	30
Delonix tomentosa	1	1
Delonix velutina	13	9
Lemuropisum edule	21	18
Delonix s.l.	416	324

The collection localities of the specimens were plotted in ArcView 3.1 and ArcGIS 9.2 to allow spatial GIS analyses using the Spatial Analyst extension (ESRI 2006), Hawth's Analysis Tools (Beyer 2004) and RBG Kew's Conservation Assessment Tools (CAT) extensions (Moat 2007; Moat 2008).

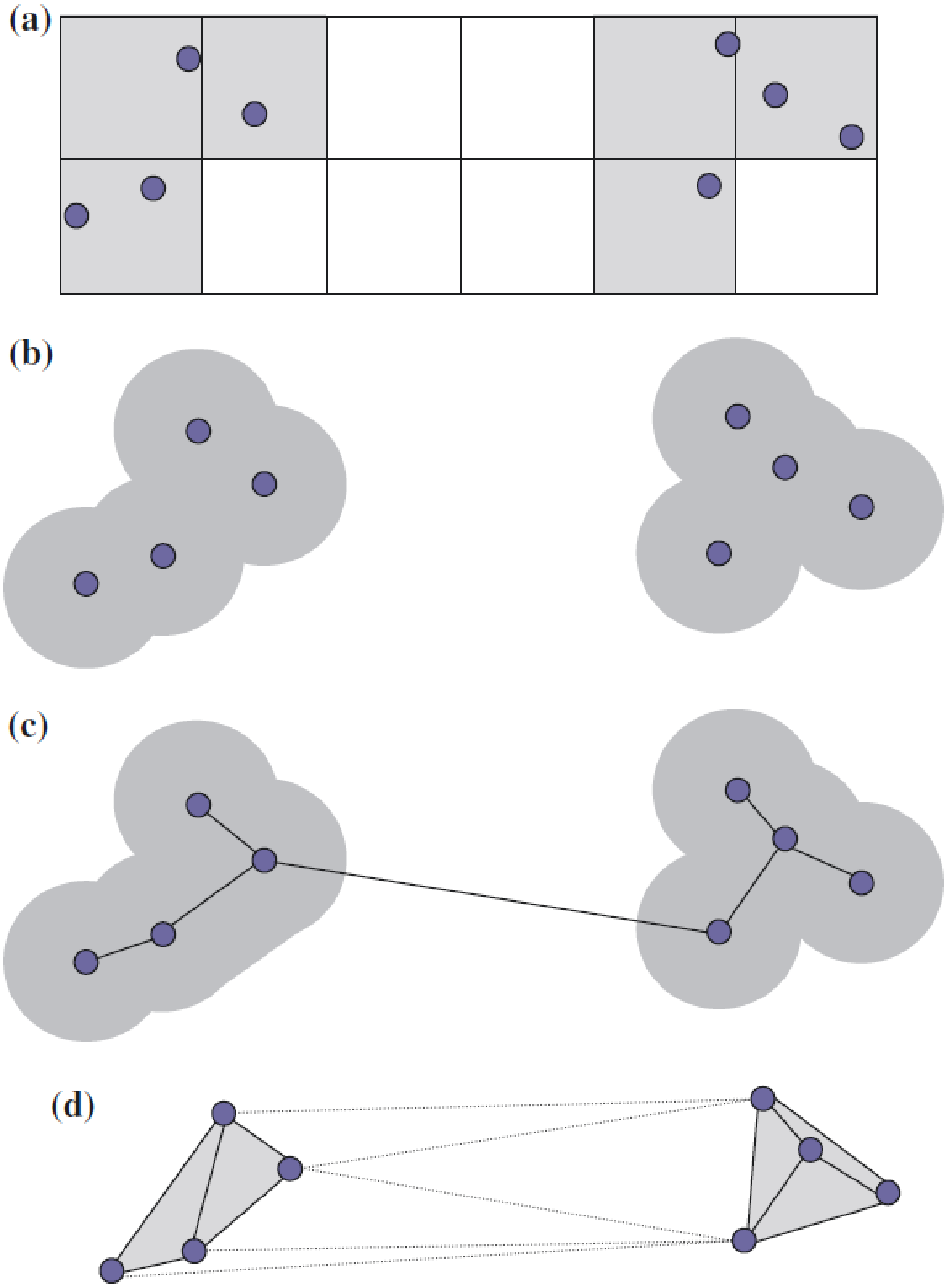
3.3.1. Measures of subpopulations

The subpopulation structure based on analysis of the geographic distribution of collection data was assessed with four methods: grid adjacency (IUCN 1994), circular buffer, Rapoport's mean propinquity (Rapoport 1982) and alpha hull (Burgman & Fox 2003; Edelsbrunner *et al.* 1983) (Figure 3.1). These methods were chosen as they have been used for estimating species range in the context of IUCN conservation assessments. Here we have adopted these methods to fit with the assessment of subpopulation structure.

Grid adjacency method: The number of subpopulations is estimated by overlaying a grid onto mapped collection localities, and contiguous occupied cells are considered to be a single subpopulation (Figure 3.1a). The area of each subpopulation is calculated as the area of the contiguous occupied cells. The grid is positioned on the mapped points in a manner that minimises the number of occupied cells (IUCN Standards and Petitions Working Group 2008). To investigate the influence of grid cell size on the structure of subpopulations, grids of 2 km, 10 km and 1/10th of the maximum distance between any pair of points ("1/10th max") were compared here. "1/10th max" is a sliding scale, based on the interpoint distances between specimen collections, that is species-specific, and so takes into account the fact that widespread species often have a lower collection density across their range than do narrowly distributed species. A 2 x 2 km grid is recommended by IUCN for estimating area of occupancy (AOO) (IUCN 2001); a 10 x 10 km grid is often used in AOO estimates in conservation assessments from Missouri Botanical Garden (Callmander *et al.* 2007; Good *et al.* 2006; Schatz 2000b); and a "1/10th max" grid is used in AOO estimates in conservation assessments at RBG Kew (Moat 2007; Willis *et al.* 2003).

Figure 3.1. Diagram of the four methods used to define subpopulations.

a) Grid adjacency method: adjacent occupied cells form a single subpopulation; b) Circular buffer method: overlapping buffered circles form a single subpopulation; c) Rapoport's mean propinquity method: the radius of the shaded buffers is equal to the mean branch length of the minimum spanning tree (black lines); d) alpha hull method: the lines represent the Delaunay triangulation; when alpha*mean side length is shorter than dashed lines, two subpopulations are formed.



Circular buffer method: Each specimen locality is buffered with a circle of a set radius. Overlapping circles are merged to form a single subpopulation, while non-overlapping circles are considered separate subpopulations (Figure 3.1b). Radii of 5.64 km (buffer area of 100 km^2 equivalent to a single cell in a 10 x 10 km grid), 10 km (equivalent to the minimum distance between two subpopulations in a 10 x 10 km grid) and "1/10th max" were used in this study.

Rapoport's mean propinquity method: An extension of the buffer method is Rapoport's mean propinquity method. All points are connected using a minimum spanning tree: a tree connecting all points together by the shortest distance (Figure 3.1c). The mean branch length (distance between points) of the minimum spanning tree is used as the radius of the buffer around the points and on both sides of the branches (if those branches are shorter than twice the mean). Again, overlapping buffers form a single subpopulation and non-overlapping buffers are considered separate subpopulations. This method was developed by Rapoport (1982) and adapted at RBG Kew (Moat 2007; Willis *et al.* 2003).

Alpha hull method: Another method based on the distances measured between points is the alpha hull method (Burgman & Fox 2003; Edelsbrunner *et al.* 1983). All points are connected using a Delaunay triangulation, where lines are drawn joining all points such that no lines are allowed to intersect, maximizing the minimum angle of all the angles of the triangles in the triangulation (Figure 3.1d). The mean length of the sides of every triangle is then calculated. Lines from the Delaunay triangulation are removed if they exceed the size of a multiple (alpha) of the mean length; the smaller the value of alpha, the finer the resolution of the hull. As lines are removed, the range is divided into subpopulations (Figure 3.1d). Alpha hulls have been used in range estimation, and for this purpose values of alpha of 2 (IUCN Standards and Petitions Working Group 2008) or 3 (Burgman and Fox, 2003) have been recommended. Alpha values of 1 (mean line length), 2 (twice mean line length) and 3 (three times mean line length) were used in this study.

3.4. Results

3.4.1. Measures of subpopulations

Estimates of the number of subpopulations from the four methods are presented in Table 3.2. The grid adjacency method using a 2 x 2 km grid gives the highest estimates of the number of subpopulations in all species analysed. Similarly the results from the 10 x 10 km grid, the circular buffer with a 5.64 km radius and a 10 km radius also show relatively high estimates of subpopulation numbers. The grid of "1/10th max" and the circular buffer of "1/10th max" show much lower estimates for all species. Rapoport's mean propinquity method gives subpopulation estimates similar to the "1/10th max" buffer for most species. The alpha hull method with both alpha = 2 and alpha = 3 show the lowest estimates of subpopulation number. However, an intermediate number of subpopulations was predicted when alpha = 1, similar in number to results from Rapoport's mean propinquity method and both "1/10th max" grid and buffer.

Table 3.2. Estimates of numbers of subpopulations for species of *Delonix s.l.* using grid adjacency, circular buffer, Rapoport's mean propinquity and alpha hull methods.

	Grid adjacency			**Circular buffer**			**Rapoport's mean propinquity**	**Alpha hull**		
	2 x 2 km	10 x 10 km	1/10th max	5.64 km	10 km	1/10th max		α = 1	α = 2	α = 3
Colvillea racemosa	34	21	3	23	19	4	5	4	3	3
Delonix boiviniana	50	32	3	36	31	4	13	7	2	1
Delonix brachycarpa	9	8	4	9	8	5	3	2	1	1
Delonix decaryi	19	12	3	15	11	8	9	4	1	1
Delonix floribunda	32	17	3	21	15	6	7	6	3	1
Delonix leucantha	15	8	4	11	7	5	3	3	2	1
Delonix pumila	6	1	6	1	1	5	2	2	1	1
Delonix regia	19	10	3	12	7	3	4	3	2	2
Delonix tomentosa	1	1	n/a	1	1	n/a	n/a	n/a	n/a	n/a
Delonix velutina	4	2	2	2	2	2	2	2	1	1
Lemuropisum edule	9	3	4	5	3	5	3	3	1	1

The relationship between number of collection localities and number of subpopulations was tested using linear regression (Table 3.3). Regression analyses show that the fixed grid adjacency and fixed circular buffer methods have a significant positive relationship between number of collection localities and subpopulations ($P<0.001$). This indicates that grid-adjacency and circular buffer methods are closely correlated with the number of collection localities at small radii and grid sizes, often with a single collection in each subpopulation. Rapoport's mean propinquity method and the alpha hull method (alpha = 1) also show a positive correlation between number of collection localities and subpopulations. However, the species-specific methods of "1/10th max" grid, "1/10th max" buffer and alpha hull method where alpha= 2 or alpha = 3 show no significant correlation between number of collection localities and number of subpopulations ($P>0.05$). Table 3.4 summarises the strengths and weaknesses of all methods investigated. It shows that the circular buffer method with the "1/10th max" sliding scale is the most desirable method, as all other methods show some weaknesses.

Table 3.3. Relationship between collection numbers (x) and subpopulation number estimates (y) for *Delonix s.l.* (n=11) using linear regression ($y = ax + b$).

Method	**a**	**b**	**R^2**	**P**
Grid 2 x 2 km	0.62	-0.35	0.92	**<0.001**
Grid 10 x 10 km	0.39	-0.91	0.86	**<0.001**
Grid "1/10th max"	0.00	3.17	0.00	0.59
Circular buffer 5.64 km	0.44	-0.51	0.87	**<0.001**
Circular buffer 10 km	0.36	-1.16	0.85	**<0.001**
Circular buffer "1/10th max"	0.03	3.52	0.12	0.29
Rapoport's mean propinquity	0.14	0.51	0.85	**<0.001**
Alpha hull ($\alpha = 1$)	0.07	1.18	0.91	**<0.001**
Alpha hull ($\alpha = 2$)	0.02	1.02	0.36	0.05
Alpha hull ($\alpha = 3$)	0.01	1.12	0.03	0.59

Table 3.4. Summary of methods to determine subpopulation structure based on GIS analysis of collection data, and how they are affected by the factors listed in the left-hand column. Desirable traits are shown in bold.

Method **Attribute**	**Grid adjacency**	**Circular buffer**	**Rapoport's mean propinquity**	**Alpha hull**
Collection number dependent	Yes (**No "1/10 max"**)	Yes (**No "1/10 max"**)	Yes	**N** (Yes α = 1)
Collection density dependent	**No**	**No**	Yes	Yes
Species-specific scale	No (**Yes "1/10 max"**)	No (**Yes "1/10 max"**)	**Yes**	**Yes**
Grid placement dependent	Yes	**No**	**No**	**No**

3.5. Discussion and Recommendations

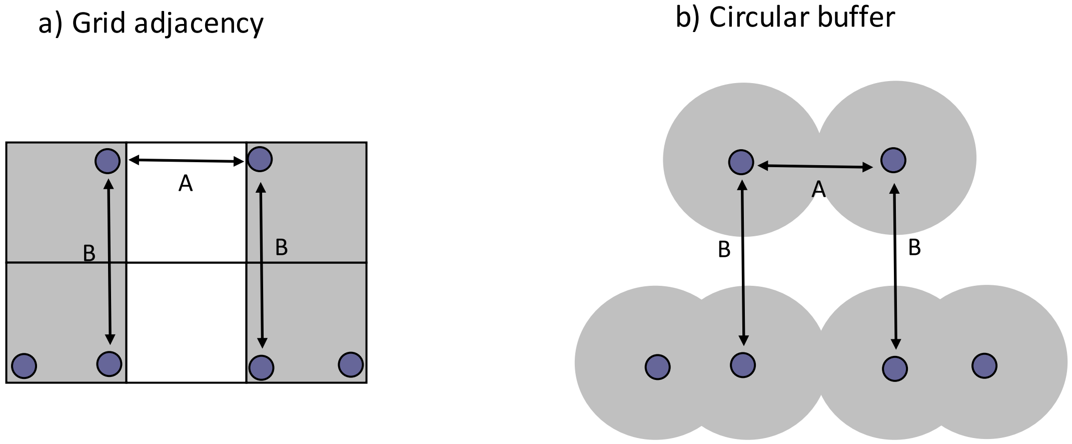
3.5.1. Measures of subpopulations

The use of herbarium specimens to generate IUCN conservation assessments is not new. However, it often relies on range estimates of the extent of occurrence (EOO) or the area of occupancy (AOO), while measures of subpopulation number and fragmentation have only occasionally been addressed (Callmander *et al.* 2007; Good *et al.* 2006; Schatz 2000b; Willis *et al.* 2003). Several comparisons exist of the different measures for calculating range using herbarium specimens (Callmander *et al.* 2007; Hernandez *et al.* 2006; Willis *et al.* 2003). However, there has previously not been a comparative analysis of different measures of the IUCN definitions of subpopulation structure, number of locations or degree of fragmentation. The strengths and weaknesses of all methods examined are summarised in Table 3.4, with detailed discussion of each below.

The grid adjacency method is a simple method widely applied to conservation assessments and recommended by IUCN for range calculations (Callmander *et al.* 2007; Good *et al.* 2006; IUCN 2001; Schatz 2000b). However, both grid size and grid placement are major determinants in the number of resulting subpopulations (Willis *et* *al.* 2003). When the grid size is too small, there may be an overestimate of the number of subpopulations, and when the grid size is too large the result may be an underestimate of subpopulations. With a 2 x 2 km grid, the estimated number of subpopulations is highly correlated to numbers of collections, and species with a higher number of collections are estimated to have a higher number of subpopulations. The 10 x 10 km grid is claimed to correspond better to the average extent of an isolated subpopulation (Callmander *et al.* 2007), but this grid size also shows a strong correlation with number of collections. From this we conclude that herbarium data are often too sparse for these grid sizes. Both 2 x 2 km and 10 x 10 km grids have been recommended in estimating AOO (Callmander *et al.* 2007; IUCN Standards and Petitions Working Group 2008; Schatz 2000b); however, due to their dependence on collection numbers they are not appropriate for the subpopulation measures used in this study. Fixed grid methods such as these generally lead to an overestimation of numbers of subpopulations for widespread taxa simply because collections are spaced further apart; this is avoided using the "1/10th max" method. The size of the "1/10th max" grid is a species-specific measure that assumes that the geographic extent of a species influences its subpopulation structure. It is not dependent on the number of collections, but instead takes into account the geographical spread of species and avoids widespread species having an artificially elevated number of subpopulations, as is seen in both the 2 x 2 km and 10 x 10 km grids. The second major factor affecting the number of subpopulations in the grid adjacency method is the placement of the grid. The grid is placed so as to minimise the number of occupied grid cells (IUCN Standards and Petitions Working Group 2008), and consequently its placement is dependent on the position of all points in the grid. This can lead to points being located at the edges of a grid cell, and two collections may be grouped together even though they are further apart than two collections that are considered to be in separate subpopulations (Figure 3.2a).

The dependency on all points for determining the number of subpopulations is avoided using the circular buffer method. The circular shape and placement of the buffer means the problem of grouping more distant collections over more closely situated collections (Figure 3.2a) is avoided with the circular buffer method (Figure 3.2b). As each point is situated in the middle of its circular buffer, slightly larger estimates of subpopulation numbers are seen when comparing equal-area grids and buffers (buffer of 5.64 km radius and a 10 x 10 km grid). As with the grid method, the circular buffer method is dependent on the choice of radius of the buffer; with buffers of small radii there is a high correlation between the number of collections and the number of subpopulations. The species-specific "1/10th max" buffer assumes that the geographic extent of a species influences its subpopulation structure and avoids widespread species having an artificially elevated number of subpopulations, as is seen with the smaller buffers. If the maximum dispersal distance for a species is known, then this can be used as the radius to mimic biological dispersal capacity.

Figure 3.2. Hypothetical diagram of collecting localities. Despite distance A being shorter than distance B, the grid placement means that the two closer points are put in separate subpopulations in the grid adjacency method (Figure 3.2a), though not with the circular buffer method (Figure 3.2b).



a) Grid adjacency

b) Circular buffer

Rapoport's mean propinquity method is an extension of the "1/10th max" buffer method, also based on species-specific buffers whose size is determined by inter-point distances. It is not surprising, therefore, that the estimates of numbers of subpopulations for both methods are similar. In both methods the placement of the buffer is unambiguous; however, the radius of the buffer in Rapoport's mean propinquity method is not based on maximum geographic range but rather on collection density. If collection density is high, the distances between points are small and therefore the radius of the buffer is small, leading to a greater number of subpopulations. Equally, when collection density is low, the distances between points are large, and consequently the buffer radius is large and the estimated number of subpopulations is small. The sensitivity of Rapoport's mean propinquity method to collection density means that poorly collected species will have fewer subpopulations than well-collected species (of the same geographic distribution). Also, species with a high density of collections from a single area may have an artificially inflated number of subpopulations. For Rapoport's mean propinquity method to work best a sufficient collection density needs to be achieved.

As with Rapoport's mean propinquity method the alpha hull method determines subpopulation structure based on the distances between collection points. However, it takes into account not only the minimum distance between all points, but all connections in the Delaunay triangulation. It is therefore less sensitive to collection density than is Rapoport's mean propinquity method. Alpha hulls have been used to estimate species ranges, but have not previously been used to estimate the number of subpopulations. In range-size estimates alpha hulls are less affected by biases due to the shape of species' ranges, errors in recording locations and sampling effort than are other measures (Burgman & Fox 2003). For the purpose of subpopulation structure, both alpha = 2 and alpha = 3 (as recommended for range studies) predict a very low degree of population subdivision, and alpha = 1 seems to be more appropriate for population subdivision. The number of subpopulations found with alpha = 1 corresponds closely to those found by grid "1/10th max", buffer "1/10th max", and Rapoport's mean propinquity measures. The high similarities between these methods indicate that geographic extent and collection density may have a similar effect on subpopulation numbers.

3.5.2. Recommendations

For species known primarily from herbarium specimens, where little or no data are available with regard to reproductive isolation, the degree of genetic exchange or dispersal ability, we recommend using a circular buffer method. This recommendation is also true for more well-studied species where more data may be available, however, the subpopulation structure may still not be implicit and spatial analysis tools are still of importance. The circular buffer method avoids the problem of point dependency of the grid adjacency method; it also allows points to be added, subtracted or moved without significantly altering the buffer placement. The most appropriate radius of the buffer is a matter of debate: an ideal size might be the maximum dispersal distance of the species. However, when dispersal characteristics are not known, as is the case for most plant species, using a sliding scale is more suitable. The sliding scale of "1/10th max" is both independent of the number of collections and also species-specific and therefore allows an appropriate spatial scale to be used for each species. The IUCN Guidelines recognise the need for species-specific analyses and state that "*methods for determining the number of subpopulations may vary according to the taxon*" (IUCN Standards and Petitions Working Group 2008). A sliding scale such as the one used here therefore allows a species-specific scale to be applied. Although only one sliding scale, "1/10th max", was investigated in this study, it was tested in both the grid adjacency and circular buffer method, where it consistently performed well. For all methods, the biological relevance of purely spatial analyses needs to be investigated, for example through population genetic analysis.

3.5.3. Further application of GIS methods to IUCN terms of population structure – location and fragmentation

Measures of the number of subpopulations can also be used to assess number of locations and degree of fragmentation, the other two terms regarding population structure used in IUCN Red List assessments.

3.5.3.1. Locations

The terms location and subpopulation are often seen together, which leads to confusion as they have independent definitions in IUCN terminology (Box 3.1). The terms are seen together in Criterion B, for example, where continuing decline and extreme fluctuations can be observed in numbers of either subpopulations or locations to fulfil subcriterion b and subcriterion c (Box 3.2). The definition of location requires a threatening event and the number of locations is based on the area covered by this threat and the species. As a location is a distinct geographic area (IUCN Standards and Petitions Working Group 2008), distribution-based geographical methods could therefore be used to estimate population subdivision in terms of number and position of locations. Furthermore, IUCN guidelines state that when parts of a species distribution are not affected by any threats, then one alternative is to set the number of locations in the unaffected areas to the number of subpopulations in those areas (IUCN Standards and Petitions Working Group 2008). This implies that, despite different definitions, numbers of subpopulations and locations are potentially closely linked, and hence we argue that when dealing with species where information is sparse and when threats are affecting the entire species range, estimates of number of locations could be made from a similar analysis of point locality data to that described for subpopulations above. As with subpopulations, it is important to document the method used to estimate the number of locations.

3.5.3.2. Fragmentation

“Severely fragmented” is the third term in the IUCN Red List that deals with population subdivision (Box 3.1). It aims to highlight species that may go locally extinct with a reduced probability of recolonisation. Although the IUCN Red List only needs a binary answer to whether a species is severely fragmented or not, this is not always straightforward. The IUCN definition of a severely fragmented species has two parts; it requires that the majority of a species range consists of both small and isolated subpopulations (see Box 3.1 and Box 3.2).

The IUCN Guidelines state that isolated subpopulations are "*isolated by distances several times greater than the (long-term) average dispersal distance of the taxon*" (IUCN Standards and Petitions Working Group 2008), while definition of a subpopulation already takes into account isolation as having "*less than one successful migrant individual or gamete per year*" (IUCN Standards and Petitions Working Group 2008). For the majority of plant species, annual and long-term average dispersal distances are not known; thus it is difficult to distinguish between "subpopulations" and "isolated subpopulations". The separation of subpopulations alone is not sufficient to qualify as fragmented: in addition, the subpopulations need to be small. If the minimum viable area for a successfully breeding subpopulation is known, then the proportion of subpopulations of a viable size can be calculated from a spatial analysis of the number of subpopulations such as that presented here. This measure can then be used with a measure of the number of isolated subpopulations to determine whether a species is considered severely fragmented. However, the minimum viable area is not known for the majority of plant species.

Geographical analysis of population structure can therefore be useful in providing information on the number of subpopulations, their size and their isolation, for use in estimating severe fragmentation. However, geographical analysis alone cannot be used to assess fragmentation. Further information on minimum viable area, dispersal distance and density are essential in order to correctly follow the rules of the IUCN Red List (Box 3.2). If these factors are known, geographical analysis can aid in estimating both the number and degree of isolation of subpopulations. As more species are being analysed with regard to their genetic diversity across their range, this new information ought to somehow be incorporated into conservation assessments to indicate the degree of genetic fragmentation of a species. Although the IUCN Red List does not directly use such information at present to establish number of genetic subpopulations or degree of fragmentation, it is likely that data of this kind will be increasingly available and increasingly useful in the future.

3.6. Conclusions

A species' range can be divided according to a continuum of different thresholds, the largest being no subdivision of the species, and the smallest being every individual constituting a separate subpopulation; the reality is likely to be found somewhere in between. Based on the limited knowledge we have of many plant species, especially those in the tropics, the use of herbarium collections and GIS techniques for estimating the degree of population subdivision often gives the best available estimates of population structure in these species to use in an IUCN conservation assessment of extinction risk.

Since different methods of analysis can result in widely divergent results, it is crucially important to document the procedures followed in any given case and, wherever possible, to make the underlying dataset available for subsequent re-analysis as new data or techniques become available, and ideally, over time, for procedures to become standardised. In going from pattern-based measures of population isolation and fragmentation to a fuller understanding of the process of extinction, details on habitat availability, dispersal ability, biotic interactions and breeding systems are needed (Hartley & Kunin 2003). In addition, genetic diversity analysis can be of crucial importance for understanding some of these factors. In the present paper, we have focused on spatial models of species' subpopulation structure and their application for IUCN conservation assessments. Our recommendation for assessing subpopulation structure is to use the circular buffer method with a species-specific sliding scale. The next stage is to determine the relationship between these spatial models and the biologically functional subpopulations, and further to address the question of how patterns of fragmentation translate into functional isolation between subpopulations along a species' potential path to extinction. Ideally, genetic analysis would be undertaken for all threatened species to help fill in these gaps in species knowledge. Such population genetic analyses for *Delonix s.l.* are currently under way and will add a functional dimension to the spatial results considered here that can help determine which of the geographical subdivision methods outlined above best correspond to the subdivision revealed by genetic analysis.

Chapter 4

Climate change and extinction risk: the impact on conservation status of legumes in Madagascar[3]

4.1. Summary

It is predicted that climate change will have many adverse effects on the world's biodiversity, but as yet few studies have investigated its potential impact in the tropics, home to the majority of species. Most species distribution modelling of plants have focussed on temperate regions of the world, or on a limited number of well-described and well-sampled floras. The opportunities and problems of species distribution modelling have been well delimited and are understood. There is now an urgent need to extend the species distribution modelling to regions with the highest biodiversity and greatest threat, outside temperate regions and model systems. This is problematic because diverse tropical regions are characterised by a lack of taxonomic knowledge and systematic ecological sampling.

This chapter evaluates the impact of climate change on conservation status for a representative plant family, the Leguminosae, from one of the world's most important biodiversity hotspots, Madagascar – applying the bioclimatic approach to the species-rich tropics. We used herbarium records for 228 species to assess the conservation status as a result of projected climate change. The low number of records for some of the rare and endemic species is off-set against ecologically-defensible patterns that emerge from the analysis of many species with contrasting life-history traits. By combining herbarium specimen data, monographic information, vegetation mapping and species distribution modelling, well-informed and scientifically-supported

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[3] This chapter will be submitted as Rivers, M.C., Brummitt, N.A., Nic Lughadha, E. & Meagher, T.R. Climate change and extinction risk: the impact on conservation status of legumes in Madagascar.

conservation assessments of the impact of future climate change on biodiversity can be developed for a broad spectrum of plant species in the tropics and beyond.

4.2. Introduction

Climate change is already having an impact on biodiversity through shifts in species' geographical distributions, phenology, community composition and species interactions (Fischlin *et al.* 2007; Parmesan 2006; Parmesan 2007; Parmesan & Yohe 2003; Walther *et al.* 2002). These trends are projected to continue and to be exacerbated in the future, and climate change is expected to become one of the major drivers of biodiversity change in the coming decades (Millennium Ecosystem Assessment 2005). Biodiversity across the globe, especially in biodiversity hotspots such as Madagascar, is likely to be severely affected.

Many studies are using species distribution modelling to assess the future impact of climate change on biodiversity. These studies suggest that biodiversity will be affected by range shifts (Midgley *et al.* 2002; Peterson *et al.* 2001), range reductions (Peterson *et al.* 2001; Peterson *et al.* 2002), species turnover (Peterson *et al.* 2002; Thuiller 2004) and species loss (Peterson *et al.* 2002; Thuiller *et al.* 2005). Most species distribution models have focussed on temperate regions of the world (Thuiller *et al.* 2005), or on a limited number of well-described and well-sampled floras in model systems (e.g. the Cape Proteaceae) (Bomhard *et al.* 2005; Midgley *et al.* 2006). Using these model systems, the opportunities and problems of species distribution modelling have been well delimited and the underlying assumptions are understood (Araujo & Pearson 2005; Davis *et al.* 1998; Pearson & Dawson 2003). However, in order to monitor the effects of climate change worldwide, there is an urgent need to extend the bioclimatic approach to regions outside temperate and model systems, examining areas with the highest biodiversity and greatest threat. This extension is problematic because tropical regions of high diversity are often also characterised by a lack of both taxonomic information and systematic sampling for ecological research. Due to the lack of data there have been few studies on the effect of climate change on plant diversity in the tropics. Herbarium specimens are a good source of presence-only distributional data for plant species (Graham *et al.* 2004; Ponder *et al.* 2001; Willis *et al.* 2003), especially when compiled from multiple herbaria (Schatz 2002), and can be used effectively to overcome the shortfall in published range data for tropical plant species. Currently, the conservation status of most plant species is poorly known. From their own figures, only about 4% of plant species worldwide have so far been assessed under IUCN Criteria and appear on the IUCN Red List (IUCN 2010). Herbarium specimens and other available data sources could be used to increase the knowledge of tropical plant diversity and to produce reliable conservation assessments. In such a way informed conservation decisions can be made in critical tropical ecosystems.

In this study, we translate results from species distribution modelling into conservation status following the IUCN Red List Categories and Criteria (IUCN 2001). We present our results in the context of the IUCN Red List as it is: (i) widely recognised as the international standard for assessing conservation status, (ii) comparable across taxonomic groups, and (iii) used extensively in setting conservation priorities by policy makers and other stakeholders. The IUCN Red List aims to be applicable to a wide range of taxa, using quantitative criteria and pre-defined thresholds to assess extinction risk. Thus, it can be used to compare future effects of climate change on biodiversity under differing biological circumstances. New developments in GIS, publically available data, and improved resolution of available bioclimatic datasets, increases the capabilities of using species distribution modelling to predict the present, past and future ranges of species (Elith *et al.* 2010; Guisan & Thuiller 2005; Heikkinen *et al.* 2006; Nogués-Bravo 2009), which in turn can inform IUCN conservation assessments.

Madagascar, the focal region for this study, is classified as one of the world's biodiversity hotspots based on the number of endemic species and loss of natural vegetation (Brummitt & Nic Lughadha 2003; Mittermeier *et al.* 2005; Myers *et al.*

2000). Species endemism in Madagascar reaches over 80% among many invertebrate and vertebrate animal groups (Goodman & Benstead 2005) as well as several plant groups, such as palms (Dransfield & Beentje 1995), orchids (Hermans *et al.* 2007) and legumes (Du Puy *et al.* 2002). However, Madagascar has a rapidly increasing human population and its unique biodiversity is under severe threat from habitat destruction, over-exploitation and erosion. A recent vegetation mapping project estimates that only 18% of primary vegetation still exists (Moat & Smith 2007), and many species are in need of effective conservation action. One of the biggest threats to biodiversity in Madagascar is anthropogenic habitat fragmentation (Ganzhorn *et al.* 2001; Harper *et al.* 2007). The already-fragmented nature of the remaining vegetation and the restricted connectivity between these primary vegetation fragments present a severe barrier to future dispersal, which means that Madagascar biodiversity is potentially at even further risk due to climate change.

In total there are approximately 12,000 species of vascular plants in Madagascar (Schatz 2000a), of which 386 (3%) are currently on the IUCN Red List (IUCN 2010). A recent monograph lists a total of 459 endemic species of Leguminosae in Madagascar (Du Puy *et al.* 2002). Leguminosae is the world's third largest angiosperm family and is well represented in tropical, temperate, dry and wet habitats (Lewis *et al.* 2006). The family has also been shown, at both a generic and specific level, to be representative of global patterns of angiosperm diversity (Nic Lughadha *et al.* 2005). It was therefore chosen as the focal sample for species to use for this study, as the results may be indicative of impacts on plant species more generally.

4.2.1. Objectives

This chapter examines the potential impact of climate change on plants in Madagascar. We use species distribution modelling of Leguminosae species to assess their conservation status as a result of projected climate change as of the year 2100. Conservation status for each species is determined using two complementary measures of species range – percentage range reduction and absolute range size. We also evaluate the usefulness of herbarium records in producing robust conservation assessments in the face of predicted climate change. We address three key questions: (1) How does projected climate change affect the conservation status of the endemic Leguminosae of Madagascar? (2) How do different dispersal scenarios affect future conservation risk? (3) Which vegetation types are likely to be most at risk?

4.3. Material and Methods

4.3.1. Species information

The species locality data used in this study come from digitised herbarium specimens predominantly from herbaria at the Royal Botanic Gardens, Kew (K), the Museum National d'Histoire Naturelle, Paris (P) and the Missouri Botanical Garden (MO). We estimate that collections of Leguminosae from K, P and MO encompass 94% of all existing legume collections from Madagascar (Chapter 1). Our initial data set comprised 10,030 specimen records covering 459 endemic species (Chapter 1, Figure 1.4). Following the recommendations of Pearson *et al.* (2007), we restricted this study to those species with five or more data points to achieve modelling accuracy, which left 7,142 specimens and 228 species for the full analysis (see Supplementary Table 4.A for a list of species). Estimates of sampling intensity for the Malagasy Leguminosae (Chapter 2) strongly suggest that species with as few as five localities reflect actual rarity rather than sampling bias. However, out of concern that five localities was a small number for modelling range size, we also compared the results from differing numbers of minimum sample sizes for range and range loss estimation to test for the trade-off between including species with low sample sizes to encompass rare species as opposed to including only species with higher sample sizes to achieve greater statistical power.

4.3.2. Modelling technique and validation

After some initial testing with a subset of the data, the geographical distributions of species of Leguminosae from Madagascar were modelled relating species occurrence points to environmental variables using Maxent 3.02 (Phillips *et al.* 2006). Maxent is a software package that uses maximum entropy to find the most uniform probability distribution from presence-only data, constrained by the approximated environmental conditions at the presence locations of the species. Maxent uses presence-only data and has performed well in several comparisons with other ecological niche modelling programs (Elith *et al.* 2006; Hernandez *et al.* 2006; Hijmans & Graham 2006; Pearson *et al.* 2007). It was also the preferred approach for a recent analysis to set conservation priorities in Madagascar (Kremen *et al.* 2008).

Our model included 21 environmental variables relating to temperature, precipitation, topography and geology (see Supplementary Table 4.B). All variables were re-sampled to an oblique Mercator projection at 2.5 arcminutes resolution (approx. 4.5 km in Madagascar). Nineteen climatic variables were extracted from the WorldClim database, a set of global climate layers generated through interpolation of climate data from weather stations (Hijmans *et al.* 2005). Since species distributions are not solely governed by climatic variables, in both present and future distribution modelling elevation (GTOP030) and geology (Du Puy & Moat 1996) were also included. The Madagascar geology map was digitized from Besaire’s (1964) geology map; the 96 original categories were reclassified into ten predominant rock types which have an important effect on the vegetation they support (Du Puy & Moat 1996).

For each species, Maxent was run five times, each time randomly selecting 75% of the presence data to train the Maxent algorithm and 25% of the presence data to test the predicted distribution. Maxent default values for the convergent threshold (0.0001), maximum number of iterations (500), and maximum number of background points (10,000) were employed. A frequently used species distribution modelling validation technique is the area under the curve (AUC) from the receiver operating characteristic (ROC) curve plot, a threshold independent measure, as recommended by Fielding and Bell (1997). Species with an average AUC of less than 0.7 over the five Maxent runs were disregarded, as there is low confidence in the accuracy of these predictions

(Swets 1988). AUC is not an absolute measure and is sensitive to the method by which absences in the evaluation data are selected (Lobo *et al.* 2008). To avoid inflated AUC values we limited our evaluation data to include absence points only from Madagascar. In addition, some of the predicted present-day distributions were successfully used in a field survey in Madagascar to identify areas of potential occupancy and then locate populations that had not been previously reported. This ground truthing exercise demonstrates the validity of the techniques employed in this study.

4.3.3. Predicted distribution maps

For the 228 species with an average AUC above 0.7, Maxent was run again using all available collection records to produce the maps of present-day predicted distribution. Using the known climatic associations from the present-day distribution maps, future distribution maps for each species were modelled using a CCM3 global climate model that simulates conditions in the year 2100 at doubled atmospheric levels of CO_2 (2 x 355ppm) (Govindasamy *et al.* 2003), downloaded from http://www.diva-gis.org/climate.htm. This was one of the highest spatial resolution datasets available (Hijmans & Graham 2006) at the time of this analysis, and it was the only dataset available for all the 19 climatic layers. It is also a model with estimates of CO_2, CH_4 and N_2O that correspond to a mid-range value of those estimated in the four scenarios by the Intergovernmental Panel on Climate Change (IPCC 2000).

The predicted maps produced by Maxent show the climatically (as well as geologically and topographically) suitable area for each species at the present day and in 2100. The output from the Maxent analysis gives a continuous map showing the relative suitability of a species across the whole range of the analysis (Madagascar). To obtain a presence/absence map, the lowest predicted value associated with any of the observed presence records (minimum presence threshold) was applied as a threshold (Liu *et al.* 2005; Pearson *et al.* 2007). This threshold is species-specific; however, for each species the same threshold was used for both present-day and future maps. Due to the high level of habitat fragmentation in Madagascar, it is unrealistic to assume that a species is occupying all of its climatically suitable range. To more accurately represent the species ranges, the present-day predicted distribution map was clipped to its known vegetation type for each species, according to the Madagascar Vegetation Atlas (Moat & Smith 2007).

In order to address a species' potential dispersal ability, three separate future prediction maps were produced. The first scenario assumes that a species is unable to disperse into any new habitat (No Dispersal). The second scenario assumes that a species is able to disperse outside its present-day range, so long as the new locality is of its known preferred vegetation type (Specific Vegetation Dispersal). The third scenario assumes that a species is able to disperse to any primary vegetation, regardless of whether or not this matches its preferred vegetation type (Full Dispersal). We assume that the vegetation types in 2100 are in the same area as at present, as in many instances the boundary of vegetation types are formed by geological features (Du Puy & Moat 1996), which are unlikely to shift over a 100 year time frame. The No Dispersal and the Full Dispersal predictions represent the worst case and best case scenarios for each species, while the Specific Vegetation predictions offer an intermediate scenario.

4.3.4. IUCN conservation assessments

IUCN conservation status was determined using the IUCN Categories and Criteria (version 3.1) (IUCN 2001). A species' conservation status is established based on any or all of five quantitative criteria (A-E). Although the IUCN Categories and Criteria were developed before climate change impacts on species were widely recognized, they remain effective for identifying species that are undergoing declines in range or population size (Foden *et al.* 2009). A new initiative aimed at examining how the IUCN Red List Criteria can be used for identifying the species most at risk from climate change is currently underway (Foden *et al.* 2009). Until then, recent publications recommend that taxa under threat from current and projected global climate change may be assessed under Criteria A and E and in certain cases under Criteria B and C

(Akçakaya *et al.* 2006; IUCN Standards and Petitions Working Group 2008). In this study the future conservation status was assessed using Criterion A and Criterion B of the IUCN Red List, which assess population reduction and geographic range (see Table 4.1). The other Criteria (C, D and E) were not used, as appropriate data for each species were not available. Evaluated species that qualify for a threatened status are listed as Critically Endangered, Endangered or Vulnerable, while species close to qualifying for a threatened status can be listed as Near Threatened. A species need only fulfil one of the five criteria to qualify for a threatened category; when several criteria are met resulting in different status assessments, the precautionary principle is applied and the most threatened category should be assigned (IUCN 2001).

Table 4.1. Thresholds set by the IUCN Categories and Criteria (2001) with regards to population reduction (Criterion A2) and geographic range (Criterion B, AOO and EOO) for determining categories of threat. The thresholds for the Near Threatened category were set by Moat (2007).

		Threatened			
	Extinct	**Critically Endangered**	**Endangered**	**Vulnerable**	**Near Threatened**
Criterion A2	100%	>80%	>50%	>30%	n/a
Criterion B – AOO	0 km^2	<10 km^2	<500 km^2	<2000 km^2	<4500 km^2
Criterion B – EOO	0 km^2	<100 km^2	<5000 km^2	<20 000 km^2	<45 000 km^2

IUCN Criteria A and B

Both Criteria A and B can be used to assess species conservation status based on estimates of range. Range, according to IUCN, is measured either as Area of Occupancy (AOO, defined as the area which is occupied by a taxon, excluding cases of vagrancy, at a scale appropriate to the taxon), or as Extent of Occurrence (EOO, defined as the smallest polygon in which no internal angle exceeds 180^{o} and contains all sites of occurrence) (IUCN 2001). Using the Spatial Analyst extension in ArcGIS software (ESRI

ArcGIS Version 9.2), AOO was calculated as the area of the predicted species distribution; EOO was calculated as the area of the minimum convex hull.

Criterion A is designed to highlight taxa that have undergone a significant reduction in population size, which may be based on a decline in range. In this study the percentage change in AOO from the present day to 2100 for each of the three dispersal scenarios was calculated, and species were grouped according to the following thresholds: 100% decline; >80% decline; >50% decline; >30% decline; >0% decline and gain/no decline (Table 4.1).

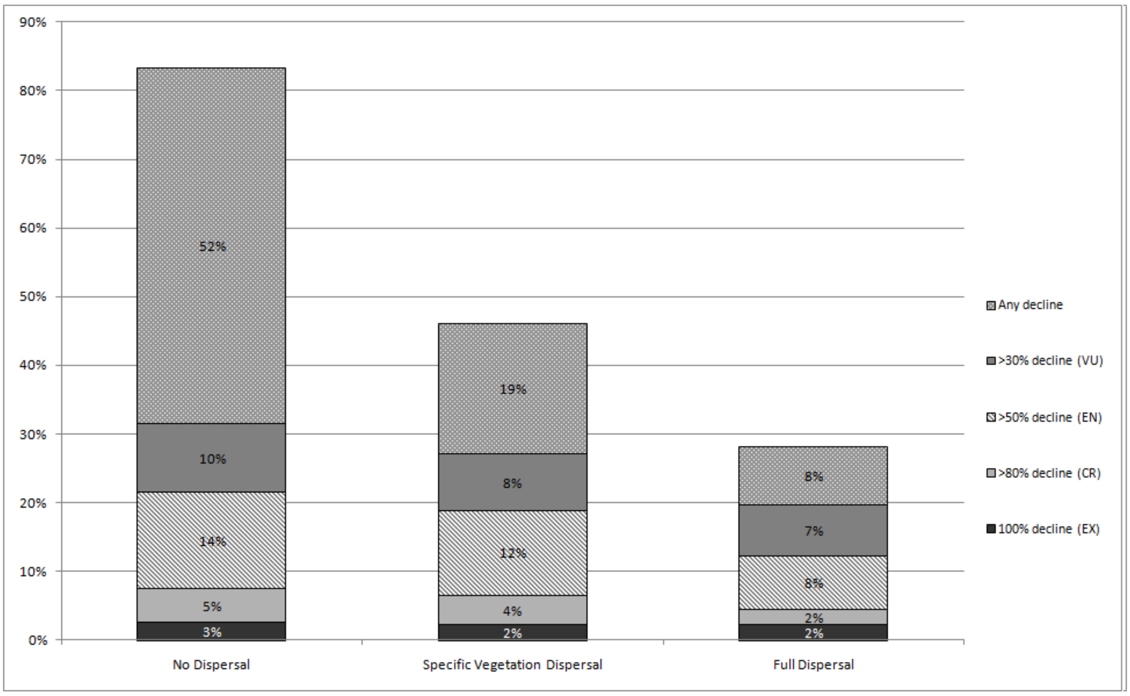
Under Criterion B, estimates of AOO and EOO are used to assess species' future conservation status in 2100 according to thresholds set by IUCN (Table 4.1). A species is considered Extinct when no climatic, geological and altitudinally suitable area remains for a species. The thresholds set for the Near Threatened category are those used by Moat (2007) at 4,500km^2 for AOO and 45,000km^2 for EOO. Species with an AOO larger than 4,500km^2 or an EOO larger than 45,000km^2 are considered Least Concern. Our estimates of future conservation status based on Criterion B should be considered preliminary assessments, as a restricted range alone is not sufficient to list a species under Criterion B. Criterion B also requires that range estimates indicate at least two of the following: severe fragmentation or low number of locations, continuing decline and/or extreme fluctuations in range size (Box 1.1, Chapter 1).

4.4. Results

4.4.1. Red List status based on predicted range reduction (Criterion A)

Using the change in predicted habitable range for the three future dispersal scenarios, the conservation status based on inferred future population reduction was assessed. Figure 4.1 shows the percentage of endemic legumes with 100% (EX), >80% (CR), >50% (EN), >30% (VU) or any decline in range under the three different dispersal models. 83% of endemic legumes are predicted to show a decrease within their present range due to climate change alone (No Dispersal, Figure 4.1). When potential dispersal into known preferred vegetation (Specific Vegetation Dispersal) or into any primary vegetation is taken into account (Full Dispersal), 46% and 28% of species respectively are expected to show an overall decline in range (Figure 4.1). Overall, 19-32% of legumes show sufficient range reduction to qualify for a threatened category (CR, EN, VU) of which 2-3% are predicted to lose their entire range.

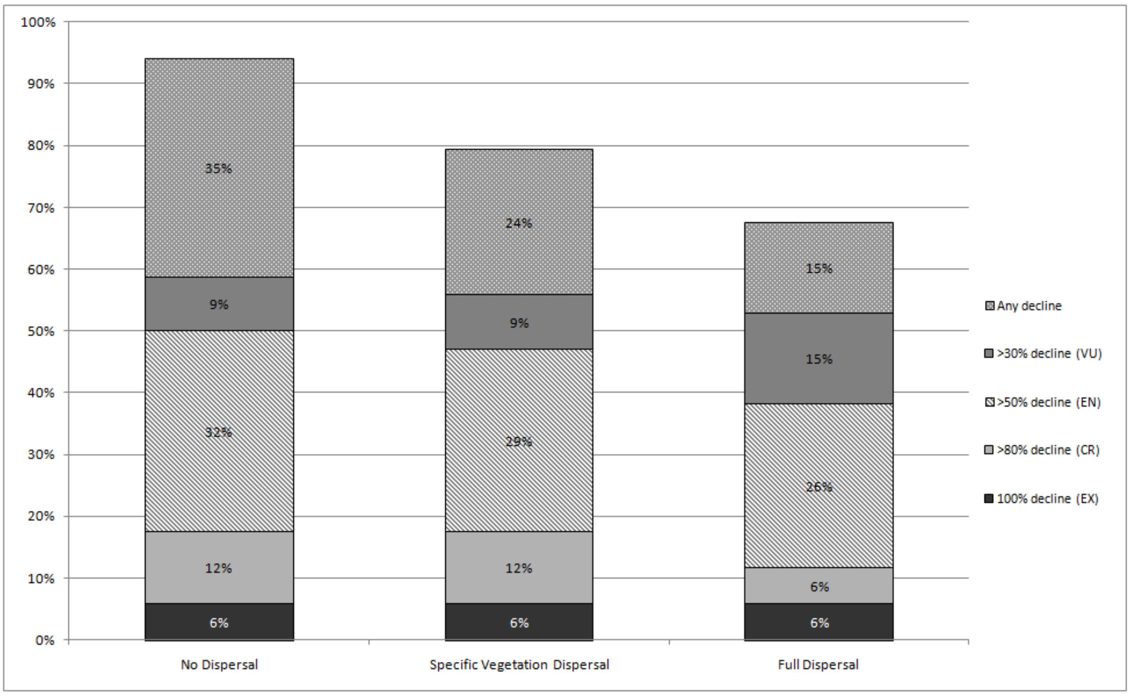
Figure 4.1. The percentage of endemic species of Leguminosae from Madagascar (n=228) showing any decline, >30%, >50%, >80% or 100% decline in range size, for the three dispersal scenarios.



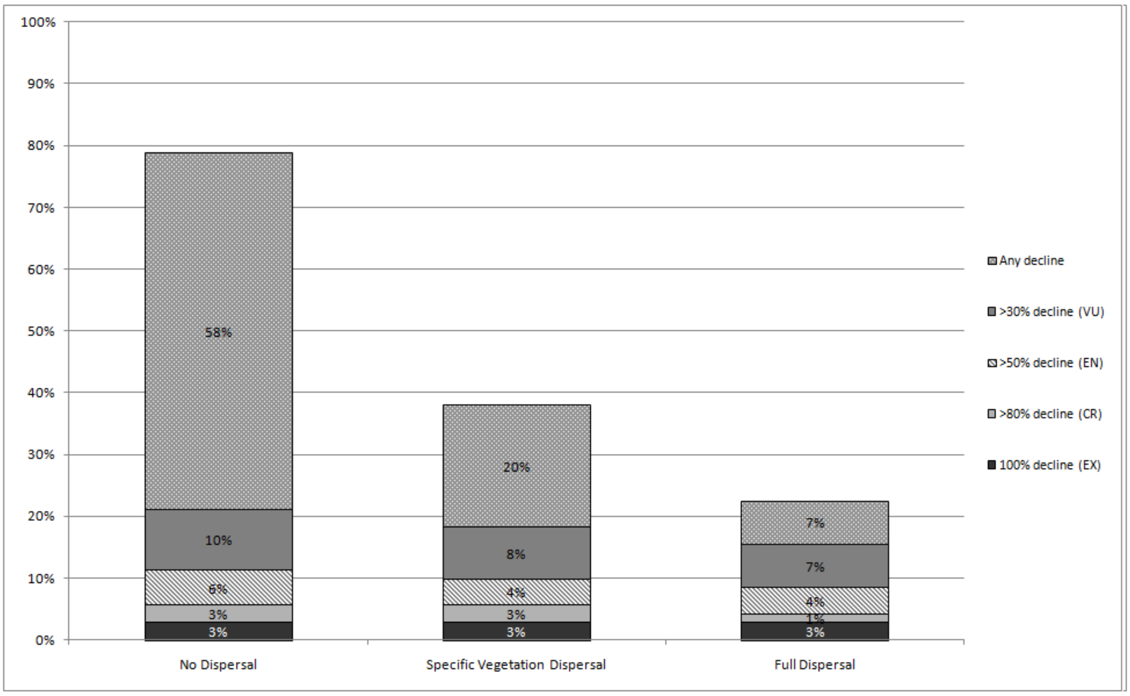
However, not all vegetation types in Madagascar are equally affected by the threat of climate change. One of the most threatened vegetation types is the humid forest, where more than half of the endemic legume species qualify for a Threatened or Extinct conservation category under all three dispersal scenarios (Figure 4.2a). In contrast, the species of the western dry forest are less threatened, with only one in five species qualifying for a Threatened or Extinct category (Figure 4.2b). However, over 79% of species in the western dry forest still show a decline in their present range. Similarly, 71% of the species in the spiny dry forest show a decline in range, and one in four species qualifies for a Threatened or Extinct category (Figure 4.2c). One might expect species found in two or more vegetation types to be more resilient to climate change as these species are less specialised in their habitat selection. However, results show that up to one third of these species still qualifies for a Threatened or Extinct category (Figure 4.2d), the same as the average (Figure 4.1). This is especially true in the No Dispersal scenario. For the two other dispersal scenarios there is a slight trend towards species found in two or more vegetation types doing better than species of a single vegetation type.

Figure 4.2. The percentage of species of Leguminosae of Madagascar showing any decline, >30%, >50%, >80% or 100% decline in range size, for the three dispersal scenarios, endemic to: a) the humid forest (n=34), b) the western dry forest (n=71), c) the dry spiny forest (n=41), d) more than one vegetation type (n=78).

a)



b)


100%
90%
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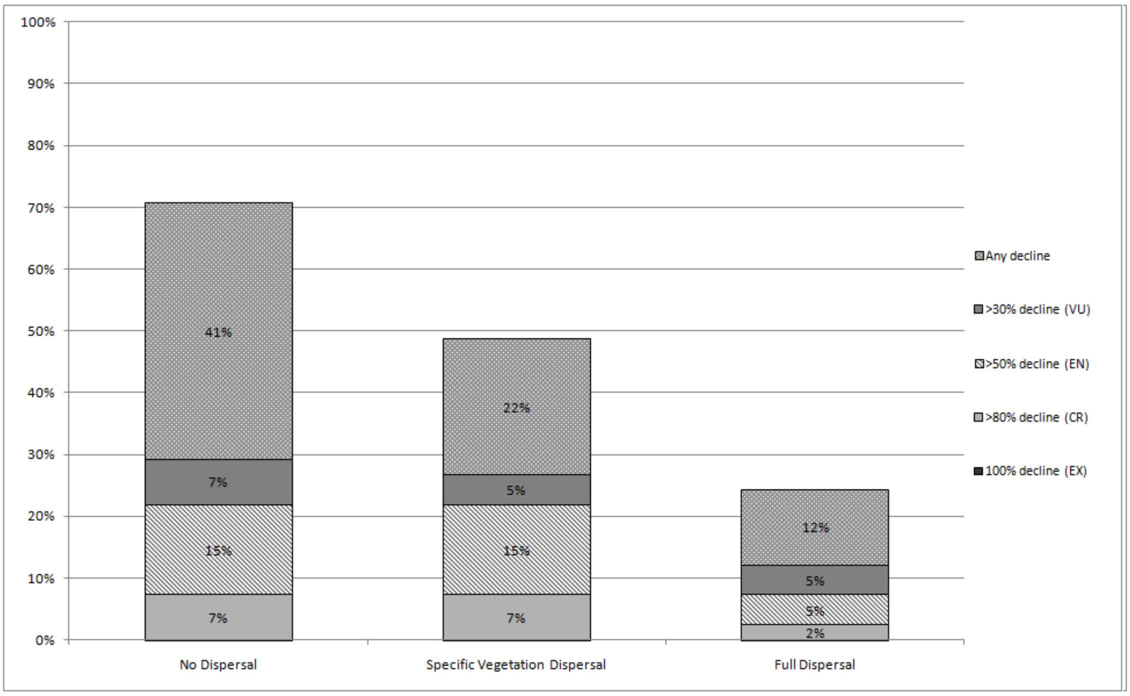
70%
60%
50%
40%
30%
20%
10%
0%
58%
10%
6%
3%
3%
20%
8%
4%
3%
3%
7%
7%
4%
1%
3%
No Dispersal
Specific Vegetation Dispersal
Full Dispersal
Any decline
>30% decline (VU)
>50% decline (EN)
>80% decline (CR)
100% decline (EX)

c)


100%
90%
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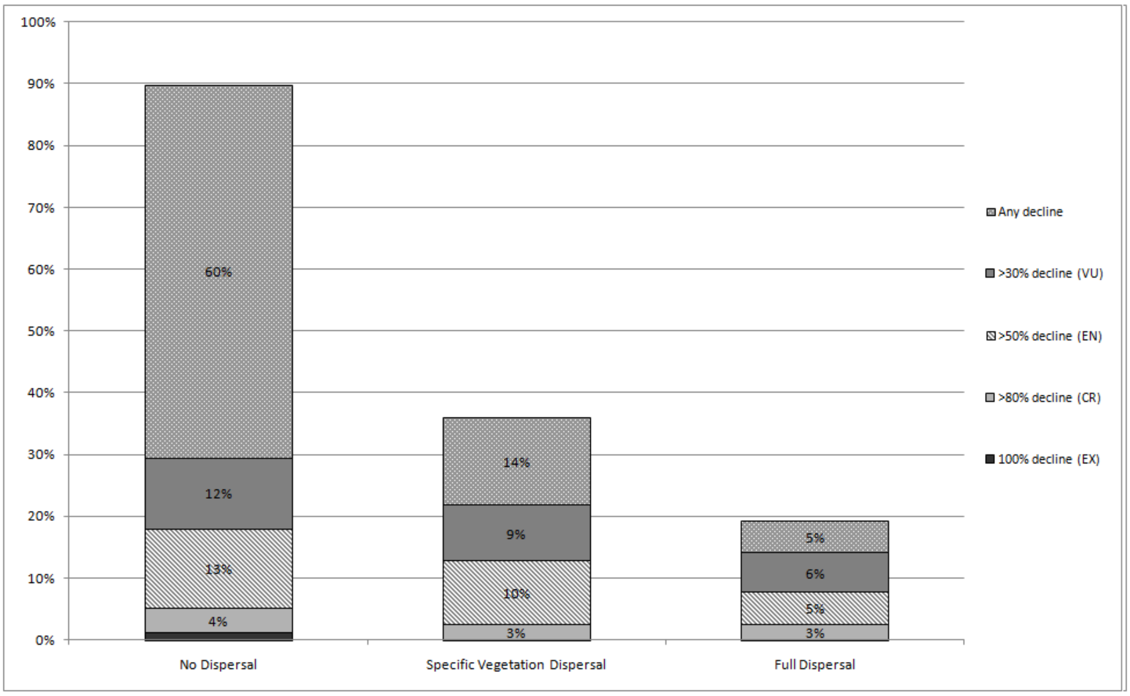
20%
10%
0%
41%
7%
15%
7%
22%
5%
15%
7%
12%
5%
5%
2%
No Dispersal
Specific Vegetation Dispersal
Full Dispersal
Any decline
>30% decline (VU)
>50% decline (EN)
>80% decline (CR)
100% decline (EX)

d)



4.4.2. Preliminary Red List status based on geographical range (Criterion B)

Table 4.2 shows the percentage of endemic legumes, under predicted future climate change by 2100, classified as Extinct, Threatened, Near Threatened or Least Concern based on preliminary Red List status under Criterion B. Six-11% of endemic legumes fall into a Threatened category based on measures of AOO, and 2-9% of species are Threatened based on EOO measures, depending on the dispersal scenario. EOO measurements give a smaller number of Threatened species, as loss of suitable habitat within the centre of distributions is not detected. When combining the threatened species from either AOO or EOO measurements, 7-15% of species will fall into a future Threatened category based on geographical range (Criterion B). Of these, one third of species qualify for a future Threatened category based both on their AOO and EOO measurements, and two thirds qualify based on one or other of the measures (AOO or EOO).

Table 4.2. The percentage of species classified as Extinct, Threatened, Near Threatened or Least Concern based on future conservation assessment under Criterion B, for estimates of Area of Occupancy (AOO), Extent of Occurrence (EOO) and the Criterion B preliminary assessment (the most threatened rating under either AOO or EOO). The variation in each category reflects the difference of the three dispersal scenarios.

	AOO	EOO	Criterion B – Preliminary assessment
Extinct	2% - 3%	2% - 3%	2% - 3%
Threatened	6% - 11%	2% - 9%	7% - 15%
Near Threatened	4% - 11%	7% - 8%	6% - 11%

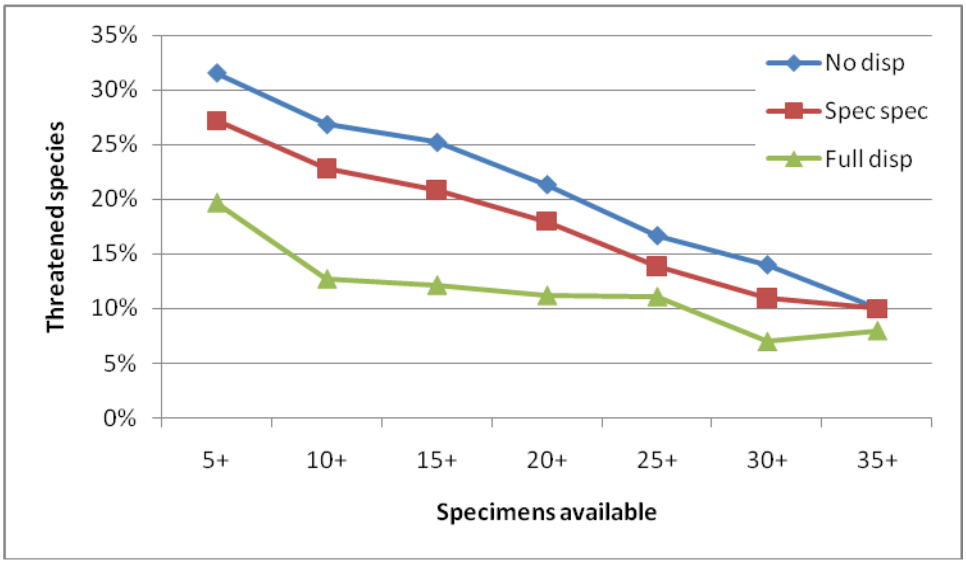
4.4.3. Precautionary principle

In summary, by applying the precautionary principle and using the most threatened ratings from both Criteria A and B, up to 79 species (35%) are predicted to become Threatened, and six species (3%) are expected to lose their entire climatically-suitable range if they cannot disperse beyond their current position (No Dispersal scenario) (Table 4.3). Many species are Threatened using both Criteria A and B; however, more species are listed as threatened under Criterion A than those listed under Criterion B (Table 4.3). This is due to species with a significant decline in habitat (>30%) being listed under Criterion A, but which do not qualify for Criterion B if the remaining predicted range is >2,000km^2 (AOO) or >20,000km^2 (EOO). A smaller proportion of species show the opposite pattern. These are range-restricted species that may show a reduction in range of less than 30% and therefore do not qualify for Criterion A, but the predicted range is small enough to qualify for Criterion B. This emphasizes the need to use both criteria, as range reduction or range size alone does not identify all the species at threat from climate change.

A comparison of the level of threat for different minimum sample sizes is seen in Figure 4.3. The percentage of threatened species declines continually with increasing specimen availability under Criterion A (Figure 4.3a). Under Criterion B, the threatened rates remain more or less stable for species with 10 to 25 specimens (no species with more than 25 specimens qualified for a threatened rating using Criterion B) (Figure 4.3b).

Figure 4.3. The change in percentage of threatened (and extinct) species in response to specimen availability for a) Criterion A and b) Criterion B.

a)



b)

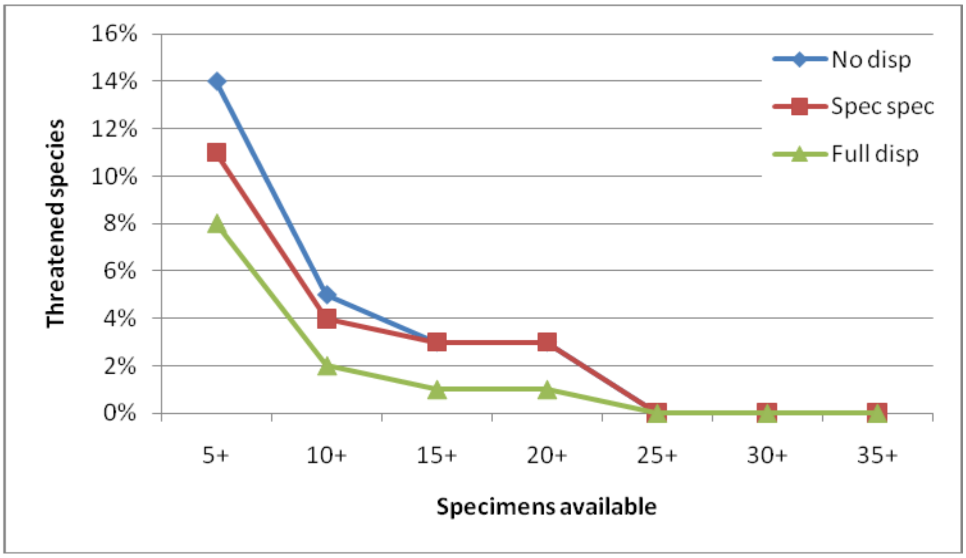


Table 4.3. Summary of the percentage of species classified as Extinct, Threatened, Near Threatened or Least Concern based on Criterion A (A), Criterion B (B) and the Precautionary Principle (PP).

	No Dispersal			**Specific Vegetation Dispersal**			**Full Dispersal**		
	A	B	**PP**	A	B	**PP**	A	B	**PP**
Extinct	3%	3%	**3%**	2%	2%	**2%**	2%	2%	**2%**
Threatened	29%	15%	**35%**	25%	11%	**27%**	18%	7%	**19%**
Near Threatened	n/a	11%	**5%**	n/a	9%	**5%**	n/a	6%	**4%**
Least Concern	68%	71%	**58%**	73%	78%	**66%**	80%	85%	**75%**

4.5. Discussion

These results suggest substantial impacts of future climate change on plant biodiversity. In 2100, due to climate change alone, 83% of 228 species are predicted to show some decline in their present range; up to one in three species are considered Threatened, and 2-3% of species are predicted to have gone extinct. Similar figures of extinction risk and declining ranges have been found in a study of Proteaceae species in the South African Cape Floristic Region where 2% extinction over 227 taxa was predicted, and 57-72% of species were predicted in the Threatened and extinct categories (Bomhard *et al.* 2005). An Australian study of *Banksia* (Proteaceae) predicted 5-25% extinction and 66% of species to have declining ranges (Fitzpatrick *et al.* 2008). In a study of European plants (Thuiller *et al.* 2005) approximately one quarter of species in the best case scenario and up to two thirds of species in the worst case scenario qualified for a Threatened category. In addition, in a recent field-survey the current models also successfully identified areas of potential occupancy where populations were located that had not been previously reported.

4.5.1. Usefulness of herbarium specimens in making conservation assessments

These studies all demonstrate the usefulness of using all available information on plants to assess the risk of climate change on plant diversity. In this case extensive collections of georeferenced herbarium specimens are used as the data source. Herbarium specimens provide us with the best available data for many plant species and have previously been used successfully to assess conservation status (Golding 2004; Willis *et al.* 2003). However, here we extended methods for herbarium specimen data to be used in species distribution modelling by combining herbarium data with monographic information, vegetation maps and GIS modelling. Herbarium specimens clearly have some pitfalls, as data are seldom collected for specific species distribution modelling purposes. However, the strengths are in their numbers and their increased availability. For many rare, tropical species there are no other data available. It is important and necessary to start using this specimen data as a source as there is not enough time to wait for all species to be systematically sampled. With digitisation projects underway in many herbaria around the world, the next step is to bring together and utilise these data to make more informed conservation decisions.

4.5.2. Number of collections

In order to ensure that the geographical distribution of species could be accurately modelled, only species represented in our dataset by at least five distinct georeferenced localities were included in the study; a threshold of five specimens has been recommended previously in published analyses (Pearson *et al.* 2007; Raes & ter Steege 2007). Many species of Malagasy Leguminosae are known from very few collection points (Figure 1.4), a situation that prevails in other tropical regions and plant families (Haripersaud *et al.* 2010; see Chapter 2). In some cases, a small number of collections might be an artefact of biased collection strategies. However, it could also reflect a biological property of the species. In this case, ignoring species with a small number of collections would result in a biased analysis, as many of the rarest and most spatially-restricted species are included in this category. By excluding species with fewer than five unique points, there is already some bias towards wider-ranged species, as is seen by the fact that no species qualifies for the Critically Endangered category in Criterion B (0-10km^2 for AOO and 0-100km^2 for EOO). The omission of these narrow endemics from the analysis means that the impact of climate change on Madagascar legumes is likely to be even larger. In addition, the percentage of threatened species declines continually with increasing specimen availability under

Criterion A (Figure 4.3a), which suggests that species with fewer specimens are more affected by declining ranges due to climate change than those species with more specimens. Under Criterion B, the threatened rates remain more or less stable for species with 10 to 25 specimens (no species with more than 25 specimens qualified for a threatened rating using Criterion B) (Figure 4.3b). This suggests that specimen availability is not related to the threat of climate change, when assessing absolute range. In summary, our statement that species with low numbers of specimens are likely to be more at risk of climate change holds as species with lower specimen numbers are more affected by declining ranges due to climate change (Criterion A); however, beyond ten specimens, specimen availability does not affect future range size (Criterion B).

4.5.3. Variation between vegetation types

Climate change will significantly increase the extinction risk for endemic legumes from Madagascar, however, species are not uniformly affected. Overall, species show variation in response in both their magnitude and direction, which is consistent with other studies (Bomhard *et al.* 2005; Fitzpatrick *et al.* 2008; Parmesan 2007; Parmesan & Yohe 2003). This study, however, did not only look at the individual species responses from climatic modelling, but gains strength from combining the effect of individual species' responses. It off-sets the low number of records for certain rare and endemic species against patterns which emerge from the analysis of vegetation types. It also shows that species are not uniformly affected across vegetation types. Species from the humid forest appear to be more affected by reduction in range than species from other primary vegetation types across Madagascar, while species from the dry forest and the spiny forest are less affected. This could be due to the drier conditions predicted for the humid forest, while the dry forest climate is predicted to remain more stable, as is found by Tadross *et al.* (2008). Their study shows the greatest warming in the south and less so in the north; rainfall is predicted to decrease on the humid southeast coast. The littoral forest only had a single endemic species in this analysis; it showed a strong decline in range, which agrees with the reported threat to this vegetation type (Consiglio *et al.* 2006).

4.5.4. Effect of dispersal

There is no guidance or recommendation on how to estimate dispersal for IUCN conservation assessments (IUCN 2001; IUCN Standards and Petitions Working Group 2008). Many studies, when the dispersal ability of a species is unknown, model two extreme scenarios: no dispersal and full dispersal (for example Malcolm *et al.* 2006; Thomas *et al.* 2004; Thuiller *et al.* 2005). Where the dispersal ability of a species is known, an average dispersal rate over time can be used (Fitzpatrick *et al.* 2008; Midgley *et al.* 2006). However, as there were no data on specific dispersal rates for the 228 species modelled here, three different scenarios were used: No Dispersal, Full Dispersal and Specific Vegetation Dispersal. The latter is modelled using the known preferred vegetation type for the species detailed in recent monographic studies (Du Puy *et al.* 2002) and recent vegetation mapping work (Moat & Smith 2007) as the limit to dispersal. As expected, the three different dispersal scenarios showed slightly different results: the No Dispersal scenario consistently results in the highest extinction risks, the Full Dispersal scenario always results in the lowest estimates of extinction risks and the Specific Vegetation Dispersal scenario shows intermediate results (Figures 4.2 and 4.3). Using three dispersal scenarios gives a range of the potential best and worst estimates of the effects of climate change, without knowing the specific dispersal capabilities of each species (as is the case for most tropical plant species). We recognise that the Full Dispersal scenario is likely an overestimate of possible dispersal, but it shows the lower end of estimated extinction risks if all of today’s primary vegetation remains intact and can be utilised. The No Dispersal scenario, however, could also be an overestimate of potential habitat as essential associated biotic interactions may have been displaced by climate change (Pearson & Dawson 2003). The Specific Vegetation Dispersal scenario allows for the fact that species often have a preference for certain vegetation types, and within those it may be more likely that important biotic relationships are maintained.

Interestingly, despite using three different dispersal scenarios, the number of species falling into a Threatened category is fairly constant in each. This is due to species in the Threatened categories generally having contracting ranges so that they may be less affected by the ability to disperse beyond their ranges. We recognise that dispersal and migration capability is a complicated subject, but in agreement with Fitzpatrick *et al.* (2008) this study shows that numbers of species falling into Threatened categories do not differ strongly under different dispersal scenarios. However, it is important to keep in mind that current and future habitat destruction and fragmentation will seriously impede not only any future potential dispersal of species in Madagascar, but also the survival of a species within its present range. Ongoing habitat destruction is probably an even bigger and more immediate threat to biodiversity in Madagascar than climate change. In addition, land use change is likely to intensify climate change impacts (Bomhard *et al.* 2005; Travis 2003). Notwithstanding this, for the purposes of this study no further habitat destruction and fragmentation has been assumed, despite it being the biggest threat to biodiversity in Madagascar today.

4.6. Conclusions

This study highlights the threat that climate change poses to biodiversity in Madagascar, but more specifically it puts the effects of climate change into the context of comparable, quantified conservation assessments, which are often the preferred way for policymakers and stakeholders to prioritise species in need of conservation. Although some model systems have been explored and studied in tropical regions, there is now a need to extend this further, and especially to include the regions of the world with highest biodiversity. In saying this, there may be uncertainties in areas such as species life-history, dispersal rates, or biotic interactions, and extrapolation of results beyond the study taxa and study area needs to be done with caution.

In this study we have incorporated the best available data for all the plant species analysed: herbarium specimens from the three largest repositories of Malagasy legume specimens, monographic information, vegetation maps and GIS modelling to produce scientifically-supported conservation assessments of the threat of future climate change. The method presented here can be used for species of other plant families or in other regions of the world to help make informed conservation decisions and ensure the survival of plants and their associated ecosystems. In addition, the results from the three different dispersal scenarios of this study indicate that if we are to mitigate the risk presented to species' by inevitable climate change it is of utmost importance to protect their available habitat today. Habitat destruction and fragmentation have been identified as the main current drivers of biodiversity change and if allowed to continue these threats will have far-reaching effects on the ways in which plants will be able to disperse and adapt to the changing climate.

4.7. Supplementary material

Supplementary Table 4.A. List of species assessed and result of assessment based on Criterion A (A), Criterion B (B) and Precautionary Principle (PP) for all three dispersal scenarios.

Species	No Dispersal			Specific Vegetation Dispersal			Full Dispersal		
	A	B	PP	A	B	PP	A	B	PP
Abrus aureus	LC	LC	LC	LC	LC	LC	LC	LC	LC
Abrus madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Abrus sambiranensis	CR	VU	CR	CR	VU	CR	EN	NT	EN
Acacia bellula	LC	LC	LC	LC	LC	LC	LC	LC	LC
Acacia hildebrandtii	CR	VU	CR	CR	VU	CR	EN	LC	EN
Acacia myrmecophila	CR	VU	CR	CR	VU	CR	CR	VU	CR
Acacia pervillei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Acacia sakalava	LC	LC	LC	LC	LC	LC	LC	LC	LC
Adenanthera mantaroa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Alantsilodendron alluaudianum	LC	LC	LC	LC	LC	LC	LC	LC	LC
Alantsilodendron decaryanum	LC	LC	LC	LC	LC	LC	LC	LC	LC
Alantsilodendron humbertii	EN	EN	EN	EN	EN	EN	LC	LC	LC
Alantsilodendron pilosum	LC	NT	NT	LC	NT	NT	LC	NT	NT
Alantsilodendron ramosum	VU	LC	VU	LC	LC	LC	LC	LC	LC
Albizia androyensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia arenicola	LC	LC	LC	LC	LC	LC	LC	LC	LC

Albizia atakataka	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia aurisparsa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia balabaka	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia bernieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia boinensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia boivinii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia commiphoroides	LC	NT	NT	LC	LC	LC	LC	LC	LC
Albizia greveana	VU	LC	VU	VU	LC	VU	LC	LC	LC
Albizia jaubertiana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia mahalao	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia masikororum	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia polyphylla	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia tulearensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Albizia viridis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Alistilus jumellei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Baudouinia fluggeiformis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Baudouinia louvelii	EX	EX	EX	EX	EX	EX	EX	EX	EX
Baudouinia rouxevillei	LC	VU	VU	LC	NT	NT	LC	NT	NT
Baudouinia sollyaeformis	LC	VU	VU	LC	NT	NT	LC	NT	NT
Bauhinia brevicalyx	LC	VU	VU	LC	NT	NT	LC	NT	NT
Bauhinia decandra	EN	NT	EN	EN	LC	EN	LC	LC	LC
Bauhinia grandidieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia grevei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia hildebrandtii	VU	LC	VU	VU	LC	VU	VU	LC	VU
Bauhinia madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia morondavensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia pervilleana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia podopetala	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bauhinia xerophyta	LC	VU	VU	LC	VU	VU	LC	NT	NT
Brandzeia filicifolia	LC	LC	LC	LC	LC	LC	LC	LC	LC
Brenierea insignis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Bussea perrieri	VU	LC	VU	VU	LC	VU	VU	LC	VU
Bussea sakalava	VU	NT	VU	LC	LC	LC	LC	LC	LC
Cadia ellisiana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cadia pedicellata	LC	LC	LC	LC	LC	LC	LC	LC	LC
Caesalpinia hildebrandtii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Caesalpinia madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Canavalia madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cassia hippophallus	LC	LC	LC	LC	LC	LC	LC	LC	LC
Chadsia coluteifolia	LC	VU	VU	LC	NT	NT	LC	NT	NT
Chadsia grevei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Chadsia racemosa	VU	VU	VU	VU	VU	VU	VU	VU	VU
Chadsia salicina	LC	LC	LC	LC	LC	LC	LC	LC	LC
Chadsia versicolor	LC	LC	LC	LC	LC	LC	LC	LC	LC
Chamaecrista arenicola	LC	LC	LC	LC	LC	LC	LC	LC	LC
Chamaecrista lateriticola	LC	LC	LC	LC	LC	LC	LC	LC	LC
Clitoria lasciva	EN	LC	EN	EN	LC	EN	EN	LC	EN
Colvillea racemosa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cordyla haraka	EN	LC	EN	EN	LC	EN	EN	LC	EN
Cordyla madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria androyensis	CR	VU	CR	CR	VU	CR	VU	LC	VU
Crotalaria ankaratrana	EN	EN	EN	EN	EN	EN	EN	EN	EN

Crotalaria capuronii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria cornu-ammonis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria coursii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria decaryana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria edmundi-bakeri	EN	LC	EN	EN	LC	EN	LC	LC	LC
Crotalaria fiherenensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria grevei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria humbertiana	EN	NT	EN	EN	NT	EN	LC	LC	LC
Crotalaria laevigata	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria mahafalensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria mandrarensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria manongarivensis	VU	NT	VU	VU	NT	VU	VU	NT	VU
Crotalaria pervillei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Crotalaria poissonii	LC	VU	VU	LC	NT	NT	LC	NT	NT
Cynometra abrahamii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cynometra ankaranensis	EX	EX	EX	EX	EX	EX	EX	EX	EX
Cynometra aurita	VU	LC	VU	VU	LC	VU	VU	LC	VU
Cynometra commersoniana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cynometra dauphinensis	VU	LC	VU	LC	LC	LC	LC	LC	LC
Cynometra lyallii	EN	LC	EN	EN	LC	EN	EN	LC	EN
Cynometra madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Cynometra pervilleana	EN	LC	EN	EN	LC	EN	EN	LC	EN
Cynometra sakalava	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia abrahamii	LC	NT	NT	LC	LC	LC	LC	LC	LC
Dalbergia baronii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia campenoni	EN	LC	EN	EN	LC	EN	VU	LC	VU
Dalbergia chapelieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia chlorocarpa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia emirnensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia glaberrima	VU	VU	VU	VU	VU	VU	VU	VU	VU
Dalbergia glaucocarpa	EX	EX	EX	EX	EX	EX	EX	EX	EX
Dalbergia greveana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia humbertii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia lemurica	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia mollis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia monticola	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia neoperrieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia orientalis	CR	NT	CR	CR	NT	CR	CR	NT	CR
Dalbergia peltieri	VU	LC	VU	VU	LC	VU	VU	LC	VU
Dalbergia pervillei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia pseudobaronii	VU	LC	VU	VU	LC	VU	LC	LC	LC
Dalbergia purpurascens	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia suaresensis	LC	NT	NT	LC	LC	LC	LC	LC	LC
Dalbergia trichocarpa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dalbergia tsaratananensis	EN	VU	EN	EN	VU	EN	EN	VU	EN
Dalbergia xerophila	LC	VU	VU	LC	VU	VU	LC	VU	VU
Decorsea grandidieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Decorsea meridionalis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Delonix boiviniana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Delonix brachycarpa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Delonix decaryi	VU	LC	VU	VU	LC	VU	LC	LC	LC

Delonix floribunda	LC	LC	LC	LC	LC	LC	LC	LC	LC
Delonix leucantha	EN	NT	EN	EN	NT	EN	VU	LC	VU
Delonix pumila	LC	NT	NT	LC	LC	LC	LC	LC	LC
Delonix regia	LC	NT	NT	LC	LC	LC	LC	LC	LC
Dialium occidentale	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dialium unifoliolatum	EN	LC	EN	EN	LC	EN	EN	LC	EN
Dichrostachys akataensis	LC	VU	VU	LC	NT	NT	LC	LC	LC
Dichrostachys arborescens	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dichrostachys bernieriana	LC	NT	NT	LC	NT	NT	LC	NT	NT
Dichrostachys dumetaria	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dichrostachys paucifoliolata	EN	NT	EN	LC	LC	LC	LC	LC	LC
Dichrostachys scottiana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dichrostachys unijuga	VU	LC	VU	LC	LC	LC	LC	LC	LC
Dichrostachys venosa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dicraeopetalum capuronianum	EN	NT	EN	EN	NT	EN	EN	LC	EN
Dicraeopetalum mahafaliense	LC	LC	LC	LC	LC	LC	LC	LC	LC
Disynstemon paullinioides	LC	LC	LC	LC	LC	LC	LC	LC	LC
Dolichos fangitsa	VU	LC	VU	LC	LC	LC	LC	LC	LC
Dolichos minutiflorus	LC	LC	LC	LC	LC	LC	LC	LC	LC
Eligmocarpus cynometroides	EX	EX	EX	LC	VU	VU	LC	VU	VU
Entada louvelii	EN	LC	EN	EN	LC	EN	VU	LC	VU
Entada pervillei	EN	LC	EN	EN	LC	EN	EN	LC	EN
Erythrophleum couminga	EN	VU	EN	EN	VU	EN	EN	VU	EN
Gagnebina calcicola	LC	LC	LC	LC	LC	LC	LC	LC	LC
Gagnebina commersoniana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Indigofera bemarahaensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Indigofera blaiseae	VU	VU	VU	VU	VU	VU	LC	LC	LC
Indigofera compressa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Kotschya perrieri	LC	VU	VU	LC	NT	NT	LC	LC	LC
Lemuropisum edule	LC	VU	VU	LC	LC	LC	LC	LC	LC
Microcharis phyllogramme	LC	NT	NT	LC	NT	NT	LC	LC	LC
Millettia aurea	LC	LC	LC	LC	LC	LC	LC	LC	LC
Millettia lenneoides	LC	LC	LC	LC	LC	LC	LC	LC	LC
Millettia nathaliae	EN	VU	EN	VU	VU	VU	VU	VU	VU
Millettia richardiana	VU	LC	VU	VU	LC	VU	LC	LC	LC
Millettia taolanaroensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mimosa delicatula	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mimosa grandidieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mimosa ikondensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mimosa onilahensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mimosa psoralea	EN	NT	EN	LC	LC	LC	LC	LC	LC
Mimosa waterlotii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mucuna humblotii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mucuna manongarivensis	EX	EX	EX	EX	EX	EX	EX	EX	EX
Mucuna paniculata	CR	VU	CR	CR	VU	CR	CR	VU	CR
Mundulea anceps	LC	EN	EN	LC	EN	EN	LC	VU	VU
Mundulea antanossarum	CR	EN	CR	EN	VU	EN	LC	LC	LC
Mundulea laxiflora	VU	LC	VU	VU	LC	VU	VU	LC	VU
Mundulea micrantha	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mundulea obovata	VU	LC	VU	VU	LC	VU	VU	LC	VU
Mundulea stenophylla	LC	LC	LC	LC	LC	LC	LC	LC	LC
Mundulea viridis	VU	LC	VU	LC	LC	LC	LC	LC	LC

Neoapaloxylon madagascariense	VU	LC	VU	LC	LC	LC	LC	LC	LC
Neoapaloxylon tuberosum	LC	LC	LC	LC	LC	LC	LC	LC	LC
Neoharmsia baronii	LC	VU	VU	LC	LC	LC	LC	LC	LC
Neoharmsia madagascariensis	EN	VU	EN	EN	VU	EN	EN	VU	EN
Ophrestia madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Ormocarpopsis parvifolia	EN	VU	EN	EN	VU	EN	EN	NT	EN
Ormocarpopsis tulearensis	LC	NT	NT	LC	LC	LC	LC	LC	LC
Ormocarpum bernierianum	LC	VU	VU	LC	NT	NT	LC	NT	NT
Ormocarpum drakei	LC	LC	LC	LC	LC	LC	LC	LC	LC
Otoptera madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Parkia madagascariensis	EX	EX	EX	EX	EX	EX	EX	EX	EX
Phylloxylon decipiens	LC	NT	NT	LC	LC	LC	LC	LC	LC
Phylloxylon perrieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Phylloxylon spinosa	CR	EN	CR	CR	EN	CR	CR	EN	CR
Phylloxylon xylophylloides	LC	LC	LC	LC	LC	LC	LC	LC	LC
Pongamiopsis amygdalina	CR	RN	CR	CR	EN	CR	LC	LC	LC
Pongamiopsis pervilleana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Pongamiopsis viguieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Pyranthus lucens	EN	VU	EN	EN	VU	EN	EN	VU	EN
Rhynchosia androyensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Rhynchosia baukea	LC	LC	LC	LC	LC	LC	LC	LC	LC
Sakoanala villosa	LC	LC	LC	LC	LC	LC	LC	LC	LC
Senna anthoxantha	EN	NT	EN	VU	LC	VU	LC	LC	LC
Senna bosseri	EN	VU	EN	EN	VU	EN	VU	LC	VU
Senna lactea	EN	LC	EN	VU	LC	VU	VU	LC	VU
Senna leandrii	LC	LC	LC	LC	LC	LC	LC	LC	LC
Senna meridionalis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Senna viguierella	LC	LC	LC	LC	LC	LC	LC	LC	LC
Strongylodon craveniae	EN	LC	EN	EN	LC	EN	EN	LC	EN
Strongylodon madagascariensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Sylvichadsia grandifolia	EN	NT	EN	EN	NT	EN	LC	LC	LC
Tephrosia alba	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tephrosia bibracteolata	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tephrosia boiviniana	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tephrosia genistoides	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tephrosia perrieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tephrosia pungens	EN	NT	EN	EN	NT	EN	LC	LC	LC
Tetrapterocarpon geayi	LC	LC	LC	LC	LC	LC	LC	LC	LC
Tetrapterocarpon septentrionalis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Vaughania cloiselii	EN	LC	EN	EN	LC	EN	LC	LC	LC
Vaughania depauperata	LC	LC	LC	LC	LC	LC	LC	LC	LC
Vaughania dionaeifolia	LC	LC	LC	LC	LC	LC	LC	LC	LC
Vaughania interrupta	LC	NT	NT	LC	NT	NT	LC	LC	LC
Vaughania longidentata	CR	EN	CR	CR	EN	CR	LC	LC	LC
Vaughania mahafalensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Vaughania perrieri	LC	LC	LC	LC	LC	LC	LC	LC	LC
Vaughania pseudocompressa	VU	LC	VU	VU	LC	VU	LC	LC	LC
Vigna keraudrenii	EN	NT	EN	EN	NT	EN	EN	NT	EN
Viguieranthus ambongensis	LC	LC	LC	LC	LC	LC	LC	LC	LC
Viguieranthus glaber	LC	LC	LC	LC	LC	LC	LC	LC	LC
Viguieranthus kony	EN	LC	EN	EN	LC	EN	EN	LC	EN
Viguieranthus pervillei	LC	LC	LC	LC	LC	LC	LC	LC	LC

Viguieranthus scottianus	LC	LC	LC	LC	LC	LC	LC	LC	LC
Xanthocercis madagascariensis	VU	LC	VU	VU	LC	VU	VU	LC	VU
Xylia fraterna	CR	EN	CR	CR	EN	CR	CR	EN	CR
Xylia hoffmannii	LC	LC	LC	LC	LC	LC	LC	LC	LC

Supplementary Table 4.B. The environmental layers used for modelling.

Environmental variables and data sources
1. Annual Mean Temperature
2. Mean Diurnal Range(Mean(period max-min))
3. Isothermality (2/7)
4. Temperature Seasonality (Coefficient of Variation)
5. Max Temperature of Warmest Period
6. Min Temperature of Coldest Period
7. Temperature Annual Range (5-6)
8. Mean Temperature of Wettest Quarter
9. Mean Temperature of Driest Quarter
10. Mean Temperature of Warmest Quarter
11. Mean Temperature of Coldest Quarter
12. Annual Precipitation
13. Precipitation of Wettest Period
14. Precipitation of Driest Period
15. Precipitation Seasonality(Coefficient of Variation)
16. Precipitation of Wettest Quarter
17. Precipitation of Driest Quarter
18. Precipitation of Warmest Quarter
19. Precipitation of Coldest Quarter
20. Geological Raster
21. DEM

Data sources: 1-19 WorldClim (Hijmans *et al*. 2005); 20 Geology map of Madagascar (Du Puy & Moat 1996); 21 GTOP030 (USGS 30-second Global Elevation Data)

Chapter 5

Genetic variation in *Delonix s.l.* (Leguminosae) in Madagascar revealed by AFLPs – fragmentation, conservation status and taxonomy[4]

5.1. Summary

The distribution of genetic diversity adds valuable information to species-level conservation but is rarely incorporated when conservation status is assigned to a species. These data can be beneficial to the conservation assessment process by providing information on subpopulations, gene flow and effective population sizes. In order to obtain a better understanding of the patterns of genetic variation and their relationship to conservation in the fragmented flora of Madagascar, this study assessed genetic diversity among and within species in the genus *Delonix s.l.* (Leguminosae) using AFLP markers. The information obtained was used to assess the genetic difference between species of different threat status and also to explore genetic implications for the effective conservation of these species.

The results show that even closely related species with the same IUCN threat status can differ in their genetic structure, likely arising from differences in life history traits, pollen and seed dispersal, and fragmentation. Such differences show that conservation assessments can greatly benefit from information on genetic diversity, in order to achieve a more complete assessment and to provide more accurate and targeted conservation recommendations. Conventional conservation assessments do not always take into account the possible threat of genetic decline, However, species that are recently affected by habitat destruction and fragmentation are likely to be at high risk of genetic erosion, potentially accelerating their decline. Thus, genetic variation

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[4] This chapter was submitted as Rivers, M.C., Brummitt, N.A., Nic Lughadha, E. & Meagher, T.R. Genetic variation in *Delonix s.l.* (Leguminosae) in Madagascar revealed by AFLPs – fragmentation, conservation status and taxonomy. Conservation Genetics. January 2011.

should be taken into consideration in conservation assessments, whenever possible, in order to achieve more successful conservation outcomes.

5.2. Introduction

Fragmentation results in smaller and more isolated populations, and the expected genetic consequences of this are: a decrease in genetic diversity due to loss of rare alleles, random drift, inbreeding within fragments and reduction in gene flow between fragments (Aguilar *et al.* 2008; Young *et al.* 1996). However, long-lived tree species are thought to be more resistant to the effects of fragmentation as they have longer generation times, high intra-population genetic diversity and the potential for high rates of pollen flow (Hamrick 2004; Kramer *et al.* 2008). Nevertheless, there can be other subtle but important impacts of fragmentation on breeding patterns, reproductive output and progeny fitness, that in turn can affect long-term population viability (Lowe *et al.* 2005; Meagher 2010). Fragmentation of a species is also important in the context of assessing conservation status, as it can lead to small and relatively isolated subpopulations with concomitant higher extinction risk (IUCN 2001).

Fragmentation and habitat destruction are major threats to the primary vegetation in Madagascar. Species endemism in Madagascar reaches over 80% in many animal (Goodman & Benstead 2005) and plant groups (Dransfield & Beentje 1995; Du Puy *et al.* 2002; Hermans *et al.* 2007); however, Madagascar has a rapidly increasing human population, which places its unique biodiversity under threat. Only 18% of natural primary vegetation is left (Moat & Smith 2007); and Malagasy forests are especially threatened, many of which are highly fragmented. Madagascar has around 12,000 species of plants (Schatz 2000a), but pattern of genetic variation have so far been studied in only five species: *Commiphora guillauminii* (Voigt *et al.* 2009), *Dalbergia monticola* (Andrianoelina *et al.* 2006; Andrianoelina *et al.* 2009), *Beccariophoenix madagascariensis, Beccariophoenix alfredii* (Shapcott *et al.* 2007) and *Prunus africana*

(Dawson & Powell 1999). The genetic consequences of fragmentation and loss of vegetation are therefore largely undocumented in Madagascar.

5.2.1. Relationship between conservation status and genetic diversity

There have been several reviews of the comparative genetic diversity of rare and common species (Cole 2003; Gitzendanner & Soltis 2000; Hamrick & Godt 1996; Karron 1987). Many different definitions of rarity exist, including restricted geographic range, small population sizes and narrow habitat specificity (Gaston 1997); due to the ambiguity of the term, here we use a threatened conservation status to designate a "rare" species. Threat status is determined by the IUCN Red List criteria and relates to aspects of population loss and decline in range size, including subpopulation structure, fragmentation, generation length, and extreme fluctuation in numbers (IUCN 2001; Mace *et al.* 2008), which are characteristics that also affect genetic diversity. In the IUCN Red List classification system there are three *threatened* categories: Critically Endangered, Endangered and Vulnerable. Species that do not qualify for one of these three threatened categories are considered *non-threatened*. We are interested in whether a genetic-based inference of risk is the same for species of similar IUCN conservation status. Does an increased risk of extinction based on the IUCN Red List correspond to an increased risk of extinction based on genetic measures?

In current IUCN Red List assessments threatened species may be determined on the basis of evidence of restricted geographical range or due to low abundance, which also relates to genetic factors. Hamrick and Godt (1996) and Karron (1987) showed that tree species with a narrow geographic distribution had less genetic variation (polymorphism and genetic diversity) than widespread species, and Frankham (1996) showed that species of small population size had less genetic variation than those of larger population size. Also, species in small, isolated populations may lose genetic diversity within populations through drift and increased inbreeding, and have increased differentiation among populations due to reduced gene flow (Aguilar *et al.* 2008; Ellstrand & Elam 1993; Young *et al.* 1996). Finally, small population sizes may limit mating opportunities in self-incompatible species, resulting in local population decline (Byers & Meagher 1992).

In contrast, non-threatened species are expected to have larger range sizes and greater abundance. They are also expected to have more genetic variation, larger subpopulations, and reduced differentiation between populations due to historical connectivity (gene flow). However, there are cases where large populations may contain very low genetic diversity due to historical genetic bottlenecks followed by expansion (see Vendramin *et al.* 2008 and references therein); and such species may therefore be at increased risk of extinction despite being abundant and/or widespread. This highlights the importance of genetic data in that species may be threatened due to low genetic diversity (within populations or overall) and/or exhibit high differentiation among populations.

5.2.2. Importance of genetic diversity studies for conservation priorities

Loss of genetic variation may affect a species' ability to adapt to changing environmental conditions and increase its risk of extinction (Keller & Waller 2002). An understanding of existing genetic diversity and differentiation within and among populations is thus a significant component of developing effective conservation strategies.

The distribution of genetic diversity adds valuable information to species conservation but is rarely incorporated when conservation status is assigned to a species. These data could be beneficial to the conservation assessment process, by providing information on subpopulations, gene flow and effective population sizes. In the US the Endangered Species Act provides the possibility of listing diversity below the species level, such as genetically distinct populations, as threatened (Laikre 2010). However the IUCN Red List does not presently incorporate genetic data explicitly.

5.2.3. Objectives

In order to obtain a better understanding of the patterns of genetic variation in the fragmented flora of Madagascar and their relationship to conservation, the objectives of the present study were: (1) to determine the level of genetic diversity, and the patterns of genetic variation among and within *Delonix s.l.* species using AFLP markers; (2) to assess the genetic difference between species with different threat status; (3) to assess how this genetic diversity analysis corresponds to current taxonomic understanding of *Delonix s.l.*; and (4) to explore genetic implications for the effective conservation of these species.

5.3. Materials and methods

5.3.1. Study species

In order to conduct a comparative investigation of patterns of genetic variation at the species level, we chose *Delonix* (Leguminosae) in Madagascar as the case genus for the present study since the taxonomy is well defined, it is easy to identify in the field, and species' ranges and threat status are typical of Leguminosae as a whole in Madagascar (Chapter 1). There are also advantages to studying an entire genus, as analysing closely related species improves the comparability of genetic data.

Delonix (Leguminosae) is a genus comprising 11 species: nine endemic to Madagascar, one restricted to East Africa and another extending from east Africa northwards through the Middle East to India. Two monospecific genera endemic to Madagascar *Lemuropisum* and *Colvillea* have been shown to be closely related to *Delonix* both genetically (Haston *et al.* 2005) and morphologically (Du Puy *et al.* 1995), and it is unclear whether or not these should remain as separate genera. The present study therefore encompasses the Malagasy species of *Delonix sensu latu*, including *Lemuropisum* and *Colvillea*.

The Malagasy species of *Delonix s.l.* are restricted to the dry forest and the spiny forest (Figure 5.1). The genus is thought to be strongly outbreeding. Although pollinators and seed dispersal agents are not well studied, observations regarding floral visitors have been made. The white flowered species (*Delonix boiviniana* (Baill.) Capuron, *Delonix brachycarpa* (R.Vig.) Capuron, *Delonix decaryi* (R.Vig.) Capuron, *Delonix leucantha* (R. Vig.) Du Puy, Phillipson & R.Rabev.*, Delonix pumila* Du Puy, Phillipson & R.Rabev. and *Lemuropisum edule* H.Perrier) possess a suite of floral characters indicative of moth pollination, including night opening, white petals with red, long-exerted stamens, and a narrowly tubular, nectariferous claw on the upper petal (Du Puy *et al.* 1995; Rivers pers. obs.). The reduction of the petals in *Delonix floribunda* (Baill.) Capuron and *Delonix velutina* Capuron is perhaps an adaptation to pollination by the Malagasy Sunbird, *Nectarinia sovimanga* (Du Puy *et al.* 1995). The sunbird is also suggested as the possible main pollinator of the large bright orange or red flowers of *Colvillea racemosa* Bojer and *Delonix regia* (Bojer ex Hook.) Raf. (Du Puy *et al.* 1995). Lemurs are reported (on herbarium sheet labels) to visit flowers of *D. boiviniana*, although they are probably not the main pollinators. The fruit pods are large and woody, containing many seeds about 10-20 mm long; they appear to be tardily dehiscent or even indehiscent until after they have fallen (Du Puy *et al.* 1995). Many species are valued locally as wood for canoes, coffins and fencing. *Delonix regia* is widely cultivated across the tropics for its attractive flowers.

The conservation status of the Malagasy species of *Delonix s.l.* was first assessed by Du Puy (1995) and included on the IUCN Red List in 1998. However, since these assessments are over 10 years old and were carried out using the old Categories and Criteria (version 2.3), for the purpose of this study, the conservation status used are those of a recent desktop assessment (see Brummitt *et al.* 2008 for methodology) using the latest Categories and Criteria (version 3.1). Full conservation assessments of *Delonix s.l.* will incorporate the information obtained from this study (Appendix 4).

5.3.2. Plant material

Tissue samples for genetic analyses were derived from leaf material collected throughout the geographical distribution of *Delonix s.l.* in Madagascar in November-December 2007 and December 2008. A total of 254 individuals were sampled at 79 sample sites covering eight species (Table 5.1, Figure 5.1).

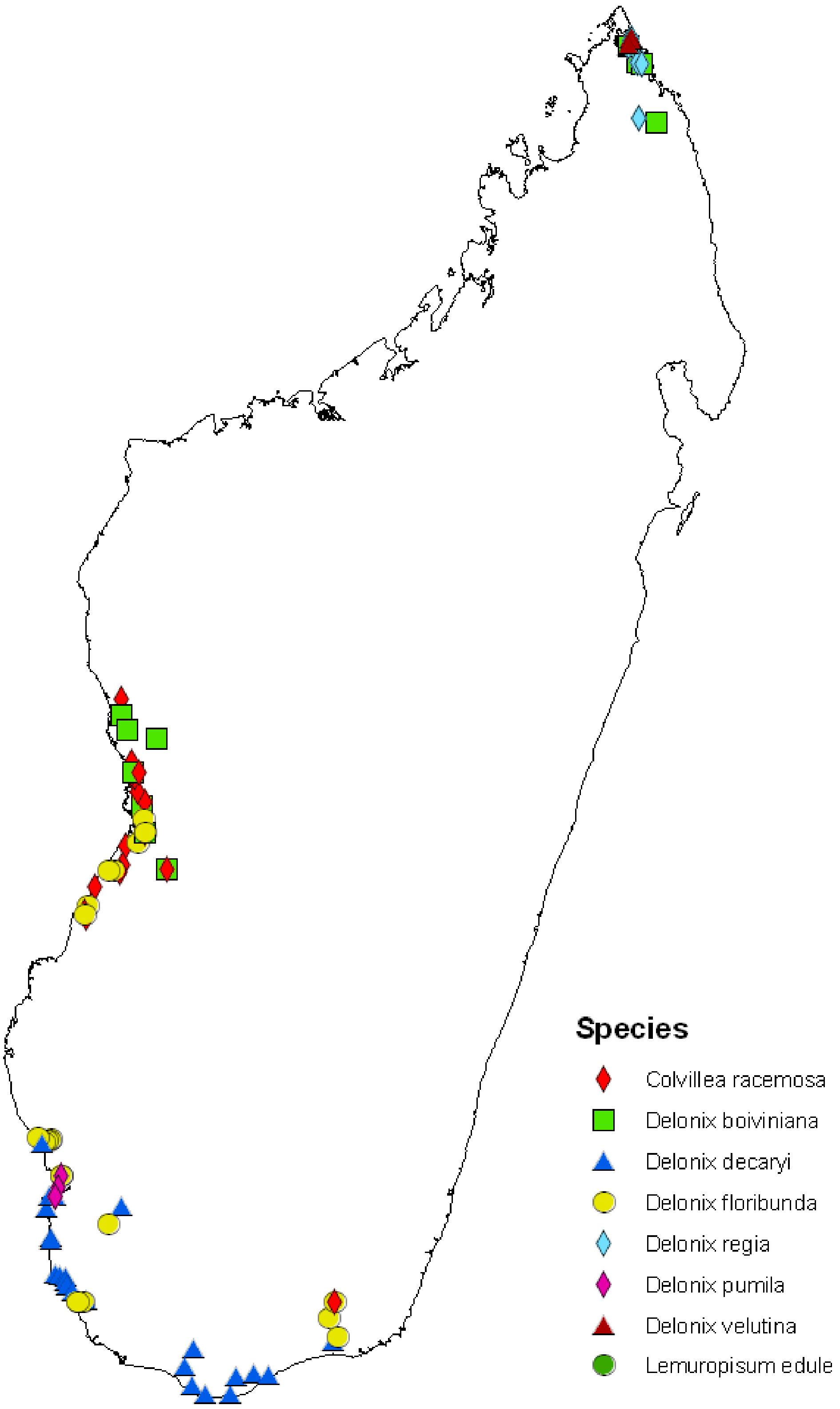
At each site all available trees were sampled. Leaf samples were desiccated in silica gel (Chase & Hills 1991). Although all species were targeted for genetic investigation, the timing of the collection trips meant that certain species were more comprehensively sampled than others. *Delonix decaryi*, *D. floribunda*, *D. pumila* were sampled throughout their known range; *D. velutina* and *L. edule* were collected across most of their known range. New localities were added to the previously known distributions of all species sampled. No collections were made of *Delonix tomentosa* (R.Vig.) Capuron*,* *D. brachycarpa* and *D. leucantha*.

Table 5.1. Number of individuals and sample sites surveyed for AFLP variation within *Delonix* species and conservation status (from Desktop assessment 2007)

Species	**Individuals**	**Sample sites**	**Conservation status**[1]
Colvillea racemosa	28	12	Least Concern
Delonix boiviniana	17	7	Least Concern
Delonix decaryi	72	22	Near Threatened
Delonix floribunda	65	21	Least Concern
Delonix regia	17	6	Least Concern
Delonix pumila	23	4	Endangered
Delonix velutina	22	4	Endangered
Lemuropisum edule	10	3	Endangered
Total *Delonix s.l.*	254	79	

[1] Conservation status is determined by desktop assessment, Appendix 2 (see Brummitt *et al.* 2008 for methodology) using the latest Categories and Criteria (version 3.1). Full conservation assessments of *Delonix s.l.* are listed in Appendix 4.

Figure 5.1. Distribution map of sites sampled (2007-08) for eight species of *Delonix s.l.*, covering 79 sites. (To compare with herbarium specimen distribution, see Appendix 1)



5.3.3. DNA extraction

DNA extractions were made using a modified CTAB method (Doyle & Doyle 1990). Dried leaf material (100-200 mg) was ground to a fine powder, 1 ml CTAB extraction buffer (with 1-2% mercaptoethanol) was added and incubated at 55°C for 30 min. The sample was cooled and then centrifuged at 13,000 rpm for 10 minutes. The supernatant was removed and 700 µl dichloromethane (or chloroform) added, then centrifuged at 13,000 rpm for 10 minutes. The supernatant was removed and this step repeated. After adding 3 µl RNase (10 mg/ml) the sample was incubated at 37°C for one hour. DNA was precipitated by 1 ml ice-cold propanol, followed by gentle mixing and incubation at -20°C for at least 30 minutes. The sample was centrifuged at 13,000 rpm for 15 minutes to form a pellet of crude DNA; the supernatant was discarded and the pellet washed with 500 µl cold 70% ethanol, hand vortexed and spun at 13,000 rpm for 7 minutes. The supernatant was discarded and the sample allowed to dry for 20 minutes. The resulting DNA pellet was finally dissolved in 200 µl TE buffer. DNA concentration was estimated by agarose gel electrophoresis with appropriate concentration standards.

5.3.4. AFLP analysis

AFLP analysis followed a standard protocol (Life Technologies, Core Reagent Kit cat no 10482-016, Starter Primer Kit cat no 10483-014) based on that originally described by Vos *et al.* (1995). DNA was digested with EcoRI and MseI restriction enzymes, and double-stranded EcoRI and MseI adaptors were ligated onto the ends of fragments. Subsequent PCR amplifications were performed in Perkin Elmer GeneAmp PCR System 9700. In the first, preselective PCR amplification, primers with a single-base-pair extension amplified a subset of the total restriction fragments. In the second, selective amplification, primers contained a 3 bp extension, which further reduced the number of fragments amplified. The selective EcoRI primer was labelled with a fluorescent dye. Three primer combinations MseI-CAA/EcoRI-AAG, MseI-CAA/EcoRI-ACT and MseI-CAC/EcoRI-AGG were used in the analysis. PCR fragments were analysed using a Beckman Coulter CEQ 8000 DNA Fragment Analyser in order to separate fragments of different lengths. A mixture of 40 µl of formamide, 0.5 µl PCR product and 0.5 µl of dye labelled size standard (60-420 bp) (Beckman Coulter-GenomeLab DNA Size Standard Kit 400 no 608098) was made and transferred to a single well on a 96 well PCR plate for analysis. The raw data were analysed using the fragment analysis module of the CEQ system software version 9.0. Signal peaks were filtered according to a number of criteria. Only signal peaks with an intensity of 5% or more of the maximum signal peak within a sample were included. Similarly, only peaks with a slope parameter >10% were retained. Fragment sizes for each peak were estimated based on an internal size standard. A binary table was constructed which recorded presence/absence of fragments ranging between 60 and 420 bp. Over 1000 polymorphic AFLP loci were identified and examined.

5.3.5. Data analysis

All statistical analyses were carried out using GenAlEx v. 6.3 (Peakall & Smouse 2006). Three descriptive measures of genetic diversity were estimated: percentage of polymorphic loci (most common allele <95%), the number of private alleles, and the Shannon index of diversity (I) (Shannon 1948). The Shannon index is not bounded by 1, is robust over a wide range of conditions and independent of hierarchical level of organization; it can thus aid the comparison and integration of diversity at the genetic and community levels (Sherwin *et al.* 2006). Pearson's correlation coefficient (r) was used to test for significant correlation between population size and gene diversity.

Principal Coordinate Analysis (PCoA) using genetic distance was conducted to visualise the genetic structure among all eight species. A hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was conducted to assess the partition of genetic diversity between genera, species and sample sites. The PCoA and AMOVA were based on a pairwise matrix where the genetic distance between individuals were estimated as squared Euclidean distance defined for dominant markers after Huff *et al*. (1993). Statistical significance of the AMOVA was determined using bootstrap results involving 999 permutations.

In four species-specific AMOVAs, the pairwise genetic variation between populations of *D. decaryi*, *D. floribunda*, *D. pumila* and *D. velutina* were calculated as $\Phi_{PT,}$ which represents the proportion of variance among populations relative to total variance (analogous to Wright's (1943) $F_{ST,}$). We restricted the analyses to sample sites with more than one individual. The levels of significance were based on 999 permutations, and the sequential Bonferroni correction used to correct for multiple comparisons.

To determine if genetic and geographic distances were significantly associated pairwise genetic distances between sites were compared with geographical distances using the Mantel test (Mantel 1967). A total of 999 random permutations were performed to test statistical significance. Significant association between genetic differentiation and geographic distance can be due to isolation by distance or to the presence of barriers to gene flow between populations that are otherwise panmictic (Guillot *et al.* 2009).

5.4. Results

Data analyses were based on eight species, 79 sample sites and 254 individuals (Table 5.1). Certain analyses and discussion will focus on *D. decaryi*, *D. floribunda*, *D. pumila* and *D. velutina*, which were sampled near exhaustively across their respective ranges and provide contrasts among IUCN threat status within the genus. The IUCN conservation status for the first two are considered non-threatened (Near Threatened and Least Concern, respectively), whereas the latter two are classified as threatened (Endangered).

5.4.1. Genetic diversity

A total of 1,005 polymorphic loci were scored. The robustness of AFLP scoring was tested by analysing replicated ALFP runs (from the same DNA extraction) for thirty samples (Bonin *et al.* 2004). The mean error rate was 4.1%, which is within reported typical error rates for AFLP scoring (Bonin *et al.* 2007), and no taxon specific responses.

Table 5.2. Estimates of genetic diversity in *Delonix s.l.*, based on a survey of AFLP variation across 1,005 loci.

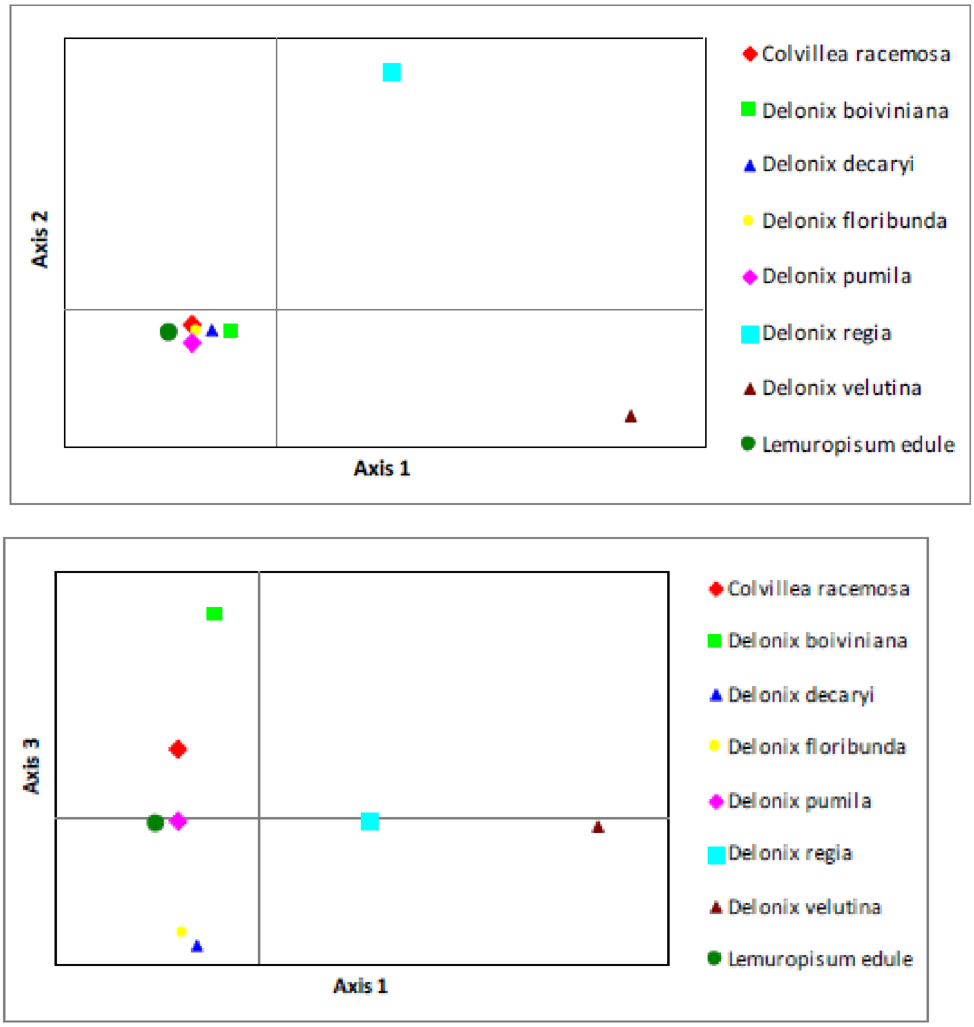
Species	**Total Alleles**	**Private alleles**	**Polymorphic loci % (95%)**	**Shannon's index (I) (s.e.)**
Colvillea racemosa	626	15	34%	0.104 (0.002)
Delonix boiviniana	547	4	54%	0.119 (0.004)
Delonix decaryi	760	11	40%	0.128 (0.004)
Delonix floribunda	735	11	34%	0.121 (0.004)
Delonix regia	618	9	61%	0.191 (0.006)
Non-threatened Delonix s.l.			*45%*	*0.132(0.004)*
Delonix pumila	456	0	25%	0.087 (0.004)
Delonix velutina	596	9	44%	0.192 (0.006)
Lemuropisum edule	266	1	26%	0.076 (0.004)
Threatened Delonix s.l.			*32%*	*0.11 (0.005)8*
Total *Delonix s.l.*	1,005		40%	0.127 (0.004)

The genetic diversity measures show that the overall polymorphism in the genus *Delonix s.l.* is 40% (Table 5.2). The highest polymorphism is found in *D. boiviniana* and *D. regia* (both non-threatened), while the two species with the lowest polymorphism are *D. pumila* and *L. edule* (both threatened). The number of private alleles ranges from 15 in *C. racemosa* to none in *D. pumila*. Shannon's diversity measure (I) shows that overall level of diversity for *Delonix s.l.* is 0.127 (Table 5.2). The lowest level of diversity is found in *D. pumila* and *L. edule* and the highest level in the two northern species *D. regia* and *D. velutina*, while *D. decaryi* and *D. floribunda* show values of genetic diversity close to the mean for the genus. There is no significant relationship between population size and the proportion of polymorphic loci at 95% level (r = -0.15, p = 0.72) or the Shannon's diversity measure (r = -0.008, p = 0.99).

5.4.2. Genetic structure

The PCoA on Nei's genetic distance between species shows that the first PCoA axis separates the two northern species, *D. regia* and *D. velutina,* from the other species found in the south and west (Figure 5.2a). The second PCoA axis separates *D. regia* from *D. velutina*, the third PCoA axis separates the southern and western species (Figure 5.2b). The two threatened species, *D. pumila* and *L. edule,* are positioned closely, and similarly *D. decaryi* and *D. floribunda* are closely related according to PCoA analysis.

Figure 5.2. Plots of the principal coordinate analysis of the eight species of *Delonix s.l.* surveyed for AFLP variation (Nei's genetic distance) a) PCoA axis 1 vs. PCoA axis 2 and b) PCoA axis 1 vs. PCoA axis 3. The percentages of total variation accounted for by PCoA1, PCoA2 and PCoA3 were 51.8%, 28.7% and 6.5%, respectively.



The AMOVA shows no significant variation among the three genera; 13% of variation is attributed to differences among the eight species while 87% of the variation is found within species (Table 5.3). For the four more intensively sampled species most of the variation is accounted for within sample sites (82-98%) rather than among sample sites (Table 5.4a-d). For the two non-threatened species (*D. decaryi* and *D. floribunda*) there is a similar division of variation within and among sample sites; both species have 15-18% of variation among sample sites (Table 5.4a-b). One of the threatened species, *D. pumila,* has very limited level of differentiation among sample sites (2%). However, *D. velutina* also classified as threatened, shows similar levels of genetic variation to the non-threatened *D. decaryi* and *D. floribunda* with 15% variation among sites. In addition, the genetic variation among sites (Φ_{PT}) in *D. pumila* is generally low and none are significantly different after the Bonferroni correction for multiple comparisons (Supplementary Table 5.Ac). On the other hand, *D. velutina* has much higher variation between pairwise comparisons of sample sites (Supplementary Table 5.Ad). The two non-threatened species also show different patterns in the pairwise comparison; in *D. decaryi* differentiation is local among sites, while in *D. floribunda* the variation is more evenly distributed across sites (Supplementary Table 5.Aa-b).

Table 5.3. Analysis of molecular variance (AMOVA) among and within species of *Delonix s.l.*, including the percentage of variation explained by the different hierarchical levels. Tests of significance were based on 999 permutations.

Source of variation	**d.f.**	**SS**	**est. var.**	**% of variation**	Φ_{PT}	**P**
Among genera	2	283.584	0.000	0%	-0.043	1.000
Among species	5	1648.087	8.256	13%	0.126	0.001
Within species	246	14029.647	57.031	87%	0.089	0.001

Table 5.4. Analysis of molecular variance (AMOVA) among and within samples sites of a) *Delonix decaryi,* b) *Delonix floribunda,* c) *Delonix pumila* and d) *Delonix velutina*, including the percentage of variation explained by the different hierarchical levels. Tests of significance were based on 999 permutations.

	d.f.	**SS**	**est. var.**	**% of variation**	Φ_{PT}	**p**
a) *Delonix decaryi*						
Among sample sites	15	1384.966	10.267	18%	0.176	0.001
Within sample sites	55	2644.612	48.084	82%		
b) *Delonix floribunda*						
Among sample sites	16	1252.977	8.321	15%	0.151	0.001
Within sample sites	48	2250.407	46.883	85%		
c) *Delonix pumila*						
Among sample sites	2	92.611	0.984	2%	0.024	0.151
Within sample sites	20	801.129	40.056	98%		
d) *Delonix velutina*						
Among sample sites	3	427.233	13.389	15%	0.152	0.001
Within sample sites	18	1343.767	74.654	85%		

5.4.3. Geographical structure

The Mantel tests revealed that the genetic differentiation is correlated to spatial distance in *C. racemosa, D. boiviniana, D. decaryi* and *D. velutina*; but this is not so in *D. floribunda*, *D. pumila, D. regia* and *L. edule* (Table 5.5). The lack of correlation in the two threatened species *D. pumila* and *L. edule* is not surprising as their ranges are very small. However, *D. velutina* has a similarly restricted range but shows significant geographical structure of its genetic diversity. The non-threatened species also vary in their geographical structure. Three non-threatened species (*C. racemosa, D. boiviniana* and *D. decaryi*) have spatial structuring of genetic variation among sites, while *D. floribunda* and *D. regia* show no geographic correlation between genetic differentiation and geographic distance.

Table 5.5. The significance of the Mantel test, comparing linear genetic distances and geographic distance between individuals, based on 999 permutations.

Species	**p**
Colvillea racemosa	0.003
Delonix boiviniana	0.047
Delonix decaryi	0.001
Delonix floribunda	n.s. (0.102)
Delonix pumila	n.s. (0.500)
Delonix regia	n.s. (0.304)
Delonix velutina	0.034
Lemuropisum edule	n.s. (0.225)

5.5. Discussion

5.5.1. Genetic diversity and structure

Despite being one of the world's most species-rich biodiversity hotspots, with high plant endemism and large scale habitat degradation (Brummitt & Nic Lughadha 2003; Mittermeier *et al.* 2005; Myers *et al.* 2000), the patterns of genetic diversity of plant species in Madagascar have been published for only five species (Andrianoelina *et al.* 2006; Andrianoelina *et al.* 2009; Dawson & Powell 1999; Shapcott *et al.* 2007; Voigt *et al.* 2009). Our study adds another eight species and therefore more than doubles the knowledge base of Madagascar's plant genetic diversity. The level of genetic variation in *Delonix s.l.* in Madagascar is in the same range as *Commiphora guillauminii* (Voigt *et al.* 2009). For the four *Delonix* species studied in detail, the majority of diversity was found within sample sites, which was also true for *C. guillauminii*. In contrast, both *Prunus africana* and *Beccariophoenix madagascariensis* exhibited more genetic variation among populations (Dawson & Powell 1999; Shapcott *et al.* 2007). The similarities between *Delonix s.l.* and *C. guillauminii* may be due to similarity in habitat, both species occur in Madagascar's dry forest, while *P. africana, B. madagascariensis, B. alfredii* and *Dalbergia monticola* occur in the island's humid or subhumid forests.

Although information on genetic variation for Malagasy plants is sparse, AFLP studies on Leguminosae from other parts of the world revealed levels of genetic diversity similar or slightly higher than those detected in *Delonix s.l.* (e.g. Baskauf & Burke 2009; Breinholt *et al.* 2009; Cardoso *et al.* 2005; Juan *et al.* 2004; Rivera-Ocasio *et al.* 2006; Song *et al.* 2008). However, direct comparisons between AFLP studies should be drawn with caution due to the variable criteria used in selecting polymorphic loci (Nybom 2004). This study, encompassing eight species using the same methodology, allows for comparisons between species using the same criteria and for studying diversity across the genus.

5.5.2. Relationship between conservation status and genetic diversity

The genus *Delonix s.l.* comprises species with both threatened and non-threatened conservation ratings, which allows comparisons to be made between differences in genetic diversity and threat status. We expected the threatened species to have lower genetic diversity than the non-threatened species, and a significant proportion of the genetic variation in threatened species to be found among populations rather than within populations. However, both the amount of genetic diversity and its distribution varied between the threatened species. The Endangered *D. pumila* had the lowest genetic diversity and no significant differentiation between samples sites. The low levels of diversity are possibly due to *D. pumila* being adapted to a narrow geographic range and having a small effective population size for many generations. The lack of genetic differentiation between sites can be attributed to the small range of this species and therefore high opportunity for gene flow between populations. In contrast, *D. velutina,* also classified as Endangered, consistently had the highest value of genetic diversity and high genetic differentiation between sample sites despite having a restricted range. High diversity within sample sites, as seen in *D. velutina,* may be a legacy of a previously more extensive species distribution and relatively few generations since population fragmentation occurred (Drummond *et al.* 2000; Shapcott *et al.* 2007). These results suggest that the same IUCN threat status can have different genetic implications.

For the non-threatened species, we expected higher genetic diversity and lower genetic distance between populations. The genetic diversity measures for both *D. decaryi* and *D. floribunda* were similar to the average for the genus. The distribution of genetic variation among and within sample sites is also similar. However, the genetic differentiation between sample sites is geographically structured in *D. decaryi*, while the genetic differentiation between sites in *D. floribunda* is not correlated to their geographic proximity. Again, the results suggest the same threat status can have different genetic implications.

These differences between genetic diversity and geographical structures may be due to differences in pollen and seed dispersal. Both *D. decaryi* and *D. pumila* are visited by moths (Du Puy *et al.* 1995), which are thought to have relatively short flight ranges, which may lead to genetic structure being related to geographical structure in these species. Such a pattern was found for *D. decaryi;* however, in *D. pumila* no spatial structure was detected, as the sample sites were all within a single population. *Delonix velutina* and *D. floribunda* are visited by sunbirds (Du Puy *et al.* 1995; Rivers pers. obs.), which are territorial birds and expected to result in genetic diversity being geographically structured. This was the case in *D. velutina,* but not in *D. floribunda*. However, Reisch *et al.* (2010) showed that the flowering season in South Africa of *Mimetes fimbriifolius* overlapped with the migration periods of the pollinating sunbirds, which lead to dispersal of pollen further than the boundary of their actual territory. Pollinator migration could likewise explain the comparatively high level of gene flow and lack of geographical structuring of genetic diversity between populations in the western and south-western distributed *D. floribunda*. However, further work is needed regarding the dispersal agents for species of *Delonix*, as information is often inferred from animal associations rather than a demonstrated role of these animals in pollination and seed dispersal.

Fragmentation has also been suggested to play a role in the distribution of genetic diversity. Due to the recent history of fragmentation in Madagascar, however, we were unlikely to detect many genetic consequences of fragmentation; the expected loss of genetic diversity due to habitat fragmentation might be delayed as long-lived tree species often show a slow response to changed environmental conditions (Hamrick 2004; Meagher 2010). A study of impacts of recent fragmentation in Madagascar on populations of *Dalbergia monticola* concluded that there was only limited impact of fragmentation on genetic parameters due to the short time scale (Andrianoelina *et al.* 2009). Anthropogenic fragmentation is a recent phenomenon in evolutionary time and this suggests that the final, long-term impacts of habitat fragmentation have yet to manifest (Ewers & Didham 2005). The risk remains that this genetic “extinction debt” can lead to the underestimation of actual threats to biodiversity.

5.5.3. Taxonomic implications

Molecular markers other than AFLP are generally preferred for phylogenetic studies. However, AFLP analysis is proving useful to support such analysis, and to add robustness, highlight anomalies and solve outliers (Koopman 2005).

Our PCoA and AMOVA analyses support previous studies on both the morphological (Du Puy *et al.* 1995) and molecular (Haston *et al.* 2005) taxonomy of *Delonix s.l*. In our study, *Colvillea* and *Lemuropisum* are nested within the *Delonix* group in many analyses, supporting their inclusion in *Delonix s.l.* Firstly, in the AMOVA analysis there was no significant variation attributable to the genus level, which would be expected if *Colvillea* and *Lemuropisum* were sufficiently different from *Delonix* to warrant recognition of three different genera. Secondly, the PCoA analysis showed both *Colvillea* and *Lemuropisum* nested within the group of southern and western species of *Delonix*, again indicating a close affinity with these species. Finally, in the genetic diversity analysis *Lemuropisum* has only one private allele; more private alleles would be expected if it was considered a separate genus. *Colvillea* on the other hand has a higher number of private alleles, which indicates more unique genetic material.

Du Puy *et al.* (1995) discussed the morphological variation within *Delonix s.l.* and suggested three subgroups within the genus: the first one comprises the two non-Malagasy species (*D. elata* and *D. baccal*), the second group comprises *D. floribunda*, *D. decaryi*, *D. leucantha* and *D. pumila* (few pinnae and leaflets on leaves, pods linear-oblong), the third group consists of *D. velutina*, *D. regia* and *D. tomentosa* (numerous pinnae and leaflets, pods strap-shaped). The molecular analyses of Haston *et al.* (2005) broadly support these groupings, although some ambiguity exists in some groupings. The results of our PCoA analysis of eight species also support the two Malagasy subgroups of *Delonix* suggested by Du Puy *et al.* (1995).

The two remaining taxa, *D. brachycarpa* and *D. boiviniana,* may be conspecific and their position in the groupings is unclear (Du Puy *et al.* 1995). Our study placed *D. boiviniana* within the second group according to the PCoA analysis, which also fits with leaf characteristics; however, the pods are not linear-oblong in *D. boiviniana* but crescent-shaped. Unfortunately, no sample for *D. brachycarpa* was included in this study. It has also been suggested that *D. boiviniana* may consist of two separate taxa as two distinct types of pods and seeds are observed (S. Rakotoarisoa pers. comm.)

5.5.4. Impact on conservation priorities

During the last century, the dry forest and the spiny forest of western and south western Madagascar have been heavily degraded by slash-and-burn agriculture, fires, charcoal production and livestock grazing (Gade 1996; Harper *et al.* 2007). At the genetic level habitat destruction has probably resulted in an immediate loss of alleles caused by a reduction in population size, and also left species in these forests, including *Delonix s.l.,* more vulnerable to the effects of genetic drift and inbreeding. This genetic analysis of *Delonix* sheds new light on the genus, and if neutral genetic diversity reflects the adaptive genetic diversity then it will have implications for conservation action regarding the management of evolutionary potential both *in situ* as well as *ex situ*.

Due to their genetic differences, management recommendations for the two threatened species differ as well. To ensure conservation of the evolutionary potential in *D. velutina,* where there are pronounced differences in genetic diversity among sites, as many sites as possible need to be conserved *in situ*. Several of the sample sites of *D. velutina* are situated within the borders of Montagne des Français and Orangea (two areas of conservation interest in northern Madagascar). Montagne des Français was afforded temporary Protected Area Status in 2006 (Sabel *et al.* 2009) and the hope is that this status will become permanent. Connectivity with the surrounding forests in Orangea is desirable to allow gene flow between subpopulations. In contrast, the lack of genetic differentiation between sites for *D. pumila* suggests the whole range of this species comprises a single breeding population that should be afforded *in situ* conservation efforts. Conservation efforts should also consider local populations at the extremes of the species distribution, as they may encompass genetic variation that may facilitate species survival during periods of environmental change.

For both the non-threatened species, *D. floribunda* and *D. decaryi,* all individuals encountered were sampled for genetic diversity. However, often trees were found singly or in small stands. The sampling intensity is therefore high for the number of sites, but, in terms of the number of individuals at each site was lower than for the threatened species. Despite *D. floribunda* and *D. decaryi* not currently being listed as threatened, our study raised three key conservation concerns, which are in fact applicable to all species of *Delonix s.l.*: Firstly, very little regeneration was observed in populations of the species sampled. This lack of recruitment and the absence of small size classes can lead to a negative feedback due to the self-reinforcing genetic bottleneck (Aldrich *et al.* 2005). Secondly, deforestation and habitat destruction in Madagascar is continuing at a rapid rate due to an expanding population in need of more resources, which may lead to more species of *Delonix* becoming threatened. Thirdly, the current protected areas network in Madagascar is impressive but sometimes limited in effectiveness; habitat destruction is still common within its boundaries. However, areas protected through local taboos or *fady* (Jones *et al.* 2008) often prove the most effective, and ensuring these traditional values are upheld may be the key to conservation success.

Conservation in protected areas also needs to be supported by *ex situ* collections, such as seed collections. Seed collection has been undertaken by the Millennium Seed Bank Partnership (http://www.kew.org/science-conservation/conservation-climate-change/millennium-seed-bank/index.htm) and the Malagasy partner Silo National des Graines Forestières for seven species of *Delonix s.l.*: *C. racemosa, D. boiviniana, D. decaryi, D. floribunda, D. pumila, D. regia* and *L. edule*. Seeds are collected and stored in Madagascar as well as in the United Kingdom, for safe keeping, research and eventual reintroduction to the field. An understanding of underlying genetic diversity can greaty inform strategies for such collections. Even on a local scale conservation and restoration efforts can benefit from estimates of genetic differentiation and levels of gene flow among plant populations to guarantee the evolutionary potential of restored populations (Millar & Libby 1991).

5.6. Conclusions

The results of this study show that even closely related species categorised with the same IUCN threat status can differ in their genetic diversity and structure arising from differences in life history traits, pollen and seed dispersal, and fragmentation. Conservation assessments can greatly benefit from the information on genetic diversity of a species, in order to achieve a more complete assessment, but also for more accurate and targeted conservation recommendations.

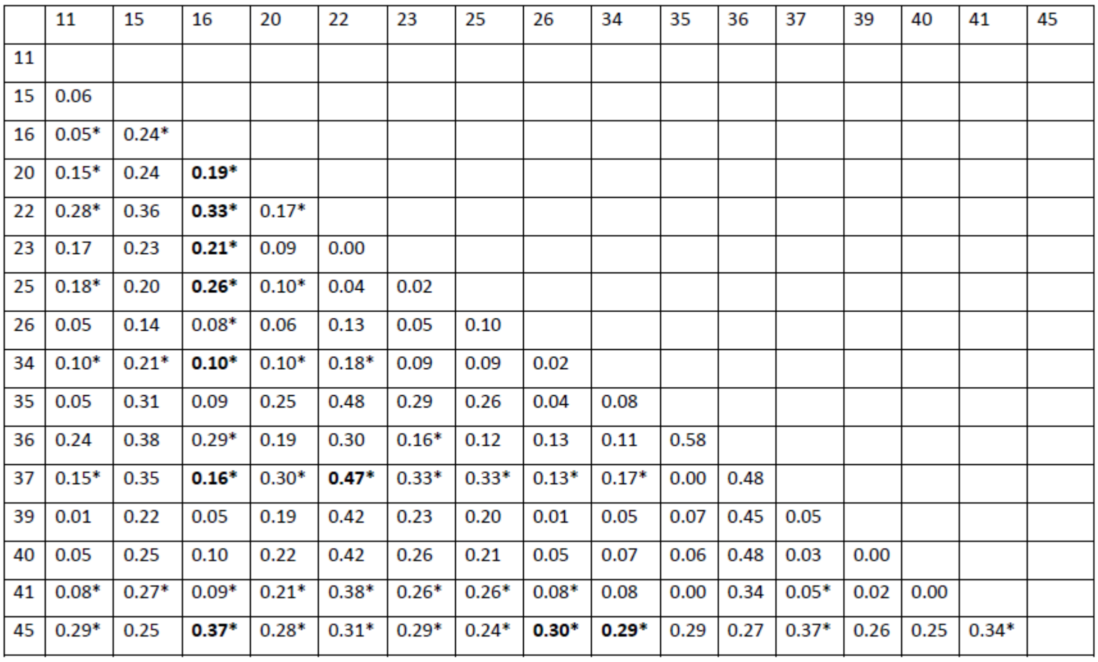
Conventional conservation assessments do not always take into account the possible threat of genetic decline, but species that have been recently affected by habitat destruction and fragmentation are likely to be at high risk of genetic erosion and possible extinction. It is, therefore, important that genetic variation is taken into consideration not only in conservation assessments but also in the development of international policy to conserve biodiversity (Laikre *et al.* 2010).

When data are unavailable, it has sometimes been appropriate to extrapolate information from closely related taxa and apply it to lesser-known taxa. However, our genetic study at the genus level has shown that such extrapolation should be undertaken with caution regarding genetic diversity, as species with the same threat status may have widely differing patterns of genetic differentiation.

5.7. Supplementary material

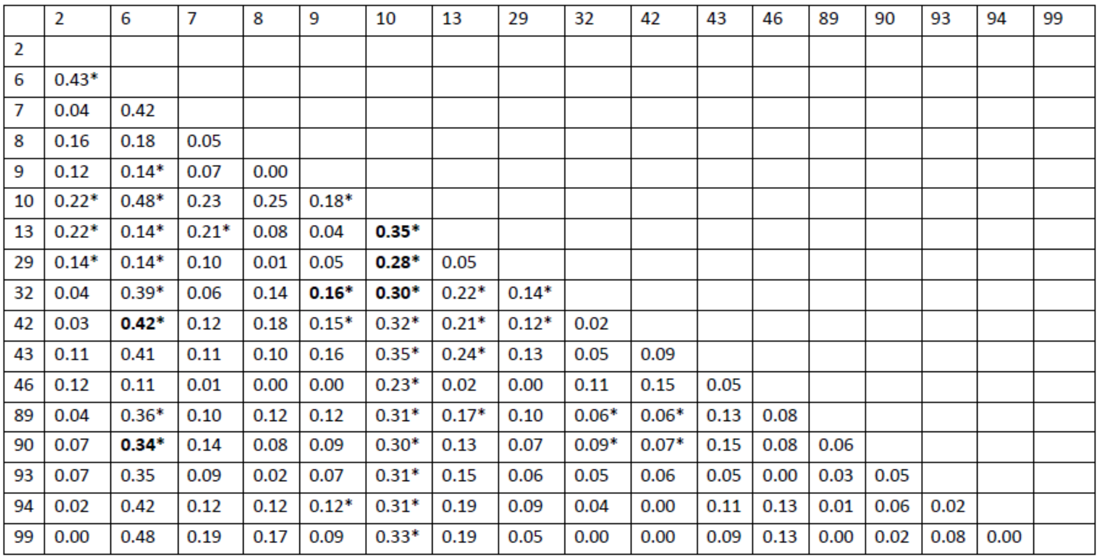
Supplementary Table 5.A. Pairwise genetic distances (Φ_{PT}) among a) *Delonix decaryi, b) Delonix floribunda, c) Delonix pumila* and *d) Delonix velutina.* Significant comparisons (p<0.05) are marked with * based on random permutations (*N*=9999). (Significant comparisons (p<0.05) after sequential Bonferroni test for multiple comparisons are seen in bold). Label numbers refers to sample site.

a)



	11	15	16	20	22	23	25	26	34	35	36	37	39	40	41	45
11																
15	0.06															
16	0.05*	0.24*														
20	0.15*	0.24	**0.19***													
22	0.28*	0.36	**0.33***	0.17*												
23	0.17	0.23	**0.21***	0.09	0.00											
25	0.18*	0.20	**0.26***	0.10*	0.04	0.02										
26	0.05	0.14	0.08*	0.06	0.13	0.05	0.10									
34	0.10*	0.21*	**0.10***	0.10*	0.18*	0.09	0.09	0.02								
35	0.05	0.31	0.09	0.25	0.48	0.29	0.26	0.04	0.08							
36	0.24	0.38	0.29*	0.19	0.30	0.16*	0.12	0.13	0.11	0.58						
37	0.15*	0.35	**0.16***	0.30*	**0.47***	0.33*	0.33*	0.13*	0.17*	0.00	0.48					
39	0.01	0.22	0.05	0.19	0.42	0.23	0.20	0.01	0.05	0.07	0.45	0.05				
40	0.05	0.25	0.10	0.22	0.42	0.26	0.21	0.05	0.07	0.06	0.48	0.03	0.00			
41	0.08*	0.27*	0.09*	0.21*	0.38*	0.26*	0.26*	0.08*	0.08	0.00	0.34	0.05*	0.02	0.00		
45	0.29*	0.25	**0.37***	0.28*	0.31*	0.29*	0.24*	**0.30***	**0.29***	0.29	0.27	0.37*	0.26	0.25	0.34*	

b)



	2	6	7	8	9	10	13	29	32	42	43	46	89	90	93	94	99
2																	
6	0.43*																
7	0.04	0.42															
8	0.16	0.18	0.05														
9	0.12	0.14*	0.07	0.00													
10	0.22*	0.48*	0.23	0.25	0.18*												
13	0.22*	0.14*	0.21*	0.08	0.04	**0.35***											
29	0.14*	0.14*	0.10	0.01	0.05	**0.28***	0.05										
32	0.04	0.39*	0.06	0.14	**0.16***	**0.30***	0.22*	0.14*									
42	0.03	**0.42***	0.12	0.18	0.15*	0.32*	0.21*	0.12*	0.02								
43	0.11	0.41	0.11	0.10	0.16	0.35*	0.24*	0.13	0.05	0.09							
46	0.12	0.11	0.01	0.00	0.00	0.23*	0.02	0.00	0.11	0.15	0.05						
89	0.04	0.36*	0.10	0.12	0.12	0.31*	0.17*	0.10	0.06*	0.06*	0.13	0.08					
90	0.07	**0.34***	0.14	0.08	0.09	0.30*	0.13	0.07	0.09*	0.07*	0.15	0.08	0.06				
93	0.07	0.35	0.09	0.02	0.07	0.31*	0.15	0.06	0.05	0.06	0.05	0.00	0.03	0.05			
94	0.02	0.42	0.12	0.12	0.12*	0.31*	0.19	0.09	0.04	0.00	0.11	0.13	0.01	0.06	0.02		
99	0.00	0.48	0.19	0.17	0.09	0.33*	0.19	0.05	0.00	0.00	0.09	0.13	0.00	0.02	0.08	0.00	

c)

	1	4	5	17
1				
4	0.006			
5	N/A	N/A		
17	0.053	0.199*	N/A	

d)

	50	52	55	58
50				
52	**0.168***			
55	**0.054***	**0.258***		
58	**0.186***	0.068	**0.230***	

Chapter 6

A comparison of genetic and spatial approaches to delineate population structure in *Delonix s.l.* (Leguminosae) from a conservation perspective

6.1. Summary

Understanding population structure, here defined as landscape-level distribution of either individuals or genetic variation, is important in the development of effective conservation strategies to species threatened with decline or even extinction. For the majority of plant species, however, there is little or no information available on population structure. A more thorough understanding of population structure would significantly enhance scientifically-based conservation initiatives.

In this study we compare the performance of population genetic clustering and spatial clustering as a means of defining landscape-level population structure. Despite some similarities between methods, there was rarely complete agreement on the ideal clustering solution due to differences in algorithms and methodology. Spatial population analyses are both simple and inexpensive and are a practical first step for population assessments, but will only detect populations formed by physical isolation. However, combining spatial analysis with population genetic data provides a more complete picture of landscape-level population dynamics. Such integration of spatial and genetic analyses has great potential to enhance conservation management and action.

6.2. Introduction

It is important to understand the population structure of species under threat in order to establish effective conservation strategies. Analysis of the spatial distribution of individuals makes it possible to identify physical groupings of individuals, but does not provide insight into whether those groupings are local breeding units or part of an extended population on a larger scale. Whether a species consists of a single interbreeding population across its entire range or several genetically distinct populations with limited genetic exchange will have consequences for conservation management. However, there is little or no information on population structure for the majority of plant species, especially for species from the species-rich but resource-poor tropics (Storfer *et al.* 2010). Species in such areas are increasingly likely to be of conservation concern. A more thorough understanding of population structure for these species would therefore significantly enhance any scientifically-based conservation initiatives.

Delineating populations is important for understanding how the movement of individuals (and gametes) influences the genetic structure of a species. Genetic differentiation occurs when gene flow between populations is limited, either naturally due to gene flow barriers such as a mountain range, or due to anthropogenic factors such as habitat destruction and fragmentation. The population structure not only affects the genetic structure of a species, but may also have an impact on its evolutionary potential. In fragmented (i.e. small and isolated) populations, the loss of genetic diversity, through inbreeding and genetic drift, may also lead to reduced reproductive fitness and decreased scope for local adaptation (Ellstrand & Elam 1993; Leimu *et al.* 2006; Young *et al.* 1996). Such impacts on the evolutionary potential of a species are clearly of conservation interest as they could increase the probability of extinction. The understanding of population delineation is also crucial in conservation management, especially for establishing effective conservation (or management) units.

6.2.1. Defining populations

Populations exist on a continuum of varying degrees of connectivity with regard to geographical isolation and gene flow (see Waples & Gaggiotti 2006). The true difficulty, however, lies in translating theoretical definitions into functional populations in reality, and accurately defining populations in the wild. Many definitions of ‘population’ have been suggested; unfortunately, few of these can be applied directly to species in the wild in order to determine how many populations exist and characterize the relationships among them (Waples & Gaggiotti 2006). The definition by the International Union for Conservation of Nature and Natural Resources (IUCN) differs from most, by defining population as the "total number of individuals of the taxon", and subpopulations as "geographically or otherwise distinct groups in the population between which there is little demographic or genetic exchange" (IUCN 2001). Therefore the IUCN definition of subpopulations can be considered the equivalent to 'populations' in the conventional sense. This chapter will use the term population in the conventional sense (and in the sense of the IUCN subpopulations). Number of populations is significant when assessing a species conservation status, due to the additional risks faced by species that are either fragmented into many small units or, indeed, the opposite situation where most individuals are concentrated into one unit (IUCN Standards and Petitions Working Group 2010). Fragmented species may consist of very small populations that are increasingly susceptible to inbreeding, drift and stochastic events. Equally, species consisting of a single population are potentially at increased risk of extinction as all individuals could be wiped out in a single stochastic event or disease outbreak.

6.2.2. Spatial models of population structure

Instances of successful dispersal between populations typically decrease with distance, and as a consequence genetic differentiation is often related to geographic distance – a concept known as "isolation by distance" (Wright 1943). When genetic data and gene flow information are unavailable, spatial analysis can be used to estimate the number of populations based on the geographical isolation of the species in the field. For plants, the connectivity of species is maintained by the exchange of both pollen and seeds. When distances of effective pollen and seed dispersal are known, geographical isolation could be calculated based on these distances and then populations could be modelled for groups of individuals between which gene flow is unlikely. Studies have shown that pollen-mediated gene flow among populations is high in many tree species, and that fragmentation has had mixed impacts on the movement of pollen, dependent on the degree of isolation of populations and on the diversity of the pollen source (Hamrick 2004; Sork & Smouse 2006). Even when distances of pollen and seed dispersal are unknown, geographically delimited populations can be estimated using spatial modelling (Chapter 3 and Rivers *et al.* 2010). Population estimates based on spatial modelling are quick, easy, low-cost and can be used as a first step in conservation assessments of population structure. However, the transition from spatial models to biologically functioning populations requires further information on habitat availability, dispersal ability, breeding systems and biotic interactions, together with an analysis of genetic diversity.

6.2.3. Genetic clustering models

Genetic diversity analysis can be used to provide more information on biologically functioning populations inferred from purely spatial models of population structure. The use of Bayesian clustering models is often preferred to traditional measures of population genetic structure (such as Wright's F-statistics (Wright 1965)), as no prior assignment of individuals to populations is necessary, and migration and admixture between populations can be detected as the analysis is based on individual genotypes, and not inferred at the population level. Several Bayesian statistical clustering approaches are currently available to assess population structure (e.g. STRUCTURE, BAPS, TESS, GENELAND). Each method has slightly different underlying assumptions and uses different methods of searching the genetic parameter space (François & Durand 2010; Wilkinson *et al.* 2011). Essentially, the genotype of each individual is assigned to a cluster of similar genotypes based on a Bayesian probabilistic model. These methods can also be applied to dominant molecular data (such as AFLPs) (Corander *et al.* 2004; Falush *et al.* 2007; Guillot & Santos 2010). Another approach to the identification of populations is by multivariate analysis such as principal coordinate analysis (PCoA) which can provide useful validation of Bayesian clustering outputs (Jombart *et al.* 2009; Patterson *et al.* 2006). PCoA can also be based on individual multi-locus genotypes and makes no prior assumption of the number or identity of clusters.

6.2.4. Objectives

In order to obtain a better understanding of the population structure of four species of *Delonix* Raf. (Leguminosae) in Madagascar and its relationship to conservation status, our objectives are: (1) to determine the genetically-based population structure of four species representing contrasting biological backgrounds and varying levels of conservation concern; (2) to assess how genetically-based population structure is reflected in spatial structuring methods; and (3) to explore the implications of these findings with respect to conservation recommendations.

6.3. Methods

6.3.1. Area and species selection

Madagascar, the focal region for this study, is one of the world's biodiversity hotspots, based on its number of endemic species and the loss of its natural vegetation (Mittermeier *et al.* 2005; Myers *et al.* 2000). Species endemism in Madagascar reaches over 80% among many animal groups (Goodman & Benstead 2005) as well as several plant groups, such as palms (Dransfield & Beentje 1995), orchids (Hermans *et al.* 2007) and legumes (Du Puy *et al.* 2002). Madagascar has a rapidly increasing human population and its unique biodiversity is under severe threat from habitat destruction and over-exploitation. The CEPF Madagascar Vegetation Mapping Project estimates that only 18% of primary vegetation still exists (Moat & Smith 2007), and many areas of the country are severely affected by habitat fragmentation (Harper *et al.* 2007).

The present study examines four species of the genus *Delonix* (Leguminosae) all endemic to Madagascar: *Delonix decaryi* (R.Vig.) Capuron*, Delonix floribunda* (Baill.) Capuron*, Delonix pumila* Du Puy, Phillipson & R.Rabev. and *Delonix velutina* Capuron. The first two are widespread, while the latter two have narrow ranges. *Delonix* is a tropical tree genus, found in the dry forest and the spiny forest of Madagascar. *Delonix decaryi* and *D. pumila* are small to medium-sized trees with large white flowers that are thought to be moth pollinated (Du Puy *et al.* 1995; Du Puy *et al.* 2002 and Rivers pers. obs.). *Delonix floribunda* and *D. velutina* are medium to large trees bearing flowers with reduced petals that are thought to be pollinated by sunbirds (Du Puy *et al.* 1995; Du Puy *et al.* 2002 and Rivers pers. obs.). The pods in all four species are large and woody. The gene flow distances of the pollinators and seed dispersal agents are largely unknown. A total of 182 plants from 51 sample sites covering most of the species' ranges were analysed (Table 6.1). The sampling strategy encompassed a range-wide sample of as many populations as could be located. The number of individuals per population varied from one to 14, limited by the number of trees available for sampling at any one site. All samples used were collected in the field (2007 and 2008) and dried immediately in silica gel. The exact latitude and longitude of each site was recorded with a GPS to an accuracy of 10m. Herbarium specimens were also collected and deposited in Madagascar (TAN), and wherever possible in Kew (K), Paris (P) and Missouri (MO).

Table 6.1. Number of individuals and sample sites surveyed for AFLP variation.

	Individuals sampled	**Sample sites**	**Conservation status**[1]
Delonix decaryi	72	22	Near Threatened
Delonix floribunda	65	21	Least Concern
Delonix pumila	23	4	Endangered
Delonix velutina	22	4	Endangered

[1] Conservation status is determined by desktop assessment, see Appendix 2 (and see Brummitt *et al.* 2008 for methodology). Full conservation assessments of *Delonix s.l.* are listed in Appendix 4.

6.3.2. DNA extraction and AFLP analysis

DNA extractions and AFLP analysis followed methodology previously outlined in detail (Chapter 5). In brief, the DNA extractions were made using a modified CTAB method (Doyle & Doyle 1990). AFLP analysis followed the protocol described by the manufacturer (Life Technologies, Core Reagent Kit cat no 10482-016, Starter Primer Kit cat no 10483-014). PCR fragments from three primer combinations (MseI-CAA/EcoRI-AAG, MseI-CAA/EcoRI-ACT and MseI-CAC/EcoRI-AGG) were analysed using a Beckman Coulter CEQ 8000 DNA Fragment Analyser in order to separate fragments of different lengths. The raw data were analysed using the fragment analysis module of the CEQ system software version 9.0. Signal peaks were filtered according to a number of criteria. Only peaks with an intensity of 5% or more of the maximum within a sample were included; similarly, only peaks with a slope parameter >10% were used. Fragment sizes for each peak were estimated based on an internal size standard. A binary table was constructed which recorded presence/absence of fragments ranging between 60 and 420bp for over 1,000 polymorphic AFLP loci examined. The robustness of AFLP scoring was tested by analysing replicated ALFP runs for thirty samples (Bonin *et al.* 2004). The mean error rate was 4.1%, which is within reported typical error rates for AFLP scoring (Bonin *et al.* 2007).

6.3.3. Population genetics analyses

Four data sets were produced, one for each species, and genetic structuring was analysed for each data set using a Bayesian clustering approach implemented in three widely available packages: STRUCTURE (Pritchard *et al.* 2000), BAPS (Corander & Marttinen 2006) and TESS (Chen *et al.* 2007). To complement the Bayesian clustering approach, a PCoA based on a matrix where the genetic distances were estimated as squared Euclidean distance defined for dominant markers (Huff *et al.* 1993) was carried out in GenAlEx v. 6.3 (Peakall & Smouse 2006). MCLUST (Fraley & Raftery 2006) was subsequently used to find structure within the PCoA. General genetic diversity statistics have been reported previously (see Chapter 5). Models of admixture were used throughout this study, as admixture among sample sites could not be ruled out.

François and Durand (2010) have shown that models without admixture are not robust when there is inclusion of admixed individuals in the sample, whereas admixture models remain robust in the absence of admixture in the sample.

6.3.3.1. STRUCTURE

STRUCTURE version 2.3.3 implements a model-based clustering method using the Markov Chain Monte Carlo algorithm (Pritchard *et al.* 2000). Recent modifications have enabled the use of dominant markers such as AFLPs (Falush *et al.* 2007). AFLP genetic marker data were analysed for different numbers of clusters (K) ranging from one to nine; with 20 replicate runs for each K and a burn-in period of 50,000 followed by 500,000 iterations. The admixture model and correlated allele frequencies were used and default values were maintained for all other parameters. Admixture proportions (ancestry coefficients) were assigned to each individual, representing the proportion of the individual genome that originates from the inferred ancestral populations.

By comparing the probability of the data estimated in different runs, it is possible to identify the optimal K, using two different methods. The first involves choosing the K with the maximum probability of the data for a given K (Ln P(D|K*)*) (Pritchard *et al.* 2000). However, this probability can continue to increase after the optimal K is reached. Therefore, a second method for estimating K used the second order rate of change of the likelihood function (from here on referred to as delta K) (Evanno *et al.* 2005). Runs from the optimal K were analysed in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) to correct for between-run discrepancies (e.g. label-switching) to obtain an optimal alignment from these replicates.

6.3.3.2. TESS

TESS version 2.3, a second Bayesian clustering model program, also uses the Markov Chain Monte Carlo algorithm; however, it incorporates geographical information for each sample (Chen *et al.* 2007). The program builds a spatial network structure indicating genetic relatedness based on individual geographical locations, which is taken into account during the admixture analysis. AFLP data were analysed for K ranging from one to nine, using 50 replicate runs for each K, with 50,000 sweeps and a 10,000 sweep burn-in. The admixture model CAR was used, the interaction parameter (ψ) was set to 0.6 and default values were maintained for the allele frequency models and all other parameters. Admixture proportions (ancestry coefficients) were assigned to each individual, representing the proportion of the individual genome that originates from the ancestral populations.

It is possible to identify the optimal K by comparing the probabilities of the Deviance Information Criterion (DIC), a statistical measure of the model prediction capabilities, which were estimated in each run. The optimal K is the one with the lowest DIC value, or where this value reaches a plateau (Chen *et al.* 2007). The method of estimating K using delta K was also used (as described under 6.3.3.1). The 20% runs with the lowest DIC values from TESS were analysed in CLUMPP 1.1.2 to correct for between-run discrepancies and obtain an optimal alignment.

6.3.3.3. BAPS

BAPS version 5.4 is a third program for Bayesian inference of population structure; it infers the optimal number of clusters as well as the cluster to which each individual belongs (Corander & Marttinen 2006). BAPS treats both the frequencies of the markers and the number of genetically divergent groups as random variables. BAPS also has an admixture model; however, the source populations have to be either sampled or inferred by the population mixture analysis. We used the latter and conducted a population mixture analysis of individuals with the geographic origin of the samples used as the informative prior ("spatial clustering of individuals"). BAPS was run with the maximum number of groups (K) set to nine. Each run was replicated ten times. The K with the minimum log likelihood was selected. The allele frequencies of genetically divergent groups identified in the mixture analysis were then used to conduct the admixture analysis (Corander & Marttinen 2006). We used 50 simulations from the posterior probability of the allele frequencies to determine admixture. We conducted the admixture analysis five times, using CLUMPP 1.1.2 to correct for between-run discrepancies, and the results were highly consistent among replicates.

6.3.3.4. MCLUST

To complement the three Bayesian clustering approaches we used a distance-based phenetic analysis, MCLUST version 3 for R (Fraley & Raftery 2006), to estimate clustering of a PCoA. PCoA is commonly used to demonstrate the relationship between samples. Groupings of points within PCoA plots, however, can be subjective and difficult to illustrate in a multidimensional space. Further analysis of PCoA results, such as by MCLUST, helps to visualize these groupings. PCoA analyses were executed using pairwise Nei's genetic distances between samples in GenAlEx v. 6.3 (Peakall & Smouse 2006). The scores from the first five principal coordinate axes (explaining 89-94% of total variation depending on species) were used as the input data matrix for MCLUST. MCLUST tests 10 models that differ in assumption regarding shape and volume of multidimensional clusters, and chooses the best-fit model based on the Bayesian Information Criterion (BIC). The number of clusters is estimated and each sample is then assigned to a cluster. This method has previously been used by Andrade *et al.* (2007) to estimate the number of groups within the PCoA.

6.3.4. Spatial population analysis

Spatial populations were estimated through GIS analysis using the 1/10th circular buffer method (Chapter 3 and Rivers *et al.* 2010). In the circular buffer method, each specimen locality is buffered by a circle with a radius of 1/10th the maximum interpoint distance between all data points of a species. Overlapping buffers are merged to form a single population, while non-overlapping buffers are considered to be separate populations. In order to place the spatial analysis into the same framework as the DNA-based population structuring, only localities for which DNA were available were included in this spatial analysis, to achieve a more accurate comparison between spatial and genetic methods. These numbers may therefore differ from those previously reported (Chapter 3 and Rivers *et al.* 2010)

6.4. Results

6.4.1. Population genetic structure

In total, 182 DNA samples and 1,005 AFLP loci from the four species of *Delonix* were used in the genetic analysis of population structure. The population genetic analyses predicted an optimal number of clusters (K) that ranged from 2 to 6 (Table 6.2). Convergence by all genetic models on the same clustering solution was observed in only one of the four species (*D. decaryi*), although the number of clusters and the assignment of individuals to clusters were similar in the other three species (Table 6.2, Supplementary Figure 6.A). Many individuals were represented by more than one ancestral population, indicating that admixture is high (Supplementary Figure 6.A). Variation between methods is seen both in terms of the number of clusters and in the level of admixture.

Table 6.2. Estimated numbers of populations for four species of *Delonix*

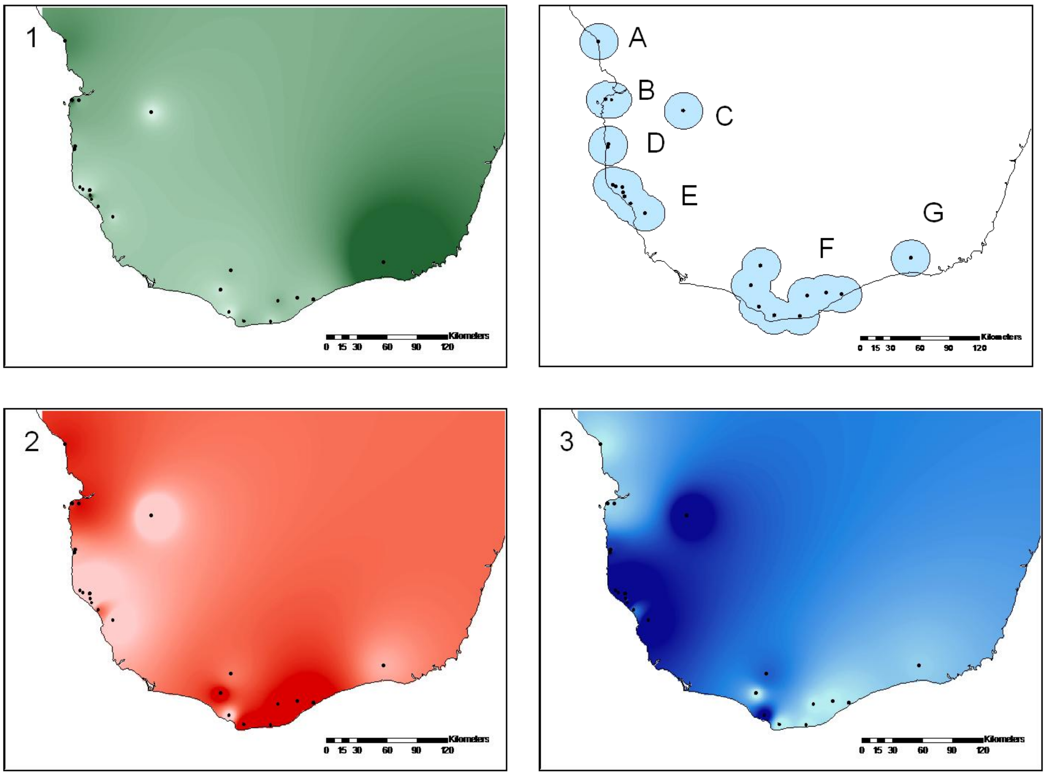
	Population genetic analysis				Spatial analysis
	STRUCTURE	BAPS	TESS	MCLUST	GIS
Delonix decaryi	3	3	3	3	7
Delonix floribunda	4	2	3	6	5
Delonix pumila	2	2	3	5	3
Delonix velutina	3	2	3	4	3

BAPS consistently estimated the lowest number of populations for all four *Delonix* species; and MCLUST consistently predicted the highest number of populations for each species. The number of clusters predicted by STRUCTURE and TESS falls between the values of BAPS and MCLUST. For *D. decaryi,* the results of STRUCTURE and TESS were very similar; however, for *D. floribunda, D. pumila* and *D. velutina,* TESS often predicted a single dominating cluster with very small proportions of the other clusters, meaning biological interpretation of its groupings is difficult (Supplementary Figure 6.A). Establishing the ideal number of K is less clear with TESS as the DIC score continued to decline beyond the ideal K (Supplementary Figure 6.B). Therefore, the preferred clustering method in this study is STRUCTURE, as it consistently gave biologically credible clustering solutions and a clear optimal number of clusters could be detected, using both posterior probabilities (Ln P (D|K)) and delta K methods (Supplementary Figure 6.C). The results from the STRUCTURE analysis are presented in Figure 6.1 and used for the comparison with spatial population estimates.

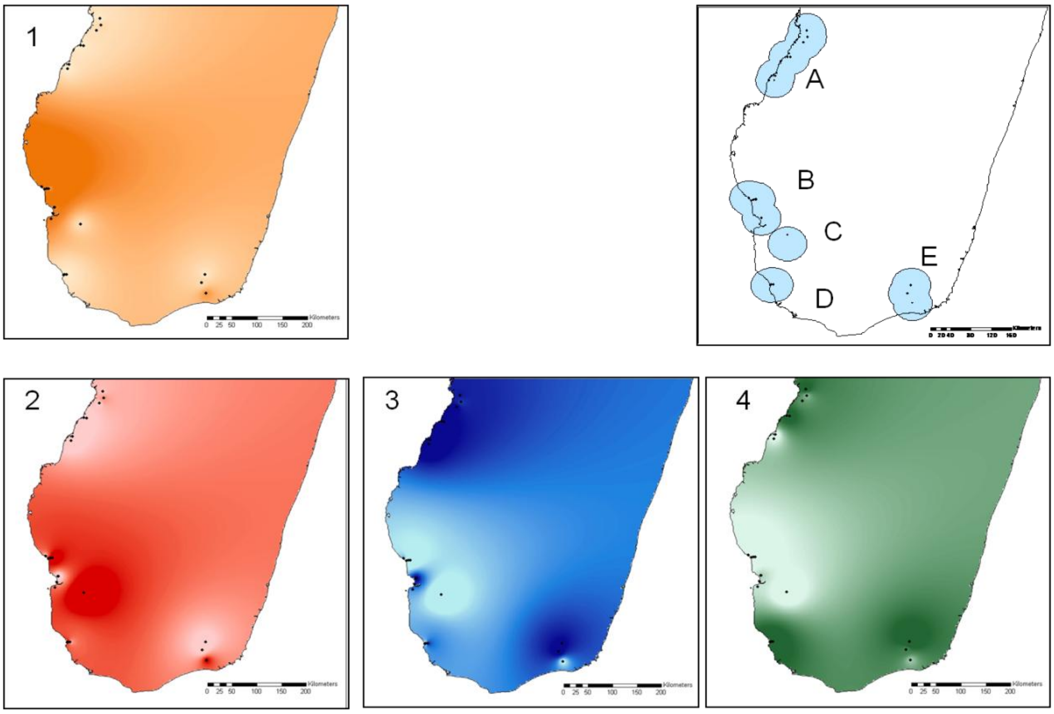
For three out of four species the spatial estimate of population numbers fell within the range of the genetic prediction. For *D. decaryi*, however, the spatial estimate (seven populations) was more than double the prediction of the number of genetically distinct populations (three populations) revealed by all four genetic clustering methods (Table 6.1, Figure 6.1).

Figure 6.1. Comparison of spatial and genetic clustering of a) *Delonix decaryi*, b) *Delonix floribunda*, c) *Delonix pumila* and d) *Delonix velutina*. The numbered maps each represent one of the different genetic clusters from the STRUCTURE analysis, presented using Inverse Distance Weighting (IDW). The top right-hand map shows the spatial clustering of each set. IDW is a method of interpolation that estimates cell values by averaging the values of sample data points in the neighbourhood of each processing cell. The closer a point is to the centre of the cell being estimated the more influence, or weight, it has in the averaging process.

a)


1
A
B
C
D
E
F
G
2
3
0 15 30 60 90 120 Kilometers

b)


1
A
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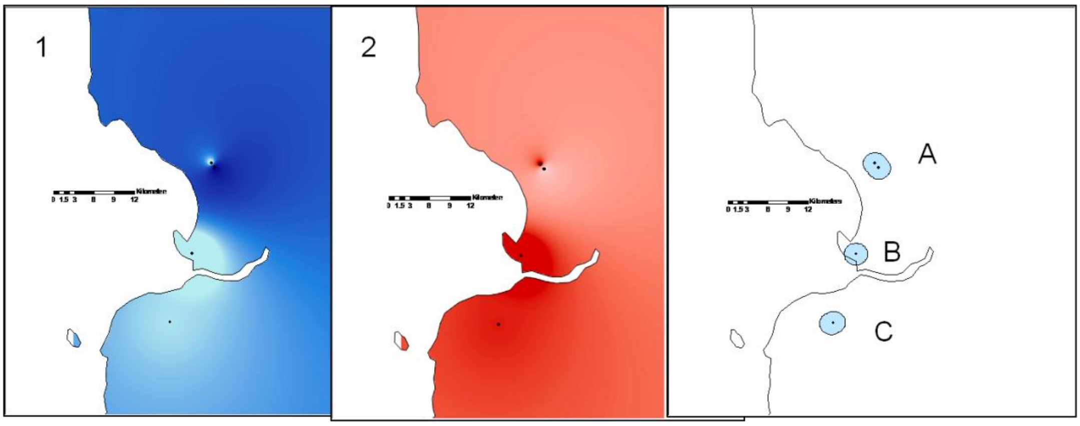
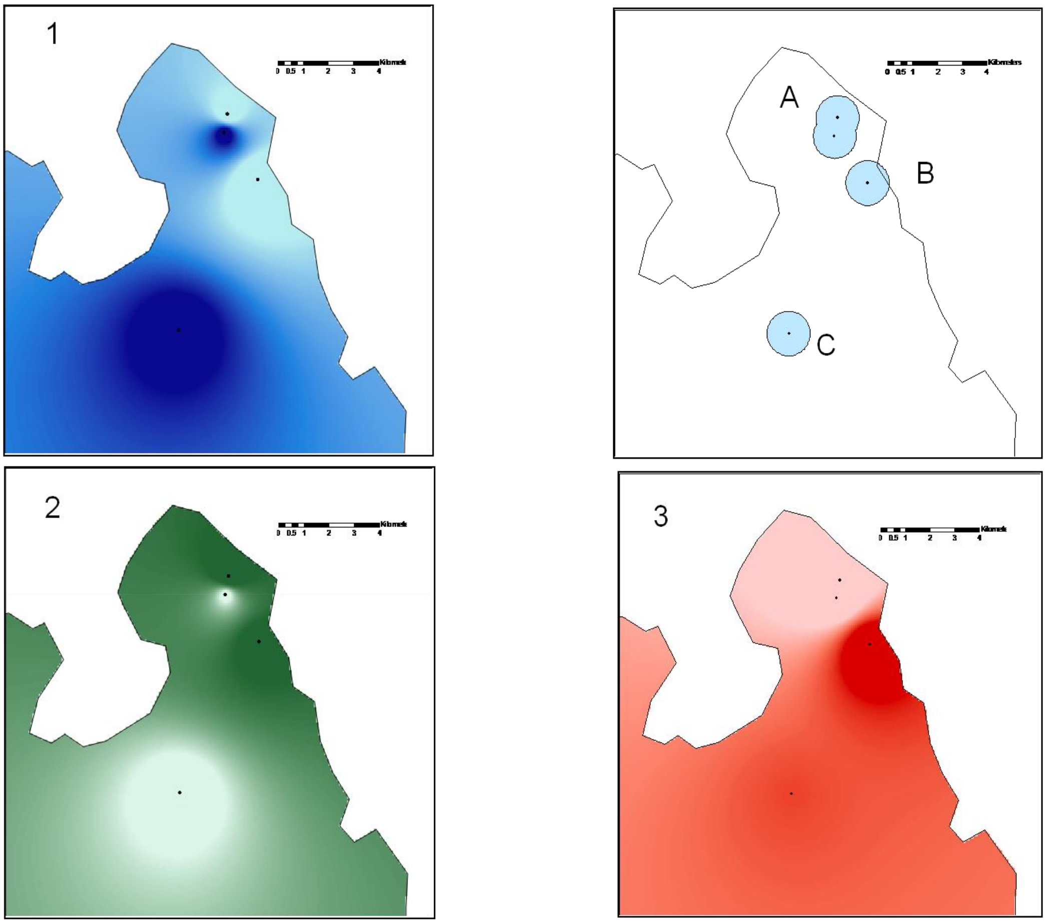
2
3
4
0 25 50 100 150 200 Kilometers
0 20 40 80 120 160 Kilometers

c)



d)



6.5. Discussion

6.5.1. Population genetics modelling

One of the most challenging problems regarding clustering models is the correct estimation of K (Guillot *et al.* 2009). Although similar numbers of clusters, and the assignment of individuals to clusters, was found in many of our models, the models converged on a single clustering solution for only one species (*D. decaryi*). The variation in the optimal value of K is not surprising as it is only optimal with respect to the particular model being used (François & Durand 2010). Many studies no longer rely on a single clustering approach to ensure robust results; it is good practice to analyse genetic data with more than one method (Ball *et al.* 2010; Guillot *et al.* 2009; Latch *et al.* 2006). Previous studies looking at the limitations and merits of various Bayesian clustering models report that non-convergence between different Bayesian clustering methods appears to be relatively common (Ball *et al.* 2010; François & Durand 2010; Frantz *et al.* 2009; Guillot *et al.* 2009; Latch *et al.* 2006; Wilkinson *et al.* 2011). By combining different analyses, one can have increased confidence in the outcomes when the same results are arrived at by different methods (Chen *et al.* 2007; Latch *et al.* 2006). BAPS and MCLUST return the optimal estimate of K as part of the analysis. However, in both STRUCTURE and TESS, the probabilities (Ln P (D|K) and DIC) are compared between multiple runs of K, either directly or through the delta K method. As previously reported by Waples and Gaggiotti (2006), the alternative approach of delta K offered no improvement over the standard approach using posterior probabilities (Ln P (D|K)) for STRUCTURE, as the same numbers of clusters were found by both methods in all instances. The delta K method proved more useful in TESS, where it offered a “second opinion” on the DIC value, which often continued to decrease even after the optimal K was reached, making it difficult to establish the ideal K (Supplementary Figure 6.B). However, a single cluster cannot be tested for in TESS. In addition, delta K is based on second order change (in probability value) and needs estimates of both K-1 and K+1; therefore, when using the delta K approach in TESS, the minimum number of clusters is three. STRUCTURE, however, can test the probability of there being a single cluster (K=1). This is of importance for species where there is no genetic structuring. In the case of *D. pumila,* where probability values for K=1 and K=2 are very similar (with STRUCTURE), a single population may be a better estimate. A single population for *D. pumila* is also supported by our previous AMOVA analysis (Chapter 5).

6.5.2. Genetic vs. spatial population structure

We also compared genetic population structure to spatial population structure. As genetic structuring is often influenced by the spatial distribution of species, we expected population estimates from genetic analysis to coincide broadly with populations estimated from spatial analysis. Also, we expected Bayesian models that take into account spatial information (TESS and BAPS) to perform better (to estimate numbers of populations that are closer to spatial analysis estimates) than non-spatial methods (STRUCTURE and MCLUST). However, these spatial genetic clustering models did not predict the population structure better than non-spatial models (Table 6.2, Supplementary Figure 6.A). Overall, there is little consensus between the estimates of genetic and spatial population number. The same number of genetic and spatial populations was seen in the clustering of *D. velutina* (STRUCTURE and TESS) and of *D. pumila* (TESS), only (Table 6.2).

The inconsistency between the estimated number of spatially- and genetically distinct populations is due to the fact that the estimates of spatial population number are based purely on geographical distance between samples, while estimates of genetic populations identify the “true” gene flow between populations. Genetic analysis distinguishes gene flow barriers that are “invisible” to an analysis based purely on spatial distance (e.g. roads, rivers). However, such barriers may not become obvious until spatial aspects of the genetic differences are explored. On the other hand, spatial estimates are simple, inexpensive and fast to generate and are therefore a first step towards incorporating population structure into conservation assessments. However, spatial separation does not reveal the full story and in order to interpret population structure in more depth and inform conservation action, genetic data should be integrated with spatial analyses. A spatial interpolation of the genetic analysis (Figure 6.1) shows the spatial distribution of populations, identifies gene flow barriers and highlights anomalies. Quémére *et al.* (2010) showed through combined spatial and genetic analysis of population structure that a major river was a barrier to gene flow in the lemur *Propithecus tattersalli*, while the main road did not restrict gene flow.

Both spatial and genetic analyses may seem to be a snapshot in time. However, in the genetic population analysis presented here, the use of an admixture model assessed genetic processes rather than just a measure of extant genetic variation. The admixture model assumes that the data originate from the admixture of ancestral populations, which may have existed at unknown times in the past. All species analysed in this study show an admixture of parental lineages, and therefore give an insight into mixing between ancestral populations. Levels of admixture between populations are consistent with predictions of long-lived tree species having high intra-population genetic diversity, due to the long generation times and the potential high rate of pollen flow (Hamrick 2004). However, population genetic analysis based on the genetic sampling of mature trees reflects the genetic situation at the time of germination and establishment of these samples (possibly several decades ago), not necessarily the current situation. The dry forest and the spiny forest of western and south western Madagascar are currently severely affected by slash-and-burn agriculture, fires, charcoal production and livestock grazing (Gade 1996; Harper *et al.* 2007; Moat & Smith 2007). Therefore, spatial analysis based on populations visited in 2007 and 2008, may better reflect current conservation concerns. Some populations that were genetically connected in the past may have become isolated due to contemporary habitat destruction. Spatial analysis should therefore not be seen as having been superseded by genetic analysis, but through a combination of spatial and genetic analysis a better understanding of the current situation is obtained. Such analyses can also be combined with species counts, predictive mapping and habitat availability, in order to gain a better picture of the conservation status of species.

Adding a spatial dimension to genetic data helps to visualise the genetic diversity within its surrounding landscape (Manel *et al.* 2003; Storfer *et al.* 2006). Simply mapping genetic populations onto geographic locations highlights the landscape utilized by species and populations, and also sheds light on population boundaries which are frequently unclear. Genetic surfacing approaches address some of the current limitations in landscape genetics by providing a statistically powerful, continuous representation of genetic variation (Murphy *et al.* 2008). Also, with the spatial dimension added to the results presented here, predictions of future population structure, based on habitat loss, climate change, and other ecological and anthropogenic factors can be made.

6.5.3. Conservation implications

With the growing concern over biodiversity loss and the associated demand for biodiversity assessment and management, genetic studies of population structure are accumulating considerable added value as an important baseline in conservation biology (Meagher 2007). One might expect species of conservation concern to have fewer populations than species of least conservation concern, as the former are often smaller in range. However, we found no correlation between conservation status and genetic structuring. In the two species of conservation concern, one (*D. velutina*) showed clear population structure, while the other (*D. pumila*) showed little (or no) structure despite similar range size; the same conservation status can therefore have different genetic implications.

Conservation status is influenced, not only by small range sizes, but also by the degree of population structure and fragmentation. While a small range could mean less population structure, a small fragmented range would lead to an increase in the degree of population structure. Having a small range has very different consequences for species in which all individuals belong to a single population than it does for species that exist in small, genetically distinct populations. The latter may occupy a network of sites that provides a resilient metapopulation structure (Mace *et al.* 2008); on the other hand, even if there is genetic diversity between populations, local populations may be too small to be viable. A small range is therefore not equivalent to a subset of the distribution of larger-ranged species. The understanding of population delineation is crucial in establishing effective conservation or management units. In order to develop effective strategies for biodiversity management, understanding not only the evolutionary impacts of future fragmentation but also the spatial aspects of the population structure of a species is critical, as they both have impacts on the probability of extinction for that species.

6.6. Conclusion

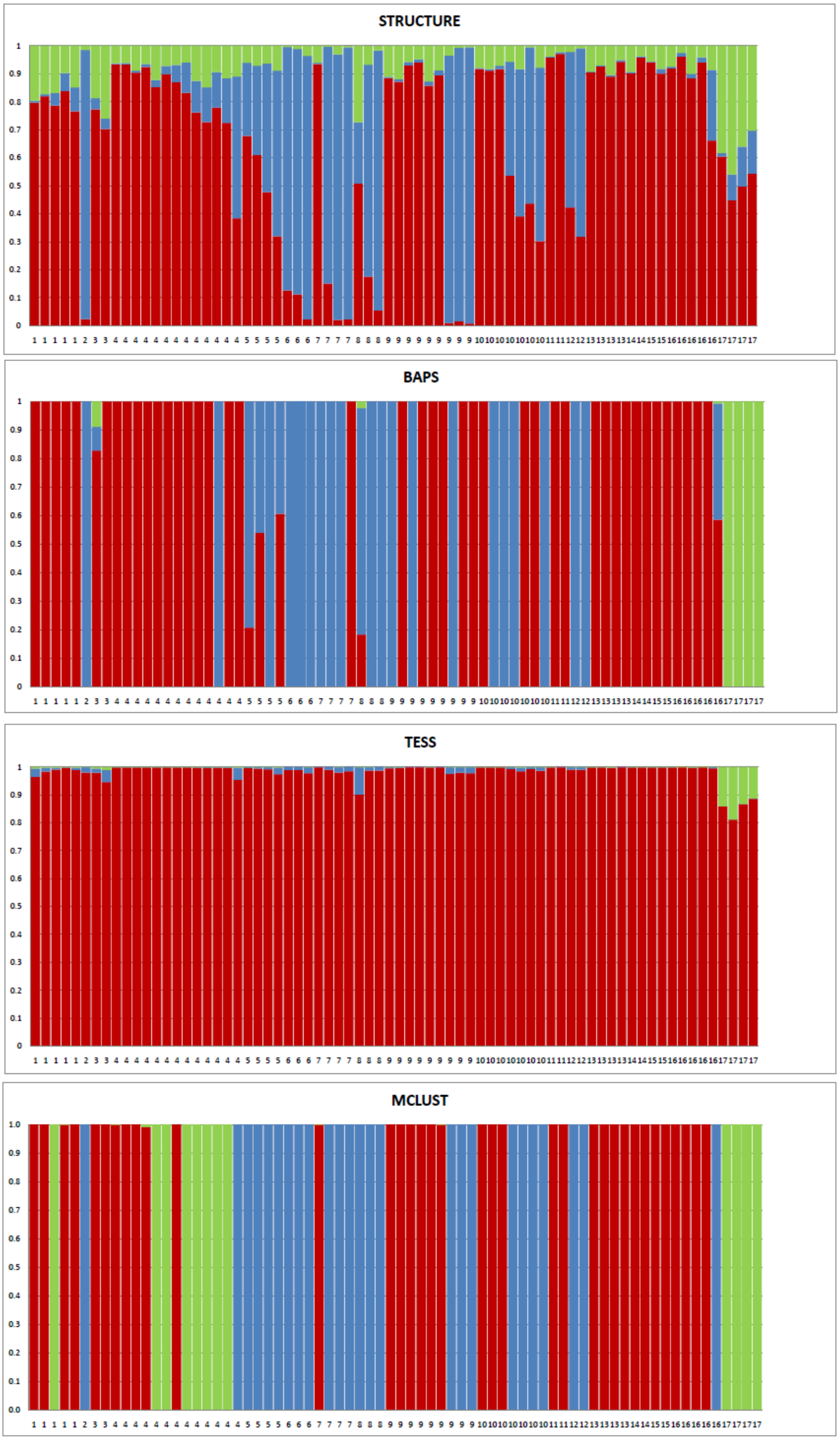
In this study we compared the performance of population genetic clustering and spatial clustering methods. Despite similarities between the methods, there was rarely complete agreement on the ideal clustering solution, due to differences between algorithms and methodology. The preferred clustering method in this study was STRUCTURE, as it consistently gave biologically credible clustering solutions and a clear optimal number of clusters could be detected. Spatial estimates of population number are a good first step for conservation assessments; however, genetic analysis is essential to complete the picture for effective conservation management. Data from two species may portray very different clustering solutions, as was found here, despite similarities in range and conservation rating. Adding a spatial dimension to genetic analysis helps to visualise genetic diversity within its surrounding landscape, providing a statistically powerful, continuous representation of genetic variation (Murphy *et al.* 2008).

Information on population structure is important for effective conservation management strategies, and as population genetic techniques and methods become cheaper and more widely available, so access to this information will improve for this purpose. In order to develop effective strategies for biodiversity management, it is critical that not only are the evolutionary impacts of future fragmentation on the population structure of a species understood, but also that the spatial aspect of these data is taken into account, as both have impacts on the probability of extinction of a species.

6.7. Supplementary material

Supplementary Figure 6.A. Genetic population structure of a) *D. decaryi*, b) *D. floribunda*, c) *D. pumila* and d) *D. velutina* for the four analyses of STRUCTURE, TESS, BAPS and MCLUST. Individuals are represented as vertical lines and their membership to genetic clusters (K) is indicated by the colours.

a)


STRUCTURE
BAPS
TESS
MCLUST
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
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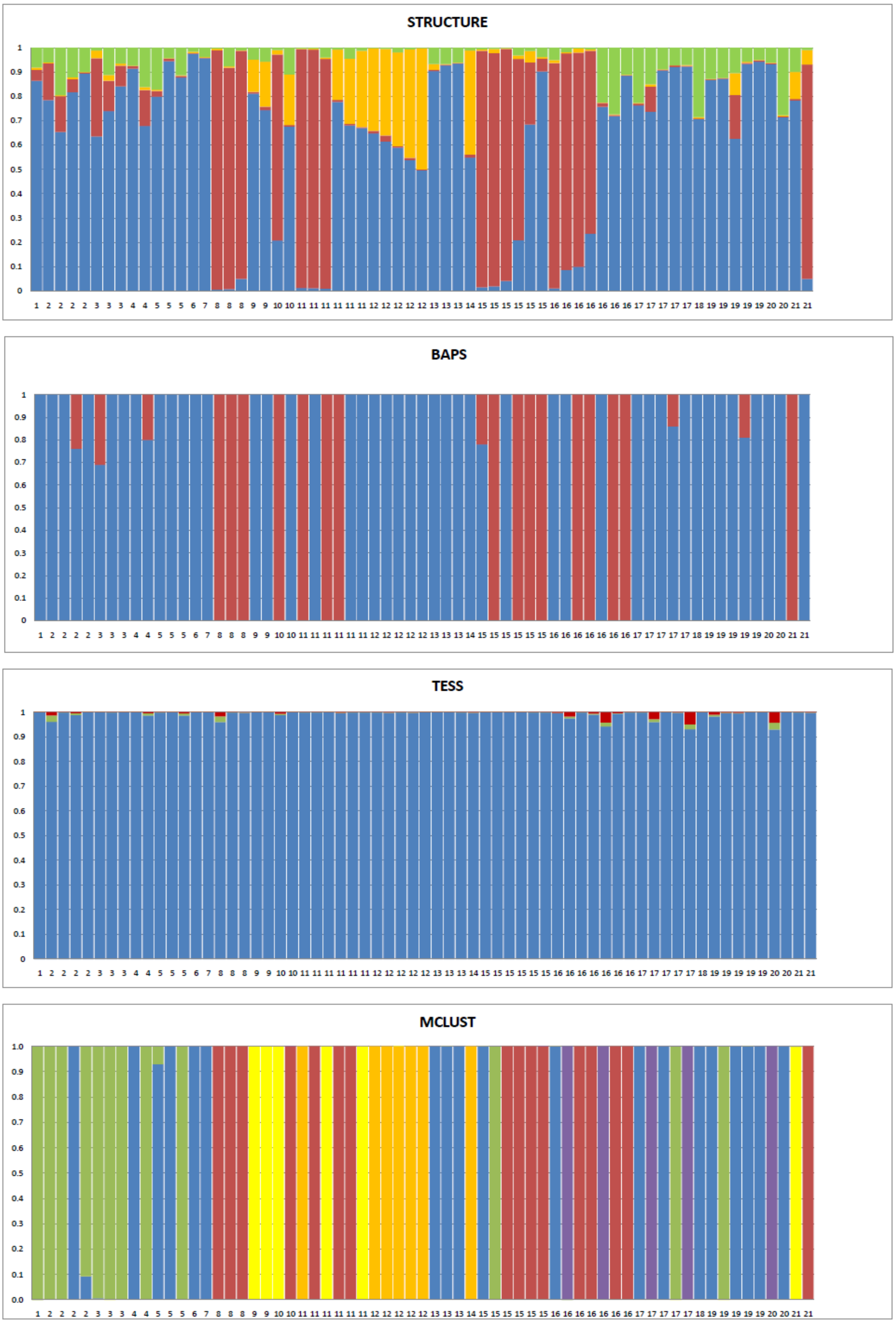
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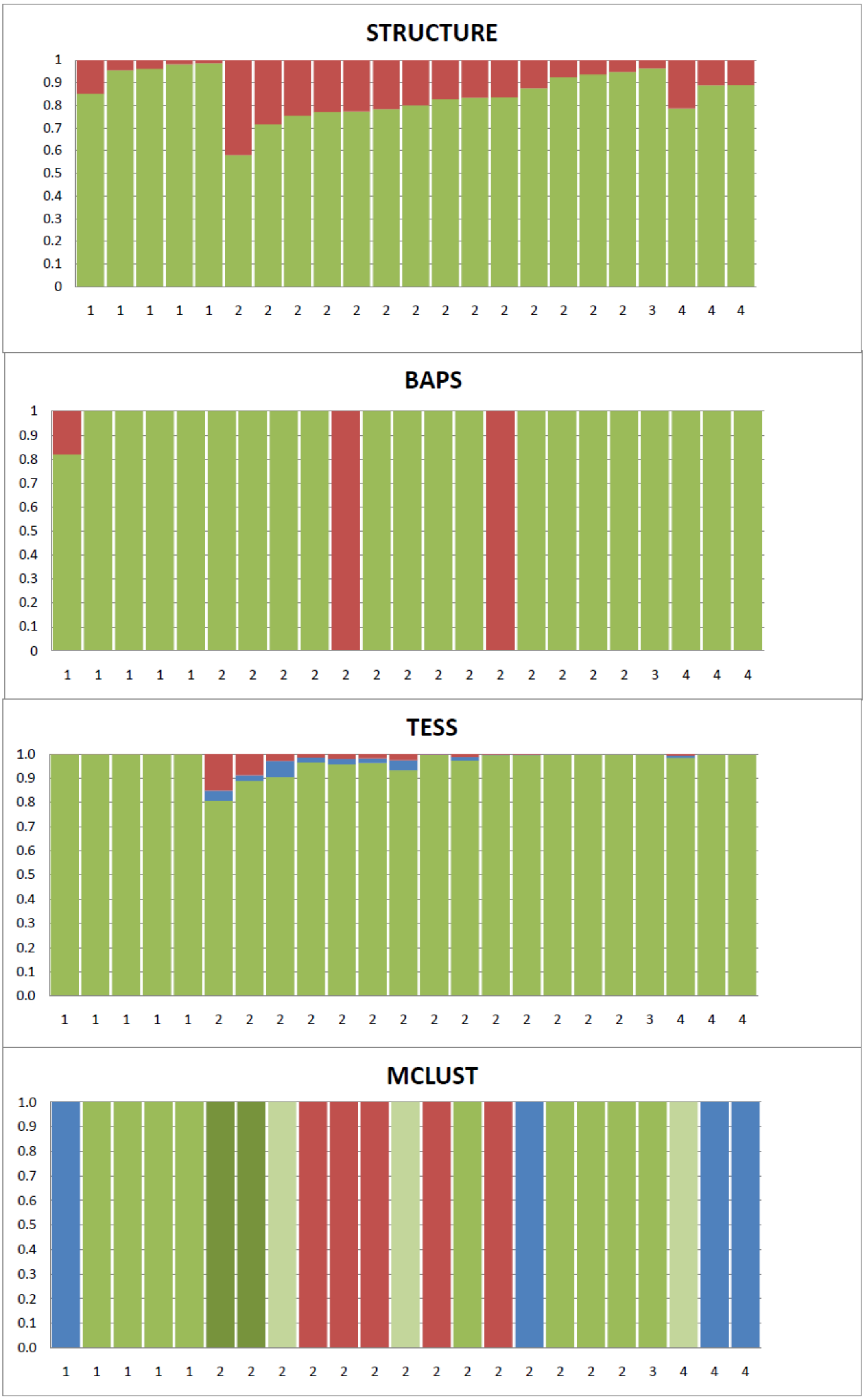
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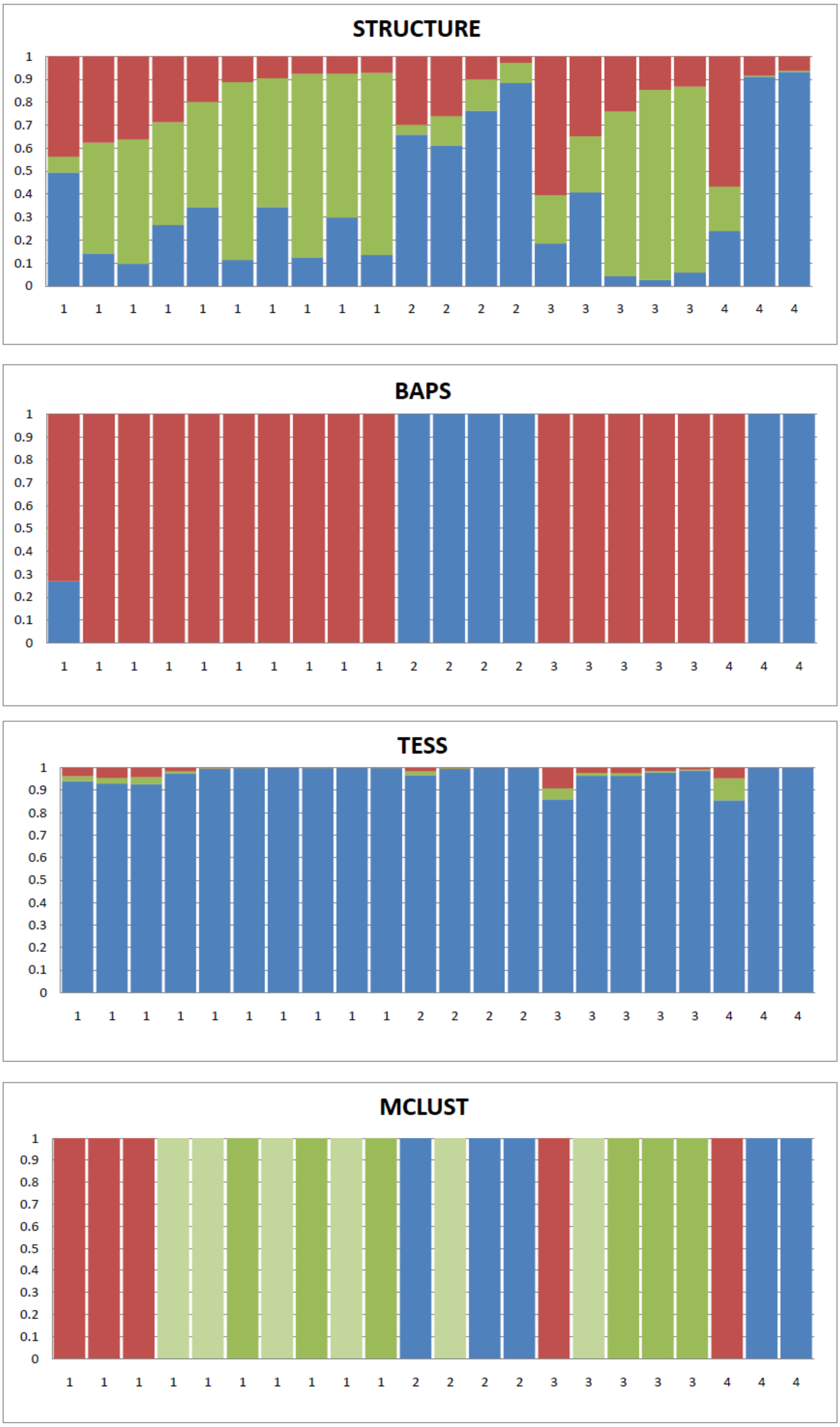
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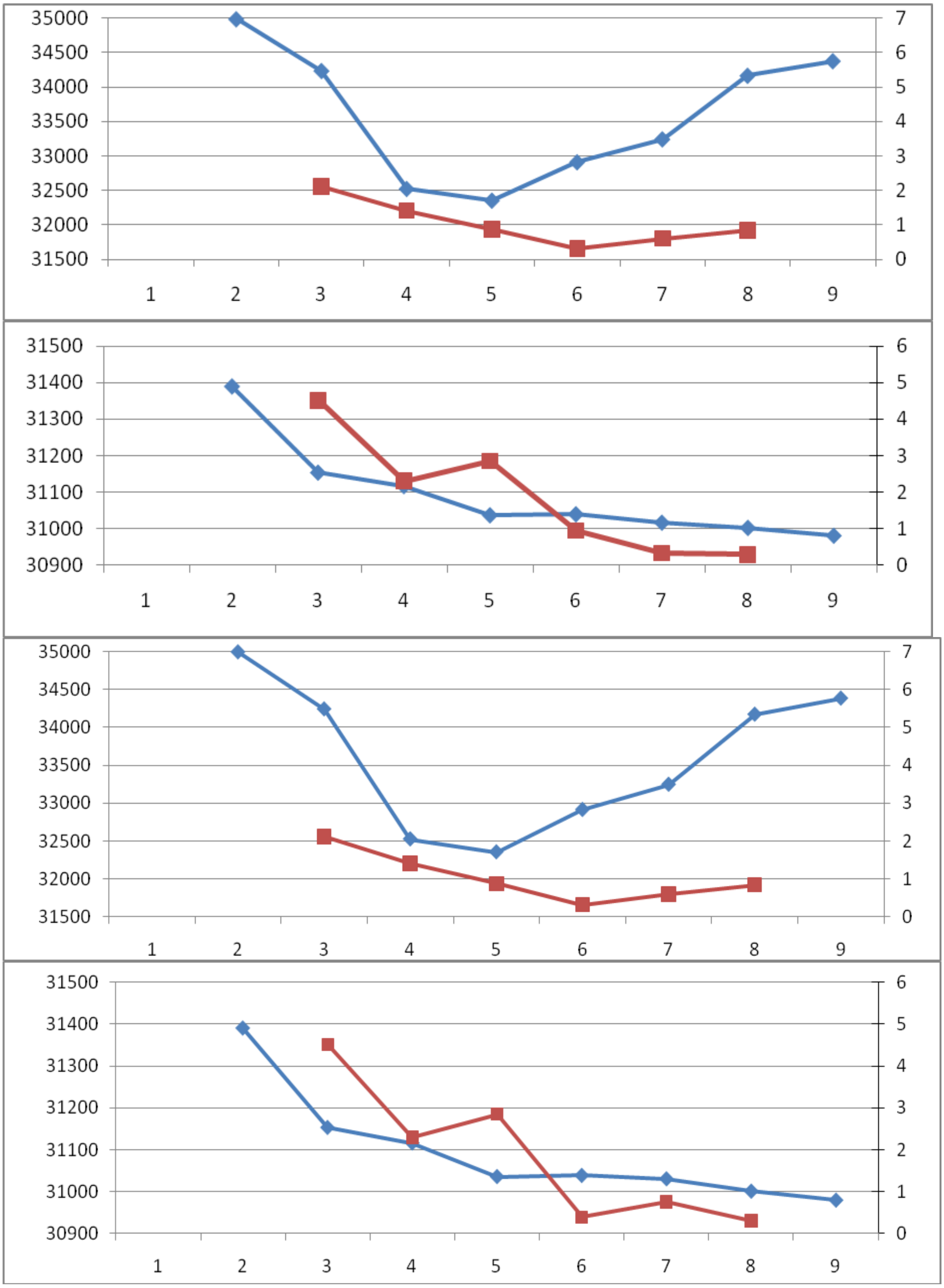
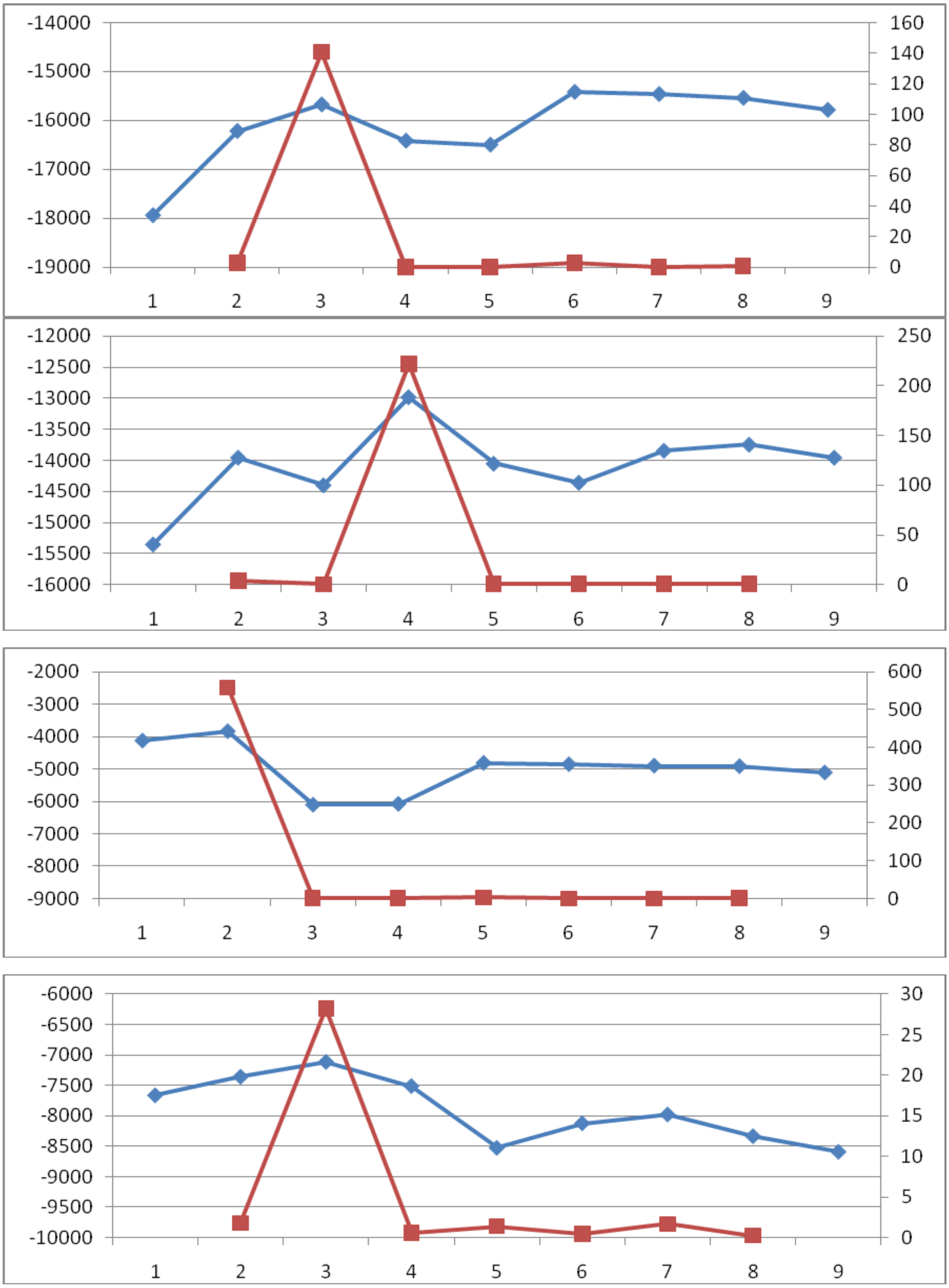
Supplementary Figure 6.B. Average DIC (diamond, left) and delta K (square, right) for the best 10 runs (20%) in TESS (K=2-9) for a) *D. decaryi,* b) *D. floribunda,* c) *D. pumila* and d) *D. velutina.*



Supplementary Figure 6.C. Average Ln P (D|K) (diamond, left) and delta K (square, right) for 100 runs in STRUCTURE (K=1-9) for a) *D. decaryi,* b) *D. floribunda,* c) *D. pumila* and d) *D. velutina.*



Chapter 7

Geographic range and genetic diversity: implications for conservation assessments

7.1. Summary

The world's most widely used system for classifying threat status of species, the IUCN Red List of Threatened Species, does not explicitly take into account genetic factors, such as inbreeding, genetic drift, and loss of genetic diversity. Although these are recognized attributes for reduced viability and increased extinction risk of populations. In this study we combined measures of range loss with measures of genetic diversity to assess how the loss of genetic diversity relates to IUCN conservation status in plants. There are very few studies of genetic diversity loss on a range-wide level. This is a range-wide simulation study on two widespread species (*D. decaryi* and *D.floribunda*) and two range-restricted species (*D. pumila* and *D. velutina)* to assess the impact of range loss on genetic diversity. Generally, there was a linear relationship between the loss of range and the loss of genetic diversity of *D. decaryi* and *D. floribunda*. The predicted losses of genetic diversity in the two range-restricted species were found at the two extremes of the subset of widespread species. Certain differences were also observed in the relationship between the loss of genetic diversity and the IUCN Criteria used. A genetic basis to the thresholds of both Criteria A and B was found with respect to threatened vs. non-threatened categories (i.e. at the threshold for Vulnerable). However, the more finely tuned thresholds within the threatened categories had less genetic basis. This provides a useful insight towards further recommendations and comments on the IUCN thresholds set in the Criteria and their relevance to genetic diversity. Clearly, further species from different taxonomic groups, different geographic areas, and with different life histories and reproductive systems should be investigated in order to fully understand the implications of IUCN thresholds on evolutionary genetic potential.

7.2. Introduction

The International Union for Conservation of Nature and Natural Resources (IUCN), recognizes the need to conserve biodiversity at three levels – genetic diversity, species diversity and ecosystem diversity (McNeely *et al.* 1990). The need to conserve genetic diversity is threefold (Frankham *et al.* 2010): (1) to allow populations to evolve and adapt to environmental change; (2) to prevent the loss of genetic diversity that is associated with inbreeding, and reduced reproduction and survival in normally outbreeding species; and (3) to preserve the genetic diversity that contributes to ecosystem diversity, making ecosystems more resilient to environmental shocks.

Previous work has shown that genetic diversity is lower in plant species with restricted ranges and with smaller population sizes (Frankham 1996; Leimu *et al.* 2006). Range size is closely correlated with population size (Gaston *et al.* 1997), and more widespread populations are able to maintain higher genetic diversity than smaller populations due to higher number of individuals, less inbreeding (i.e. maintenance of heterozygotes) and higher potential for rare alleles to be maintained rather than lost by random genetic drift. A species which undergoes a significant range reduction may suffer from a genetic bottleneck, where a large proportion of individuals are killed (or otherwise prevented from reproducing). The loss of genetic variability concomitant with a bottleneck event is due to increased genetic drift and increased inbreeding – which all ultimately leads to a loss of genetic diversity, especially of rare alleles (Frankel & Soulé 1981).

Range loss can take two different forms and has a significant impact on the genetic structure of a species. Range loss can occur either through a reduction of geographical spread (range lost from the edge) or through fragmentation (range lost from within the interior of the range), which may have very different genetic consequences. Loss of geographic extent decreases the number of individuals and the loss of genetic diversity of such individuals; as populations become smaller genetic drift and increased inbreeding lead to further loss of genetic diversity. In addition, fragmentation can cause further loss of genetic diversity and increased differentiation between fragments due to the restriction of gene flow and exacerbated inbreeding (Ellstrand & Elam 1993; Young *et al.* 1996). It has been shown that when a species becomes threatened, its geographical range does not necessarily contract inwards with the core populations persisting; instead, most species examined persisted in the periphery of their historical geographical ranges, but these ranges tend to be more fragmented (Channell & Lomolino 2000).

7.2.1. Conservation assessments

The world's most authoritative and widely used system for classifying the threat status of species, the IUCN Red List of Threatened Species, does not explicitly take genetic analysis into account in the red listing process. Threat status, as determined by the IUCN Red List Criteria, relates to aspects of population loss and decline in range size, including subpopulation structure, fragmentation, generation length, and extreme fluctuation in numbers (IUCN 2001; Mace *et al.* 2008). These factors also affect genetic diversity; inbreeding, genetic drift, and loss of genetic diversity are recognized as reducing viability and increasing extinction risk of populations but are not explicitly considered in the IUCN Red List Criteria (Laikre *et al.* 2009; Laikre 2010).

Numerous studies have described the genetic diversity of single threatened species, and the genetic history and structure of these species are important factors in the design of effective conservation strategies and management plans (for example Smith & Waldren 2010). There have also been comparisons of the overall genetic diversity of threatened and non-threatened species in the same genera (Cole 2003; Gitzendanner & Soltis 2000; Karron 1987). Spielman *et al.* (2004) compared heterozygosity between threatened and non-threatened species and their results (in their supplementary material) indicate that the level of heterozygosity decreased with increasing threat category. Comparative studies of genetic diversity in species across the IUCN Red List categories are, however, still lacking. Such studies would lead to improved conservation recommendations through a better understanding of the relationship between genetic diversity and threat category. There are very few studies of genetic diversity loss on a range-wide level; here we report on range-wide estimates of genetic decline in four species of *Delonix* (Leguminosae) in Madagascar. We investigated whether or not there is a genetic basis to the inference of risk according to the species' IUCN conservation status. By combining conservation assessments based on range loss with measures of genetic diversity we assessed how genetic diversity is affected by range loss, and more specifically assessed how genetic diversity relates to the IUCN categories and criteria.

7.3. Methods

We used genetic and range data from four species in the genus *Delonix* Raf. (Leguminosae) - *Delonix decaryi* (R.Vig.) Capuron, *Delonix floribunda* (Baill.) Capuron, *Delonix pumila* Du Puy, Phillipson & R.Rabev. and *Delonix velutina* Capuron. The genetic data were obtained by amplified fragment length polymorphism (AFLP) analysis of 182 samples from the four species (for methods see Chapter 5) (Table 7.1).

7.3.1. Allelic richness estimation by multiple subsampling

The number of unique alleles in a population (private allelic richness) is a simple measure of genetic distinctiveness. However, estimation of allelic richness is dependent on sample size because larger samples are likely to capture more alleles than smaller ones (Kalinowski 2004; Leberg 2002; Szpiech *et al.* 2008). As the number of samples for *Delonix* varied across localities depending on the availability of trees to be sampled, a procedure involving multiple random subsampling within each locality was used to estimate allelic richness (*sensu* Leberg 2002). For each species in our study, allelic richness was estimated using a subset of the available data from each locality, restricting the subsample so that the number of individuals included for each locality was no more than the number of individuals present in the locality with the smallest number of individuals sampled. This procedure was applied 1,000 times, taking different random subsamples of each site, in order to obtain a mean and standard error for the estimate of allelic richness.

Table 7.1. The number of individuals, localities, alleles and the range estimates for four species of *Delonix*.

	Individuals	**Localities**	**Total number of alleles**	**Range (km^2) (EOO / AOO)**
Delonix decaryi	72	18	760	32,862 / 16,856
Delonix floribunda	65	18	735	94,258 / 28,339
Delonix pumila	23	4	459	11 / 18
Delonix velutina	22	4	596	7 / 3
TOTAL	182	51		

7.3.2. Simulated range size reduction

For each species, we performed a simulation study to assess the extent to which range loss impacts genetic diversity. We assumed that the present known range represents the full range of the species and that the present number of alleles, estimated by multiple subsampling, represents the maximum genetic diversity. We then simulated the effect of range reduction on genetic diversity by applying multiple subsampling procedures (7.3.1) to randomly selected subsets of the species range, dropping numbers of localities from the full range at random. The minimum number of localities included in a simulated reduced range was three, as this is the lowest number needed to estimate the extent of occurrence (see Chapter 2 and below).

For the full range-wide genetic dataset and for each of 1,000 simulated range reductions, genetic diversity was measured as the percentage of alleles remaining (total number of alleles minus the private alleles lost through the loss of localities). In addition, for each of the 1,000 simulations, the range was calculated in terms of extent of occurrence (EOO) and area of occupancy (AOO) using the Conservation Assessment Tool (http://www.kew.org/gis/projects/cats) (Moat 2007) in ArcView 3.2. EOO is the total geographical spread of a species and is calculated by the area of the minimum convex polygon encompassing all included localities. AOO is the occupied range of a species and is calculated by summing the area of the occupied cells in a grid system, using a cell size (side length) equal to 1/10th of the maximum distance between the most distant pair of included localities (Rivers *et al.* 2010; Willis *et al.* 2003). The IUCN states that the appropriate scale to measure AOO will depend on the taxon in question (IUCN Standards and Petitions Working Group 2010), and a grid size of 1/10th of the maximum distance allows the size of the grid to be adjusted to the geographical range of the species. Also, estimates of the numbers of (sub)populations/locations were carried out in all of the 1,000 simulations, following methods recommended in Chapter 3 and Rivers *et al.* (2010). The simulation of range loss and associated calculations were repeated for all four species. However, due to the low number of localities for *D. pumila* and *D. velutina*, and the requirement of having three localities for the calculation of range measures, only four simulations (subsampled 1,000 times) were made.

7.3.3. Conservation assessments

In the IUCN Red List for Threatened Species there are three threatened categories: Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) (IUCN 2001). Species that do not qualify for one of these three threatened categories are considered non-threatened (Least Concern (LC) or Near Threatened (NT)). Without information on the total number of individuals of a species, only a subset of the IUCN Red List Criteria could be applied here: Criteria A (population reduction), B (geographic range) and D (very small or restricted population). For each simulation, the conservation rating was calculated using Criteria A, B and D according to thresholds set out in the Categories and Criteria v. 3.1 (IUCN 2001) and summarised in Table 7.2.

Criterion A assesses species with significant population reductions over three generations or up to 100 years (whichever is shorter). A time-frame of 100 years is recommended for species with long generation times, and was employed for the world list of threatened trees (Oldfield *et al.* 1998). For Criterion A, when the decline has not ceased, the thresholds for population decline are 80%, 50% and 30% respectively for the three threatened categories (CR, EN, VU). A decline in EOO, AOO and/or quality of habitat can be used as a surrogate for population decline (IUCN 2001), as was the case here. The exact relationship between population decline and range loss is not known. We tested the relationship between loss of genetic diversity and range loss (as a surrogate for population decline) using Pearson's correlation coefficient (r).

Under Criterion B, a species' range needs to fall within the absolute range thresholds given in the criteria, either for EOO or for AOO, as well as fulfilling two out of three subcriteria: i) severely fragmented range/low number of locations, ii) extreme fluctuation in range, habitat or numbers of individuals and iii) continuing decline in range, habitat or numbers of individuals. In this study the number of subpopulations/locations was estimated following methods recommended in Chapter 3 (see also Rivers *et al*. 2010), and continuing decline in range was modelled.

Criterion D2 can be used without information on numbers of individuals; however, using this criterion only a Vulnerable rating can be assigned. A taxon qualifies for Vulnerable under D2 if the AOO is highly restricted (typically less than 20 km^2) or it exists at five or fewer locations, and if there is a plausible natural or anthropogenic threat (IUCN Standards and Petitions Working Group 2010). The IUCN stresses that restricted range alone should not be used to list species under this category; rather, it is evidence that an actual threat to the species is more likely to have an impact on its survival because of its very restricted distribution (Mace *et al.* 2008).

Table 7.2. The range thresholds for Criteria A2-4, B and D2 in the IUCN Red List Categories and Criteria v. 3.1 (IUCN 2001)

	Critically Endangered	**Endangered**	**Vulnerable**	**Not threatened**
Criterion A2-4 EOO & AOO	Reduction >80%	Reduction >50%	Reduction >30%	Reduction <30%
Criterion B1 EOO*	<100 km^2	<5,000 km^2	<20,000 km^2	>20,000 km^2
Criterion B2 AOO*	<10 km^2	<500 km^2	<2,000 km^2	>2,000 km^2
Criterion D2 AOO	n/a	n/a	<20 km^2 or ≤5 locations	>20 km^2 or >5 locations

* two out of the following three subcriteria also need to be fulfilled: i) severely fragmented range/low number of locations, ii) extreme fluctuation in range, habitat or numbers of individuals and iii) continuing decline in range, habitat or numbers of individuals.

For each species and range estimate, the differences in genetic diversity and criteria were tested in SPSS (SPSS Statistics version 17.0) using analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. Finally, we examined how already range-restricted species compare to the pattern of modelled range reduction. For this purpose *D. pumila* and *D. velutina*, the two species with a restricted range, were compared with the modelled range restriction of the more widespread species *D. decaryi* and *D. floribunda*.

7.4. Results

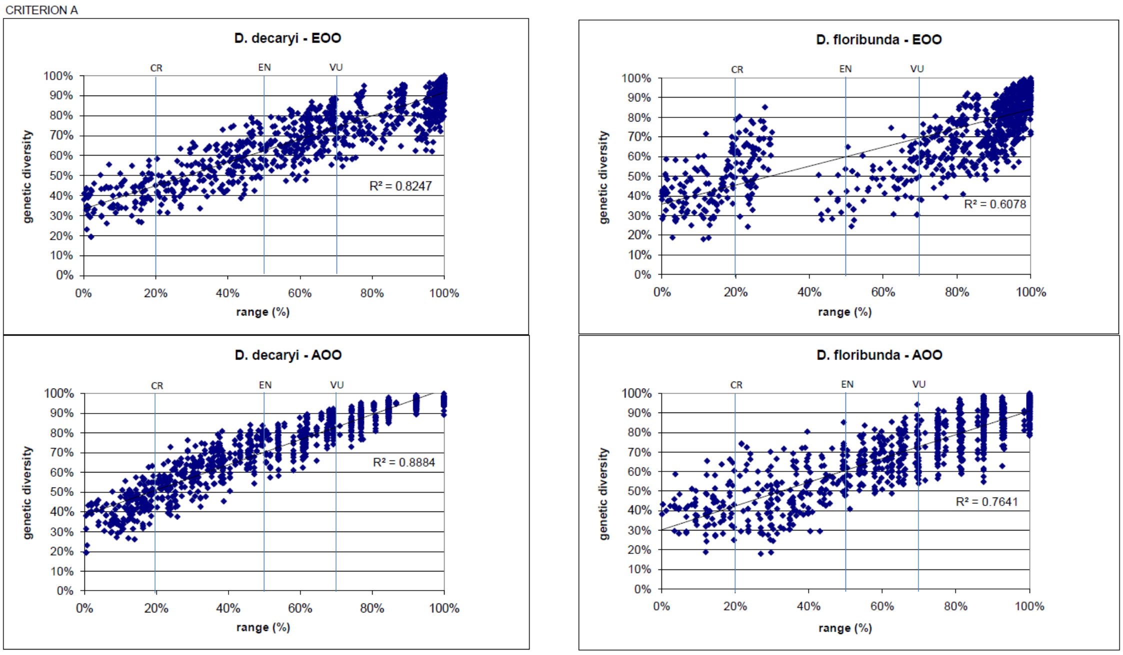
The simulated range reduction and its impact on genetic diversity is plotted in Figure 7.1. There was a linear relationship between genetic diversity and decline in range, as measured by EOO and AOO, for both *D. decaryi* and *D. floribunda* (Figure 7.1). There was also a decrease in the number of subpopulations/locations with decreasing range and genetic diversity (Figure 7.1b). The correlation between genetic diversity and range size was high for both range measures and for both species ($r^2 = 0.61 – 0.89$, all $p<0.001$).

When the modelled range reduction and range sizes were translated into conservation ratings, decreasing genetic diversity were correlated to increasing levels of threat in conservation assessment categories (Figure 7. 2 and Supplementary Figure 7.A). The ANOVA with multiple comparisons showed consistently significant differences between the level of genetic diversity for a threatened category vs. non-threatened category, for both IUCN Criteria (A and B), both range estimates (EOO and AOO), and both species tested (*D. decaryi* and *D. floribunda*) (Figure 7.2). The ANOVA of the results under Criterion A also showed significant differences between the levels of genetic diversity among the three threatened categories. However, this distinction was less clear in the results under Criterion B, where differences between the threatened categories due to genetic diversity are mostly absent (Figure 7.2).

The range size threshold for Criterion D2 was reached by only a single simulation of *D. floribunda* and no simulations of *D. decaryi*, thus it was impossible to determine the degree of genetic diversity loss associated with this threshold. To obtain an estimate of genetic diversity for Criterion D2, therefore, targeted simulations of restricted range were performed (using the same methodology outlined in 7.3.2, but with the AOO range set to <20km^2). In addition, these extra data points were used to highlight different genetic histories of species already restricted in their range (Figure 7.3). Very few combinations of sample sites produced an AOO of less than 20km^2 and therefore the results only reflect the genetic diversity of 4 or 5 sample sites for each of the four species. However, the results show a difference between the two widespread species (*D. decaryi* and *D. floribunda*) and the two range-restricted species (*D. pumila* and *D. velutina*): the two range-restricted species show genetic diversity at the extreme ends of the subset of the two more widespread species. *Delonix velutina* has the highest genetic diversity despite having the smallest range; in contrast, *D. pumila* consistently has the lowest genetic diversity. The ANOVA of the genetic diversity where AOO is <20km^2 showed significant differences between all four species (Figure 7.3). However, as already mentioned, the subsample is small.

Figure 7.1. The decline in genetic diversity (allelic diversity) of *Delonix decaryi* and *Delonix floribunda* with modelled range reduction in EOO and AOO, in terms of a) Criterion A and b) Criterion B. The vertical lines symbolize the thresholds for the threatened categories (Vulnerable, Endangered and Critically Endangered from right to left) under each Criterion. The colour coding in b) represents the number of (sub)populations/locations identified by spatial analysis (Chapter3).

a)



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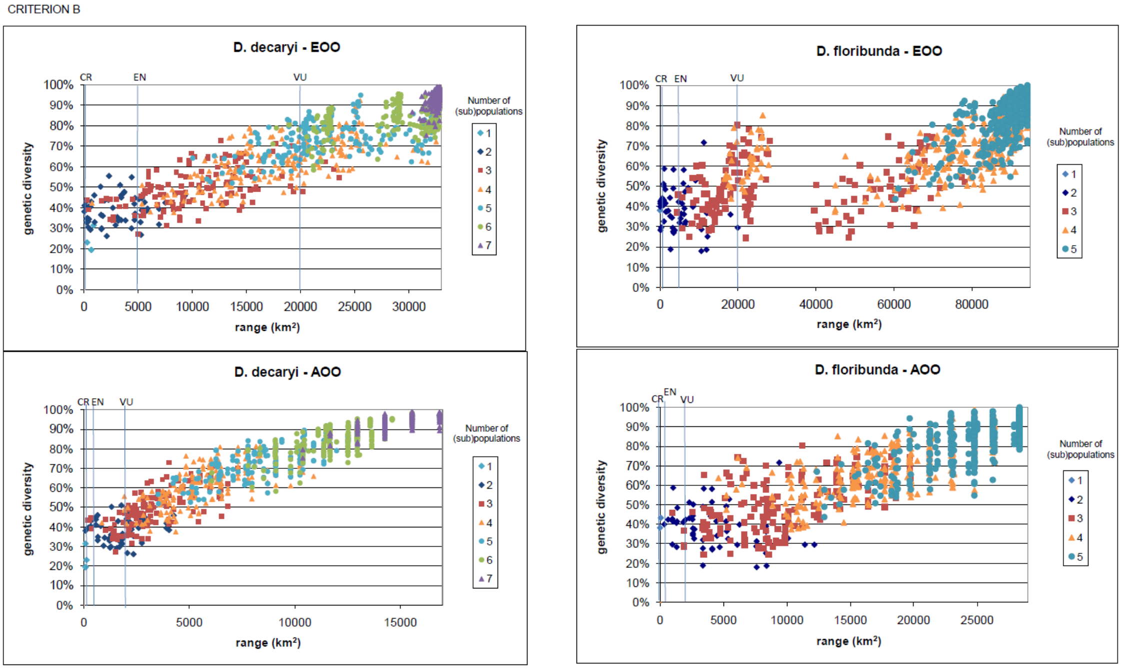
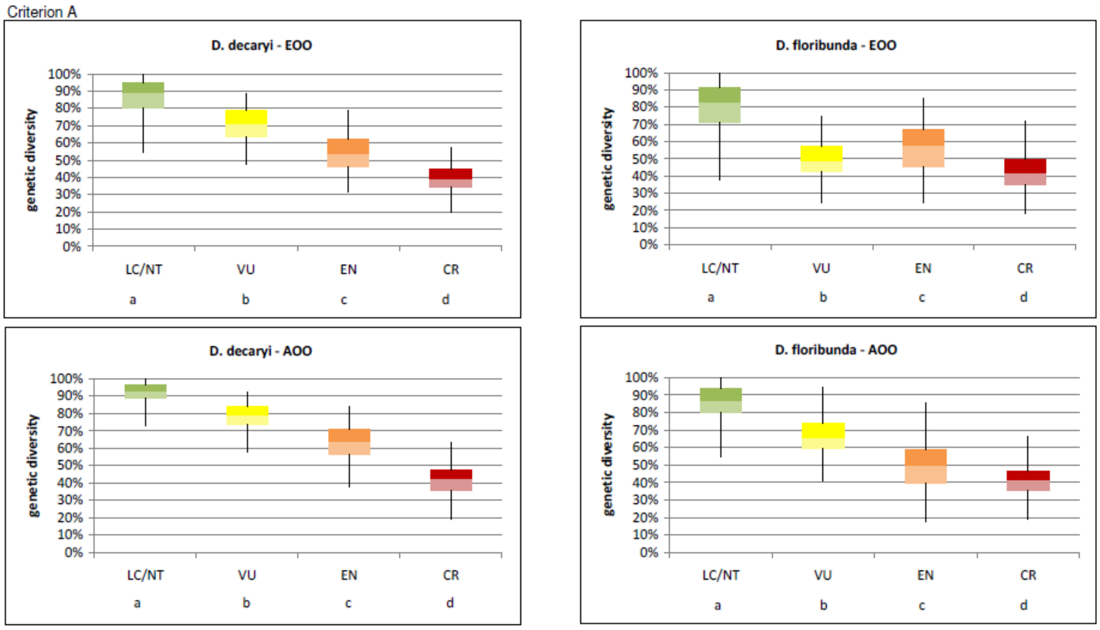


Figure 7.2. Box-plots of the percentage genetic diversity (allelic diversity) for different IUCN categories of threat for *Delonix decaryi* and *Delonix floribunda*, based on range estimates of EOO and AOO, under a) Criterion A and b) Criterion B. The bottom and top of the box are the 25th and 75th percentiles, the band near the middle is the mean, and the whiskers represent the minimum and maximum of all the data. Different letters (a-d), underneath each box, indicate a significant difference between the categories using Tukey's test.

a)



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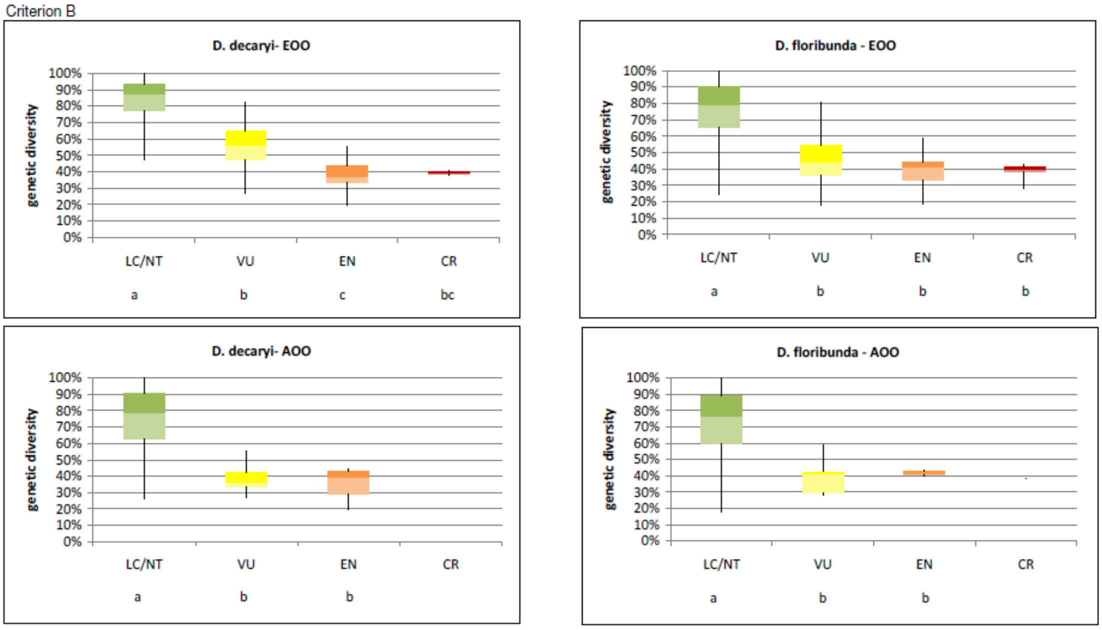
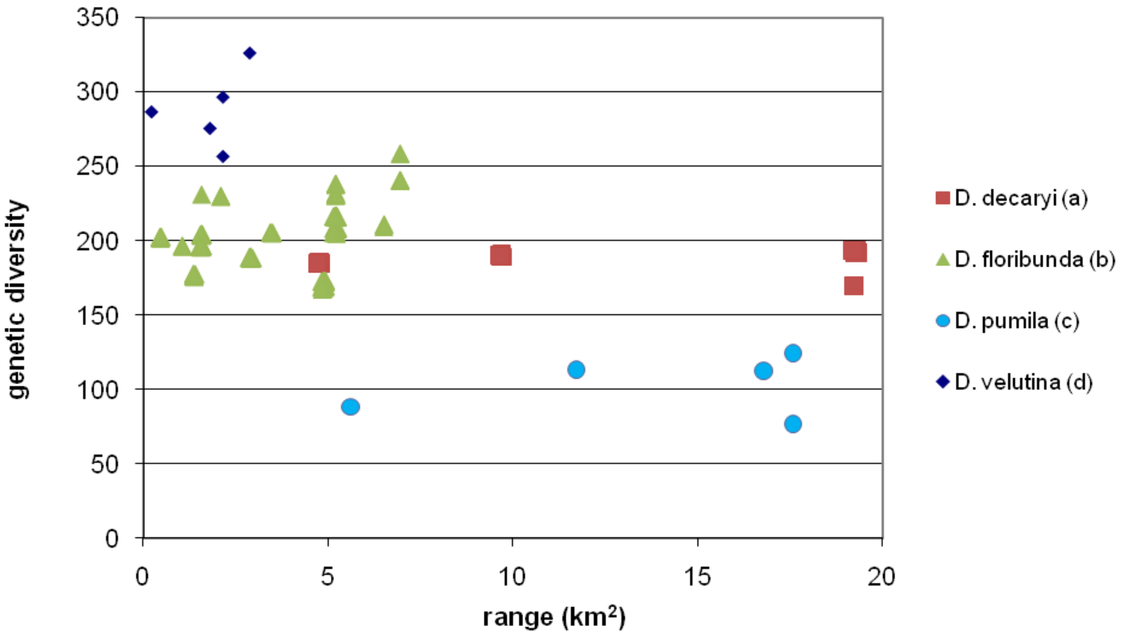


Figure 7.3. The response in genetic diversity (number of alleles) of *Delonix decaryi*, *Delonix floribunda*, *Delonix pumila* and *Delonix velutina* to modelled range reduction when AOO ≤20km^2. The different letters (after each species name) indicate a significant difference between the species using ANOVA, and Tukey's test.



7.5. Discussion

Generally, there is a linear relationship between the loss of genetic diversity and range loss for both *D. decaryi* and *D. floribunda*. The predicted losses in genetic diversity of the two range-restricted species, *D. pumila* and *D. velutina*, are seen at the two extremes of the two widespread species, *D. decaryi* and *D. floribunda*. The pattern of genetic diversity loss was also consistent across both measures of range loss – EOO and AOO. However, some differences are observed in the loss of genetic diversity under different IUCN Criteria, as is set out below.

7.5.1. Criterion A vs. Criterion B vs. Criterion D2

Under both Criteria A and B, there are significant differences in the levels of genetic diversity observed between threatened and non-threatened categories. This indicates that the Vulnerable threshold, which distinguishes a non-threatened category from a threatened category, is set at a biologically, or at least genetically, meaningful level. There is generally a higher level of genetic diversity in species classified as threatened under Criterion A than in those classified as threatened under Criteria B and D. The level of genetic diversity at a certain threat category is therefore not consistent across criteria. This is not surprising, as the criteria are measuring different aspects of threat: under Criteria B and D absolute range size is measured, while under Criterion A a reduction in range is assessed.

For *D. decaryi* and *D. floribunda*, a less conservative rating was obtained under Criterion A than either Criteria B or D, i.e. a species may be considered threatened under Criterion A but not under Criteria B or D. This is due to the fact that their current range (100%) falls outside the threatened threshold of Criteria B and D; these species cannot qualify for a threatened category using these criteria until more than 30% of their current range is lost. However, the two range-restricted species (*D. velutina* and *D. pumila*) both qualified for a threatened category under Criteria B or D at their current range (i.e. before 30% of the current range was lost) and a more conservative rating is obtained under Criterion A than Criteria B and D. Only species with an EOO of 28,571km^2 (and/or AOO of 2,857km^2) qualify for a threatened category for Criterion A at the same time as Criterion B (i.e. 30% reduction in range is equal to 20,000 km^2 (EOO) or 2,000km^2 (AOO)). This shows that a threatened rating under different criteria is based on different aspects of threat and depending on the biological history of a species, different criteria will be more or less conservative.

Under Criterion A, a significant difference between the levels of genetic diversity was also observed among individual threat categories, whereas this difference in genetic diversity between categories was not detected under Criterion B. The thresholds within the threatened categories (i.e. those for Endangered and Critically Endangered) can therefore be considered more genetically meaningful under Criterion A than under Criterion B. This consideration does not apply to Criterion D2, as only the Vulnerable category can be assigned here. Criterion D2 has been criticised for being too inclusive

(IUCN Standards and Petitions Working Group 2010; Mace *et al.* 2008); however, this small study demonstrates the opposite. The two species that qualify for Vulnerable under Criterion D2 did so after qualifying for Critically Endangered using Criteria A and B.

7.5.2. Temporal aspects of range loss

As mentioned, a reduction in range over a timeframe of three generations (up to a maximum of 100 years, whichever is longer) is needed for a species to qualify as threatened under Criterion A. The temporal aspect of this range loss can be incorporated into the simulations presented here based on projected habitat destruction in Madagascar. Recent estimates of the deforestation rate of the dry spiny forest in Madagascar are around 1% per annum (Harper *et al.* 2007; MEFT *et al.* 2009; Moat & Smith 2007). Therefore the percentage of range lost (Figure 1) can be interpreted over a 100 year timeline (from right to left), assuming a continued deforestation rate of 1% a year from the current extent.

This study models the loss of genetic diversity through the loss of individuals from the known range, and does not model genetic drift or inbreeding. Although drift and inbreeding are critically important processes to the evolution and survival of species, these genetic processes are unlikely to have any major genetic impact over the 100 year time frame on which this habitat loss is predicted, as the generation times for *Delonix* are likely to be measured in decades. Drift and inbreeding may, however, be the factors that have led to the differences in the two range-restricted species. *Delonix pumila* is likely to have been a historically range restricted species, subject to drift and inbreeding, and to have adapted to its low levels of genetic diversity. On the other hand, *D. velutina* has higher genetic diversity than would be expected based on its range, which may indicate a recent range reduction that has not yet affected its degree of genetic diversity, or possibly, there has been a recent increase in its genetic diversity. This result provides further support to range restricted species having very different genetic histories (see also Chapter 5).

7.5.3. Range size vs. population size

Census population size is the "holy grail" for plant conservation assessments, as it is almost always lacking. If more was known about plant population sizes and their fluctuations, conservation assessments could make use of all of the categories and a more complete estimate of the extinction risks facing plant species could be achieved. Criticism has highlighted that a decline in habitat area or in range cannot be straight-forwardly translated into a decline in population size, especially if this involves the loss of edges or lower-quality habitat (Akçakaya *et al.* 2006; Rodríguez 2002). However, this study has found that the correlation between genetic diversity and modelled range is high for both widespread species (*D. decaryi* and *D. floribunda*) and both range measures (EOO and AOO) ($r^2 = 0.61 – 0.89$, for all $p<0.001$). In comparison, in a meta-analysis, Leimu *et al.* (2006) found that a mean significantly positive correlation between population size and genetic variation in the studies examined was lower ($r =$ approx. 0.4) than the correlation between genetic diversity and range in this present study. Although Leimu *et al.* (2006) did not test for a relationship between genetic diversity and range size, they did find that the relationship between population size, plant fitness and genetic diversity implies that negative effects of habitat fragmentation on plant fitness and genetic variation are common. The strong correlation between genetic diversity and range found here may, therefore, support plant conservation assessments to be based on genetic diversity rather than population size data, whenever such information is available.

7.6. Conclusions

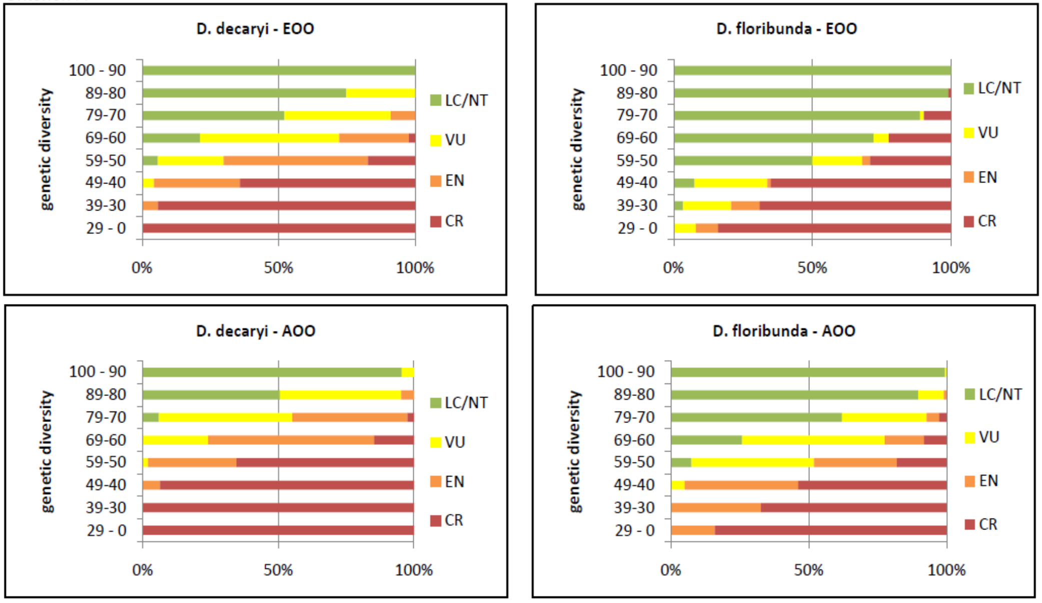
Clearly, both genetic and demographic factors influence the risk of extinction for a small population – demographic stochasticity, environmental stochasticity, genetic drift and inbreeding. These factors are often looked at in isolation; however, combining genetic analysis with the very real threat of current habitat loss and fragmentation adds new, and much needed, information about the genetic robustness of the IUCN Red List categories.

Studies such as this help set the tone for more informed species conservation assessments and the integration of population genetics into the IUCN Red List. Despite its limited taxonomic scope, clear messages are evident from the work presented here. There is a genetic basis to the threatened IUCN Red List categories versus the non-threatened categories using different criteria (i.e. at the Vulnerable thresholds). However, the genetic basis of the thresholds between the threatened categories is less obvious. We would welcome further range-wide genetic data on species from different taxonomic groups, from different geographic areas, with different life histories and reproductive systems in order to make further recommendations on the IUCN thresholds set in Criteria A, B and D, and their relevance to genetic diversity. Such studies will be fundamentally important for understanding how the IUCN thresholds can be interpreted in terms of evolutionary genetic potential, and the prospects for plant conservation improved.

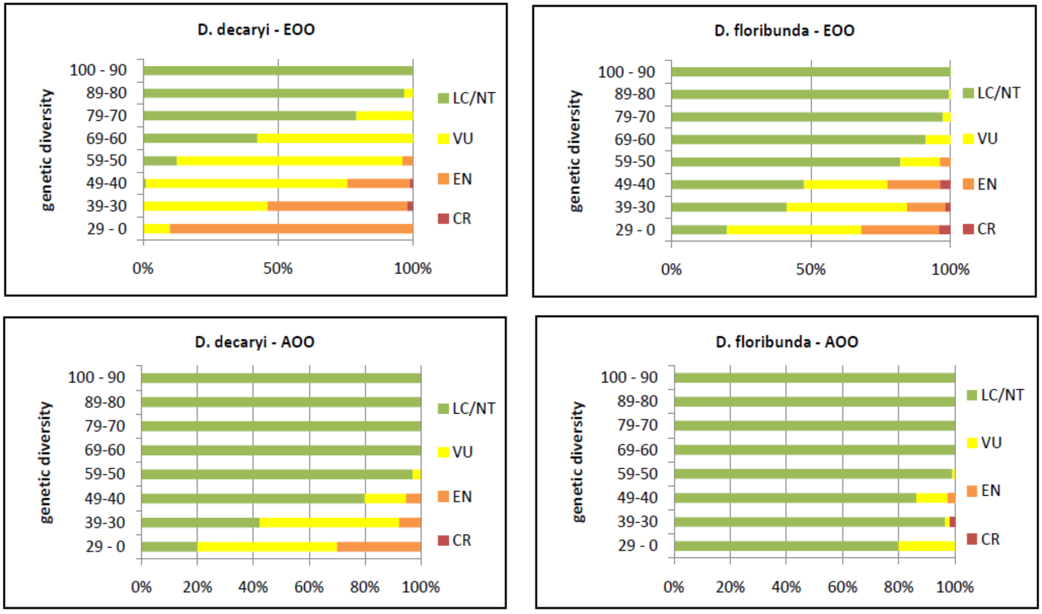
7.7. Supplementary material

Supplementary Figure 7.A. The distribution of Red List categories for different levels of genetic diversity, for the two range measures (EOO and AOO), for *Delonix decaryi* and *Delonix floribunda,* in terms of Criteria A and B.

Criterion A



Criterion B



Chapter 8

Conclusions

This thesis has confirmed that the information held in herbaria around the world is invaluable for producing conservation assessments for some of the world's most poorly known plants. Herbarium-based conservation assessments provide essential information for prioritisation purposes. Furthermore, herbarium-based assessments can be used with confidence to assign a species to a threatened or a non-threatened rating, and often to the correct specific IUCN Red List category of threat. Field-based information and genetic analysis provide further detail that is important in conservation management decisions, although the latter is currently not incorporated in the assessment process directly. This thesis has shown that combining spatial analysis with range-wide population genetic data provides a more complete picture of landscape-level population dynamics and the impacts on conservation status.

8.1. Main findings

The main aims of this thesis were: (1) to investigate how the quality of herbarium-based conservation assessments can be optimised; (2) to assess the extent to which herbarium-based conservation assessments reflect the reality on the ground; and (3) to scientifically validate genetic and spatial underpinning of the IUCN criteria. The main findings are listed below.

1. Optimising the quality of herbarium-based conservation assessments (Chapters 2-4)

- Using as many relevant data sources as possible will ensure that the most accurate range-based preliminary assessments are made.

- Preliminary range-based assessments achieved a rating consistent with the final conservation status for over 95% (90%) of species when using up to fifteen (ten) herbarium specimens.
- Species that are assigned a threatened status based on fewer than 15 specimens should be flagged to indicate a possible under-representation when published.
- Subpopulation, location and fragmentation analyses using herbarium records can help fulfil the required subcriteria in IUCN Criterion B.
- For the estimation of population numbers using GIS techniques, results support the application of a circular buffer method with a species-specific sliding-scale, such as 1/10th the maximum interpoint distance.
- Herbarium-based desktop analysis can effectively assess species using criteria other than Criterion B. For example, species distribution modelling of species habitat and the effect of future climate change can assess species under Criterion A.
- Species distribution modelling of species habitat (both current and future predictions) is useful for fieldwork planning.

2. Assessing whether herbarium-based conservation assessments reflect the reality on the ground (Chapters 5-6)

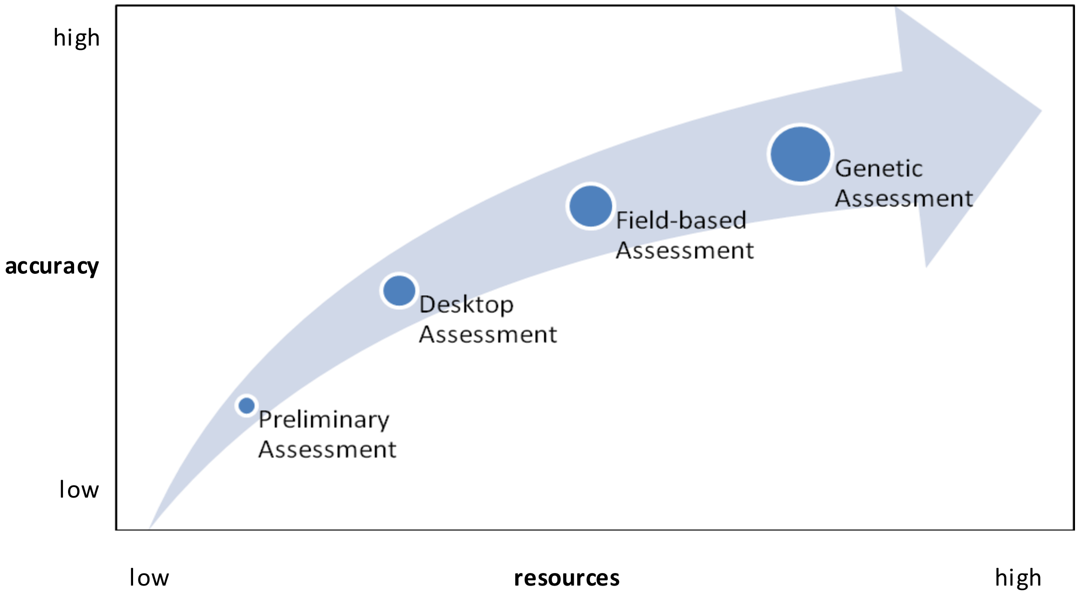
- Species classified as threatened by IUCN Red List Criteria may differ widely in their genetic diversity and genetic structure.
- In terms of genetic structure, a restricted-range species may differ significantly from a subset of a widespread species of equivalent range.
- The number of populations determined by GIS methods are broadly comparable to those determined by genetic analysis, but the geographic limitations of the populations identified by the two methods differ.
- Herbarium-based conservation assessments provide enough information for IUCN Red List listing and prioritisation. For conservation management and

specific conservation action, however, more data (e.g. field data and genetic analysis) are desirable.

3. Scientifically validating the genetic and spatial underpinning of the IUCN criteria (Chapter 7)

- IUCN range thresholds for Criteria A and B have a genetic basis at the threatened vs. not threatened level.
- The IUCN thresholds in Criterion A have a genetic basis between the threatened categories (Vulnerable, Endangered and Critically Endangered).
- The level of genetic diversity of a species qualifying as threatened under one criterion will not necessarily qualify as threatened under another criterion.

Figure 8.1. Relationship of the four different stages of conservation assessments



8.2. Relationship between the four stages of conservation assessments

8.2.1. Preliminary → Desktop → Field-based → Genetic assessments

To assess the accuracy and reliability of the herbarium-based assessments, the ratings from the two stages (preliminary assessments and desktop assessments) were compared with the ratings from the conservation assessments based on the reality on the ground (field-based assessments and genetic assessments). With increasing resources such as time, effort, money, expertise and data the assessments are an increasingly good reflection of the reality of threat (Figure 8.1). At each assessment stage, new information becomes available and improves the level of knowledge of a species. This is discussed below in general terms followed by specific examples taken from the *Delonix s.l.* data (Table 8.1).

Table 8.1. Table of the four assessment types for *Delonix s.l.* – preliminary, desktop, field-based and genetic. Changes at each stage are marked in bold. See also Appendices 1-4.

Species	Preliminary Assessment	Desktop Assessment	Field-based Assessment	Genetic Assessment
Colvillea racemosa	LC	LC	LC	LC
Delonix boiviniana	LC	LC	LC	LC
Delonix brachycarpa	LC	**NT**	*	*
Delonix decaryi	LC	**NT**	NT	**VU A3c**
Delonix floribunda	LC	LC	LC	LC
Delonix leucantha	LC	**NT**	*	*
Delonix pumila	EN B1+2	EN B1**ab(iii)+2ab(iii)**	EN B1ab(iii,**v**)+2ab(iii,**v**)	EN B1ab(iii,**iv**,v) +2ab(iii,**iv**,v)
Delonix regia	LC	LC	LC	LC
Delonix tomentosa	DD	**CR B1ab(iii); D**	*	*
Delonix velutina	EN B1+2	EN B1**ab(iii)**+2**ab(iii)**	EN B1ab(iii,**v**)+2ab(iii,**v**)	EN B1ab(iii,v)+2ab(iii,v)
Lemuropisum edule	EN B1	EN B1**ab(iii)**	EN B1ab(iii,**iv,v**)	EN B1ab(iii,iv,v)

*No updates were made for the field-based or genetic assessments for *D. brachycarpa, D. leucantha* and *D. tomentos*a as they were not observed in the field.

8.2.2. Preliminary → Desktop assessments

Moving from range-based preliminary assessments to desktop assessments, more information is gathered about the species from specimen labels, literature sources and taxonomic or regional experts. This information adds to the preliminary assessments of range, and either supports or suggests amendments to these ratings.

Species known from a single locality cannot be given a preliminary assessment, since these assessments require a minimum of two data points (for AOO estimates, and at least three for EOO estimates). This means that 11% (47 species) of endemic Malagasy legumes are considered Data Deficient (DD) based on a preliminary assessment (Appendix 1). However, guidelines are available on how to assess these species (IUCN Standards and Petitions Working Group 2010), and the desktop assessment process can often assign a conservation rating for many hitherto DD species.

- *Delonix tomentosa* was only known from the type locality; in the desktop assessment a category of Critically Endangered (CR) was assigned, as the species has not been refound at the type locality despite an active search (following recommendations of Callmander *et al.* 2005; IUCN Standards and Petitions Working Group 2010).

Desktop assessments can provide true information needed to qualify as a full IUCN assessment under Criterion B by addressing the subcriteria of severe fragmentation, number of locations, continuing decline and extreme fluctuation.

- In *Delonix s.l.* the desktop assessments allowed the preliminary conservation assessments to be supplemented with the subcriteria of severe fragmentation, number of locations and continuing decline, thus upgrading the preliminary assessments to full assessments for *D. pumila, D. velutina* and *L. edule.*

If the subcriteria under Criterion B are not fulfilled, then a species can be rated as threatened based on its restricted range under Criterion D2 (Vulnerable), if the AOO is < 20km^2 or there are five or fewer subpopulations and there is a plausible threat (IUCN 2001).

- No species of *Delonix s.l.* qualified as Vulnerable under Criterion D2.

Desktop assessments can provide information on non-threatened species and place them in either the Near Threatened (NT) or Least Concern (LC) categories, depending on whether the species is likely to qualify for a threatened category in the near future (NT), or if the range is widespread or naturally restricted but not at risk (LC), respectively.

- Within *Delonix s.l.,* three species (*D. leucantha, D. decaryi* and *D. brachycarpa*) classified as LC at the preliminary assessment stage were considered NT after the desktop analysis. The range measures were not below the thresholds of Criterion B, but there is high risk of continued decline (due to climate change and habitat destruction) and severe fragmentation of their habitat.

Using modelling in the desktop assessment process, criteria other than Criterion B can be used for assessment. One example is species distribution modelling of species habitat and projecting this into the future to model the impacts of climate change. Climate change modelling allows an assessment based on habitat decline, using Criterion A. However, the link between the predicted suitable habitat loss and the population loss needs to be verified.

- Among *Delonix s.l.*, two species included in the species distribution modelling exercise (*D. decaryi* and *D. leucantha*) showed a predicted decline in species range that could qualify for a threatened category. However, in the desktop analysis, the link between predicted loss in habitat and the actual impact on the species could not be established (see population genetics section for further information) and the species were classified as NT in this instance.

Desktop assessments can also classify a species according to Criteria A, C, D and E if information on population size (number of individuals) is available.

- Accurate population size estimates were not available from herbarium specimen data for *Delonix s.l.*

8.2.3. Desktop → Field-based assessments

Field-based assessments incorporate direct observations of the species in its habitat with the information previously used in the desktop assessment. Assessments based on observations in the field are often considered complete assessments. The changes in ratings from the desktop assessment process to field assessments are due to updated, extended and enhanced information, such as population size and structure, range, threats and conservation measures.

Range estimates from preliminary assessments can be updated with respect to the current situation on the ground – including new populations found and excluding extinct populations.

- In *Delonix s.l.,* field assessments extended the known range for several species but this did not lead to a change in the threatened rating for any of the species found in the field.

The direct threats to a species and the related conservation measures can be more apparent when the species is observed in the field. Information obtained during the desktop assessment may be anecdotal or no longer valid. Alternatively, it could be that the information is relevant to an area, but not necessarily to all species in that area.

- Previously unrecorded threats were observed for many of the species, due to expanding human settlements, new mining areas and insufficient maintenance of officially protected areas. In contrast, one population of *L. edule* was well protected (outside a formally protected area) by a village-based scheme to protect the nearby forest.

During fieldwork, an estimate of population numbers, density and structure can be made. Ideally, recurrent field assessments would measure changes in population size and structure more accurately (Criterion A).

- Initial estimates of population size were made for *Delonix s.l.*; however, without a repeat visit changes in population numbers cannot be calculated. Seedlings, in any significant number, were only observed in *Colvillea racemosa.*

8.2.4. Field-based → Genetic assessments

As genetic information is not directly incorporated into the IUCN Categories and Criteria, few changes in conservation rating are expected between the field assessments and the genetic assessments. However, genetic analysis can support and give important background information to many of the species assessments. The general genetic diversity and structure of a species can provide information on the historical context of a species and help to complete the full picture for the final conservation rating.

One instance where genetic information can be used directly is the estimate of population number (Criteria B and C).

- In *D. pumila,* the genetic analysis indicated that there was a single interbreeding population. For the other species, the estimates of number of genetic subpopulations did not differ significantly from those estimated by GIS analysis (but did differ in delimitation).

Another area where genetic data can prove useful is to support findings from climate change analysis, by linking predicted range loss to genetic diversity loss.

- In *D. decaryi* a modelled loss in range showed a significant decrease in genetic diversity. This means a Vulnerable rating was given based on the climate change modelling (using Criterion A), as a predicted 46% of its current range would be lost due to climate change, which in turn would lead to an approximately 30% decrease in genetic diversity.

Genetic analysis also provides information on genetic diversity within and among species, which reflects the historical gene flow. Although such data are not currently easily incorporated into the IUCN Red Listing process, the information on levels of genetic diversity can give estimates of “genetic threat”.

- *D. pumila* and *L. edule* both showed very low levels of genetic diversity, whereas the levels of genetic diversity are considerably higher for *D. velutina* and *D. regia*.

8.2.5. Feedback between the four stages of conservation assessments

It is important to allow feedback loops in the conservation assessment process. Information obtained at a later stage can often help to update the conservation assessments from an earlier stage. For example, the desktop assessment might shed light on an area that had been completely cleared of its vegetation (e.g. due to volcanic activity or forest plantation), and herbarium specimens from this area then ought to be excluded in the range estimates obtained in the preliminary assessments. At another level, when genetic information on population structure is available, then field-based assessments can be targeted on these populations.

8.3. Recommendations

A recurring theme throughout this thesis is the need to use the best available information for many of the poorest known plant species. Even species known from just one specimen can have their conservation status assessed using methodologies documented here. Table 8.2 outlines the possibilities for herbarium-based conservation assessments when a limited number of specimens are available. It shows that, for most species, assessments will have to be made based on a limited number of specimens, since less than half (42%) of the endemic legumes of Madagascar have 15 or more specimens.

Table 8.2. Possibilities for herbarium-based conservation assessments (preliminary and desktop assessments) with a limited number of herbarium specimens.

Herbarium specimens	Preliminary assessment		Desktop assessment	% endemic legumes in Madagascar
	AOO	EOO		
1	No	No	Using results from recent surveys (threats, conservation measures, habitat quality)	9%
2	Yes	No	Threats, conservation measures, habitat quality	7%
3-4	Yes	Yes	Subpopulations, threats, conservation measures, habitat quality	10%
5-14	Yes	Yes	As above, and species distribution modelling of impacts of climate change	32%
15 +	Yes, with >95% accuracy		As above	42%

A set of practical recommendations for herbarium-based conservation assessments is listed below to facilitate the process of using herbarium specimens in IUCN conservation assessments (Table 8.3). This list is aimed at those carrying out herbarium-based assessments, or those using and supporting these assessments. In addition, specific recommendations for the IUCN Red List are given in Table 8.4. Although changes to the IUCN categories and criteria are unlikely (and arguably undesirable), the guidelines for how the categories and criteria ought to be interpreted are continually updated.

Table 8.3. Recommendations for herbarium-based conservation assessments.

General recommendations	
Preliminary assessments	• Use data from as many (relevant) herbaria as possible for a representative dataset. • Flag herbarium-based assessments based on range estimates from fewer than 15 specimens. • Include subpopulation analysis of a circular buffer (1/10th) in the preliminary analysis. • Use both EOO and AOO range measures, as a single range measure does not identify all species at threat.
Desktop assessments	• Encourage modelling and GIS analysis of herbarium specimen data, as this can provide information on the subcriteria needed under Criterion B to qualify a preliminary range-based assessment as a full IUCN assessment. • Modelling and GIS analysis of herbarium specimen data can also be used for assessments using Criteria A and D. • Allow (GIS) modelling with the best available data, even if data points are few. • Reference all literature sources (and experts) consulted for the assessments. • Allow grey and unpublished literature for desktop analysis, as long as this information can be referenced. • Area-specific information from other species may also be utilised.
Field-based assessments	• A protocol is needed for the ground truthing of herbarium-based conservation assessments in the field. • In the field, population counts or estimates should be recorded, as this information is useful for many of the IUCN Red Listing criteria. • Field studies should ideally be repeated regularly. • Records of pollination and seed dispersal are highly valuable and should be recorded whenever possible.
Genetic assessments	• Utilise as many samples as possible (financially and practically) both from the geographical range and from within populations, when undertaking genetic studies. • Species information should to be used sparingly for closely related species, as they may differ in genetic history and population structure.

Table 8.4. Specific recommendations for the IUCN Red Listing guidelines regarding herbarium-based conservation assessments.

Specific recommendations for IUCN Red Listing guidelines
• For range estimates, the AOO grid size of 2 km is not suitable for herbarium specimen data; instead the use of a flexible grid size is recommended (Criteria A and B). • For subpopulation estimates, a grid is not suitable; instead the use of a circular buffer of a species specific, flexible size is recommended. • Allow the number of subpopulations to be used as a surrogate of the number of locations (Criterion B). • A clarification is needed regarding the relationship between the threatened categories (VU, EN, CR) and the Near Threatened category under Criterion B, as well as other Criteria. • It has sometimes been argued that Criterion D2 is too inclusive and results in over-listing; there is no such evidence from the herbarium-based assessments. • It is unclear if the decline under Criterion C refers to range, habitat, population numbers or genetic diversity. • Genetic diversity data should be accommodated in the IUCN Red List assessment process, for example by allowing genetic decline to be used as a surrogate for decline in population size (Criterion A). • More analysis is needed on the genetic basis of the IUCN thresholds, covering more taxa and geographic regions. • Although the precautionary principle should still hold, it is beneficial to note which categories were used for the assessments even if their results did not contribute to the final rating. This would be especially useful when assessing changes in ratings between repeat assessments.

8.4. Final words

Through the Global Strategy for Plant Conservation, the CBD has called for a complete list of conservation assessments for plants (Convention on Biological Diversity 2010). However, in the last ten years only 5,319 species of plants have been added to the IUCN Red List (IUCN 2010). In addition, assessments should be revised every ten years according to the rules of the IUCN Red List (IUCN 2009). If this slow incorporation of plant assessments continues, the IUCN Red List will never have a complete list of conservation assessments for plants.

There is clearly a need to increase the number of plant conservation assessments. Such an increase is only realistic if assessments based on herbarium specimens are carried out and accepted. Hence, this thesis has highlighted the strengths and weaknesses of herbarium-based conservation assessments ensuring their validity by comparing them with ground truthed conservation assessments and incorporating genetic data. Combining spatial analysis with population genetic data provides a more complete picture of landscape-level population dynamics and the impact on conservation status. Although genetic data are currently not often incorporated into conservation assessments, it is vital in making accurate management decisions and creating effective action plans for conservation. Only by using all available scientific resources can informed conservation decisions be made, and the survival of plants and their associated ecosystems be ensured.

References

1. Aguilar R., Quesada M., Ashworth L., Herrerias-Diego Y. & Lobo J. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17: 5177-5188.

2. Akçakaya H.R., Butchart S.H.M., Mace G.M., Stuart S.N. & Hilton-Taylor C. 2006. Use and misuse of the IUCN Red List Criteria in projecting climate change impacts on biodiversity. Global Change Biology 12: 2037-2043.

3. Aldrich P.R., Glaubitz J.C., Parker G.R., Rhodes Jr O.E. & Michler C.H. 2005. Genetic structure inside a declining red oak community in old-growth forest. Journal of Heredity 96: 627-634.

4. Allendorf F.W. & Luikart G. 2007. Conservation and the Genetics of Populations. Blackwell, Malden, USA.

5. Andrade I.M., Mayo S.J., Van den Berg C., Fay M.F., Chester M., Lexer C. & Kirkup D. 2007. A preliminary study of genetic variation in populations of *Monstera adansonii* var. *klotzschiana* (Araceae) from north-east Brazil, estimated with AFLP molecular markers. Annals of Botany 100: 1143-1154.

6. Andrianoelina O., Favreau B., Ramamonjisoa L. & Bouvet J.M. 2009. Small effect of fragmentation on the genetic diversity of *Dalbergia monticola*, an endangered tree species of the eastern forest of Madagascar, detected by chloroplast and nuclear microsatellites. Annals of Botany 104: 1231-1242.

7. Andrianoelina O., Rakotondraoelina H., Ramamonjisoa L., Maley J., Danthu P. & Bouvet J.M. 2006. Genetic diversity of *Dalbergia monticola* (Fabaceae) an endangered tree species in the fragmented oriental forest of Madagascar. Biodiversity and Conservation 15: 1109-1128.

8. Araujo M.B. & Pearson R.G. 2005. Equilibrium of species' distributions with climate. Ecography 28: 693-695.

9. Ball M.C., Finnegan L., Manseau M. & Wilson P. 2010. Integrating multiple analytical approaches to spatially delineate and characterize genetic population structure: an application to boreal caribou (*Rangifer tarandus caribou*) in central Canada. Conservation Genetics 11: 2131-2143.

10. Banks H. 1997. The pollen of *Delonix* (Leguminosae: Caesalpinioideae: Caesalpinieae). Kew Bulletin 52: 417-434.

11. Barthlott W., Lauer W. & Placke A. 1996. Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. Erdkunde 50: 317-328.

12. Baskauf C.J. & Burke J.M. 2009. Population genetics of *Astragalus bibullatus* (Fabaceae) using AFLPs. Journal of Heredity 100: 424-431.

13. Besaire, H. H. Madagascar Carte Geologique, Echelle 1:1,000,000. 1964. Antananarivo, Societe des Petroles de Madagascar, Bur. Rech. Geologiques Minieres. Commissariat Energie Atomique, Inst. Rech. Scient., Soc. Ugine Pechiney.

14. Beyer, H. L. Hawth's Analysis Tools for ArcGIS 3.26. 2004. Available at http://www.spatialecology.com/htools.

15. Blanc-Pamard C. 2002. La forêt et l'arbre en pays masikoro (Madagascar): un paradoxe environnemental. Bois et forêts des Tropiques 271: 5-22.

16. Bomhard B., Richardson D.M., Donaldson J.S., Hughes G.O., Midgley G.F., Raimondo D.C., Rebelo A.G., Rouget M. & Thuiller W. 2005. Potential impacts of future land use and climate change on the Red List status of the Proteaceae in the Cape Floristic Region, South Africa. Global Change Biology 11: 1452-1468.

17. Bonin A., Bellemain E., Eidesen P.B., Pompanon F., Brochmann C. & Taberlet P. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology 13: 3261-3273.

18. Bonin A., Ehrich D. & Manel S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Molecular Ecology 16: 3737-3758.

19. Bosch C.H. 2004. *Lemuropisum edule*. In: Grubben GJH and Denton OA (eds), Plant Resources of Tropical Africa 2. Vegetables. pp. 364-365. Prota Foundation, Wageningen, Netherlands.

20. Breinholt J.W., Van Buren R., Kopp O.R. & Stephen C.L. 2009. Population genetic structure of an endangered Utah endemic, *Astragalus ampullarioides* (Fabaceae). American Journal of Botany 96: 661-667.

21. Brummitt N., Bachman S.P. & Moat J. 2008. Applications of the IUCN Red List: towards a global barometer for plant diversity. Endangered Species Research 6: 127-135.

22. Brummitt N. & Nic Lughadha E. 2003. Biodiversity: Where's hot and where's not. Conservation Biology 17: 1442-1448.

23. Bruneau A., Forest F., Herendeen P.S., Klitgaard B.B. & Lewis G.P. 2001. Phylogenetic relationships in the Caesalpinioideae (Leguminosae) as inferred from chloroplast *trnL* intron sequences. Systematic Botany 26: 487-514.

24. Burgman M.A. & Fox J.C. 2003. Bias in species range estimates from minimum convex polygons: implications for conservation and options for improved planning. Animal Conservation 6: 19-28.

25. Byers D.L. & Meagher T.R. 1992. Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. Heredity 68: 353-359.

26. CAB International 2000. Delonix regia . Forestry Compendium CAB International, Wallingford , UK.

27. Callmander M.W., Schatz G.E., Lowry II P.P., Laivao M.O., Raharimampionona J., Andriambololonera S., Raminosoa T. & Consiglio T.K. 2007. Identification of priority areas for plant conservation in Madagascar using Red List criteria: rare and threatened Pandanaceae indicate sites in need of protection. Oryx 41: 168-176.

28. Callmander M.W., Schatz G.E. & Lowry P.P. 2005. IUCN Red List assessment and the Global Strategy for Plant Conservation: taxonomists must act now. Taxon 54: 1047-1050.

29. Cardoso S.R.S., Provan J., Lira C.D.F., Pereira L.D.O.R., Ferreira P.C.G. & Cardoso M.A. 2005. High levels of genetic structuring as a result of population fragmentation in the tropical tree species *Caesalpinia echinata* Lam. Biodiversity and Conservation 14: 1047-1057.

30. Channell R. & Lomolino M.V. 2000. Dynamic biogeography and conservation of endangered species. Nature 403: 84-86.

31. Chase M.W. & Hills H.H. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon 40: 215-220.

32. Chen C., Durand E., Forbes F. & François O. 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Molecular Ecology Notes 7: 747-756.

33. Cole C.T. 2003. Genetic variation in rare and common plants. Annual Review of Ecology, Evolution and Systematics 34: 213-237.

34. Consiglio T., Schatz G.E., Mcpherson G., Lowry P.P., Rabenantoandro J., Rogers Z.S., Rabevohitra R. & Rabehevitra D. 2006. Deforestation and plant diversity of Madagascar's littoral forests. Conservation Biology 20: 1799-1803.

35. Convention on Biological Diversity. Proposals for a consolidated update of the Global Strategy for Plant Conservation 2011-2020. 2-11-2010. Conference of the Parties to the Convention on Biological Diversity, Tenth Meeting, Nagoya, Japan 18-29 October 2010, Agenda item 4.7.

36. Corander J. & Marttinen P. 2006. Bayesian identification of admixture events using multilocus molecular markers. Molecular Ecology 15: 2833-2843.

37. Corander J., Waldmann P., Marttinen P. & Sillanpaa M.J. 2004. BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics 20: 2363-2369.

38. Corvalan C., Hales S. & McMichael A.J. 2005. Ecosystems and human well-being: health synthesis. Report of the Millennium Ecosystem Assessment. World Health Organization, Geneva.

39. Davis A.J., Jenkins L.S., Lawton J.H., Shorrocks B. & Wood S. 1998. Making mistakes when predicting shifts in species range in response to global warming. Nature 391: 783-786.

40. Davis A.P., Govaerts R., Bridson D.M. & Stoffelen P. 2006. An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). Botanical Journal of the Linnean Society 152: 465-512.

41. Dawson I.K. & Powell W. 1999. Genetic variation in the Afromontane tree *Prunus africana*, an endangered medicinal species. Molecular Ecology 8: 151-156.

42. Donaldson J.S.ed. 2003. *Cycads. Status Survey and Conservation Action Plan*. IUCN/SSC Cycad Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.

43. Doyle J.J. & Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

44. Dransfield J. & Beentje H. 1995. The Palms of Madagascar. Royal Botanic Gardens, Kew, UK.

45. Drummond R.S.M., Keeling D.J., Richardson T.E., Gardner R.C. & Wright S.D. 2000. Genetic analysis and conservation of 31 surviving individuals of a rare New Zealand tree, *Metrosideros bartlettii* (Myrtaceae). Molecular Ecology 9: 1149-1157.

46. Du Puy D.J., Labat J.-N., Rabevohitra R., Villiers J.-F., Bosser J. & Moat J. 2002. The Leguminosae of Madagascar. Royal Botanic Gardens, Kew, UK.

47. Du Puy D.J. & Moat J. 1996. A refined classification of the primary vegetation of Madagascar based on the underlying geology: using GIS to map its distribution

and to assess its conservation status. In: Lourenco WR (ed), Proceedings of the International Symposium on the Biogeography of Madagascar pp. 205-218. Editions de l'ORSTOM, Paris, France.

48. Du Puy D.J., Phillipson P. & Rabevohitra R. 1995. The genus *Delonix* (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar. Kew Bulletin 50: 445-475.

49. Edelsbrunner H., Kirkpatrick D. & Seidel R. 1983. On the shape of a set of points in the plane. IEEE Transactions on Information Theory 29: 551-559.

50. Elith J., Graham C.H., Anderson R.P., Dudik M., Ferrier S., Guisan A., Hijmans R.J., Huettmann F., Leathwick J.R. & Lehmann A. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29: 129-151.

51. Elith J., Kearney M. & Phillips S. 2010. The art of modelling range-shifting species. Methods in Ecology and Evolution 1: 330-342.

52. Ellstrand N.C. & Elam D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24: 217-242.

53. ESRI. Spatial Analyst 9.2 Copyright ©1999-2006. 2006. ESRI Inc.

54. Evanno G., Regnaut S. & Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.

55. Ewers R.M. & Didham R.K. 2005. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81: 117-142.

56. Excoffier L., Smouse P.E. & Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

57. Falush D., Stephens M. & Pritchard J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7: 574-578.

58. Farjon A., Bachman S., Gardner M., Luscombe D., Reynolds C. & Thomas P. 2006. Conservation assessments of data deficient (DD) conifers, using herbarium and GIS data. The Royal Botanic Gardens, Kew, UK.

59. Farjon A. & Page C.N. 1999. Conifers: Status Survey and Conservation Action Plan. IUCN/SSC Conifer Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.

60. Fielding A. & Bell J. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24: 38-49.

61. Fischlin A., Midgley G.F., Price J.T., Leemans R., Gopal B., Turley C., Rounsevell M.D.A., Dube O.P., Tarazona J. & Velichko A.A. 2007. Ecosystems, their properties, goods, and services. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ and Hanson CE (eds), Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change pp. 211-272. Cambridge University Press, Cambridge, UK.

62. Fitzpatrick M., Gove A., Sanders N. & Dunn R. 2008. Climate change, plant migration, and range collapse in a global biodiversity hotspot: the *Banksia* (Proteaceae) of Western Australia. Global Change Biology 14: 1-16.

63. Foden W.B., Mace G.M., Vie J.-C., Angulo A., Butchart S.H.M., DeVantier L., Dublin H.T., Gutsche A., Stuart S.N. & Turak E. 2009. Species susceptibility to climate change impacts. In: Vie J-C, Hilton-Taylor C and Stuart SN (eds), Wildlife in a Changing World - An Analysis of the 2008 IUCN Red List of Threatened Species pp. 77-87. IUCN, Gland, Switzerland.

64. Fraley, C. & Raftery, A. E. MCLUST version 3 for R: Normal mixture modeling and model-based clustering. Technical Report No. 504. 2006. Department of Statistics, University of Washington.

65. François O. & Durand E. 2010. Spatially explicit Bayesian clustering models in population genetics. Molecular Ecology Resources 10: 773-784.

66. Frankel O.H. 1974. Genetic conservation: our evolutionary responsibility. Genetics 78: 53-65.

67. Frankel O.H. & Soulé M.E. 1981. Conservation and evolution. Cambridge University Press, Cambridge, UK.

68. Frankham R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500-1508.

69. Frankham R., Ballou J.D. & Briscoe D.A. 2010. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

70. Frantz A.C., Cellina S., Krier A., Schley L. & Burke T. 2009. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? Journal of Applied Ecology 46: 493-505.

71. Gade D.W. 1996. Deforestation and its effects in highland Madagascar. Mountain Research and Development 16: 101-116.

72. Ganzhorn J.U., Lowry P.P., Schatz G.E. & Sommer S. 2001. The biodiversity of Madagascar: one of the world's hottest hotspots on its way out. Oryx 35: 346-348.

73. Gaston K.J. 1997. What is rarity? In: Kunin WE and Gaston KJ (eds), The biology of rarity: causes and consequences of rare-common differences pp. 30-47. Chapman & Hall, London, UK.

74. Gaston K.J., Blackburn T.M. & Lawton J.H. 1997. Interspecific abundance-range size relationships: an appraisal of mechanisms. Journal of Animal Ecology 66: 579-601.

75. Gaston K.J. & Fuller R.A. 2009. The sizes of species' geographic ranges. Journal of Applied Ecology 46: 1-9.

76. Gitzendanner M.A. & Soltis P.S. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 783-792.

77. Golding J.S. 2004. The use of specimen information influences the outcomes of Red List assessments: the case of southern African plant specimens. Biodiversity and Conservation 13: 773-780.

78. Good T., Zjhra M. & Kremen C. 2006. Addressing data deficiency in classifying extinction risk: a case study of a radiation of Bignoniaceae from Madagascar. Conservation Biology 20: 1099-1110.

79. Goodman S.M. & Benstead J.P. 2005. Updated estimates of biotic diversity and endemism for Madagascar. Oryx 39: 73-77.

80. Govindasamy B., Duffy P.B. & Coquard J. 2003. High-resolution simulations of global climate, part 2: effects of increased greenhouse cases. Climate Dynamics 21: 391-404.

81. Graham C.H., Ferrier S., Huetman F., Moritz C. & Peterson A.T. 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology and Evolution 19: 497-503.

82. Guillot G., Leblois R., Coulon A. & Frantz A.C. 2009. Statistical methods in spatial genetics. Molecular Ecology 18: 4734-4756.

83. Guillot G. & Santos F. 2010. Using AFLP markers and the Geneland program for the inference of population genetic structure. Molecular Ecology Resources 10: 1082-1084.

84. Guisan A. & Thuiller W. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters 8: 993-1009.

85. Hamrick J.L. 2004. Response of forest trees to global environmental changes. Forest Ecology and Management 197: 323-335.

86. Hamrick J.L. & Godt M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions: Biological Sciences 351: 1291-1298.

87. Harcourt C. & Thornback J. 1990. Lemurs of Madagascar and the Comoros: the IUCN red data book. IUCN, Gland, Switzerland and Cambridge, UK.

88. Haripersaud P., Steege H., Granville J.J., Chevillotte H. & Hoff M. 2010. Species abundance, distribution and diversity in time and space after centuries of botanical collecting in the Guianas. Taxon 59: 592-597.

89. Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F. 2007. Fifty years of deforestation and forest fragmentation in Madagascar. Environmental Conservation 34: 325-333.

90. Hartley S. & Kunin W.E. 2003. Scale dependency of rarity, extinction risk, and conservation priority. Conservation Biology 17: 1559-1570.

91. Haston E.M., Lewis G.P. & Hawkins J.A. 2005. A phylogenetic reappraisal of the *Peltophorum* group (Caesalpinieae: Leguminosae) based on the chloroplast *trnL-F*, *rbcL* and *rps16* sequence data. American Journal of Botany 92: 1359-1371.

92. Heikkinen R.K., Luoto M., Araujo M.B., Virkkala R., Thuiller W. & Sykes M.T. 2006. Methods and uncertainties in bioclimatic envelope modelling under climate change. Progress in Physical Geography 30: 751.

93. Hermans J., Hermans C., Du Puy D.J., Cribb P.J. & Bosser J. 2007. Orchids of Madagascar. Royal Botanic Gardens, Kew, UK.

94. Hernandez P.A., Graham C.H., Master L.L. & Albert D.L. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography 29: 773-785.

95. Hijmans R.J., Cameron S.E., Parra J.L. & Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

96. Hijmans R.J. & Graham C.H. 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. Global Change Biology 12: 2272-2281.

97. Hoffmann M., Brooks T.M., da Fonseca G.A.B., Gascon C., Hawkins A.F.A., James R.E., Langhammer P., Mittermeier R.A., Pilgrim J.D. & Rodrigues A.S.L. 2008. Conservation planning and the IUCN Red List. Endangered Species Research 6: 113-125.

98. Huff D.R., Peakall R. & Smouse P.E. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). TAG Theoretical and Applied Genetics 86: 927-934.

99. IPCC. 2000. IPCC Special Report: Emissions Scenarios. Cambridge University Press, Cambridge, UK.

100. IUCN. 1963. Animals and Plants Threatened with Extinction. Survival Service Commission, IUCN, Switzerland.

101. IUCN. 1994. IUCN Red List Categories: Version 2.3. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland.

102. IUCN. 2001. IUCN Red List Categories: Version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

103. IUCN. Terms of Reference or Red List Authorities. 2009. IUCN. Downloaded from http://cmsdata.iucn.org/downloads/rla_tor_2009_2012_revised_final.pdf.

104. IUCN. 2010. 2010 IUCN Red List of Threatened Species. http://www.iucnredlist.org Downloaded October 2010.

105. IUCN Standards and Petitions Working Group. 2008. Guidelines for using the IUCN Red List Categories and Criteria Version 7.0. Prepared by the Standards and Petitions Working Group of the IUCN SSC Biodiversity Assessments Sub-Committee in August 2008. Downloadable from http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf.

106. IUCN Standards and Petitions Working Group. 2010. Guidelines for using the IUCN Red List Categories and Criteria Version 8.0. Prepared by the Standards and Petitions Working Group of the IUCN SSC Biodiversity Assessments Sub-Committee in March 2010. Downloaded from http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf.

107. Jakobsson M. & Rosenberg N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23: 1801-1806.

108. Jombart T., Pontier D. & Dufour A.B. 2009. Genetic markers in the playground of multivariate analysis. Heredity 102: 330-341.

109. Jones J.P.G., Andriamarovololona M.M. & Hockley N. 2008. The importance of taboos and social norms to conservation in Madagascar. Conservation Biology 22: 976-986.

110. Juan A., Crespo M.B., Cowan R.S., Lexer C. & Fay M.F. 2004. Patterns of variability and gene flow in *Medicago citrina*, an endangered endemic of islands in the western Mediterranean, as revealed by amplified fragment length polymorphism (AFLP). Molecular Ecology 13: 2679-2690.

111. Kalinowski S.T. 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conservation Genetics 5: 539-543.

112. Karron J.D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology 1: 47-58.

113. Keller L.F. & Waller D.M. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17: 230-241.

114. Kite G.C., Plant A.C., Burke A., Simmonds M.J.S., Blaney W.M. & Fellows L.E. 1995. Accumulation of trans-3-hydroxy-L-proline by seeds and leaves of the edible Madagascan legume *Lemuropisum edule* H. Perrier. Kew Bulletin 50: 585-590.

115. Koopman W.J.M. 2005. Phylogenetic signal in AFLP data sets. Systematic Biology 54: 197-217.

116. Kramer A.T. & Havens K. 2009. Plant conservation genetics in a changing world. Trends in Plant Science 14: 599-607.

117. Kramer A.T., Ison J.L., Ashley M.V. & Howe H.F. 2008. The paradox of forest fragmentation genetics. Conservation Biology 22: 878-885.

118. Kremen C., Cameron A., Moilanen A., Phillips S.J., Thomas C.D., Beentje H., Dransfield J., Fisher B.L., Glaw F., Good T.C., Harper G.J., Hijmans R.J., Lees D.C., Louis E.Jr., Nussbaum R.A., Raxworthy C.J., Razafimpahanana A., Schatz G.E., Vences M., Vietes D.R., Wright P.C. & Zjhra M.L. 2008. Aligning conservation priorities across taxa in Madagascar with high-resolution planning tools. Science 320: 222-226.

119. Laikre L. 2010. Genetic diversity is overlooked in international conservation policy implementation. Conservation Genetics 11: 349-354.

120. Laikre L., Allendorf F.W., Aroner L.C., Baker C.S., Gregovich D.P., Hansen M.M., Jackson J.A., Kendall K.C., McKelvey K. & Neel M.C. 2010. Neglect of genetic diversity in implementation of the Convention on Biological Diversity. Conservation Biology 24: 86-88.

121. Laikre L., Nilsson T., Primmer C.R., Ryman N. & Allendorf F.W. 2009. Importance of genetics in the interpretation of Favourable Conservation Status. Conservation Biology 23: 1378-1381.

122. Lamoreux J., Akcakaya H.R., Bennun L., Collar N.J., Boitani L., Brackett D., Brautigam A., Brooks T.M., da Fonseca G.A.B., Mittermeier R.A., Rylands A.B., Gardenfors U., Hilton-Taylor C., Mace G.M., Stein B.A. & Stuart S.N. 2003. Value of the IUCN Red List. Trends in Ecology and Evolution 18: 214-215.

123. Latch E.K., Dharmarajan G., Glaubitz J.C. & Rhodes O.E. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conservation Genetics 7: 295-302.

124. Leberg P.L. 2002. Estimating allelic richness: effects of sample size and bottlenecks. Molecular Ecology 11: 2445-2449.

125. Leimu R., Mutikainen P.I.A., Koricheva J. & Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94: 942-952.

126. Lewis G., Schrire B., Mackinder B. & Lock M. 2006. Legumes of the World. Royal Botanic Gardens, Kew, UK.

127. Lindsay D. 2009. Improving the targeting of seed collecting programmes for *ex situ* conservation. In: van der Burgt X, van der Maesen J and Onana J-M (eds), Systematics and conservation of African plants pp. 487-497. Royal Botanic Gardens, Kew.

128. Liu C., Berry P.M., Dawson T.P. & Pearson R.G. 2005. Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28: 385-393.

129. Lobo J.M., Jimenez-Valverde A. & Real R. 2008. AUC: a misleading measure of the performance of predictive distribution models. Global Ecology and Biogeography 17: 145-151.

130. Loiselle B.A., Jørgensen P.M., Consiglio T., Jiménez I., Blake J.G., Lohmann L.G. & Montiel O.M. 2008. Predicting species distributions from herbarium collections: does climate bias in collection sampling influence model outcomes? Journal of Biogeography 35: 105-116.

131. Lowe A.J., Boshier D., Ward M., Bacles C.F.E. & Navarro C. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95: 255-273.

132. Mace G.M., Collar N.J., Cooke J., Gaston K.J., Ginsberg J.R., Leader-Williams N., Maunder M. & Milner-Gulland E.J. 1992. The development of new criteria for listing species on the IUCN Red List. Species 19: 16-22.

133. Mace G.M., Collar N.J., Gaston K.J., Hilton-Taylor C., Akçakaya H.R., Leader-Williams N., Milner-Gulland E.J. & Stuart S.N. 2008. Quantification of extinction risk: IUCN's system for classifying threatened species. Conservation Biology 22: 1424-1442.

134. Mace G.M. & Lande R. 1994. Draft IUCN Red List categories, Version 2.2. Species 21-22: 13-24.

135. Mace G.M. & Lande R. 1991. Assessing extinction threats: Toward a reevaluation of IUCN threatened species categories. Conservation Biology 5: 148-157.

136. Malcolm J.R., Liu C., Neilson R.P., Hansen L. & Hannah L. 2006. Global warming and extinctions of endemic species from biodiversity hotspots. Conservation Biology 20: 538-548.

137. Manel S., Schwartz M.K., Luikart G. & Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution 18: 189-197.

138. Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209-220.

139. McNeely J.A., Miller K.R., Reid W.V., Mittermeier R.A. & Werner T.B. 1990. Conserving the world's biological diversity. IUCN, Gland, Switzerland and Washington, DC, USA.

140. Meagher T.R. 2007. Plant population biology: Paternity analysis in a fragmented landscape. Heredity 99: 563-564.

141. Meagher T.R. 2010. A fragmented future for the forest flora. Heredity 105: 163-164.

142. MEFT, UNEP & CI. 2009. Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005.

143. Midgley G.F., Hannah L., Millar D., Rutherford M.C. & Powrie L.W. 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. Global Ecology and Biogeography 11: 445-451.

144. Midgley G.F., Hughes G.O., Thuiller W. & Rebelo A.G. 2006. Migration rate limitations on climate change-induced range shifts in Cape Proteaceae. Diversity and Distributions 12: 555-562.

145. Millar C.I. & Libby W.J. 1991. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. In: Falk DA and Holsinger KE (eds), Genetics and conservation of rare plants pp. 149-170. Oxford University Press, New York, USA.

146. Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being Synthesis. Island Press, Washington DC, USA.

147. Mittermeier R.A., Gil P.R., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. & da Fonseca G.A.B. 2005. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Ecoregions. Conservation International, Washington DC, USA.

148. Moat, J. Conservation assessment tools extension for ArcView 3.x, version 1.2. 2007. GIS Unit, Royal Botanic Gardens, Kew, UK. Available at: http://www.rbgkew.org.uk/gis/cats.

149. Moat J. 2008. (unpublished) Conservation assessment tools extension for ArcGIS. GIS Unit, Royal Botanic Gardens, Kew, UK.

150. Moat J. & Smith P. 2007. Atlas of the Vegetation of Madagascar. Royal Botanic Gardens, Kew, UK.

151. Murphy M.A., Evans J.S., Cushman S.A. & Storfer A. 2008. Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. Ecography 31: 685-697.

152. Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B. & Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

153. Nic Lughadha E., Baillie J., Barthlott W., Brummitt N.A., Cheek M.R., Farjon A., Govaerts R., Hardwick K.A., Hilton-Taylor C., Meagher T.R., Moat J., Mutke J., Paton A.J., Pleasants L.J., Savolainen V., Schatz G.E., Smith P., Turner I., Wyse-Jackson P. & Crane P.R. 2005. Measuring the fate of plant diversity: towards a foundation for future monitoring and opportunities for urgent action. Philosophical Transaction of the Royal Society B 360: 359-372.

154. Nogués-Bravo D. 2009. Predicting the past distribution of species climatic niches. Global Ecology and Biogeography 18: 521-531.

155. Norscia I., Carrai V. & Borgognini-Tarli S.M. 2006. Influence of dry season and food quality and quantity on behavior and feeding strategy of *Propithecus verreauxi* in Kirindy, Madagascar. International Journal of Primatology 27: 1001-1022.

156. Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13: 1143-1155.

157. Oldfield S.F., Lusty C. & Mackinven A. 1998. The World List of Threatened Trees. World Conservation Press, Cambridge, UK.

158. Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics 37: 637-669.

159. Parmesan C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology 13: 1860-1872.

160. Parmesan C. & Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

161. Parnell J.A.N., Simpson D.A., Moat J., Kirkup D.W., Chantaranothai P., Boyce P.C., Bygrave P., Dransfield S., Jebb M.H.P., Macklin J., Meade C., Middleton D.J., Muasya A.M., Prajaksood A., Pendry C.A., Pooma R., Suddee S. & Wilkin P. 2003. Plant collecting spread and densities: their potential impact on biogeographical studies in Thailand. Journal of Biogeography 30: 193-209.

162. Paton A.J., Brummitt N., Govaerts R., Harman K., Hinchcliffe S., Allkin B. & Nic Lughadha E. 2008. Towards Target 1 of the Global Strategy for Plant Conservation: a working list of all known plant species - progress and prospects. Taxon 57: 602-611.

163. Patterson N., Price A.L. & Reich D. 2006. Population structure and eigenanalysis. PLoS Genetics 2: e190.

164. Peakall R.O.D. & Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

165. Pearson R.G. & Dawson T.P. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography 12: 361-371.

166. Pearson R.G., Raxworthy C.J., Nakamura M. & Peterson A.T. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of Biogeography 34: 102-117.

167. Peterson A.T., Ortega-Huerta M.A., Bartley J., Sanchez-Cordero V., Soberon J., Buddemeier R.H. & Stockwell D.R.B. 2002. Future projections for Mexican faunas under global climate change scenarios. Nature 416: 626-629.

168. Peterson A.T., Sanchez-Cordero V., Soberon J., Bartley J., Buddemeier R.W. & Navarro-Siguenza A.G. 2001. Effects of global climate change on geographic distributions of Mexican Cracidae. Ecological Modelling 144: 21-30.

169. Phillips S.J., Anderson R.P. & Schapire R.E. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231-259.

170. Phillipson P.B. 1992. *Colvillea racemosa*. Flowering Plants of Africa 52: pl. 2055.

171. Ponder W.F., Carter G.A., Flemons P. & Chapman R.R. 2001. Evaluation of museum collection data for use in biodiversity assessment. Conservation Biology 15: 648-657.

172. Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

173. Quéméré E., Crouau-Roy B., Rabarivola C., Louis Jr E.E. & Chikhi L. 2010. Landscape genetics of an endangered lemur (*Propithecus tattersalli*) within its entire fragmented range. Molecular Ecology 19: 1606-1621.

174. Rabinowitz P.D., Coffin M.F. & Falvey D. 1983. The separation of Madagascar and Africa. Science 220: 67-69.

175. Raes N. & ter Steege H. 2007. A null-model for significance testing of presence-only species distribution models. Ecography 30: 727-736.

176. Randrianasolo A., Miller J.S. & Consiglio T.K. 2002. Application of IUCN criteria and Red List categories to species of five Anacardiaceae genera in Madagascar. Biodiversity and Conservation 11: 1289-1300.

177. Rapoport E.H. 1982. Areography: geographical strategies of species. Published on behalf of the Fundacion Bariloche by Pergamon Press.

178. RBG Kew. 2010. Plants under pressure a global assessment. The first report of the IUCN Sampled Red List Index for Plants. Royal Botanic Gardens, Kew, UK.

179. Reisch C., Kaiser A.J., Horn A. & Poschlod P. 2010. Genetic variation of *Mimetes hirtus* and *Mimetes fimbriifolius* (Proteaceae) - a comparative analysis of two closely related fynbos species. Plant Biology 12: 537-544.

180. Rico Arce M.L. & Bachman S. 2006. A taxonomic revision of *Acaciella* (Leguminosae, Mimosoideae). Anales del Jardín Botánico de Madrid 63: 189-244.

181. Rivera-Ocasio E., Aide T.M. & McMillan W.O. 2006. The influence of spatial scale on the genetic structure of a widespread tropical wetland tree, *Pterocarpus officinalis* (Fabaceae). Conservation Genetics 7: 251-266.

182. Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. & Brummitt N.A. 2010. Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data. Biodiversity and Conservation 19: 2071-2085.

183. Roberts D.L. & Dixon K.W. 2008. Orchids. Current Biology 18: R325-R329 (invited Primer).

184. Rockström J., Steffen W., Noone K., Persson Å., Chapin F.S., Lambin E.F., Lenton T.M., Scheffer M., Folke C., Schellnhuber H.J., Nykvist B., De Wit C.A., Hughes T., van der Leeuw S., Rodhe H., Sörlin S., Snyder P.K., Costanza R., Svedin U., Falkenmark M., Karlberg L., Corell R.W., Fabry V.J., Hansen J., Walker B., Liverman D., Richardon K., Crutzen P. & Foley J. 2009. A safe operating space for humanity. Nature 461: 472-475.

185. Rodrigues A.S.L., Pilgrim J.D., Lamoreux J.F., Hoffmann M. & Brooks T.M. 2006. The value of the IUCN Red List for conservation. Trends in Ecology & Evolution 21: 71-76.

186. Rodríguez J.P. 2002. Range contraction in declining North American bird populations. Ecological Applications 12: 238-248.

187. Sabel J., Green K., Dawson J., Robinson J., Gardner C., Starkie G. & D'Cruze N. 2009. The conservation status of mammals and avifauna in the Montagne des Français massif, Madagascar. Madagascar Conservation & Development 4: 44-51.

188. Schatz G.E. 2009. Plants on the IUCN Red List: Setting priorities to inform conservation. Trends in Plant Science 14: 638-642.

189. Schatz G.E. 2000a. Endemism in the Malagasy tree flora. In: Lourenco R and Goodman SM (eds), Diversity and Endemism in Madagascar pp. 1-11. Memoires de la Societe de Biogeographie, Paris, France.

190. Schatz G.E. 1996. Malagasy/Indo-austral-malesian phytogeographic connections. In: Lourenco WR (ed), Biogeographie de Madagascar pp. 73-83. ORSTROM, Paris, France.

191. Schatz G.E. 2000b. The endemic plant families of Madagascar project: integrating taxonomy and conservation. In: Lourenco R and Goodman SM (eds), Diversity and Endemism in Madagascar pp. 11-24. Memoires de la Societe de Biogeographie, Paris, France.

192. Schatz G.E. 2002. Taxonomy and herbaria in service of plant conservation: Lessons from Madagascar's endemic families. Annals of the Missouri Botanical Garden 89: 145-152.

193. Shannon C.E. 1948. A mathematical theory of communication. Bell System Technical Journal 27: 379-423.

194. Shapcott A., Rakotoarinivo M., Smith R.J., LysakovA G., Fay M.F. & Dransfield J. 2007. Can we bring Madagascar's critically endangered palms back from the

brink? Genetics, ecology and conservation of the critically endangered palm *Beccariophoenix madagascariensis*. Botanical Journal of the Linnean Society 154: 589-608.

195. Sherwin W.B., Jabot F., Rush R. & Rossetto M. 2006. Measurement of biological information with applications from genes to landscapes. Molecular Ecology 15: 2857-2869.

196. Sheth S.N., Lohmann L.G., Consiglio T. & Jiménez I.A.N. 2008. Effects of detectability on estimates of geographic range size in Bignonieae. Conservation Biology 22: 200-211.

197. Smith R.J. & Waldren S. 2010. Patterns of genetic variation in *Colchicum autumnale* L. and its conservation status in Ireland: a broader perspective on local plant conservation. Conservation Genetics 11: 1351-1361.

198. Song J., Murdoch J., Gardiner S.E., Young A., Jameson P.E. & Clemens J. 2008. Molecular markers and a sequence deletion in intron 2 of the putative partial homologue of *LEAFY* reveal geographical structure to genetic diversity in the acutely threatened legume genus Clianthus. Biological Conservation 141: 2041-2053.

199. Sork V.L. & Smouse P.E. 2006. Genetic analysis of landscape connectivity in tree populations. Landscape Ecology 21: 821-836.

200. Spielman D., Brook B.W. & Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101: 15261-15264.

201. Storfer A., Murphy M.A., Evans J.S., Goldberg C.S., Robinson S., Spear S.F., Dezzani R., Delmelle E., Vierling L. & Waits L.P. 2006. Putting the 'landscape' in landscape genetics. Heredity 98: 128-142.

202. Storfer A., Murphy M.A., Spear S.F., Holderegger R. & Waits L.P. 2010. Landscape genetics: where are we now? Molecular Ecology 19: 3496-3514.

203. Stuart S.N., Wilson E.O., McNeely J.A., Mittermeier R.A. & Rodriguez J.P. 2010. The Barometer of Life. Science 328: 177.

204. Swets J.A. 1988. Measuring the accuracy of diagnostic systems. Science 240: 1285.

205. Szpiech Z.A., Jakobsson M. & Rosenberg N.A. 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24: 2498.

206. Tadross M., Randriamarolaza L., Rabefitia Z. & Zheng K.Y. 2008. Climate change in Madagascar; recent past and future. World Bank, Washington, DC, USA.

207. The Plant List. 2010. Version 1. Published on the Internet. http://www.theplantlist.org/ (accessed 5th January).

208. Thiers, B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/. 2009.

209. Thomas C.D., Cameron A., Green R.E., Bakkenes M., Beaumont L.J., Collingham Y.C., Erasmus B.F.N., Siqueira M.F.D., Grainger A., Hannah L., Hughes L., Huntley B., van Jaarsveld A.S., Midgley G.F., Miles L., Ortega-Huerta M.A., Peterson A.T., Phillips O.L. & Williams S.E. 2004. Extinction risk from climate change. Nature 427: 145-148.

210. Thuiller W. 2004. Patterns and uncertainties of species' range shifts under climate change. Global Change Biology 10: 2020-2027.

211. Thuiller W., Lavorel S., Araujo M.B., Sykes M.T. & Prentice I.C. 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences 102: 8245-8250.

212. Travis J.M.J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proceedings of the Royal Society B: Biological Sciences 270: 467-473.

213. Tremblay R.L., Ackerman J.D., Zimmerman J.K. & Calvo R.N. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biological Journal of the Linnean Society 84: 1-54.

214. UN. 1992. Convention on Biological Diversity. United Nations. http://www.cbd.int/doc/legal/cbd-en.pdf.

215. UNDP. 2010. Human development report 2010. United Nations Development Programme, New York, USA.

216. UNEP. 2002. Global Strategy for Plant Conservation. COP Decision VI/9, CBD Secretariat, Montreal. http://www.cbd.int/decision/cop/?id=7183.

217. UNEP. 2010. (United Nations Environment Programme) Progress in the implementation of the global strategy for plant conservation and development of a consolidated update beyond 2010. UNEP/CBD/SBSTTA/14/INF/16.

218. Vendramin G.G., Fady B., González Martínez S.C., Hu F.S., Scotti I., Sebastiani F., Soto Á. & Petit R.J. 2008. Genetically depauperate but widespread: the case of an emblematic Mediterranean pine. Evolution 62: 680-688.

219. Voigt F.A., Arafeh R., Farwig N., Griebeler E.M. & Böhning-Gaese K. 2009. Linking seed dispersal and genetic structure of trees: a biogeographical approach. Journal of Biogeography 36: 242-254.

220. Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Friters A., Pot J., Paleman J., Kuiper M. & Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

221. Walter K.S. & Gillett H.J. 1998. The 1997 IUCN Red List of Threatened Plants. IUCN, Gland, Switzerland.

222. Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O. & Bairlein F. 2002. Ecological responses to recent climate change. Nature 416: 389-395.

223. Waples R.S. & Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15: 1419-1439.

224. Wilkinson S., Haley C., Alderson L. & Wiener P. 2011. An empirical assessment of individual-based population genetic statistical techniques: application to British pig breeds. Heredity 106: 261-269.

225. Willis F., Moat J. & Paton A. 2003. Defining a role for herbarium data in Red List assessments: a case study of *Plectranthus* from eastern and southern tropical Africa. Biodiversity and Conservation 12: 1537-1552.

226. World Bank. Country profile: Madagascar. World Development Indicators (WDI). 2010. Accessed on 13 December 2010 at www.data.worldbank.org/country/Madagascar.

227. World Wildlife Fund (WWF). Terrestrial Ecoregions, WWF. http://www.worldwildlife.org/science/ecoregions/terrestrial.cfm . 2001.

228. Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.

229. Wright S. 1943. Isolation by distance. Genetics 28: 114-138.

230. Young A., Boyle T. & Brown T. 1996. The population genetic consequences of habitat fragmentation for plans. Trends in Ecology and Evolution 11: 413-418.

Appendix 1

Preliminary conservation assessments for *Delonix s.l.* and endemic Leguminosae in Madagascar

A.1.1. Preliminary conservation assessments for *Delonix s.l.*

Preliminary herbarium-based conservation assessments are based on estimates of geographic range calculated from georeferenced localities of herbarium specimens. The two range measures used by the IUCN Categories and Criteria (IUCN 2001) are the extent of occurrence (EOO) and the area of occupancy (AOO). These can be calculated using geographic information systems (GIS) methods outlined in Willis *et al.* (2003). EOO and AOO require at least three and two data points respectively. By comparing these measures of range against the thresholds under IUCN Criterion B, a preliminary category of threat for each species was obtained. These GIS assessments are described as preliminary, since Criterion B also requires at least two out of three subcriteria (severe fragmentation or number of locations; continuing decline; extreme fluctuation) to be fulfilled (Box 1.1., Chapter 1). The range measures and preliminary conservation assessments for species of *Delonix s.l.* were calculated using the Conservation Assessment Tool (http://www.kew.org/gis/projects/cats) (Moat 2007) in ArcView (ESRI), using specimens collected prior to 2007 (Table A.1.1). AOO was calculated using a grid size of 1/10th maximum interpoint distances (1/10th max) and a grid size of 2km. IUCN recommends the use of a 2km grid, but accepts that the grid size should be species-specific (IUCN Standards and Petitions Working Group 2010). In addition, three other measures of range, alpha hulls (α=2 and α=3) and Rapoport's mean propinquity method (Rapoport 1982), were calculated using the Conservation Assessment Tool for ArcGIS (ESRI) (Moat 2008).

A.1.2. Preliminary conservation assessments for all endemic legumes in Madagascar.

Preliminary herbarium-based conservation assessments were also produced for all endemic legumes of Madagascar. Only species endemic to Madagascar were included, as non-endemic species will be distributed in countries outside Madagascar, so their full geographic range could not be assessed in this study. All species placed into the Data Deficient (DD) category are species with a single collection, which is too few to calculate either EOO or AOO. The preliminary conservation ratings for all endemic legumes of Madagascar are based on estimates of EOO and AOO (1/10th max), using the precautionary principle (Figure A.1.1). The preliminary conservation ratings indicate that nearly half (49%) of the endemic legumes of Madagascar qualify for a threatened category (or are DD). A quarter of all endemic species have ranges sufficiently small to place them into the two categories Critically Endangered (CR) and Endangered (EN), showing a very high potential risk to the future of legumes in Madagascar.

Figure A.1.1. The preliminary conservation rating for endemic legumes of Madagascar based on estimates of extent of occurrence (EOO) and area of occupancy (AOO) (n=453). Category, number of species and percentage of species in each category are given.

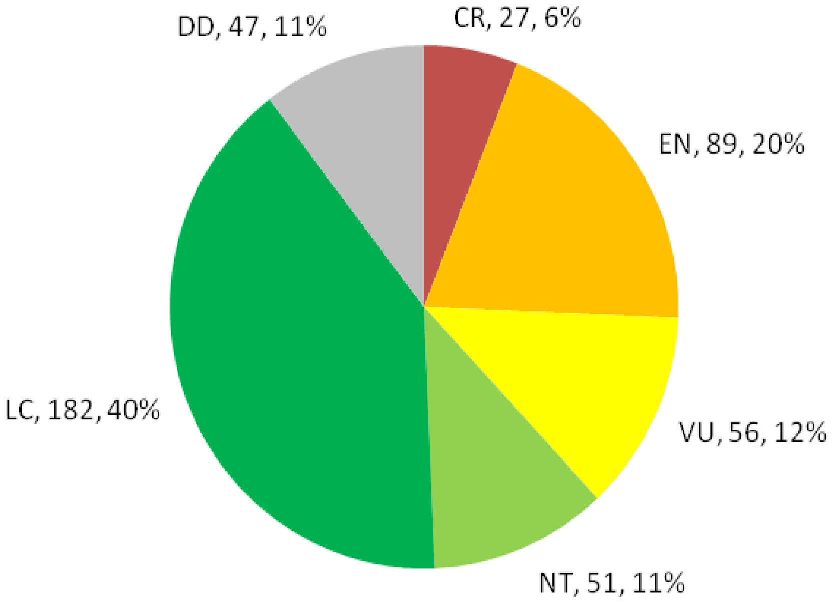


Table A.1.1. Range size measures and preliminary conservation assessments for *Delonix s.l.* based on herbarium data (prior to 2007)

	# collections	**# localities**	**EOO (km^2)**	**AOO** 1/10th max (km^2)	**Preliminary conservation rating**	**AOO** 2km grid (km^2)	***Rapoport area*** (km^2)	***Alpha 2x*** (km^2)	***Alpha 3x*** (km^2)
Colvillea racemosa	45	39	325,399	188,263	LC	*152*	*100,724*	*20,603*	*20,603*
Delonix boiviniana	84	65	456,346	315,942	LC	*256*	*78,850*	*24,895*	*92,670*
Delonix brachycarpa	9	9	190,398	51,427	LC	*36*	*358,787*	*32,465*	*163,531*
Delonix decaryi	40	27	49,974	22,789	LC	*108*	*12,015*	*8,317*	*24,711*
Delonix floribunda	54	38	124,693	64,283	LC	*148*	*30,198*	*13,330*	*16,787*
Delonix leucantha	21	18	157,088	60,667	LC	*72*	*82,273*	*30,544*	*30,544*
Delonix pumila	23	12	166	44	EN B1+2	*40*	*89*	*760*	*760*
Delonix regia	35	28	66,708	58,156	LC	*100*	*28,547*	*2,898*	*2,898*
Delonix tomentosa	1	1			DD	*0*	*0*	*n/a*	*n/a*
Delonix velutina	9	7	271	198	EN B1+2	*24*	*929*	*2*	*667*
Lemuropisum edule	18	13	923	540	EN B1	*52*	*985*	*767*	*1,186*

*colours indicates where a range measure would fall into a threatened category: Vulnerable- yellow, Endangered – orange and Critically Endangered – red.

See also Figure A.1.1.2.

 # collections - number of collections

 # localities - number of unique georeferenced localities

EOO - extent of occurrence, measured as the area of the minimum convex hull (a polygon around all distribution points with no internal angle exceeding 180°).

AOO 1/10th max - area of occupancy, measured as the area of the occupied cells on a grid with a cell side-length of 1/10th the maximum interpoint distance between any two distribution points (Willis *et al.* 2003).

Preliminary conservation rating - The conservation rating based on the worst-case rating from EOO or AOO 1/10th measures using the range-size thresholds of Criterion B (IUCN 2001).

AOO 2km - area of occupancy, measured as the area occupied by a species on a grid of 2x2km cells, as recommended by the IUCN (IUCN Standards and Petitions Working Group 2010).

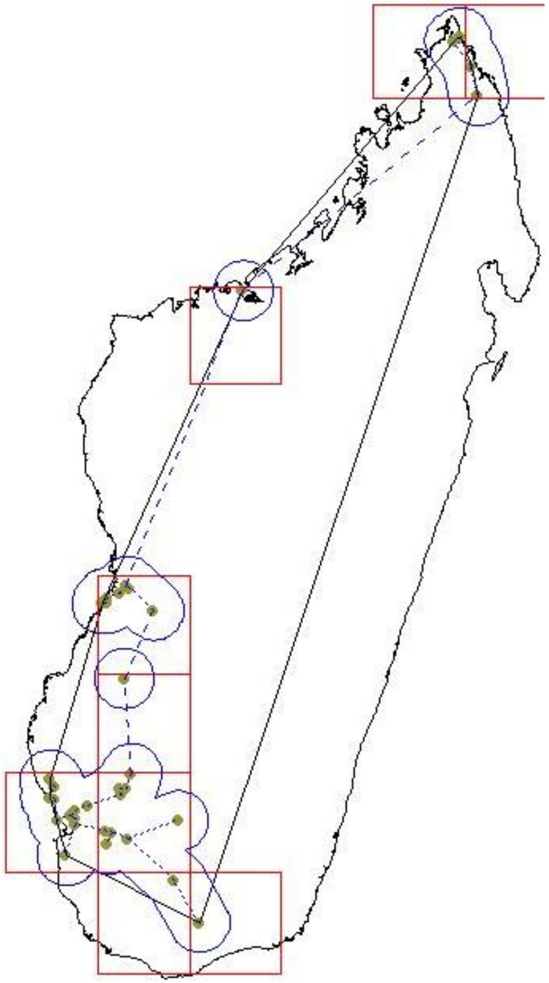
Rapoport area - the area represented by the buffer used in defining subpopulations by Rapoport's method. These are estimated by the Rapoport mean propinquity method (Rapoport 1982), where all points are connected using a minimum spanning tree (a tree connecting all points together by the shortest distance). The mean branch length (distance between points) of the minimum spanning tree is used as the radius of the buffer around the points (and branches if they are shorter than twice the mean). Isolated points are also buffered to the mean branch length.

Alpha 2x – alpha hull with an alpha value of 2. Suggested as an alternative measures of EOO by IUCN, calculated using (Moat 2008).

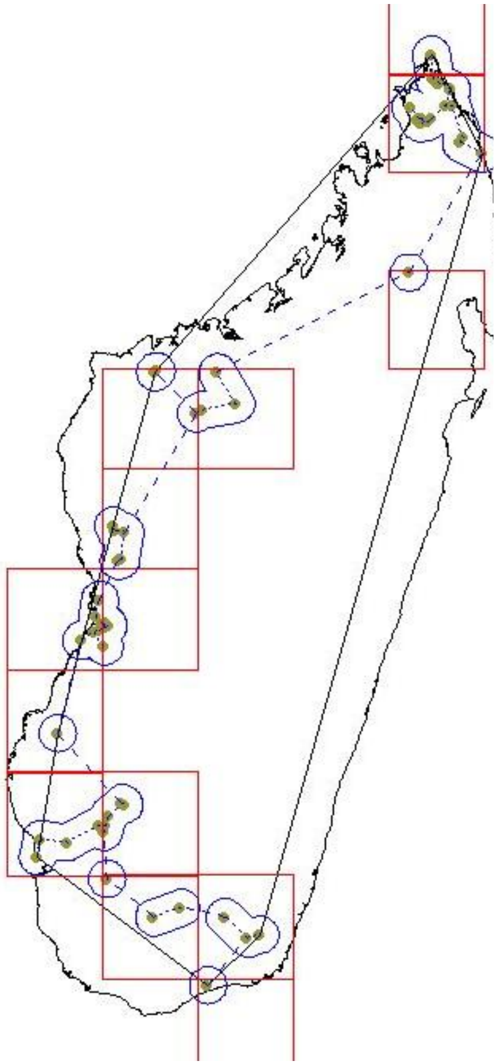
Alpha 3x – alpha hull with an alpha value of 3. Suggested as an alternative measures of EOO by IUCN, calculated using (Moat 2008).

Figure A.1.2. The distribution of herbarium specimens for Delonix s.l. Green circles represent point, black line EOO, red squares, AOO and blue lines Rapoport's propinquity method.

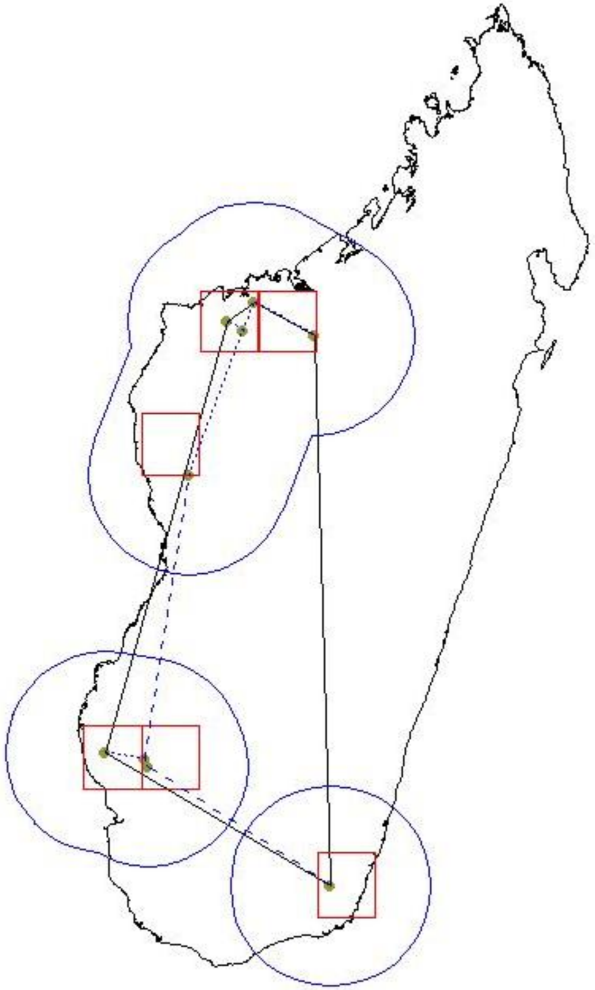
Colvillea racemosa



Delonix boiviniana

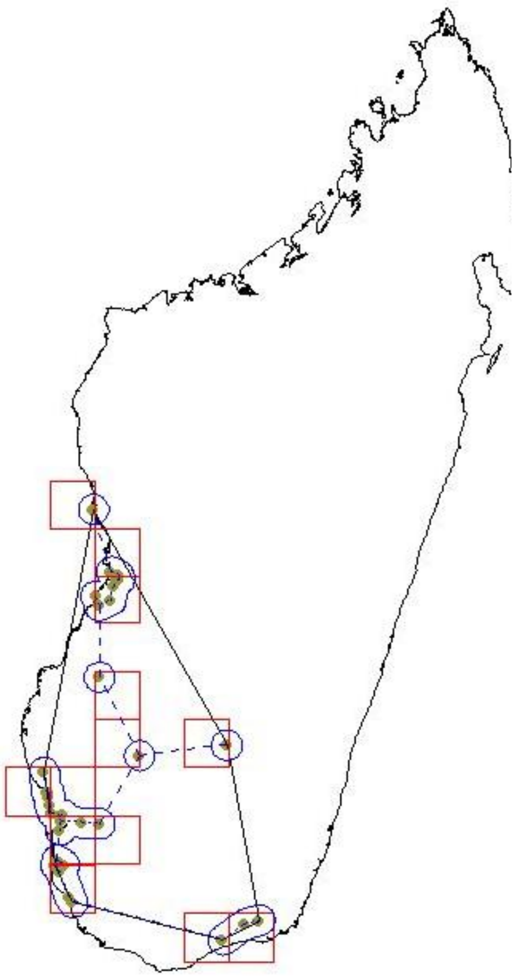


Delonix brachycarpa

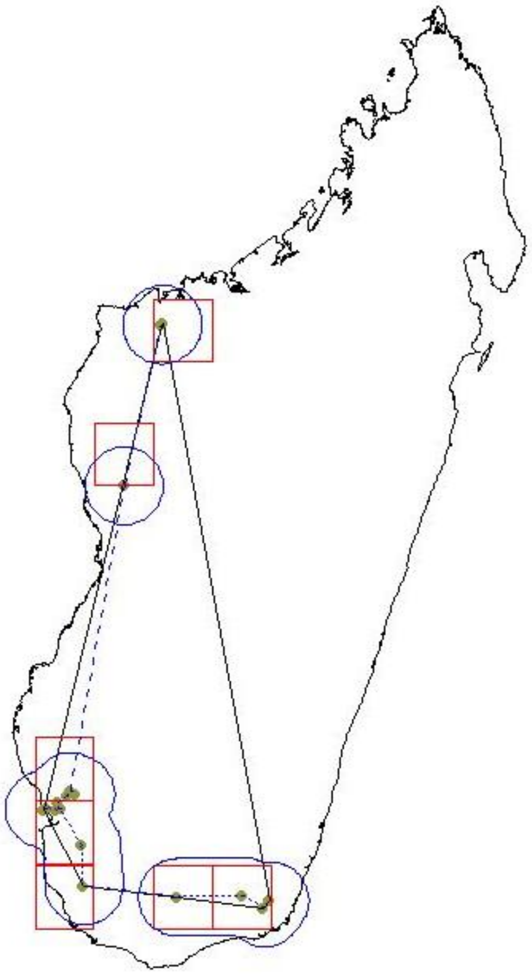


Delonix decaryi

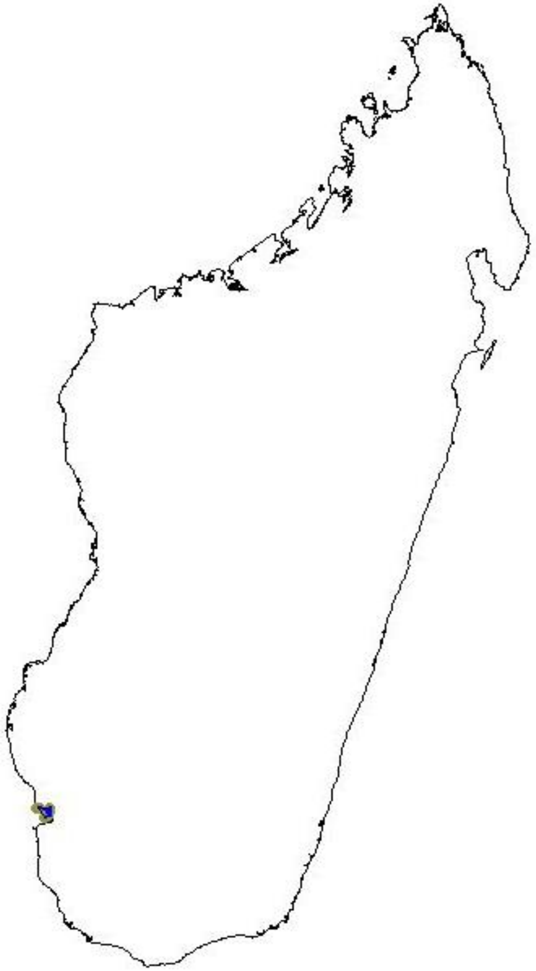
Delonix floribunda



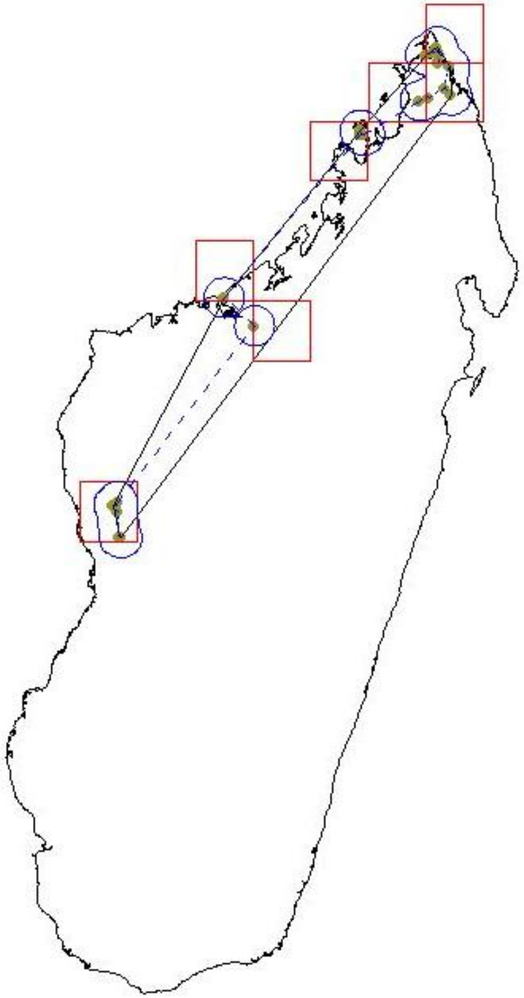
Delonix leucantha



Delonix pumila

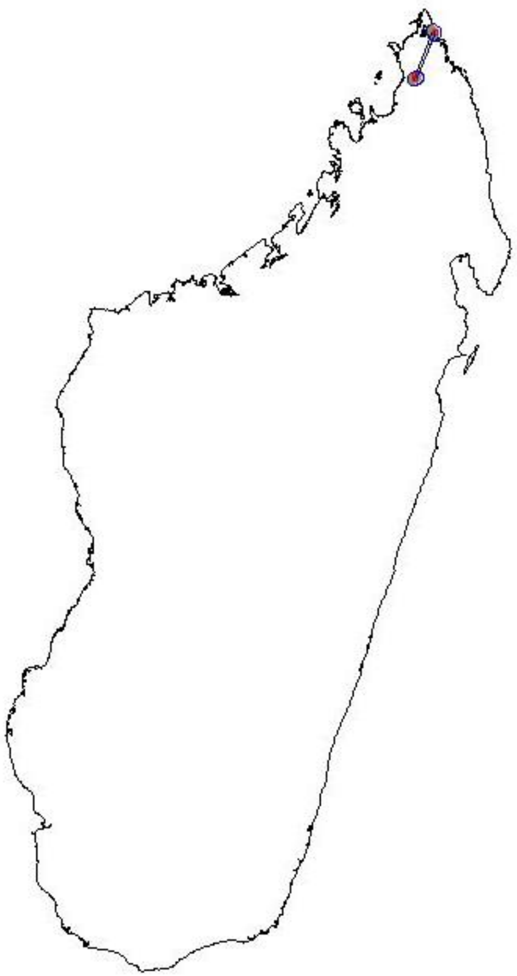


Delonix regia



Delonix tomentosa

Delonix velutina



Lemuropisum edule

Appendix 2

Desktop conservation assessments for *Delonix s.l.*

For desktop assessments relevant available information for a species is gathered by searching the literature, examining information from specimen labels, incorporating GIS techniques and (where data permits) statistical models, as well as contacting experts. The information assembled for the desktop assessments is organised according to the "Species Information Service Data Entry Module" database supplied by IUCN for recording conservation assessments. This data entry module requires information under the following headings:

- *Systematics*
- *Distribution*
- *Habitat & Ecology*
- *Population information*
- *Threat*
- *Conservation measures*
- *Uses*

Such information was gathered for all species of *Delonix s.l.* and used to allocate a conservation rating based on the IUCN Red List Categories and Criteria (version 3.1) (IUCN 2001). A summary of the desktop assessment ratings for *Delonix s.l.* is found in Table A.2.1, followed by full desktop assessments for each of the species.

Table A.2.1. Conservation ratings of *Delonix s.l.* based on desktop assessments.

Species	Desktop Assessment
Colvillea racemosa	LC
Delonix boiviniana	LC
Delonix brachycarpa	NT
Delonix decaryi	NT
Delonix floribunda	LC
Delonix leucantha	NT
Delonix pumila	EN B1ab(iii)+2ab(iii)
Delonix regia	LC
Delonix tomentosa	CR B1ab(iii); D
Delonix velutina	EN B1ab(iii)+2ab(iii)
Lemuropisum edule	EN B1ab(iii)

Colvillea racemosa

Distribution

C. racemosa is endemic to Madagascar, found in the southern, western and northern parts of the country – from Antsiranana in the north, the Bemaraha massive in the west to the extreme south of Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 397,012km^2 and the area of occupancy is 230,099km^2.

Habitat and Ecology

C. racemosa is a deciduous tree up to 20 m tall, with distinctive, large panicles of bright orange flowers. It is found within the dry forest and is thought to be pollinated by Sovimanga Sunbirds (Du Puy *et al.* 2002; Phillipson 1992).

Population information

GIS analysis estimates that *C. racemosa* has four populations (Rivers *et al.* 2010, Chapter 3). Total population numbers, number of mature individuals and population density estimates are unknown. Although the species is sometimes recorded as locally common (Phillipson 1992)

Threats

The native vegetation of *C. racemosa* is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted no change in the climatically suitable range by 2100 (Chapter 4).

Conservation measures

C. racemosa is found in five protected areas (Bezaha Mahafaly, Kirindi Mitea, Menabe, Onilahy, Tsingy de Bemaraha), although the effectiveness of this official protection is variable. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections also exist according to BGCI (www.bgci.org). In addition, *C. racemosa* is found cultivated elsewhere in the tropics, including South Africa (Phillipson 1992).

Uses

C. racemosa is used for construction, fences (Blanc-Pamard 2002) and trunks are hollowed out as canoes (Du Puy *et al.* 2002). It is also sometimes used as an ornamental shade tree (Phillipson 1992). Herbarium specimens also report the seeds to be used in occult sciences (Du Puy M304).

Rationale for the Red List Assessment

Colvillea racemosa is listed as Least Concern as it has a wide distribution, sometimes locally common and there are no specific threats associated with the species. There have been recent collections made, and it is known from five protected areas. *Colvillea racemosa* does not qualify for a threatened category based on geographic range (EOO and AOO), nor is it likely to be declining quickly enough to qualify for a threatened rating. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

LC

[Criteria used for assessment A, B and D2]

Delonix boiviniana

Distribution
D. boiviniana is endemic to Madagascar, found in the southern, western and northern parts of the country. It is distributed from the Mandrare River in the southeast to Antsiranana in the north. Based on the distribution of herbarium specimens, the extent of occurrence is 464,797km^2 and the area of occupancy is 338,508km^2.

Habitat and Ecology
D. boiviniana is a deciduous tree up to 30m tall. It is found within the dry forest especially on limestone outcrops but also on sand (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002). Sifaka (*Propithecus*) and Mouse-lemurs (*Microcebus*) are both recorded as visiting the flowers (Du Puy *et al.* 2002) and occasionally eating the leaves (Norscia *et al.* 2006).

Population information
GIS analysis estimates that *D. boiviniana* has four populations (Rivers *et al.* 2010, Chapter 3).

Threats
The natural vegetation of Madagascar is under threat from fragmentation and habitat loss, as a result of the conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted a loss of 5% of its present climatically suitable range by 2100 (Chapter 4).

Conservation measures
D. boiviniana can be found in several protected areas (Analamerana, Ankarana, Daraina, Kasijy, Menabe, Montagne des Français, Tsingy de Bemaraha, Tsingy de Namoroka, Vohibasia, Zombitse), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections exist according to BGCI (www.bgci.org)

Uses
Trunks of *D. boiviniana* are hollowed out to make canoes and coffins (Rivers, pers. obs.), and the resin is used as glue (Du Puy *et al.* 2002).

Rationale for the Red List Assessment
Delonix boiviniana is listed as Least Concern, as it has a wide distribution, there have been recent collections made, and it is known from several protected areas. *Delonix boiviniana* does not qualify for a threatened category based on geographic range (EOO or AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

LC

[Criteria used for assessment A, B and D2]

Delonix brachycarpa

Distribution
D. brachycarpa is endemic to Madagascar, found in the southern and western parts of the country. Although found from Ankara Plateau, Tsingy de Bemaraha and the upper Mandrare River basin, areas widely separated geographically, it is scattered and rare where found (Du Puy *et al.* 1995). Based on the distribution of herbarium specimens, the extent of occurrence is 190,398km^2 and the area of occupancy is 51,428km^2.

Habitat and Ecology
D. brachycarpa is a deciduous tree. It is found within the dry forest especially on limestone (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information
GIS analysis estimates that *D. brachycarpa* has five populations (Rivers *et al.* 2010, Chapter 3). Population numbers and density are largely unknown, however it has been recorded as scattered (Du Puy *et al.* 1995).

Threats
The natural vegetation of *D. brachycarpa* is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling is showing little change in climatically suitable range by 2100 (Chapter 4).

Conservation measures
D. brachycarpa can be found in three protected areas (Mahavavy Kinkony, Tsingy de Bemaraha, Tsingy de Namoroka), although the effectiveness of this official protection is not always adequate. No botanic garden collections exist according to BGCI (www.bgci.org)

Rationale for the Red List Assessment
D. brachycarpa is listed as Near Threatened, as it has a very low number of recent collections, it is reported to have a scattered distribution and to be rare when found. *Delonix brachycarpa* does not qualify for a threatened category based on geographic range (EOO and AOO), but the range is scattered, and its habitat is fragmented and degraded, and the habitat quality and extent continues to decline. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

NT

[Criteria used for assessment A, B and D2]

Delonix decaryi

Distribution
D. decaryi is endemic to the southern parts of Madagascar. It is found in a narrow band along the coast from north of Toliara to Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 51,902km^2 and the area of occupancy is 28,048km^2.

Habitat and Ecology
D. decaryi is a deciduous tree reaching up to 10m. It is found within the spiny forest and coastal bushland often with Didieriaceae and succulent species of *Euphorbia* on limestone and sand (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information
GIS analysis estimates that *D. decaryi* has eight populations (Rivers *et al.* 2010, Chapter 3).

Threats
Major threats to the spiny forest (natural vegetation of *D. decaryi*) are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat. The degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration is putting the species endemic to the area at particular risk (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The spiny forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 1.2% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted a 46% loss (by 2100) of its present climatically suitable range (Chapter 4).

Conservation measures
D. decaryi can be found in two protected areas (Cap Ste Marie and Tsimanampetsotsa), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections exist according to BGCI (www.bgci.org)

Uses
Cuttings of *D. decaryi* are often planted in villages as a “living fence”. The trunks are sometimes hollowed out to make canoes; the seeds are reported to be edible; and the resin used as glue (Du Puy *et al.* 2002) for sealing canoes. Herbarium collections also report that crushed leaves are put on a baby’s head to harden the scull (Du Puy M94).

Rationale for the Red List Assessment
D. decaryi is listed as Near Threatened, as it is expected to be severely affected by future climate change, with a loss of more than 30% of its current range. However, the impact of this predicted loss on population size and/or genetics has not yet been demonstrated. *Delonix decaryi* is known from several localities, however, rarely in substantial populations. It does not qualify for a threatened category based on geographic range (EOO or AOO), but its habitat is fragmented and degraded, and its native habitat continues to decrease in quality and extent. These trends should be monitored to determine whether the population of this species is declining. Its three subspecies are isolated and are likely to be severely affected by future habitat degradation.

NT

[Criteria used for assessment A, B and D2]

Delonix floribunda

Distribution
D. floribunda is endemic to the western and southern parts of Madagascar. It is found mainly along the coast from the Bemaraha massive to the Mandrare River, near Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 125,372km^2 and the area of occupancy is 79,118km^2.

Habitat and Ecology
D. floribunda is a deciduous tree reaching up to 15m. It is found within the dry forest as well as the spiny forest and coastal bushland on limestone or sand (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by sunbirds due to its flowers with highly reduced petals and copious amount of nectar (Du Puy *et al.* 2002). Lemurs (*Cheirogaleus medius*) have been recorded to feed on nectar (Harcourt & Thornback 1990).

Population information
GIS analysis estimates that *D. floribunda* has six populations (Rivers *et al.* 2010, Chapter 3).

Threats
Major threats to the spiny forest and the dry forest (the natural vegetation of *D. floribunda*) are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The rate of degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration in the spiny forest is putting the species endemic to the area at particular risk (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The spiny forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 1.2% per year; the dry forest is also declining significantly with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted little change in climatically suitable range by 2100 (Chapter 4).

Conservation measures
D. floribunda can be found in some protected areas (Andohahela, Ankodida, Kirindi Mitea, Menabe, Onilahy, Tsimanampetsotsa), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections exist according to BGCI (www.bgci.org)

Uses
D. floribunda is sometimes planted in villages, and trunks hollowed out to make canoes; the resin is also used as glue (Du Puy *et al.* 2002).

Rationale for the Red List Assessment
Delonix floribunda is listed as Least Concern, as it is known from a several localities, covering a wide distribution, however, rarely in substantial populations. It grows in two vegetation types (dry forest and spiny forest), and is found in several protected areas. *Delonix floribunda* does not qualify for a threatened category based on geographic range (EOO or AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

LC

[Criteria used for assessment A, B and D2]

Delonix leucantha

Distribution
D. leucantha is endemic to Madagascar, where it is found in the southern and western parts of the country, including Namoroka and Bemaraha in the west, and around Toliara in the south. Based on the distribution of herbarium specimens, the extent of occurrence is 157,089km^2 and the area of occupancy is 60,668km^2.

Habitat and Ecology
D. leucantha is a deciduous tree measuring up to 15m. It is found within the dry forest especially on limestone (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information
GIS analysis estimates that *D. leucantha* has five populations (Rivers *et al.* 2010, Chapter 3). Population numbers and density are largely unknown, however, the species has been recorded as uncommon (Du Puy *et al.* 1995).

Threats
Major threats to the spiny forest and the dry deciduous forests, where *D. leucantha* is found, are the widespread exploitation for firewood and charcoal production (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat. The rate of degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration is putting the species endemic to the area at particular risk (WWF 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar, with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted a 77% decrease in its present climatically suitable range by 2100 (Chapter 4).

In the South, subspecies *gracilis* is threatened by charcoal production. The subspecies *D. leucantha* subsp. *leucantha* is known from a small population in a fairly inaccessible location within a protected area, where it is not susceptible to exploitation or fires (Du Puy *et al.* 2002). Insufficient information is available regarding the threats to subspecies *bemarahensis*.

Conservation measures
D. leucantha can be found in four protected areas (Andohahela, Onilahy, Tsingy de Bemaraha and Tsingy de Namoroka), although the effectiveness of this official protection is not always adequate. Botanic garden collections exist according to BGCI (www.bgci.org)

Rationale for the Red List Assessment
D. leucantha is listed as Near Threatened, as it is expected to be severely affected by climate change, with a loss of more than 70% of its current range. However, the impact of this predicted range loss on population size and/or genetics has not yet been demonstrated. *Delonix leucantha* does not qualify for a threatened category based on current geographic range (EOO and AOO), but its occurrence is scattered, its habitat is fragmented and degraded, and the habitat quality and extent continues to decline. These trends should be monitored to determine whether the population of this species is declining. Its three subspecies are isolated and are likely to be severely affected by future habitat degradation.

NT

[Criteria used for assessment A, B and D2]

Delonix pumila

Distribution
D. pumila is endemic to a small region around Toliara in the southwestern part of Madagascar. It is found near the hill 'La Table' approximately 25km east of Toliara and along the adjacent escarpment edge of the Mahafaly Plateau extending to the plateau above Saint Augustine. A collection from 2006 from the Zombitse area (Tefy 919) has been misidentified as *D. pumila*. Based on the distribution of herbarium specimens, the extent of occurrence is 311km^2 and the area of occupancy is 93km^2.

Habitat and Ecology
D. pumila is a dwarf, deciduous shrub-like tree less than 3m tall. It is found within the spiny forest and coastal bushland with succulent species of *Euphorbia* on limestone rock (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is slow-growing, and thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information
GIS population analysis estimates five populations of *D. pumila* (Rivers *et al.* 2010, Chapter 3).

Threats
The natural vegetation where *D. pumila* is found is under threat from widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007). The rate of degradation has been exacerbated in recent years and the naturally slow growth and regeneration of the spiny forest is putting the endemic species of the area at particular risk (WWF 2001). The spiny forest is one of the primary vegetation types in Madagascar that is declining significantly with an estimated rate of loss of 1.2% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Also, the main population of the species exist close to one of the largest and expanding towns in Madagascar. Climate change modelling predicted little change in climatically suitable range by 2100 (Chapter 4).

Conservation measures
D. pumila is found in a proposed protected area (Onilahy), although the effectiveness of this proposed protection is not known. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections exist according to BGCI (www.bgci.org)

Rationale for the Red List Assessment
D. pumila is listed as Endangered since its EOO is less than 5,000km^2 and its AOO is less than 500km^2; its habitat is severely fragmented and exists in fewer than five locations; and its native habitat, the spiny forest, continues to decline in quality and extent, due to the effect of harvesting, human population expansion and habitat destruction. It is also known to be very slow-growing, and so will have less cgabce if adapting to these pressures.

EN B1ab(iii)+2ab(iii)

[Criteria used for assessment A, B and D2]

Delonix regia

Distribution

D. regia is endemic to Madagascar. In the wild, it is found in the west (Tsingy de Bemaraha) and the north (including Orangea, Cap d'Ambre peninsulas and around Baie de Diego, the Ankarana and Analamerana Massifs) and possibly from Nosy Be. It is also cultivated in most areas of Madagascar and across much of the tropics as a street tree. Based on the distribution of herbarium specimens, the extent of occurrence of the wild populations is 68,334km^2 and the area of occupancy is 58,156km^2.

Habitat and Ecology

D. regia is a deciduous tree up to 30m tall. It is found within the dry forest especially on limestone (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It has distinctive large, bright red flowers, and is thought to be pollinated by sunbirds (Du Puy *et al.* 2002).

Population information

GIS analysis estimates that *D. regia* has three populations (Rivers *et al.* 2010, Chapter 3).

Threats

D. regia is thought to be rare in its native habitat, although this seems to be an exaggeration (Du Puy *et al.* 2002 and Rivers pers. obs.). The natural vegetation of Madagascar is under threat from habitat loss and fragmentation, as a result of conversion of land for slash-and-burn agriculture, grazing, charcoal production and collection of firewood (Moat & Smith 2007). The Madagascar dry forests are severely fragmented and often in small blocks. Expanding rural populations and selective logging is also adding to the pressure (WWF 2001). The dry forest is one of the vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted that approximately 10% of the present climatically suitable range will be lost by 2100 (Chapter 4).

Conservation measures

D. regia is widely cultivated across Madagascar and in the rest of the tropics as an ornamental tree. It can be found in some protected areas (Analamerana, Ankarana, Lokobe, Montagne des Français, Tsingy de Bemaraha), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK). Botanic garden collections exist according to BGCI (www.bgci.org)

Uses

*D. regia is u*sed across the tropics for firewood, woodware, gum, pesticide and as a cultivated ornamental. The pods are possibly also edible by humans and livestock (CAB International 2000).

Rationale for the Red List Assessment

Delonix regia is listed as Least Concern as it has a wide distribution, sometimes being locally common. There have been collections made recently, and it is known from several protected areas. *Delonix regia* does not qualify for a threatened category based on geographic range (EOO and AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. There is no precise information to assess the population size and trends of the species. However, its native habitat the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

LC

[Criteria used for assessment A, B and D2]

Delonix tomentosa

Distribution
D. tomentosa is endemic to Madagascar. Only known from type specimen collected on the Ankara Plateau in the Boina, western Madagascar in 1901.

Habitat and Ecology
D. tomentosa is a deciduous tree 10-15m tall. It is found within the dry forest especially on limestone (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information
Only one population of *D. tomentosa* can be estimated by GIS analysis (Rivers *et al.* 2010, Chapter 3). Population numbers and density are largely unknown.

Threats
D. tomentosa is only known from the type collection on the Ankara Plateau, where the small fragments of remaining native vegetation are under threat from annual fires (Du Puy *et al.* 2002).

Conservation measures
None known

Rationale for the Red List Assessment
Delonix tomentosa is listed as Critically Endangered as it is only known from one collection made in 1901, and attempts to find it since have failed. It is not found in a protected area and its single location, the continuing fragmentation and deforestation of the western dry forest habitat is a cause for concern. In agreement with the IUCN Red List guidelines (IUCN Standards and Petitions Working Group 2010), the rating given for *D. tomentosa* is CR B1ab(iii), D.

CR B1ab(iii), D

[Criteria used for assessment B and D]

Delonix velutina

Distribution
D. velutina is endemic to the very north of Madagascar, found only on the Orangea Peninsula and in the Ankarana Massif. Based on the distribution of herbarium specimens, the extent of occurrence is 270km^2 and the area of occupancy is 264km^2.

Habitat and Ecology
D. velutina is a deciduous tree up to 15 m tall. It is found within the dry forest especially on limestone rocks and tsingy (Du Puy *et al.* 1995; Du Puy *et al.* 2002). It is thought to be pollinated by sunbirds due to its reduced petals and copious amount of nectar (Du Puy *et al.* 2002).

Population information
GIS analysis of population structure estimates that *D. velutina* has two populations (Rivers *et al.* 2010, Chapter 3).

Threats
The dry forest of Madagascar is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper *et al.* 2007; MEFT *et al.* 2009). The main known populations consists of very few individuals and under imminent threat from clearing for charcoal production (Du Puy *et al.* 2002). Also the proximity of the populations to Antsiranana means the habitat is under added pressure from the expanding rural populations (Sabel *et al.* 2009).

Conservation measures
D. velutina is found in one protected areas (Ankarana), although the main population in Orangea is currently under high threat of extinxtion and is not protected. Botanic garden collections exist according to BGCI (www.bgci.org)

Uses
The trunks of *D. velutina* are sometimes hollowed out to make canoes (Du Puy *et al.* 2002).

Rationale for the Red List Assessment
Delonix velutina is listed as Endangered since its EOO is less than 5,000km^2 and its AOO is less than 500km^2; its habitat is severely fragmented and consists of a fewer than five population; and its native habitat, the dry forest, continues to decline in quality and extent, due to the effect of habitat destruction, population expansion and harvesting for firewood and charcoal protduction. There is no precise information to assess the population size and trends of the species. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

EN B1ab(iii)+ 2ab(iii)

[Criteria used for assessment B and D2]

Lemuropisum edule

Distribution

L. edule is endemic to a small area in the southwest of Madagascar, along a narrow zone following the escarpment edge of the Mahafaly Plateau and the narrow coastal plain from Lake Tsimanampetsotsa to Itampolo. Based on the distribution of herbarium specimens, the extent of occurrence is 1,003km^2 and the area of occupancy is 540km^2.

Habitat and Ecology

L. edule is a shrub reaching less than 3m. It is found within the spiny forest and coastal bushland with *Allaudia* and succulent species of *Euphorbia* on limestone or sand (Du Puy *et al.* 1995); (Du Puy *et al.* 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Population information

GIS analysis estimates that *L. edule* has five populations (Rivers *et al.* 2010)

Threats

The species has a restricted and fragmented distribution and is threatened by intensifying grazing especially in the main population site around Itampolo (Du Puy *et al.* 2002). Major threats to the habitat of *L. edule* are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The rate of degradation has been exacerbated in recent years and the naturally slow growth and regeneration of the spiny forest is putting the species endemic to the area at particular risk (WWF 2001). The spiny forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 1.2% per year (Harper *et al.* 2007; MEFT *et al.* 2009). Climate change modelling predicted a 4% loss of current climatically suitable range by 2100 (Chapter 4).

Conservation measures

Lemuropisum edule is found in a protected area (Tsimanampetsotsa – all these collections were made before 1965). Seed collections have been made and are held in-country with Silo National des Graines Forestières (SNGF) and by the Millennium Seed Bank (MSB, Wakehurst Place, UK).

Use

The seeds are also known to be eaten raw locally. They are not cultivated or sold locally but an investigation into its possible commercial use has been carried out in Western Australia (Bosch 2004). Also a study into possible toxic compound found in the seeds have also been made (Kite *et al.* 1995).

Rationale for the Red List Assessment

Lemuropisum edule is listed as Endangered since its EOO is less than 5,000km^2; its habitat is severely fragmented and the species exists at fewer than five locations; and its native habitat, the spiny forest, continues to decline in quality and extent, due to the effect of habitat destruction and population expansion. There is no precise information to assess the population size and trends of the species. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

EN B1ab(iii)

[Criteria used for assessment A, B and D2]

Appendix 3

Field-based conservation assessment for *Delonix s.l.* and guidelines for ground truthing herbarium-based assessments in the field

A.3.1. Field-based conservation assessments of *Delonix s.l.*

To verify the herbarium-based conservation assessments with the reality of threat to these species on the ground, the species need to be visited in their natural habitat. Fieldwork can provide relevant and up to date information on the status of a species. Unfortunately fieldwork is often labour intensive, requires significant resources and is potentially subject to political or logistical restrictions. However, where field data do exist it contributes valuable information for a conservation assessment. Simply visiting an area and noting that a population is still extant (or not) is useful. To add further value other types of information could be gathered, including population size and structure, status of its habitat, environmental preferences, and level of protection.

In order to verify the herbarium-based conservation assessments for *Delonix s.l.*, fieldwork was carried out in 2007 (Nov-Dec, six weeks) and 2008 (Nov, three weeks). All specimen locality data and predicted current distributions from species distribution modelling were taken into the field. Building on the information from the preliminary and desktop assessments, and incorporating new up-to-date information from the field trips, field-based assessments were carried out (Table A.3.1). There are two parts to the ground truthing: firstly, the data used in the herbarium-based assessments were verified; secondly, further data were collected to improve the assessments and ensure their accuracy. In addition, DNA samples were collected in order to perform population genetic analysis.

There were no protocols or established methodology available for the ground truthing herbarium-based conservation assessments, as this has not previously been carried out in a systematic way. It would be beneficial to develop such guidelines to make the ground truthing of herbarium-based assessments easier and more accessible, and a routine activity for botanical field work. The methodology used for *Delonix s.l.* were used to develop the protocol to that suggested below.

Table A.3.1. Field-based conservation assessments of *Delonix s.l.*

Species	**Field-based Assessment**
Colvillea racemosa	LC
Delonix boiviniana	LC
Delonix brachycarpa	*
Delonix decaryi	NT
Delonix floribunda	LC
Delonix leucantha	*
Delonix pumila	EN B1ab(iii,v)+2ab(iii,v)
Delonix regia	LC
Delonix tomentosa	*
Delonix velutina	EN B1ab(iii,v)+2ab(iii,v)
Lemuropisum edule	EN B1ab(iii,iv,v)

* no field-based assessment, as the species was not observed in the field (2007-2008)

A.3.2. Guidelines for ground truthing herbarium-based conservation assessments in the field

** Check known locations* – Locations known from observations or herbarium records need to be checked, especially locations where no records have been made for a substantial period of time or where the species is suspected to no longer exist. It is important to record both the absence of a species as well its presence.

** Check new locations* – Species distribution modelling (and other types of modelling) can be used to predict areas of suitable climatic niche, habitat, etc. Such predictions can form the basis for predicting previously unrecorded locations where a species may be found. Again, it is important to record the absence as well as the presence of a species.

* *Population size (or estimates)* – Estimates of population size will allow more criteria to be used in the IUCN Red Listing process. The "gold standard" is a complete count of the number of individuals of a species; however, in practise this is not achievable for most plant species. Despite this, an estimate such as "x number of plants in x m^2" when the species is encountered, is generally possible. For long term monitoring it would be useful to set up permanent plots across the species' range.

* *Population structure* – It is valuable to record the counts (or estimates) of the number of seedlings (new recruits) vs. the number of mature individuals.

* *Current threats* – Updates on the threats (direct and indirect) to the species and its habitat in the field should also be recorded. This includes the general condition of the habitat/species, the effects of habitat conversion, fire, grazing, etc. The exploitation of the species due to local, national or international use/trade should be recorded.

* *Conservation measures* - It is important to note the present level of protection for the population. Does the population occur many kilometres within a well managed park or is it on the outskirts of a populated town where it is un-protected and under pressure from development? How effective are these conservation measures?

* *Pollinators and dispersal agents* – Any information on the pollination and dispersal of the species is important to note.

* *Recording observations* – If herbarium specimens (a verifiable reference collection) are not collected, then observation records can be made recording locations of a species using photographs and GPS coordinates. It can be useful to collect a DNA sample (often leaf) in silica gel for future studies.

* *Environmental conditions* - Recording the environmental conditions is important to help understand factors controlling the distribution of the species. Variables to note

include: climate, soil, substrate, habitat/vegetation type, elevation, slope, aspect and landform. These data can also feed into modelling of habitat suitability.

A.3.3. Example of information needed for ground truthing – *Delonix leucantha*

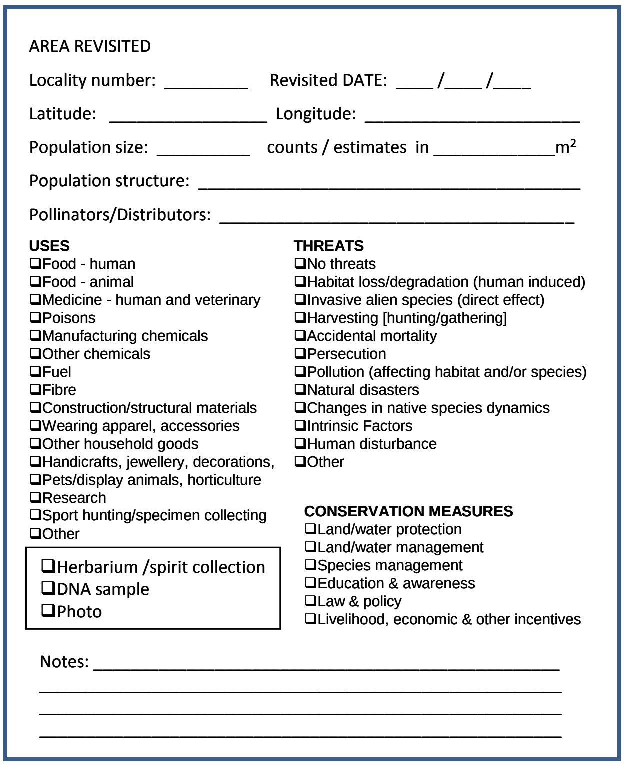
A ground truthing form may be handed to botanists about to visit a region where a species is found (and for which a herbarium-based assessment has been made). This form would need to contain all information needed, to ensure that the required information is gathered, and to make the ground truthing process as easy and accessible as possible.

Species: *Delonix leucantha*

General description*:* *D. leucantha* is a deciduous tree measuring up to 15m. It is found within the dry forest especially on limestone (Du Puy *et al.* 2002). Night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy *et al.* 2002).

Herbarium-based conservation rating: *NT*

Examples of ground truthing forms:



AREA REVISITED

Locality number: _________ Revisited DATE: ____/____/____

Latitude: _________________ Longitude: _______________________

Population size: __________ counts / estimates in _____________m^2

Population structure: ____

Pollinators/Distributors: ______________________________________

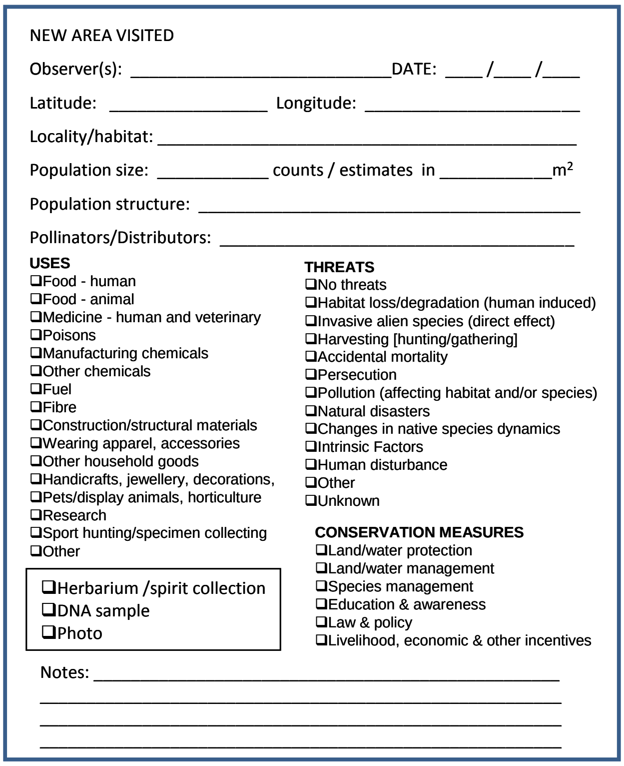
USES
- ☐Food - human
- ☐Food - animal
- ☐Medicine - human and veterinary
- ☐Poisons
- ☐Manufacturing chemicals
- ☐Other chemicals
- ☐Fuel
- ☐Fibre
- ☐Construction/structural materials
- ☐Wearing apparel, accessories
- ☐Other household goods
- ☐Handicrafts, jewellery, decorations,
- ☐Pets/display animals, horticulture
- ☐Research
- ☐Sport hunting/specimen collecting
- ☐Other

☐Herbarium /spirit collection
☐DNA sample
☐Photo

THREATS
- ☐No threats
- ☐Habitat loss/degradation (human induced)
- ☐Invasive alien species (direct effect)
- ☐Harvesting [hunting/gathering]
- ☐Accidental mortality
- ☐Persecution
- ☐Pollution (affecting habitat and/or species)
- ☐Natural disasters
- ☐Changes in native species dynamics
- ☐Intrinsic Factors
- ☐Human disturbance
- ☐Other

CONSERVATION MEASURES
- ☐Land/water protection
- ☐Land/water management
- ☐Species management
- ☐Education & awareness
- ☐Law & policy
- ☐Livelihood, economic & other incentives

Notes: ____



NEW AREA VISITED

Observer(s): ____________________________DATE: ____/____/____

Latitude: _________________ Longitude: _______________________

Locality/habitat: ____

Population size: ____________ counts / estimates in ____________m^2

Population structure: ____

Pollinators/Distributors: ______________________________________

USES
- ☐Food - human
- ☐Food - animal
- ☐Medicine - human and veterinary
- ☐Poisons
- ☐Manufacturing chemicals
- ☐Other chemicals
- ☐Fuel
- ☐Fibre
- ☐Construction/structural materials
- ☐Wearing apparel, accessories
- ☐Other household goods
- ☐Handicrafts, jewellery, decorations,
- ☐Pets/display animals, horticulture
- ☐Research
- ☐Sport hunting/specimen collecting
- ☐Other

☐Herbarium /spirit collection
☐DNA sample
☐Photo

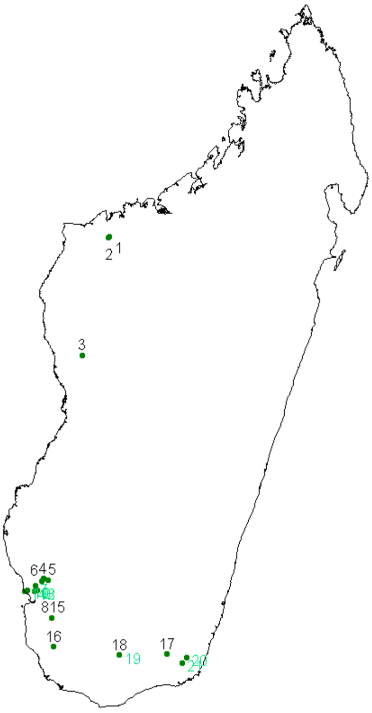
THREATS
- ☐No threats
- ☐Habitat loss/degradation (human induced)
- ☐Invasive alien species (direct effect)
- ☐Harvesting [hunting/gathering]
- ☐Accidental mortality
- ☐Persecution
- ☐Pollution (affecting habitat and/or species)
- ☐Natural disasters
- ☐Changes in native species dynamics
- ☐Intrinsic Factors
- ☐Human disturbance
- ☐Other
- ☐Unknown

CONSERVATION MEASURES
- ☐Land/water protection
- ☐Land/water management
- ☐Species management
- ☐Education & awareness
- ☐Law & policy
- ☐Livelihood, economic & other incentives

Notes: ____

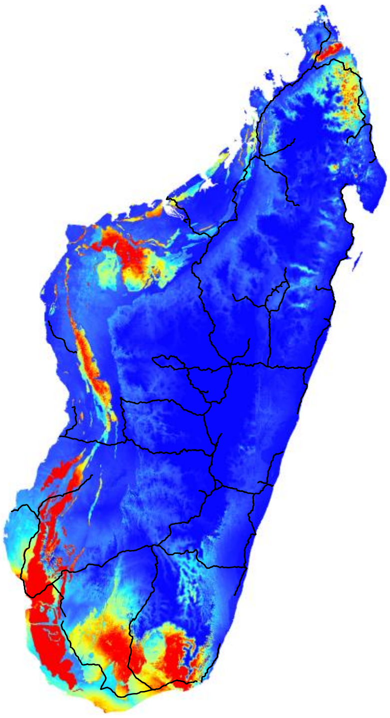
Revisited, species not found	Revisited, species found		Latitude				Longitude				Locality	Locality notes	Alt. (m)	Collector	Number	Date	Flower /Fruit
☐	☐	1	16°	27’	0’’	S	45°	21’	0’’	E	Vilanandro	Réserve N_ 8, Village le plus proche: Vilanandro (RN 8) Soalala; Canton Andranomavo, Dt Soalala	71	Service Forestier,	21385 sf	12/10/1963	Flower
☐	☐	2	16°	28’	0’’	S	45°	20’	0’’	E	Namoroka	Tsingy de Namoroka (Ambongo)	150	Perrier de la Bâthie, H.	4760	00/09/1914	Flower
☐	☐	3	18°	45’	0’’	S	44°	48’	0’’	E	Antsalova	Reserve Naturelle de Bemaraha S.E. of Antsalova	400	Phillipson, P.B.	2252	23/08/1987	Flower
☐	☐	4	23°	6’	0’’	S	44°	2’	0’’	E	Fiherenana	Falaises calcaires du Fiherenana	0	Bosser, J.	15712	00/02/1962	
☐	☐	5	23°	8’	0’’	S	44°	7’	0’’	E	Andranovory	A l'Ouest d'Andranovory (au PK 66 de la Route Tuléar-Sakaraha)	0	Service Forestier,	29093 sf	27/02/1970	Fruit
☐	☐	6	23°	9’	0’’	S	44°	0’	0’’	E	Fiherenana	Gorges du Fiherenana	0	Keraudren, M.	1343	03/02/1962	Flower
☐	☐	7	23°	15’	0’’	S	43°	52’	0’’	E	Behompy	30 km NE of Toliara, the Fiherenana River valley, around the village of Beantsy, c. 5 km NE of Behompy	125	Du Puy, D.J.	M 87	21/01/1989	Flower
☐	☐	8	23°	20’	0’’	S	43°	53’	0’’	E	Toliary	Km 30 Route Tulear-Sakaraha	0	Poupon,	2 [a]	00/01/1953	Flower
☐	☐	9	23°	20’	0’’	S	43°	55’	0’’	E	Toliary	SW: Province of Toliara (Tulear), c. 25 km NE of Tulear, Route Nationale 7, just north of the radio masts	200	Du Puy, D.J.	M 419	27/01/1990	Flower
☐	☐	10	23°	20’	0’’	S	43°	43’	0’’	E	Sakaraha	environs de Tulear, km 30 route de Sakaraha	0	Descoings, B.	2267	05/02/1957	Flower
☐	☐	11	23°	20’	0’’	S	43°	43’	0’’	E	Sakaraha	environs de Tulear, km 30 route de Sakaraha	0	Descoings, B.	2291	05/02/1957	Flower
☐	☐	12	23°	21’	0’’	S	43°	51’	0’’	E	Toliary	RN 7. 27 km from Tulear	100	Phillipson, P.B.	2764	30/12/1987	Flower
☐	☐	13	23°	21’	0’’	S	43°	51’	0’’	E	Toliary	27 km from Tulear, along Route Nationale 7, near radio mast	100	Phillipson, P.B.	3760	12/10/1990	Fruit
☐	☐	14	23°	21’	0’’	S	43°	40’	0’’	E	Toliary	Tulear	0	Poupon,	2 b	00/03/1953	Fruit
☐	☐	15	23°	52’	0’’	S	44°	12’	0’’	E	Betioky	Itambono Corridor between Betioky and Beheloka, 18 km from R.N. 10	250	Phillipson, P.B.	2743	30/12/1987	Flower
☐	☐	16	24°	26’	0’’	S	44°	14’	0’’	E	Itampolo	km 46 de la piste d'Itampolo à Ejeda	0	Labat, J.N.	2101	09/02/1990	Flower
☐	☐	17	24°	35’	0’’	S	46°	29’	0’’	E	Toliary		0	Allorge, L.	904	21/10/1993	Flower
☐	☐	18	24°	36’	0’’	S	45°	33’	0’’	E	Vohipary	Massif granitique du Vohipary, au N.W. d'Andalatanosy (Entre Antanimora et Beraketa)	0	Service Forestier,	28295 sf	15/09/1968	Flower
☐	☐	19	24°	36’	0’’	S	45°	33’	0’’	E	Vohipary		400	McWhirter, J.H.	237	14/09/1968	Flower
☐	☐	20	24°	39’	0’’	S	46°	52’	0’’	E	Enaniliha	Enaniliha, Fort-Dauphin	0	Réserves Naturelles Madagascar,	8526 rn	08/01/1956	Flower
☐	☐	21	24°	45’	0’’	S	46°	47’	0’’	E	Andohahela	Andohahela RNI. Côte sud de la Reserve vers Ambohitra.	0	Rakotomalaza, P.J.	297	04/10/1994	

Available collections



Predicted distribution

red (warm colours), predicted high suitability,
blue (cold), predicted low suitability.



Identification guide *(Du Puy et al. 2002)*

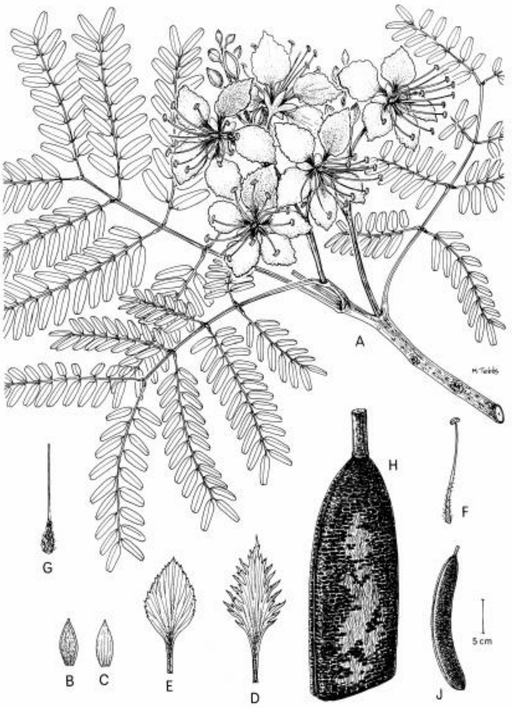


FIG. 3. **A–C & E–J** *Delonix leucantha* subsp. *gracilis*: **A** flowering shoot × ⅔; **B** sepal inner surface × 1; **C** sepal outer surface × 1; **D** petal of subsp. *leucantha* × 1; **E** petal of subsp. *gracilis* × 1; **F** stamen × 1; **G** ovary and style × 1; **H** pod base × ⅔; **J** pod (scale bar = 5 cm). **A–C & E–G** from *Du Puy, Du Puy, Labat & Phillipson* M419, **D** from *Service Forestier* 21385-SF, **H–J** from *Phillipson, Randriarimalala & Andriantiana* 3760. Drawn by Margaret Tebbs.

Herbarium specimen of *D. leucantha*

FLORA OF MADAGASCAR

Delonix leucantha subsp gracilis Du Puy, Phillipson & Rabevohitra

HOLOTYPE

SW Madagascar, Province of Toliara (Tulear), c. 25 km NE of Tulear, Route Nationale 7, just north of the radio masts.

Map Ref.: 23°20'S 43°55'E

In scrubby, spiny vegetation in very thin gravelly soil over limestone rock outcrop.

Alt. c. 200 m

An attractive small tree c. 9 m tall; trunk unbranched for c. 6 m, inflated and cigar shaped, narrowing at the base and apex; bark smooth, greyish, peeling in thin, papery red-brown strips; twigs grey with few fawn lenticels. Leaves clustered near the twig tips, with (2)3(4) pairs of pinnae, no glands visible; leaflets c. 12–18, alternate to subopposite, bluish green above, much paler beneath, somewhat thickened and thinly coriaceous with the veins hardly raised. Flowers numerous, in axillary and terminal racemes c. 6–10 cm long, c. 4–5 cm in diameter, not scented, white fading to salmon pink; calyx 5-lobed, green, the lobes reflexed and white hairy within; upper petal largest, c. 2.8 cm long, 1.8 cm broad, with a large pale yellow central area and a white crisped margin, the claw vermillion, the other petals similar but smaller and white lacking the yellow, all fading to salmon pink; stamen filaments pink, the anthers orange-brown, medifixed; style pink, the stigma green, the ovary densely white-woolly.

D.J. & B.P. Du Puy, J.-N. Labat &
P. Phillipson
29.1.90 M419

Appendix 4

Full conservation assessment for *Delonix s.l.*

The full conservation assessments of all eleven species of *Delonix s.l.* from Madagascar as submitted to the IUCN Red List for Threatened Species.

Colvillea racemosa **LC**

Taxonomic Authority: Bojer ex Hook.

☑ Global Assessment ☐ Regional

Region: Global ☐ Endemic to region

No synonyms available

Common names

FINGOKO	Unknown
SARONGAZA(O)	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name:
☐ Plant Hybrid
Subpopulation:
Authority:

General Information

Distribution

C. racemosa is endemic to Madagascar, found in the southern, western and northern parts of the country – from Antsiranana in the north, the Bemaraha massive in the west to the extreme south of Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 397,012km2 and the area of occupancy is 230,099km2.

Range Size

Area of Occupancy: 230099
Extent of Occurrence: 397012
Map Status:

Elevation

Upper limit: 300(-700)
Lower limit: 0

Depth

Upper limit:
Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

GIS analysis estimates that Colvillea racemosa has four subpopulations (Rivers et al. 2010). Total population numbers, number of mature individuals and population density estimates are unknown. Although the species is sometimes recorded as locally common (Phillipson 1992). Genetically, C. racemosa contains levels of genetic diversity that are slightly below the average for Delonix s.l. (Rivers in prep.). The variation is distributed geographically.

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

C. racemosa is a deciduous tree up to 20 m tall, with distinctive, large panicles of bright orange flowers. It is found within the dry forest and is thought to be pollinated by Sovimanga Sunbirds (Du Puy et al. 1995; Du Puy et al. 2002; Phillipson 1992).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

The native vegetation of C. racemosa is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted no change in the climatically suitable range by 2100 (Rivers, in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

C. racemosa is found in five protected areas (Bezaha Mahafaly, Kirindi Mitea, Menabe, Onilahy, Tsingy de Bemaraha), although the effectiveness of this official protection is variable. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country with Silo National des Graines Forestières (SNGF). Botanic garden collections also exist according to BGCI (www.bgci.org). In addition, C. racemosa is found cultivated elsewhere in the tropics, including South Africa (Phillipson 1992).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐
South Africa	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐

General Habitats	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
11. Other household goods	☑	☐	☐
3. Medicine - human and veterinary	☑	☐	☐
7. Fuel	☑	☐	☐
9. Construction/structural materials	☑	☐	☐

C. racemosa is used for construction, fences (Blanc-Pamard 2002) and trunks are hollowed out as canoes (Du Puy et al. 2002). It is also sometimes used as an ornamental shade tree (Phillipson 1992). Herbarium specimens also report the seeds to be used in occult sciences (Du Puy M304).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Least Concern (LC)

Red List Criteria:

Date Last Seen (only for EX, EW or Possibly EX species):

Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Colvillea racemosa is listed as Least Concern as it has a wide distribution, it is sometimes locally common and there are no specific threats associated with the species. There have been recent collections made, and it is known from five protected areas. C. racemosa does not qualify for a threatened category based on geographic range (EOO and AOO), nor is it likely to be declining quickly enough to qualify for a threatened rating. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment: A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

☐ Genuine Change
- ☐ Genuine (recent)
- ☐ Genuine (since first assessment)

☑ Nongenuine Change
- ☐ New information
- ☐ Knowledge of Criteria
- ☐ Incorrect data used previously
- ☐ Taxonomy
- ☑ Criteria
- ☑ Other

☐ No Change
- ☐ Same category and criteria
- ☐ Same category but change in criteria

Current Population Trend: Unknown

Date of Assessment: 13/1/2011

Name(s) of the Assessor(s): Malin Rivers

Evaluator(s):

Notes:

Currently listed as LR/nt (1998) version 2.3. The change in category is due to criteria revision (as LR/nt no longer exists) and a change in the recommendations in the guidelines.

Criterion A	Criterion B	Criterion C	Criterion D
A1a ☐ A1b ☐ A1c ☐ A1d ☐	B1a ☐	C1 ☐	D ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐	B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐	C2a(i) ☐ C2a(i) ☐	D1 ☐
A3b ☐ A3c ☐ A3d ☐	B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐	C2b ☐	D2 ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐	B2a ☐		
	B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐	Criterion E	
	B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐	E ☐	

Generation Length:

% population decline in the past:

Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):

% population decline in the future:

Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):

Number of Locations: 4

Severely fragmented ☐

Number of Mature Individuals:

Bibliography

Blanc-Pamard C., 2002, La forêt et l'arbre en pays masikoro (Madagascar): un paradoxe environnemental, Bois et forêts des Tropiques, 5-22, ,

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Phillipson P.B., 1992, Colvillea racemosa, Flowering Plants of Africa52, pl. 2055, ,

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Delonix boiviniana

LC

Taxonomic Authority: (Baill.) Capuron

☑ Global Assessment ☐ Regional

Region: Global ☐ Endemic to region

Synonyms	
Poinciana boiviniana	Baill.
Poinciana lutea	Regel

Common names	
ARIVORAVY	Unknown
FANDRIANAKANDRA	Unknown
FARAFAHATSA	Unknown
FARAFANA	Unknown
FENGOKY	Unknown
HARAKA	Unknown
HIDY	Unknown
HINTSINA	Unknown
KIDROA	Unknown
MAFANGALOTRA	Unknown
MALAMASAFOY	Unknown
SARIFANY	Unknown
SEKATSA	Unknown
TSIPELATSY	Unknown
VOANKAZOMELOKA	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE

Class: MAGNOLIOPSIDA

Family: LEGUMINOSAE

Phylum: TRACHEOPHYTA

Order: FABALES

Lower Level Taxonomy

Rank:

Infra- rank name:

☐ Plant Hybrid

Subpopulation:

Authority:

General Information

Distribution

D. boiviniana is endemic to Madagascar, found in the southern, western and northern parts of the country. It is distributed from the Mandrare River in the southeast to Antsiranana in the north. Based on the distribution of herbarium specimens, the extent of occurrence is 464,797km2 and the area of occupancy is 338,508km2.

Range Size

Area of Occupancy: 338508

Extent of Occurrence: 464797

Map Status:

Elevation

Upper limit: 600 (1100)

Lower limit: 0

Depth

Upper limit:

Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal

☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical

☐ Antarctic

☐ Australasian

☐ Neotropical

☐ Oceanian

☐ Palearctic

☐ Indomalayan

☐ Nearctic

Population

GIS analysis estimates that D. boiviniana has four subpopulations (Rivers et al. 2010). The level of genetic diversity for D. boiviniana is similar to the average for Delonix s.l. (Rivers in prep.) and this variation is distributed geographically. New collections (from 2007/2008) show that the species is widespread, but only locally common in the area in which it is found.

Total Population Size

Minimum Population Size:

Maximum Population

Habitat and Ecology

D. boiviniana is a deciduous tree up to 30m tall. It is found within the dry forest especially on limestone outcrops but also on sand (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002). Sifaka (Propithecus) and Mouse-lemurs (Microcebus) are both recorded as visiting the flowers (Du Puy et al. 2002) and occasionally eating the leaves (Norscia et al. 2006).

System
☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern
☐ Congregatory ☐ Migratory

Crop Wild Relative
☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

The natural vegetation of D. boiviniana is under threat from fragmentation and habitat loss, as a result of the conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted a loss of 5% of its present climatically suitable range by 2100 (Rivers, in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.3.3.2 Selective logging	☐	☑	☐
1.3.7 Other	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐
11 Other	☐	☐	☑

Conservation Measures

D. boiviniana can be found in several protected areas (Analamerana, Ankarana, Daraina, Kasijy, Menabe, Montagne des Français, Tsingy de Bemaraha, Tsingy de Namoroka, Vohibasia, Zombitse), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
11. Other household goods	☑	☐	☐
5. Manufacturing chemicals	☑	☐	☐
9. Construction/structural materials	☑	☐	☐

Trunks of D. boiviniana are hollowed out to make canoes and coffins (Rivers, pers. obs.), the resin is used as glue (Du Puy et al. 2002).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Least Concern (LC)

Red List Criteria:
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Delonix boiviniana is listed as Least Concern, as it has a wide distribution, there have been recent collections made, and it is known from several protected areas. D. boiviniana does not qualify for a threatened category based on geographic range (EOO or AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☑ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☑ Criteria
  - ☑ Other
- ☐ No Change
  - ☐ Same category and criteria
  - ☐ Same category but change in criteria

Current Population Trend:
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as LR/nt (1998) version 2.3. The change in category is due to criteria revision (as LR/nt no longer exists) and a change in the recommendations in the guidelines.

Criterion A
A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☐ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B
B1a ☐
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☐
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C
C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion E
E ☐

Criterion D
D ☐
D1 ☐
D2 ☐

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 4 Severely fragmented ☐
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Norscia I., Carrai V. and Borgognini-Tarli S.M., 2006, Influence of dry season and food quality and quantity on behavior and feeding strategy of Propithecus verreauxi in Kirindy, Madagascar, International Journal of Primatology, 1001-1022, ,

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Delonix brachycarpa **NT**

Taxonomic Authority: (R.Vig.) Capuron

☑ Global Assessment ☐ Regional

Region: Global ☐ Endemic to region

Synonyms		Common names	
Poinciana	R.Vig.	BONARANALA	Unknown
		FENGOKY	Unknown
		KOMANGAVATO	Unknown
		SARIKOMANGA	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name:
☐ Plant Hybrid
Subpopulation:
Authority:

Potentially not a distinct taxon from Delonix boiviniana, which it closely resembles, but Delonix brachycarpa has a fine dense velvety indementum (Du Puy et al 2002).

General Information

Distribution

D. brachycarpa is endemic to Madagascar, found in the southern and western parts of the country. Although found from Ankara Plateau, Tsingy de Bemaraha and the upper Mandrare River basin, areas widely separated geographically, it is scattered and rare where found (Du Puy et al. 1995). Based on the distribution of herbarium specimens, the extent of occurrence is 190,398km2 and the area of occupancy is 51,428km2.

Range Size

Area of Occupancy: 51428
Extent of Occurrence: 190398
Map Status:

Elevation

Upper limit: 300 (1200)
Lower limit: 0

Depth

Upper limit:
Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

GIS analysis estimates that D. brachycarpa has five subpopulations (Rivers et al. 2010).
Population numbers and density are largely unknown, however it has been recorded as scattered (Du Puy et al. 1995).

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

D. brachycarpa is a deciduous tree. It is found within the dry forest especially on limestone (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

The natural vegetation of D. brachycarpa is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling is showing little change in climatically suitable range by 2100 (Rivers in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

D. brachycarpa can be found in three protected areas (Mahavavy Kinkony, Tsingy de Bemaraha, Tsingy de Namoroka), although the effectiveness of this official protection is not always adequate. No botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☑ Species is not utilised at all

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Near Threatened (NT)

Red List Criteria:
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

D. brachycarpa is listed as Near Threatened, as it has a very low number of recent collections, it is reported to have a scattered distribution and to be rare when found. D. brachycarpa does not qualifying for a threatened category based on geographic range (EOO and AOO), but the range is scattered, and its habitat is fragmented and degraded, and the habitat quality and extent continues to decline. There is not enough information to assess the population size and trends of the species. However, its native habitat, the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☑ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☑ Criteria
  - ☐ Other
- ☐ No Change
  - ☐ Same category and criteria
  - ☐ Same category but change in criteria

Current Population Trend:
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:
Currently listed as LR/nt (1998) version 2.3. The change in category is due to criteria revision.

Criterion A
A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☐ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B
B1a ☐
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☐
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C
C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion E
E ☐

Criterion D
D ☐
D1 ☐
D2 ☐

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):

% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 5
Severely fragmented ☐
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Delonix decaryi **VU**

Taxonomic Authority: (R.Vig.) Capuron

☑ Global Assessment ☐ Regional

Region: Global

☐ Endemic to region

Synonyms

Poinciana	R.Vig.
Poinciana decaryi	R.Vig.

Common names

FENGOKY(A)	Unknown
FENGOPASY	Unknown
MALAMASAFOY	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE

Phylum: TRACHEOPHYTA

Class: MAGNOLIOPSIDA

Order: FABALES

Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:

Infra- rank name:

☐ Plant Hybrid

Subpopulation:

Authority:

General Information

Distribution

D. decaryi is endemic to the southern parts of Madagascar. It is found in a narrow band along the coast from north of Toliara to Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 51,902km2 and the area of occupancy is 28,048km2.

Range Size

Area of Occupancy: 28048

Extent of Occurrence: 51902

Map Status:

Elevation

Upper limit: 260

Lower limit: 0

Depth

Upper limit:

Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal

☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical

☐ Antarctic

☐ Australasian

☐ Neotropical

☐ Oceanian

☐ Palearctic

☐ Indomalayan

☐ Nearctic

Population

GIS analysis estimates that D. decaryi has eight subpopulations (Rivers et al. 2010). However, genetic subpopulation analyses show that the species consist of three genetic subpopulations. One in the south-eastern corner, one mainly in the north-western corner and one in the middle (Rivers in prep). D. decaryi contains levels of genetic diversity that are similar to the average for Delonix s.l. (Rivers in prep.). About one fifth of the genetic variation is distributed within sample sites, and four fifths are distributed between different sample sites. The genetic variation is distributed geographically.

Signs of regeneration were rarely found, and the stands often consisted of single or very few mature trees.

Total Population Size

Minimum Population Size:

Maximum Population

Habitat and Ecology

D. decaryi is a deciduous tree reaching up to 10m. It is found within the spiny forest and coastal bushland often with Didieriaceae and succulent species of Euphorbia on limestone and sand (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

Major threats to the spiny forest (natural vegetation of D. decaryi) are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat. The degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration is putting the species endemic to the area at particular risk (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The spiny forest is one of the primary vegetation types that are declining significantly in Madagascar with an estimated rate of loss of 1.2% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted a 46% loss (by 2100) of its present climatically suitable range (Rivers in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.3.7 Other	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐
11 Other	☐	☐	☑

Conservation Measures

D. decaryi can be found in two protected areas (Cap Ste Marie and Tsimanampetsotsa), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
1. Food - human	☑	☐	☐
11. Other household goods	☑	☐	☐
3. Medicine - human and veterinary	☑	☐	☐
5. Manufacturing chemicals	☑	☐	☐
9. Construction/structural materials	☑	☐	☐

Cuttings of D. decaryi are often planted in villages as a "living fence". The trunks are sometimes hollowed out to make canoes; the seeds are reported to be edible; and the resin used as glue (Du Puy et al. 2002) for sealing canoes. Herbarium collections also report that crushed leaves are put on a baby's head to harden the scull (Du Puy M94).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Vulnerable (VU)

Red List Criteria: A3c

Date Last Seen (only for EX, EW or Possibly EX species):

Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Delonix decaryi is listed as Vulnerable because it is predicted to lose more than 30% of its present range in the next 100 years (approximately 3 generations) due to climate change. Genetic-range studies also found that the loss in range is strongly correlated to genetic diversity, so this decline is likely to have a serious impact on the species, and would therefore qualify for a threatened (Vulnerable) rating under Criterion A3.

D. decaryi is known from several localities, however, rarely in substantial populations. It does not qualify for a threatened category based on geographic range (EOO or AOO), but its habitat is fragmented and degraded, and its native habitat continues to decrease in quality and extent.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☑ Nongenuine Change
  - ☑ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☐ Criteria
  - ☐ Other
- ☐ No Change
  - ☐ Same category and criteria
  - ☐ Same category but change in criteria

Current Population Trend:

Date of Assessment: 13/1/2011

Name(s) of the Assessor(s): Malin Rivers

Evaluator(s):

Notes:

Currently listed as LR/nt (1998) version 2.3. The change in category is due to the fact new information have become available.

Criterion A

A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☑ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B

B1a ☐
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☐
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C

C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion E

E ☐

Criterion D

D ☐
D1 ☐
D2 ☐

Generation Length:

% population decline in the past:

Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):

% population decline in the future: 46%

Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations): 100 years

Number of Locations: 3-8

Severely fragmented ☐

Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar spiny thickets (AT1311)19 Sept, , WWF,

Delonix floribunda

LC

Taxonomic Authority: (Baill.) Capuron

☑ Global Assessment ☐ Regional

Region: Global

☐ Endemic to region

Synonyms

Aprevalia floribunda	Baill.
Aprevalia perrierii	R.Vig.
Delonix adansonioides	
Poinciana	R.Vig.

Common names

BOY	Unknown	
FENGOBOHITSY	Unknown	
(R.Vig.) Capuron	FENGOKA	Unknown
FENGOKY	Unknown	
HAROFO	Unknown	
HAZOMASEFOY	Unknown	
MALAMASAFOY	Unknown	
MALAMASOFOHIHY	Unknown	
SARINGAZA	Unknown	

Upper Level Taxonomy

Kingdom: PLANTAE

Phylum: TRACHEOPHYTA

Class: MAGNOLIOPSIDA

Order: FABALES

Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:

Infra- rank name:

☐ Plant Hybrid

Subpopulation:

Authority:

General Information

Distribution

D. floribunda is endemic to the western and southern parts of Madagascar. It is found mainly along the coast from the Bemaraha massive to the Mandrare River, near Tolagnaro (Fort Dauphin). Based on the distribution of herbarium specimens, the extent of occurrence is 125,372km2 and the area of occupancy is 79,118km2.

Range Size

Area of Occupancy: 79118

Extent of Occurrence: 125372

Map Status:

Elevation

Upper limit: 250

Lower limit: 0

Depth

Upper limit:

Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal

☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical

☐ Antarctic

☐ Australasian

☐ Neotropical

☐ Oceanian

☐ Palearctic

☐ Indomalayan

☐ Nearctic

Population

GIS analysis and genetic population analysis estimate that D. floribunda has 6 subpopulations (Rivers et al. 2010). Genetically D. floribunda contains levels of genetic diversity that are similar to the average levels for Delonix s.l. (Rivers in prep.). 15% of the genetic variation is distributed within sample sites, and 85% are distributed between different sample sites. The genetic variation is not distributed geographically. Signs of regeneration were rarely found, and the stands often consisted of single or very few mature trees (M. Rivers pers. obs.).

Total Population Size

Minimum Population Size:

Maximum Population

Habitat and Ecology

D. floribunda is a deciduous tree reaching up to 15m. It is found within the dry forest as well as the spiny forest and coastal bushland on limestone or sand (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by sunbirds due to its flowers with highly reduced petals and copious amount of nectar (Du Puy et al. 2002). Lemurs (Cheirogaleus medius) have been recorded to feed on nectar (Harcourt & Thornback 1990).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

Major threats to the spiny forest and the dry forest (the natural vegetation of D. floribunda) are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The rate of degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration in the spiny forest is putting the species endemic to the area at particular risk (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The spiny forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 1.2% per year; the dry forest is also declining significantly with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted little change in climatically suitable range by 2100 (Rivers in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

D. floribunda can be found in some protected areas (Andohahela, Ankodida, Kirindi Mitea, Menabe, Onilahy, Tsimanampetsotsa), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
11. Other household goods	☑	☐	☐
12. Handicrafts, jewellery, decorations, curios, etc.	☑	☐	☐
5. Manufacturing chemicals	☑	☐	☐
9. Construction/structural materials	☑	☐	☐

D. floribunda is sometimes planted in villages, and trunks hollowed out to make canoes; the resin is also used as glue (Du Puy et al. 2002).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Least Concern (LC)

Red List Criteria:
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Delonix floribunda is listed as Least Concern, as it is known from a several localities, covering a wide distribution, however, rarely in substantial populations. It grows in two vegetation types (dry forest and spiny forest), and is found in several protected areas. D. floribunda does not qualify for a threatened category based on geographic range (EOO or AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☑ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☑ Criteria
  - ☑ Other
- ☐ No Change
  - ☐ Same category and criteria
  - ☐ Same category but change in criteria

Current Population Trend:
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as LR/nt (1998) version 2.3. The change in category is due to criteria revision (as LR/nt no longer exists) and a change in the recommendations in the guidelines.

Criterion A

A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☐ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B

B1a ☐
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☐
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C

C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion E

E ☐

Criterion D

D ☐
D1 ☐
D2 ☐

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 4-6
Severely fragmented ☐

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harcourt C. and Thornback J., 1990, Lemurs of Madagascar and the Comoros: the IUCN red data book, , IUCN, Gland, Switzerland and Cambridge, UK.

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar spiny thickets (AT1311)19 Sept, , WWF,

Delonix leucantha **NT**

Taxonomic Authority: (R.Vig.) Du Puy, Phillipson & R. Rabev.

☑ Global Assessment ☐ Regional Region: Global ☐ Endemic to region

Synonyms		Common names	
Poinciana leucantha	R.Vig.	FENGOKY	Unknown

Upper Level Taxonomy

Kingdom:	PLANTAE	Phylum:	TRACHEOPHYTA
Class:	MAGNOLIOPSIDA	Order:	FABALES
Family:	LEGUMINOSAE		

Lower Level Taxonomy

Rank: Infra- rank name: ☐ Plant Hybrid

Subpopulation: Authority:

Includes three subspecies: ssp. leucantha, ssp. gracilis ssp. bemaharensis

General Information

Distribution

D. leucantha is endemic to Madagascar, where it is found in the southern and western parts of the country, including Namoroka and Bemaraha in the west, and around Toliara in the south. Based on the distribution of herbarium specimens, the extent of occurrence is 157,089km2 and the area of occupancy is 60,668km2.

Range Size

Area of Occupancy: 60668

Extent of Occurrence: 157089

Map Status:

Elevation

Upper limit: 400 (700)

Lower limit: 0

Depth

Upper limit:

Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal

☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical

☐ Antarctic

☐ Australasian

☐ Neotropical

☐ Oceanian

☐ Palearctic

☐ Indomalayan

☐ Nearctic

Population

GIS analysis estimates that D. leucantha has five subpopulations (Rivers et al. 2010). Population numbers and density are largely unknown, however, the species has been recorded as uncommon (Du Puy et al. 1995).

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

D. leucantha is a deciduous tree measuring up to 15m. It is found within the dry forest especially on limestone (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

Major threats to the spiny forest and the dry deciduous forests, where D. leucantha is found, are the widespread exploitation for firewood and charcoal production (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat. The rate of degradation has been exacerbated in recent years and the naturally slow rate of growth and regeneration is putting the species endemic to the area at particular risk (WWF 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar, with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted a 77% decrease in its present climatically suitable range by 2100 (Rivers in prep.).

In the South, subspecies gracilis is threatened by charcoal production. The subspecies D. leucantha subsp. leucantha is known from a small population in a fairly inaccessible location within a protected area, where it is not susceptible to exploitation or fires (Du Puy et al 2002). Insufficient information is available regarding the threats to subspecies bemarahensis.

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐
11 Other	☐	☐	☑

Conservation Measures

D. leucantha can be found in four protected areas (Andohahela, Onilahy, Tsingy de Bemaraha and Tsingy de Namoroka), although the effectiveness of this official protection is not always adequate. Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☑ Species is not utilised at all

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Near Threatened (NT)

Red List Criteria:
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

D. leucantha is listed as Near Threatened, as it is expected to be severely affected by climate change, with a loss of more than 70% of its current range. However, the impact of this predicted range loss on population size and/or genetics has not yet been demonstrated. D. leucantha does not qualify for a threatened category based on current geographic range (EOO and AOO), but its occurrence is scattered, its habitat is fragmented and degraded, and the habitat quality and extent continues to decline. These trends should be monitored to determine whether the population of this species is declining. Its three subspecies are isolated and are likely to be severely affected by future habitat degradation.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

☐ Genuine Change	☐ Nongenuine Change		☐ No Change
☐ Genuine (recent)	☐ New information	☐ Taxonomy	☐ Same category and criteria
☐ Genuine (since first assessment)	☐ Knowledge of Criteria	☐ Criteria	
	☐ Incorrect data used previously	☐ Other	☐ Same category but change in criteria

Current Population Trend: Unknown
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently not listed on species level

Criterion A	Criterion B	Criterion C	Criterion D
A1a ☐ A1b ☐ A1c ☐ A1d ☐	B1a ☐	C1 ☐	D ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐	B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐	C2a(i) ☐ C2a(i) ☐	D1 ☐
A3b ☐ A3c ☐ A3d ☐	B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐	C2b ☐	D2 ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐	B2a ☐		
	B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐	Criterion E	
	B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐	E ☐	

Generation Length:

% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future: 77%
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations): 100 years
Number of Locations: 5
Severely fragmented ☐
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar spiny thickets (AT1311)19 Sept, , WWF,

Delonix pumila **EN**

Taxonomic Authority: Du Puy, Phillipson & R. Rabev.

☑ Global Assessment ☐ Regional

Region: Global

☐ Endemic to region

Synonyms

Poinciana R.Vig.

Common names

FENGODIVA	Unknown
FENGOKO	Unknown
MALAMASAFOY	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name:
☐ Plant Hybrid
Subpopulation:
Authority:

General Information

Distribution

D. pumila is endemic to a small region around Toliara in the southwestern part of Madagascar. It is found near the hill 'La Table' approximately 25km east of Toliara and along the adjacent escarpment edge of the Mahafaly Plateau extending to the area just south of Onilahy river. A collection from 2006 from the Zombitse area (Tefy 919) has been misidentified as D. pumila. Based on the distribution of herbarium specimens, the extent of occurrence is 311km2 and the area of occupancy is

Range Size

Area of Occupancy: 93
Extent of Occurrence: 311
Map Status:

Elevation

Upper limit: 160
Lower limit: 0

Depth

Upper limit:
Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

Genetic population analysis shows that the species consist of a single interbreeding population. Only 2% of the genetic variation is distributed within sample sites, and 98% are distributed between different sample sites. Genetically D. pumila contains low levels of genetic diversity compared to other species in Delonix s.l. (Rivers in prep.). New collections (from 2007/2008) show that the largest subpopulation exist along the main road into Toliara; the other localities much smaller

Total Population Size

Minimum Population Size:
Maximum Population

Habitat and Ecology

D. pumila is a dwarf, deciduous shrub-like tree less than 3m tall. It is found within the spiny forest and coastal bushland with succulent species of Euphorbia on limestone rock (Du Puy et al. 1995; Du Puy et al. 2002). It is slow-growing, and thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - small	Small tree, also termed a Phanerophyte (>1m)

Threats

The natural vegetation where D. pumila is found is under threat from widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007). The rate of degradation has been exacerbated in recent years and the naturally slow growth and regeneration of the spiny forest is putting the endemic species of the area at particular risk (WWF 2001). The spiny forest is one of the primary vegetation types in Madagascar that is declining significantly with an estimated rate of loss of 1.2% per year (Harper et al. 2007; MEFT et al. 2009). Also, the main population of the species exist close to one of the largest and expanding towns in Madagascar. Climate change modelling predicted little change in climatically suitable range by 2100 (Rivers

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.7 Other	☐	☑	☐
1.4 Infrastructure development	☐	☑	☐
1.4.4 Transport - land/air	☐	☑	☐
1.7 Fires	☐	☑	☐
3 Harvesting (hunting/gathering)	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

D. pumila is found in a proposed protected area (Onilahy), although the effectiveness of this proposed protection is not known. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☑ Species is not utilised at all

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Endangered (EN)

Red List Criteria: B1ab(iii,iv,v)+2ab(iii,iv,v)
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

D. pumila is listed as Endangered since its EOO is less than 5,000km2 and its AOO is less than 500km2; its habitat is severely fragmented and genetic studies show that the species consist of a single subpopulation; and habitat continues to decline in quality and extent, and probably in the number of mature individuals due to the effect of harvesting, population expansion and habitat destruction. It is also known to be very slow-growing, and so will have less chance of adapting to these pressures.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☐ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☐ Criteria
  - ☐ Other
- ☑ No Change
  - ☐ Same category and criteria
  - ☑ Same category but change in criteria

Current Population Trend: Unknown
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as EN B1+2cde (1998) version 2.3. There is no change in category but the assessment is updated due to criteria revision.

Criterion A
A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☐ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B
B1a ☑
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☑ B1b(iv) ☑ B1b(v) ☑
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☑
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☑ B2b(iv) ☑ B2b(v) ☑
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C
C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion D
D ☐
D1 ☐
D2 ☐

Criterion E
E ☐

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 1
Severely fragmented ☑
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar spiny thickets (AT1311)19 Sept, , WWF,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar succulent woodlands (AT1312)19 Sept, , WWF,

Delonix regia **LC**

Taxonomic Authority: (Bojer ex Hook.) Raf.

☑ Global Assessment ☐ Regional Region: Global ☐ Endemic to region

Synonyms

Delonix regia var.	Stehle
Delonix regia var.	Stehle
Poinciana regia	Bojer ex Hook.

Common names

ALAMBORONALA	Unknown
FANNOU	Unknown
FLAMBOYANT	French
FLAME TREE	English
GOLD MOHAR	English
HARONGADRA	Unknown
HINTSAKINSA	Unknown
HINTSAKINSAN	Unknown
KITSAKITSABE	Unknown
MONOGO	Unknown
SARONGADRA	Unknown
TANAHOU	Unknown
TSIOMBIVOSITRA	Unknown
VOLOBARA	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name:
☐ Plant Hybrid
Subpopulation:
Authority:

General Information

Distribution

D. regia is endemic to Madagascar. In the wild, it is found in the west (Tsingy de Bemaraha) and the north (including Orangea, Cap d'Ambre peninsulas and around Baie de Diego, the Ankarana and Analamerana Massifs) and possibly from Nosy Be. It is also cultivated in most areas of Madagascar and across much of the tropics as a street tree. Based on the distribution of herbarium specimens, the extent of occurrence of the wild populations is 68,334km2 and the area of occupancy is 58,156km2.

Range Size

Area of Occupancy: 58156
Extent of Occurrence: 68334
Map Status:

Elevation

Upper limit: 400 (750)
Lower limit: 0

Depth

Upper limit:
Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

GIS analysis estimates that D. regia has three subpopulations (Rivers et al. 2010). Genetically (only samples from the northern distribution) D. regia contains levels of genetic diversity that are high compared to the average for Delonix s.l. (Rivers in prep.). The variation is not distributed in geographically. During collection trip in 2007 D. regia was spotted in several locations that were previously unrecorded for the species. However, little regeneration was seen.

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

D. regia is a deciduous tree up to 30m tall. It is found within the dry forest especially on limestone (Du Puy et al. 1995; Du Puy et al. 2002). It has distinctive large, bright red flowers, and is thought to be pollinated by sunbirds (Du Puy et al. 2002).

System			Movement pattern		Crop Wild Relative
☑ Terrestrial	☐ Freshwater	☐ Marine	☐ Congregatory	☐ Migratory	☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

D. regia is thought to be rare in its native habitat, although this seems to be an exaggeration (Du Puy et al. 2002 and Rivers pers. obs.). The natural vegetation of Madagascar is under threat from habitat loss and fragmentation, as a result of conversion of land for slash-and-burn agriculture, grazing, charcoal production and collection of firewood (Moat & Smith 2007). The Madagascar dry forests are severely fragmented and often in small blocks. Expanding rural populations and selective logging is also adding to the pressure (WWF 2001). The dry forest is one of the vegetation types that are declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted that approximately 10% of the present climatically suitable range will be lost by 2100 (Rivers in

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.3.3.2 Selective logging	☐	☑	☐
1.3.7 Other	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐
11 Other	☐	☐	☑

Conservation Measures

D. regia is widely cultivated across Madagascar and in the rest of the tropics as an ornamental tree. It can be found in some protected areas (Analamerana, Ankarana, Lokobe, Montagne des Français, Tsingy de Bemaraha), although the effectiveness of this official protection is not always adequate. Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Antigua and Barbuda	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Argentina	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Australia	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Bangladesh	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Bermuda	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Brazil	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Burundi	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Congo, The Democratic Republic of the	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Cuba	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Dominica	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Dominican Republic	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Ecuador	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Egypt	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Ethiopia	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Ghana	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐
Malaysia	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peninsular Malaysia	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Paraguay	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Puerto Rico	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Sierra Leone	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Sri Lanka	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Tanzania	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Trinidad and Tobago	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Uganda	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
United States of America	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐
Zimbabwe	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐

General Habitats

General Habitats	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
11. Other household goods	☑	☐	☐
16. Other	☑	☐	☐
5. Manufacturing chemicals	☑	☐	☐
7. Fuel	☑	☐	☐
9. Construction/structural materials	☑	☐	☐

D. regia is used across the tropics for firewood, woodware, gum, pesticide and as a cultivated ornamental. The pods are possibly also edible by humans and livestock (CAB International 2000).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Least Concern (LC)

Red List Criteria:
Date Last Seen (only for EX, EW or Possibly EX species):

Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Delonix regia is listed as Least Concern as it has a wide distribution, sometimes being locally common. There have been collections made recently, and it is known from several protected areas. D. regia does not qualify for a threatened category based on geographic range (EOO and AOO), nor is it likely to be declining fast enough to qualify for a threatened rating. There is no precise information to assess the population size and trends of the species. However, its native habitat the dry forest, is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

☐ Genuine Change	☑ Nongenuine Change		☐ No Change
☐ Genuine (recent)	☑ New information	☐ Taxonomy	☐ Same category and criteria
☐ Genuine (since first assessment)	☑ Knowledge of Criteria	☐ Criteria	
	☐ Incorrect data used previously	☑ Other	☐ Same category but change in criteria

Current Population Trend: Unknown
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as VU B1+2c (1998) version 2.3. The change in category is due to more information has become available (range size), also the criteria are revised and the guidelines have been updated.

Criterion A	Criterion B	Criterion C	Criterion D
A1a ☐ A1b ☐ A1c ☐ A1d ☐	B1a ☐	C1 ☐	D ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐	B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☐ B1b(iv) ☐ B1b(v) ☐	C2a(i) ☐ C2a(i) ☐	D1 ☐
A3b ☐ A3c ☐ A3d ☐	B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐	C2b ☐	D2 ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐	B2a ☐		
	B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐	Criterion E	
	B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐	E ☐	

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for

% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 3
Severely fragmented ☐
Number of Mature Individuals:

Bibliography

CAB International, 2000, Forestry compendium - global module, , CAB International, Wallingford, UK

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Delonix tomentosa **CR**

Taxonomic Authority: (R.Vig.) Capuron

☑ Global Assessment ☐ Regional Region: Global ☐ Endemic to region

Synonyms
Poinciana tomentosa R.Vig.

No common names available

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name:
☐ Plant Hybrid
Subpopulation:
Authority:

General Information

Distribution

D. tomentosa is endemic to Madagascar. Only known from type specimen collected from the Ankara plateau in the Boina, western Madagascar in 1901.

Range Size
Area of Occupancy: n/a
Extent of Occurrence: n/a
Map Status:

Elevation
Upper limit: 500
Lower limit: 200

Depth
Upper limit:
Lower limit:

Depth Zones
☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm
☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

Only one subpopulation of D. tomentosa can be estimated by GIS analysis (Rivers et al. 2010). Population numbers and density are largely unknown.

Total Population Size
Minimum Population Size: Maximum Population

Habitat and Ecology

D. tomentosa is a deciduous tree 10-15m tall. It is found within the dry forest especially on limestone (Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and the upper petal with a narrow tubular nectariferous claw (Du Puy et al. 1995; Du Puy et al. 2002).

System
☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern
☐ Congregatory ☐ Migratory

Crop Wild Relative
☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

D. tomentosa is only known from the type collection on the Ankara Plateau, where the small fragments of remaining native vegetation are under threat from annual fires (Du Puy et al. 2002; WWF 2001).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.7 Fires	☐	☑	☐
7 Natural disasters	☐	☑	☐
7.4 Wildfire	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

None known

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☐	☑	☐	☑	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☑ Species is not utilised at all

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Critically Endangered (CR)

Red List Criteria: B1ab(iii); D
Date Last Seen (only for EX, EW or Possibly EX species): 1901

Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☑

Rationale for the Red List Assessment

Delonix tomentosa is listed as Critically Endangered as it is only known from one collection made in 1901, and attempts to find it since have failed. It is not found in a protected area and its single location, the continuing fragmentation and deforestation of the western dry forest habitat is a cause for concern. In agreement with the IUCN Red List guidelines (IUCN Standards and Petitions Working Group 2010), the rating given for D. tomentosa is CR B1ab(iii), D.

[Criteria used for assessment B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☑ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☑ Criteria
  - ☑ Other
- ☐ No Change
  - ☐ Same category and criteria
  - ☐ Same category but change in criteria

Current Population Trend: Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as EN B1+2c (1998) version 2.3. The change in category is due to criteria revision and changes to guideline recommendations.

Criterion A	Criterion B	Criterion C	Criterion D
A1a ☐ A1b ☐ A1c ☐ A1d ☐	B1a ☑	C1 ☐	D ☑
A2a ☐ A2b ☐ A2c ☐ A2d ☐	B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☑ B1b(iv) ☐ B1b(v) ☐	C2a(i) ☐ C2a(i) ☐	D1 ☐
A3b ☐ A3c ☐ A3d ☐	B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐	C2b ☐	D2 ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐	B2a ☐		
	B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☐ B2b(iv) ☐ B2b(v) ☐	Criterion E	
	B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐	E ☐	

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for

% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: 1 Severely fragmented ☑
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

IUCN Standards and Petitions Subcommittee, 2010, Guidelines for using the IUCN Red List Categories and Criteria Version 8.1., , Prepared by the Standards and Petitions Subcommittee in March 2010. Dowloadable from http://www.iucnredlist.org/documents/RedListGuidelines.pdf,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Delonix velutina **EN**

Taxonomic Authority: Capuron

☑ Global Assessment ☐ Regional

Region: Global ☐ Endemic to region

No synonyms available

Common names

FENGOKO	Unknown

Upper Level Taxonomy

Kingdom: PLANTAE
Phylum: TRACHEOPHYTA
Class: MAGNOLIOPSIDA
Order: FABALES
Family: LEGUMINOSAE

Lower Level Taxonomy

Rank:
Infra- rank name: ☐ Plant Hybrid
Subpopulation:
Authority:

General Information

Distribution

D. velutina is endemic to the very north of Madagascar, found only on the Orangea Peninsula and in the Ankarana Massif. Based on the distribution of herbarium specimens, the extent of occurrence is 270km2 and the area of occupancy is 264km2 .

Range Size

Area of Occupancy: 264
Extent of Occurrence: 270
Map Status:

Elevation

Upper limit: 200 (500)
Lower limit: 0

Depth

Upper limit:
Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal
☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical
☐ Antarctic
☐ Australasian
☐ Neotropical
☐ Oceanian
☐ Palearctic
☐ Indomalayan
☐ Nearctic

Population

GIS analysis show that D. velutina has two subpopulations (Rivers et al. 2010); and, genetic subpopulation analysis show that the species consist of three genetic subpopulations (Rivers in prep). Despite being distributed over a small geographic range, there is clear genetic population structure. Genetically D. velutina contains high levels of genetic diversity compared to the average levels for Delonix s.l. (Rivers in prep.). 15% of the genetic variation is distributed within sample sites, and 85% are distributed between different sample sites. The genetic variation is distributed geographically.

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

D. velutina is a deciduous tree up to 15m tall. It is found within the dry forest especially on limestone rocks and tsingy (Du Puy et al. 1995; Du Puy et al. 2002). It is thought to be pollinated by sunbirds due to its reduced petals and copious amount of nectar (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Tree - size unknown	Tree (any size), also termed a Phanerophyte (>1m)

Threats

The dry forest of Madagascar is under threat from fragmentation and habitat loss, as a result of conversion of land for slash-and-burn agriculture, grazing of livestock, charcoal production and collection of firewood (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The dry forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 0.4-0.7% per year (Harper et al. 2007; MEFT et al. 2009). The main known populations consists of very few individuals and under imminent threat from clearing for charcoal production (Du Puy et al. 2002). Also the proximity of the populations to Antsiranana means the habitat is under added pressure from the expanding rural populations (Sabel et

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☑
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☑
1.3.1 Mining	☐	☐	☑
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.3.3.2 Selective logging	☐	☑	☐
1.3.7 Other	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

D. velutina is found in one protected areas (Ankarana), although the main population in Orangea is currently under high threat of extinxtion and is not protected. Botanic garden collections exist according to BGCI (www.bgci.org).

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐

Countries of Occurrence

	Native Presence Introduced Confirmed	Native - PresenceP Vagrant ossible	Extinct Possibly Extinct	Possibly Vagrant	introduced	Re- Reintroduced	Possibly	Reintroduced Introduced	Introduced	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats

	Score	Description
1 Forest	1	Suitable
1.5 Forest - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
11. Other household goods	☑	☐	☐

The trunks of D. velutina are sometimes hollowed out to make canoes (Du Puy et al. 2002).

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Endangered (EN)

Red List Criteria: B1ab(iii,v)+2ab(iii,v)
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Delonix velutina is listed as Endangered since its EOO is less than 5,000km2 and its AOO is less than 500km2; its habitat is severely fragmented and genetic studies show that the species consist of a fewer than five subpopulation; and its habitat continues to decline in quality and extent, and probably in the number of mature individuals due to the effect of habitat destruction, population expansion and harvesting.

There is no precise information to assess the population size and trends of the species. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

☐ Genuine Change
- ☐ Genuine (recent)
- ☐ Genuine (since first assessment)

☐ Nongenuine Change
- ☐ New information
- ☐ Knowledge of Criteria
- ☐ Incorrect data used previously
- ☐ Taxonomy
- ☐ Criteria
- ☐ Other

☑ No Change
- ☐ Same category and criteria
- ☑ Same category but change in criteria

Current Population Trend: Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:
Currently listed as EN B1+2c (1998) version 2.3. There is no change in category but the assessment is updated due to criteria revision.

Criterion A
A1a ☐ A1b ☐ A1c ☐ A1d ☐
A2a ☐ A2b ☐ A2c ☐ A2d ☐
A3b ☐ A3c ☐ A3d ☐
A4a ☐ A4b ☐ A4c ☐ A4d ☐

Criterion B
B1a ☑
B1b(i) ☐ B1b(ii) ☐ B1b(iii) ☑ B1b(iv) ☐ B1b(v) ☑
B1c(i) ☐ B1c(ii) ☐ B1c(iii) ☐ B1c(iv) ☐
B2a ☑
B2b(i) ☐ B2b(ii) ☐ B2b(iii) ☑ B2b(iv) ☐ B2b(v) ☑
B2c(i) ☐ B2c(ii) ☐ B2c(iii) ☐ B2c(iv) ☐

Criterion C
C1 ☐
C2a(i) ☐ C2a(i) ☐
C2b ☐

Criterion E
E ☐

Criterion D
D ☐
D1 ☐
D2 ☐

Generation Length:
% population decline in the past:
Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: <5 Severely fragmented ☑
Number of Mature Individuals:

Bibliography

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Du Puy D.J., Phillipson P. & Rabevohitra R., 1995, The genus Delonix (Leguminosae: Caesalpinioideae: Caesalpinieae) in Madagascar, Kew Bulletin, 445-475, ,

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

Sabel J., Green K., Dawson J. et al., 2009, The conservation status of mammals and avifauna in the Montagne des Français massif, Madagascar., Madagascar Conservation & Development, 44-51, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar dry deciduous forests (AT0202)19 Sept, , WWF,

Lemuropisum edule **EN**

Taxonomic Authority: H.Perrier

☑ Global Assessment ☐ Regional

Region: Global ☐ Endemic to region

No synonyms available

Common names

TARA	Unknown (Primary)
LALONAMBARIKA	Unknown

Upper Level Taxonomy

Kingdom:	PLANTAE	Phylum:	TRACHEOPHYTA
Class:	MAGNOLIOPSIDA	Order:	FABALES
Family:	LEGUMINOSAE		

Lower Level Taxonomy

Rank:

Infra- rank name: ☐ Plant Hybrid

Subpopulation:

Authority:

General Information

Distribution

L. edule is endemic to a small area in the southwest of Madagascar, along a narrow zone following the escarpment edge of the Mahafaly Plateau and the narrow coastal plain from Lake Tsimanampetsotsa to Itampolo. Based on the distribution of herbarium specimens, the extent of occurrence is 1,003km2 and the area of occupancy is 540km2.

Range Size

Area of Occupancy: 540

Extent of Occurrence: 1003

Map Status:

Elevation

Upper limit: 100

Lower limit: 0

Depth

Upper limit:

Lower limit:

Depth Zones

☐ Shallow photic ☐ Bathyl ☐ Hadal

☐ Photic ☐ Abyssal

Biogeographic Realm

☑ Afrotropical

☐ Antarctic

☐ Australasian

☐ Neotropical

☐ Oceanian

☐ Palearctic

☐ Indomalayan

☐ Nearctic

Population

GIS analysis show that L. edule has five subpopulations (Rivers et al. 2010). Genetically, L. edule contains levels of genetic diversity that are low compared to the average for Delonix s.l. (Rivers in prep.). The variation is not distributed in

Total Population Size

Minimum Population Size: Maximum Population

Habitat and Ecology

L. edule is a shrub reaching less than 3m. It is found within the spiny forest and coastal bushland with Allaudia and succulent species of Euphorbia on limestone or sand (Du Puy et al. 1995); (Du Puy et al. 2002). It is thought to be pollinated by moths due to its night opening flowers, white petals with long dark stamens and an upper petal with a narrow tubular nectariferous claw (Du Puy et al. 2002).

System

☑ Terrestrial ☐ Freshwater ☐ Marine

Movement pattern

☐ Congregatory ☐ Migratory

Crop Wild Relative

☐ Is the species a wild relative of a crop?

Growth From	Definition
Shrub - large	Perennial shrub (>1m), also termed a Phanerophyte (>1m)

Threats

The species has a restricted and fragmented distribution and is threatened by intensifying grazing especially in the main population site around Itampolo (Du Puy et al. 2002). Major threats to the habitat of L. edule are the widespread exploitation for firewood and charcoal production. Selective logging, increased cultivation and grazing of livestock are also leading to further degradation of the habitat (Moat & Smith 2007; World Wildlife Fund (WWF) 2001). The rate of degradation has been exacerbated in recent years and the naturally slow growth and regeneration of the spiny forest is putting the species endemic to the area at particular risk (WWF 2001). The spiny forest is one of the primary vegetation types that is declining significantly in Madagascar with an estimated rate of loss of 1.2% per year (Harper et al. 2007; MEFT et al. 2009). Climate change modelling predicted a 4% loss of current climatically suitable range by 2100 (Rivers in prep.).

	Past	Present	Future
1 Habitat Loss/Degradation (human induced)	☐	☑	☐
1.1 Agriculture	☐	☑	☐
1.1.1 Crops	☐	☑	☐
1.1.4 Livestock	☐	☑	☐
1.3 Extraction	☐	☑	☐
1.3.3 Wood	☐	☑	☐
1.3.3.1 Small-scale subsistence	☐	☑	☐
1.7 Fires	☐	☑	☐
10 Human disturbance	☐	☑	☐
10.5 Fire	☐	☑	☐

Conservation Measures

Lemuropisum edule is found in a protected area (Tsimanampetsotsa – all these collections were made before 1965). Seed collections have been made and are held by the Millennium Seed Bank (MSB, Wakehurst Place, UK) as well as in-country by Silo National des Graines Forestières (SNGF). The area around Itampolo is protected through a community-based initiative.

	In Place	Needed
4 Habitat and site-based actions	☑	☐
4.4 Protected areas	☑	☐
4.5 Community-based initiatives	☑	☐
5 Species-based actions	☑	☐
5.7 Ex situ conservation actions	☑	☐
5.7.2 Genome resource bank	☑	☐

Countries of Occurrence

	Native Presence Confirmed	Native - Presence Possible	Extinct	Possibly Extinct	Re-introduced	Possibly Reintroduced	Introduced	Possibly Introduced	Vagrant	Possibly Vagrant
Madagascar	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐

General Habitats	Score	Description
3 Shrubland	1	Suitable
3.5 Shrubland - Subtropical/Tropical Dry	1	Suitable

Ecosystem Services

☑ Insufficient Information available ☐ Species provides no ecosystem services

Species Utilisation

☐ Species is not utilised at all

Purpose / Type of Use	Subsistence	National	International
1. Food - human	☑	☐	☐
7. Fuel	☑	☐	☐

The seeds are also known to be eaten raw locally. They are not cultivated or sold locally but an investigation into its possible commercial use has been carried out in Western Australia (Bosch 2004). Also a study into possible toxic compound found in the seeds have also been made (Kite et al. 1995).

Trend in the level of wild offtake/harvest in relation to total wild population numbers over the last five years:

Trend in the amount of offtake/harvest produced through domestication/cultivation over the last five years:

CITES status: Not listed

Livelihood Value

☑ There is no information for this species regarding its livelihood

IUCN Red Listing

Red List Assessment: (using 2001 IUCN system) Endangered (EN)

Red List Criteria: B1ab(iii,iv,v)
Date Last Seen (only for EX, EW or Possibly EX species):
Is the species Possibly Extinct? ☐ Possibly Extinct Candidate? ☐

Rationale for the Red List Assessment

Lemuropisum edule is listed as Endangered since its EOO is less than 5,000km2; its habitat is severely fragmented and the species exists at fewer than five locations; and its native habitat, the spiny forest, continues to decline in quality and extent, due to the effect of habitat destruction and population expansion. There is no precise information to assess the population size and trends of the species. However, its native habitat is fragmented and degraded, and continues to decrease in quality and extent; these trends should be monitored to determine whether the population of this species is declining.

[Criteria used for assessment A, B and D2]

Reason(s) for Change in Red List Category from the Previous Assessment:

- ☐ Genuine Change
  - ☐ Genuine (recent)
  - ☐ Genuine (since first assessment)
- ☐ Nongenuine Change
  - ☐ New information
  - ☐ Knowledge of Criteria
  - ☐ Incorrect data used previously
  - ☐ Taxonomy
  - ☐ Criteria
  - ☐ Other
- ☑ No Change
  - ☐ Same category and criteria
  - ☑ Same category but change in criteria

Current Population Trend:
Date of Assessment: 13/1/2011
Name(s) of the Assessor(s): Malin Rivers
Evaluator(s):
Notes:

Currently listed as EN (B1+2ce) version 2.3. There is no change in category but the assessment is updated due to criteria revision.

Criterion A				Criterion B					Criterion C		Criterion D
A1a ☐	A1b ☐	A1c ☐	A1d ☐	B1a ☑					C1 ☐		D ☐
A2a ☐	A2b ☐	A2c ☐	A2d ☐	B1b(i) ☐	B1b(ii) ☐	B1b(iii) ☑	B1b(iv) ☑	B1b(v) ☑	C2a(i) ☐	C2a(i) ☐	D1 ☐
	A3b ☐	A3c ☐	A3d ☐	B1c(i) ☐	B1c(ii) ☐	B1c(iii) ☐	B1c(iv) ☐		C2b ☐		D2 ☐
A4a ☐	A4b ☐	A4c ☐	A4d ☐	B2a ☐							
				B2b(i) ☐	B2b(ii) ☐	B2b(iii) ☐	B2b(iv) ☐	B2b(v) ☐	Criterion E		
				B2c(i) ☐	B2c(ii) ☐	B2c(iii) ☐	B2c(iv) ☐		E ☐		

Generation Length:
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Time period over which the past decline has been measured for applying Criterion A or C1 (in years or generations):
% population decline in the future:
Time period over which the future decline has been measured for applying Criterion A or C1 (in years or generations):
Number of Locations: <5
Severely fragmented ☑
Number of Mature Individuals:

Bibliography

Bosch C.H., 2004, Lemuropisum edule, Plant Resources of Tropical Africa 2 - Vegetables, Grubben G.J.H. & Denton O.A., 364-365, Prota Foundation, Wageningen

Du Puy D.J., J.-N. Labat, R. Rabevohitra, J.-F. Villiers, J. Bosser, J. Moat, 2002, The Leguminosae of Madagascar, , Royal Botanic Gardens, Kew, Kew

Harper G.J., Steininger M.K., Tucker C.J., Juhn D. & Hawkins F., 2007, Fifty years of deforestation and forest fragmentation in Madagascar, Environmental Conservation, 325-333, ,

Kite G.C., Plant A.C., Burke A. et a., 1995, Accumulation of trans-3-hydroxy-L-proline by seeds and leaves of the edible Madagascan legume Lemuropisum edule, Kew Bulletin, 585-590, ,

MEFT, UNEP, and CI., 2009, Evolution de la couverture de forêts naturelles a Madagascar, 1990-2000-2005., , ,

Moat J. & Smith P., 2007, Atlas of the Vegetation of Madagascar, , Royal Botanic Gardens, Kew, UK

Rivers M.C., Bachman S., Meagher T.R., Nic Lughadha E. and Brummitt N.A., 2010, Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data, Biodiversity and Conservation, 2071-2085, ,

World Wildlife Fund (WWF), 2001, Terrestrial Ecoregions - Madagascar spiny thickets (AT1311)19 Sept, , WWF,