Endemic Plants of St. Helena: Potential for Rescue, Inherent Variation within Species, and the Future of Babies' Toes and the Lowland Desert Habitat

https://sthelenaisland.info/flora/scrubwood/

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Abstract

St. Helena is a remote volcanic island in the South Atlantic Ocean with a diverse topology and a rich endemic flora. Due to anthropogenic factors, many plant species have been reduced to small, severely fragmented populations, resulting in genetic erosion and a more limited ability to adapt to environmental pressures. This thesis addresses conservation of St. Helena's endemic flora relating to themes of genetic variation and climate change. Chapter one introduces the island and its flora. Chapter two reviews the literature on genetic rescue in plants and shows that it can be beneficial for small, fragmented populations, though additional research is needed. Chapter three conceptually applies the genetic rescue approach to St. Helena's critically endangered endemic plants, many of which are promising candidates for the strategy and are predicted to be heading rapidly towards extinction. Chapter four assesses historical levels of variation through morphometric analysis using herbarium specimens of three endemic St. Helena genera: *Carex* L., *Phylica* L., and *Wahlenbergia* Schrad. ex Roth; proposes areas for further research and conservation, and addresses taxonomic issues for *Carex*. Chapter five discusses potential impacts from climate change and increased tourism in St. Helena's lowland desert habitat and proposes conservation actions and future research directions. Chapter six provides a brief conclusion to the thesis, which will contribute valuable insights for informing conservation decisions on St. Helena.

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Chapter 1: Introduction and Aims

Oceanic islands have fascinated scientists for centuries due to their remote locations, interesting flora and fauna, and high numbers of endemic species. Islands serve as "natural laboratories", where species evolved in isolation, and often with different functional characteristics than related continental species (Whittaker et al., 2017). The high level of endemism on islands is at risk as the planet is in the midst of an extinction crisis which could see irreversible losses in biodiversity (Ceballos et al., 2015, Pimm et al., 2014). Species on islands face a higher risk of extinction compared to those on the continents, and the impact from climate change is expected to put many of these species even more at risk (Gray, 2018, Harter et al., 2015, Humphreys et al., 2019). The high levels of unique and threatened endemic species coupled with their interesting evolution in an isolated system, highlight the importance of research focus and conservation efforts on oceanic islands.

The Island of St. Helena

St. Helena is a small and remote volcanic island in the South Atlantic Ocean (latitude 15° 56' S, longitude 5° 43' W) (**Figure 1.1**). It is 1,931 km west of continental Africa and 1,127 km northwest of the next closest island, Ascension Island. St. Helena was formed in the Miocene, approximately 14.5 million years ago, with the last volcanic activity ceasing approximately six million years ago (Cronk, 2000). Subsequent volcanic eruptions on the island were localized, thus enabling a continuity of life after initial colonization (Cronk, 2000). Despite its small size of 121.7 km^2, St. Helena's topology is considerably varied due to is volcanic origins, and is characterized by steep cliffs, deep valleys locally known as guts, and a central ridge containing Diana's Peak, the highest point on the island (826 m) (**Figure 1.2**).

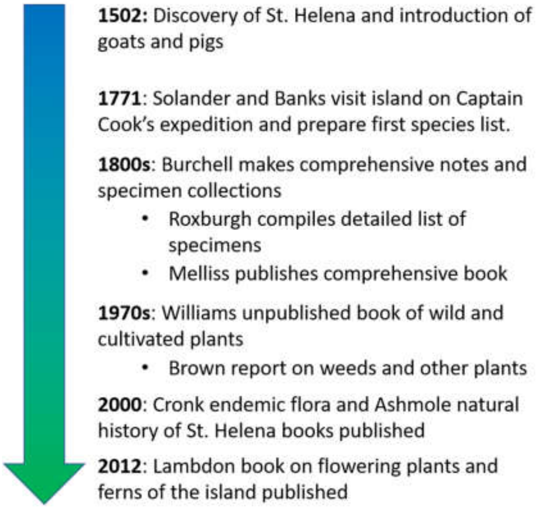
Figure 1.1 Map showing the location of St. Helena, 1.931 km west of the African coast. Image credit: TUBS

Due to its position in the South Atlantic and the island's topological features, St. Helena experiences several microclimates. The weather on the island is controlled by the South Atlantic Anticyclone and influenced by the Benguela upwelling system and the south-east trade winds (Feistel et al., 2003). These factors give St. Helena a more temperate climate compared to other locations at this latitude, with an average temperature ranging between 20°C and 24°C at the main coastal settlement of Jamestown, and cooler temperatures at higher altitudes, averaging 16°C at an elevation of 600 m (Cronk, 2000). Precipitation is also varied with altitude and topography, with the mist-shrouded peaks at the highest elevation receiving the most rainfall, and the arid coast receiving the least precipitation.

Figure 1.2. Satellite view of St. Helena showing select locations mentioned in this study as well as the island's rugged topography, green peaks, and barren coast, generated using Google Earth.

Flora of St. Helena

Despite discovery of the uninhabited island in 1502, detailed historical records of St. Helena's flora are lacking, with few early accounts describing the endemic plants in detail (**Figure 1.3**) (Cronk, 2000, Lambdon, 2012). Daniel Solander compiled a comprehensive list of the island's species in 1771, more than 250 years after St. Helena's discovery and the introduction of goats and pigs, logging, and clearing of the land. This leaves the island's full extent of endemic species prior to the large-scale destruction of their habitat up to speculation. Additional records of the flora were not produced until the 1800s, and the most recent comprehensive accounts were published within the last 20 years by Cronk (2000) and Lambdon (2012).



1502: Discovery of St. Helena and introduction of goats and pigs

1771: Solander and Banks visit island on Captain Cook's expedition and prepare first species list.

1800s: Burchell makes comprehensive notes and specimen collections
- Roxburgh compiles detailed list of specimens
- Melliss publishes comprehensive book

1970s: Williams unpublished book of wild and cultivated plants
- Brown report on weeds and other plants

2000: Cronk endemic flora and Ashmole natural history of St. Helena books published

2012: Lambdon book on flowering plants and ferns of the island published

Figure 1.3. Timeline of key botanical records for St. Helena. Adapted from Lambdon, 2012.

The major habitats on St. Helena today include shrubland, desert, and a small amount of remaining forest concentrated on the central ridge (**Figure 1.4**). The lush cloud forest on the peaks contrasts with the arid, severely eroded, and barren land bordering the desert at lower altitudes. The uplands are covered in plantations of New Zealand flax (*Phormium tenax* Forst.), a crop no longer of economic value but that takes precious space away from endemic plants (Cronk, 1989). Before the arrival of humans, St. Helena had been thickly forested with species of the endemic *Commidendrum* Burch. ex DC. genus, a unique genus of woody Asteraceae with four extant species on the island, now mostly present only in sparse numbers. **Figure 1.5** illustrates the predicted changes of vegetation zones from the initially untouched island to the modern day, and encapsulates the subsequent degradation, erosion, and shift toward non-native and invasive species (Cronk, 2000). These transitions are more complex than simply moving from one type of vegetation to another, and as Cronk (1989) puts it, St. Helena's natural vegetation has been replaced by a "complicated mosaic resulting from the island's historical ecology".

Today there 46 endemic plant species on St. Helena, which support a host of lichens and endemic invertebrates on the island. Of the extant endemic plants, there are 26 eudicots, 15 ferns, and five monocots, and all but eight are vulnerable, endangered, or critically endangered according to the International Union for Conservation of Nature (IUCN) Red List criteria (Lambdon and Cronk, 2020). Four species are estimated to have less than ten mature individuals surviving in the wild (**Figure 1.6**). Many of the endemic plant and invertebrate species reside within the island's last remaining vestiges of cloud forest and tree fern thicket, which represents only 0.3% of the total land mass (Lambdon, 2012). These forests are now limited to two areas on the island, at Diana’s Peak and High Peak (**Figure 1.2**). The pressures of grazing, logging, and clearing have resulted in pushing many of the endemic plants to inaccessible areas such as steep cliffsides and mountain tops.

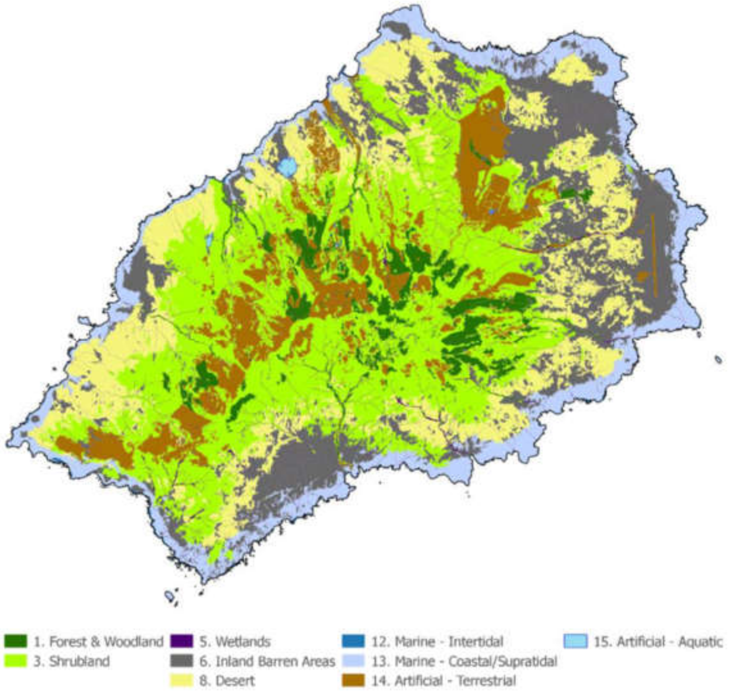


Figure 1.4. Map of habitats on St. Helena according to IUCN level one classification. Image credit: reproduced from Pike et al., 2018.

Extinction of and Threats to St. Helena’s Flora

As with many of the world’s island plants, St. Helena’s unique endemic flora is gravely threatened, and several species have already become extinct. Since the first species list for the island produced in 1771, eight endemic plants have been met with extinction (Lambdon and Cronk, 2020). Between the discovery of the island in 1502 and 1771, an estimated ten plants that have never been recorded also likely faced a so-called “dark extinction”, and four plants are predicted to face extinction within the next 50 years (Lambdon and Cronk, 2020). The most recent loss was *Nesiota elliptica* (Roxb.) Hook.f., the St. Helena Olive, when both the last remaining plant in the wild died in 1994, and the last cultivated specimen died in 2003 (**Figure 1.7**).

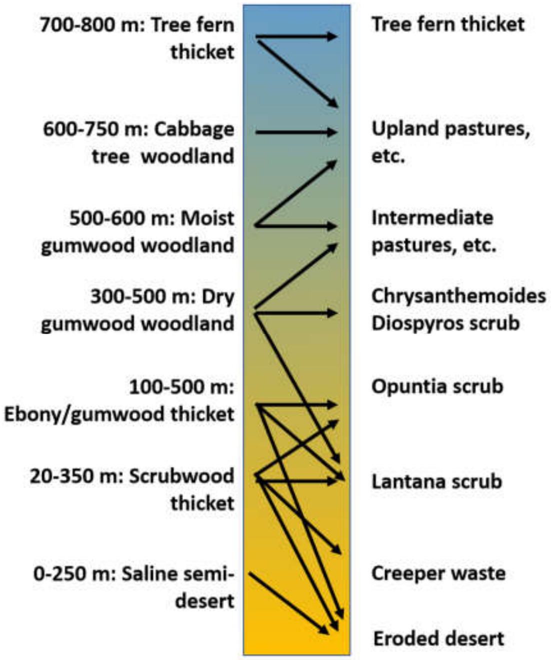


Figure 1.5. Transitions of past vegetation zones to the current state due to grazing, erosion, cutting, plant invasions, and clearance. Redrawn and adapted from Figure 9 of Cronk, 2000.

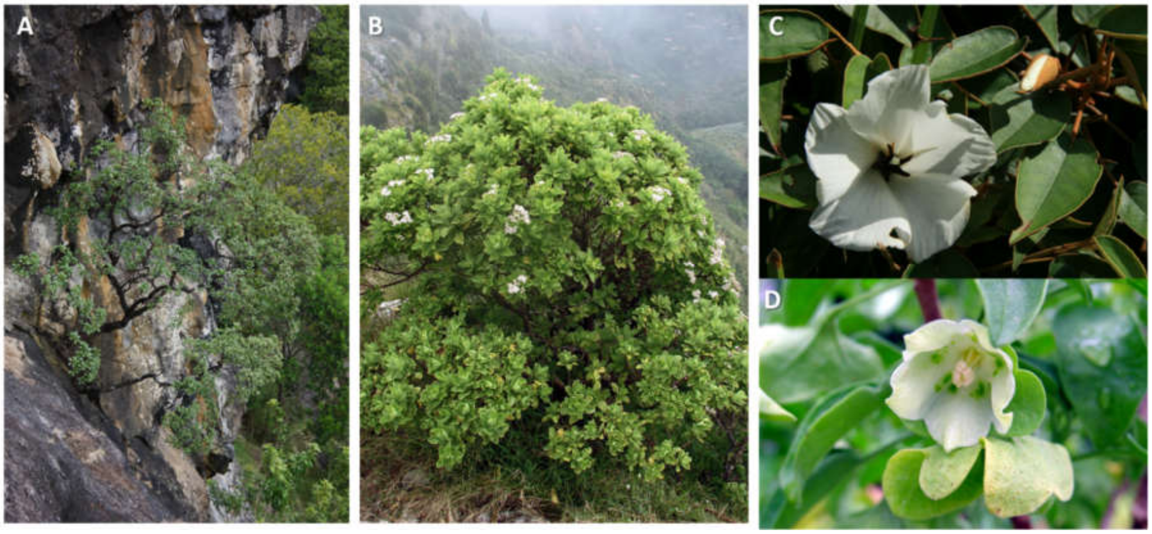


Figure 1.6. Endemic St. Helena species with less than ten mature individuals in the wild: A) *Commidendrum rotundifolium* (Roxb.) DC., Bastard Gumwood, one individual B) *Commidendrum spurium* DC., False Gumwood, six individuals, C) *Trochetiopsis ebenus* Cronk, Dwarf Ebony, five individuals, D) *Withania begoniifolia* (Roxb.) Hunz. & Barboza, St. Helena Boxwood, two individuals. Image credits: A) © P. Lambdon, 2009, B) © V.E. Thomas, 2011), C) © P. Lambdon, 2008, D) © Rebecca Cairns-Wicks.

Figure 1.7. The recently extinct St. Helena Olive, *Nesiota elliptica*. Image credits: Drawing, Melliss, 1875, photo, © Rebecca Cairns-Wicks.

The discovery of the island in 1502, and its use as a refueling station for ships and their crew brought logging, farming, and the arrival of grazing animals and foreign or invasive species, resulting in devastating habitat destruction and soil erosion. The introduction of goats on the island was responsible for massive amounts of plant and habitat loss as well as exacerbating erosion, lending to the description of these animals as "scourges of green crops, these horned and four-legged locusts" (Gosse, 1938). Goats were replaced by non-native and invasive plants as the prime threat to the native flora, remaining a major issue today. Melliss (1875) describes with dismay the large-scale effort by Governor Major-General Beatson to introduce many foreign plants to the island. He lamented that these plants did so well that the endemic species could not compete with them (Melliss, 1875). Non-native and invasive species account for an estimated 99% of the biomass on St. Helena, and continue to represent one of the biggest threats to the endemic vegetation on the island (Lambdon, 2012). Some of the problematic invasive species include *Lantana camara* L., *Carpobrotus edulis* (L.) N.E.Br., *Cenchrus setaceus* (Forssk.) Morrone, and *Fuchsia boliviana* Carrière. All four of these species are of global concern and are listed on the Global Invasive Species Database (Global Invasive Species Database, 2020).

In addition to loss of species or habitat, Cronk (2000) describes the genetic erosion of the endemic flora, including inbreeding, selective elimination of biotypes both within and between populations, and hybridization between species. All of these issues are introduced and exacerbated by the destruction of the native habitat, and they result in diminished diversity and resilience of the endemic plants (Cronk, 2000). Currently, many species populations are fragmented on the island, increasing inbreeding and reducing genetic variation, thus rendering these plants more susceptible to environmental perturbances.

Conservation Efforts on St. Helena

Conservation on St. Helena is essential to protect its rich heritage of biodiversity. The earliest account of conservation efforts on the island go back as far as the late 1600s to protect St. Helena's source of timber (Cronk, 2000). By 1700, St. Helena already experienced major erosion and much of the island was largely deforested. Its importance as a stop for provisions for the East India Company prompted early conservation actions, such as removal of goats and afforestation attempts to preserve the island's natural resources (Grove, 1993). Today there are several conservation bodies or those dedicated to scientific research and sustainability on St. Helena, including the St. Helena National Trust, UK Overseas Territories Conservation Forum, the recently established St. Helena Research Institute, and biodiversity projects funded by multiple Darwin Awards from the UK government. In an effort to partly restore the Great Wood that covered much of the eastern part of the island, the Millennium Forest was launched in 2000, with the planting of thousands of *Commidendrum robustum* (Roxb.) DC. trees and active engagement from the local community (Thompson, 2008). St. Helena also has 23 National Conservation Areas, including three National Parks, six Nature Reserves, and five Important Wirebird Areas, all of which are managed by the Environmental Management Division of St. Helena's Government (**Figure 1.8**). The remaining nine are Historic Conservation Areas. Although these protected areas cover various parts of the island, they are also disconnected and leave important gaps in species continuity.

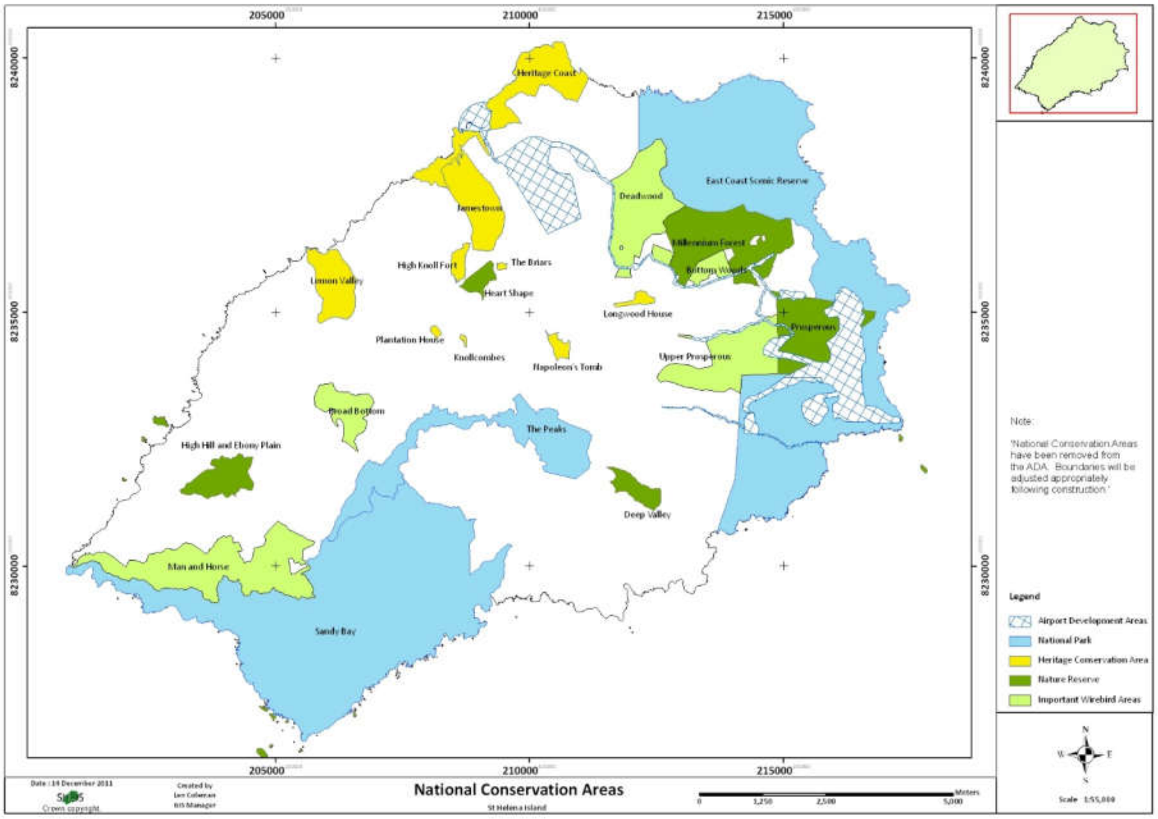


Figure 1.8: Map showing the National Conservation Areas of St. Helena. Image downloaded from https://www.sainthelena.gov.sh/wp-content/uploads/2013/10/NCAs-map.jpg.

Thesis Aims

The overarching theme of this thesis is conservation of St. Helena's endemic flora, as approached from the angles of variation within populations and environmental challenges such as climate change. The importance of variation and the ability to adapt to changing environmental pressures tie together the three aims:

Aim 1: Review the available evidence on genetic rescue in plants and assess the potential for using this strategy to restore genetic variation in St. Helena's critically threatened endemic flora (Chapters 2 and 3).

Aim 2: Assess and quantify inherent morphological variation within historical endemic plant specimens and develop character sets for future morphological study of these species (Chapter 4).

Aim 3: Assess the potential impact of climate change and increased tourism on *Hydrodea cryptantha* and other species in St. Helena's lowland arid habitats, and propose relevant conservation actions to address these issues and areas for further research (Chapter 5).

Chapter 2: Genetic Rescue as a Conservation Strategy

Introduction

The world faces a sixth mass extinction, and a crisis that will result in major losses of global biodiversity (Ceballos et al., 2015, Pimm et al., 2014). Extinction rates are 1000 times higher than the predicted background rate, and islands face an even higher extinction rate compared to continents (Pimm et al., 2014, Gray, 2018). Plants frequently experience a substantial lag in extinction due to their long generation times and presence of seedbanks in the soil, providing a potential opportunity to rescue them from completely disappearing (Cronk, 2016). Small populations are especially at risk for becoming extinct. They are particularly vulnerable to stochastic factors, such as a single landslide or hurricane that can eliminate an entire population, as well as negative impacts from genetic drift. Small populations may also more readily form hybrids with closely related species due to the lack of mate availability. Additionally, small, fragmented populations often have high levels of inbreeding, and thus suffer from inbreeding depression (Hedrick and Kalinowski, 2000). Inbreeding depression can occur when, due to lack of genetic diversity, recessive detrimental alleles become homozygous and therefore fully expressed (Hedrick and Garcia-Dorado, 2016). Inbreeding depression tends to be higher in more stressful environments (Hedrick and Garcia-Dorado, 2016). Various factors play a role in whether or not a population experiences inbreeding depression, such as the evolutionary history of the species and the particular mating system. In selfing plants, deleterious alleles may be purged from the population, and therefore no longer problematic. This occurs when recessive deleterious mutations are exposed in the homozygous state and selected out of the population (Arunkumar et al., 2015). For example, researchers found no evidence for inbreeding depression in small, isolated populations of a primarily self-fertilizing herb, *Geum urbanum* L., and outcrossing did not offer any fitness advantages (Vandepitte et al., 2010). Although *G. urbanum* is known to produce hybrids with *G. rivale* L., this was not addressed in the study.

The aim of this chapter is to review the available evidence on genetic rescue in plants. The following chapter (Chapter 3) applies this knowledge to the potential use of genetic rescue on St. Helena.

Genetic Rescue: One Potential Solution

One tool to combat the extinction crisis and tackle the issue of poor genetic diversity in small, isolated populations is genetic rescue. The concept of “rescue” was coined in the 1970’s to describe the effect of higher immigration on reducing extinction and turnover rates in island populations (Brown and Kodric-Brown, 1977). Several types of “rescue” have since been described, including genetic, demographic, and evolutionary. Demographic rescue refers to the addition of immigrants to increase the size of a population, while evolutionary rescue relates to the adaptation of a population to environmental changes (Bell et al., 2019, Hufbauer et al., 2015). Broadly speaking, genetic rescue involves the introduction of gene flow into small, inbred populations to recover the fitness of the target population (Frankham, 2015, Ingvarsson, 2001, Tallmon et al., 2004, Whiteley et al., 2015). The introduction of new gene flow into a population often results in heterosis, or hybrid vigor, in which the hybrid offspring are fitter than the inbred parents and deleterious alleles are masked (Whiteley et al., 2015, Hedrick and Fredrickson, 2009). Adaptive evolution may also rescue populations by selection of the best phenotype for the current environment (Whiteley et al., 2015). All three types of rescue can overlap and occur naturally through migration of individuals, or through human-mediated conservation efforts. Experiments in invertebrates have suggested that a combination of demographic and genetic rescue provides the strongest countermeasure against extinction (Hufbauer et al., 2015).

Genetic rescue has been defined and measured in various ways, and authors have continued to refine their definitions over time (Bell et al., 2019, Hedrick et al., 2011, Tallmon et al., 2004, Richards, 2000, Frankham, 2015). Some authors focus on population fitness, while others stress the population growth component (Bell et al., 2019, Frankham, 2015). Tallmon et al., (2004) defined genetic rescue as “when population fitness, inferred from some demographic vital rate or phenotypic trait, increases by more than can be attributed to the demographic contribution of immigrants”. Hedrick et al., (2011) expanded on this definition by adding a corollary, “or when the environmental conditions are thought to be limiting or deteriorating and genetic analysis of ancestry demonstrates a contribution of recent immigration significantly greater than expected by chance from genetic drift”, to bring the genetic component to the foreground. Frankham’s definition centers on fitness: “improvement in reproductive fitness and increase in genetic diversity due to crossing a population previously suffering from inbreeding and low genetic diversity to another distinct population” (Frankham, 2015, Frankham et al., 2017). The most recently published definition of genetic rescue is “a decrease in population extinction probability owing to gene flow, best measured as an increase in population growth rate” (Bell et al., 2019). This interpretation expands on the authors’ previous definition to account for the observation that populations cannot grow if their environment does not support their expansion, and to allow for other outcomes besides population growth rate to be measured (Bell et al., 2019). The authors proposed a hierarchy of evidence for evaluating genetic rescue success, ranging from an increase in heterozygosity (weak evidence), to an increase in population growth rate due to gene flow (strong evidence) (**Figure 2.1**) (Bell et al., 2019).

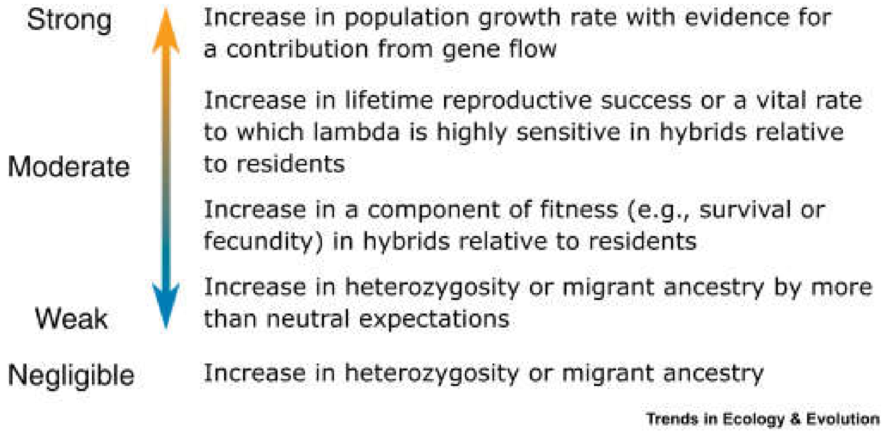


Figure 2.1. The relative strength of different types of evidence for genetic rescue. Few if any studies meet the criteria for strong evidence. Reproduced from Bell et al., 2019.

The format for how to measure genetic rescue varies, but the primary concept that populations are “saved” due to the influx of gene flow is consistent. Since the aim of genetic rescue is to prevent extinction, population growth is an important component of measuring its success. Although the idea of genetic rescue has gained traction in the research field, it has not been implemented many times in the real world for conservation efforts. Many researchers have called for its increased use as well as for additional research to further understand the underlying genetic mechanisms and long-term fitness and population outcomes of the strategy (Bell et al., 2019, Frankham, 2015, Hedrick and Fredrickson, 2009, Ralls et al., 2018, Tallmon, 2017, Whiteley et al., 2015). Recently, an entire book has been dedicated to the topic, with an aim towards promoting uptake of genetic rescue and helping conservation practitioners apply it (Frankham et al., 2017).

What Can Go Wrong? Potential Issues and Shortcomings of Genetic Rescue

Although genetic rescue has the potential to save vulnerable populations from potential extinction by introducing beneficial gene flow, there are potential risks associated with it. Outbreeding depression is the most prominent concern, resulting in hybrid offspring having lower fitness. It is believed to occur when there are genetic incompatibilities between loci or maladaptation (Frankham et al., 2011). Effects of outbreeding depression may not be evident until later generations, F2 and beyond (Edmands, 2007). Introducing too much gene flow could also result in genetic swamping, where the unique alleles of the target population are diluted out by large numbers of the introduced migrants. Ensuring that the introduced population is adapted to a similar environment can help to avoid this issue, as can aiming to introduce the minimum amount of gene flow required to rescue the target population (Frankham et al., 2017). A recent pre-print modeling study suggested that introducing migrants from large populations into small, inbred populations could raise the extinction risk for the small population rather than mitigate it (Kyriazis et al., 2019). However, introductions from other small populations were shown to decrease the extinction risk. This is explained by the presence of deleterious alleles in large populations that have been purged in small populations (donor or recipient) (Kyriazis et al., 2019). Note that this paper has not been peer-reviewed, and results should be interpreted with caution, especially since ecological systems are far more complex than can be accounted for in mathematical models alone.

Beyond providing genetic diversity, the success of genetic rescue depends on the continued availability of a suitable habitat for the population to expand. Although gene flow may be restored, if the carrying capacity of the local environment is at its limit, the population will be unable to grow (Bell et al., 2019). This has led several authors to describe genetic rescue as a way to buy time for the species, preventing immediate extinction while more suitable habitat can be secured (Hedrick and Fredrickson, 2009, Gewin, 2017). Similarly, if the environment changes rapidly due to human-mediated causes or climate change, the species must be able to adapt to the new conditions to avoid extinction.

The Role of Genomics

As next-generation sequencing techniques become more accessible, quicker, and less expensive, the role of genomics is increasingly important in all of biology, including conservation. Genetics has been useful in conservation research for many years, however, genomics allows for the analysis of a much greater magnitude of data and yields more precise insights into populations (Supple and Shapiro, 2018). Genomics may be useful both at the beginning stages for identifying suitable candidates for genetic rescue and the most appropriate source for introducing gene flow, as well as for evaluating the outcomes in genetic rescue trials (Whiteley et al., 2015). Deciding whether to use a genetic rescue approach includes assessing the level of inbreeding and its impact on the fitness of the population, and genomics may help to answer some questions about the underlying genomic mechanism of inbreeding depression and outbreeding depression (Kardos et al., 2016). It may also provide insight into important alleles for local adaptations or pressures that the population might be facing, such as pathogens, thus allowing selection from source populations that provide the best influx of new alleles (Harrisson et al., 2014, Shafer et al., 2015). Temporal genomics can determine the level of genetic erosion that a population has experienced when compared to historical specimens, which can have conservation implications (Díez-Del-Molino et al., 2018). For example, observing a substantial decrease in genetic diversity in current populations compared to historical specimens might warrant reclassification of the species on the IUCN Red List to a more threatened status (Díez-Del-Molino et al., 2018).

Flanagan et al., (2018) have developed guidelines for incorporating next generation sequencing into conservation efforts, focusing on local adaptation. They provide structure for researchers designing studies to address adaptive variation using genomics, and their guidance covers all stages of conservation efforts, from planning to monitoring for success, including considerations for genetic rescue (Flanagan et al., 2018). Although the use of genomics in conservation efforts can be valuable, it may be limited due to cost and the lack of available reference genomes (Supple and Shapiro, 2018). Since plants have especially large and complex genomes, further complicated by polyploidy in some instances, these issues may be particularly problematic in plants. A recent article discussed the use of genomics for conservation, but did not mention a single plant study in the midst of many examples of animal studies (Pennisi, 2019).

Genetic Rescue Trials in Conservation

There are very few published genetic rescue attempts to save endangered species in the wild (Bell et al., 2019, Frankham et al., 2017, Whiteley et al., 2015). Most studies touch on aspects of genetic rescue in controlled settings, which could inform potential application of this strategy in the future; and many of the studies are in animals. In addition to studies on model systems such as *Drosophila*, reports range from other invertebrates, fish, and birds, to various mammals (Akesson et al., 2016, Bijlsma et al., 2010, Heber et al., 2013, Miller et al., 2020, Quinn et al., 2019, Robinson et al., 2017). One of the most iconic examples of successful genetic rescue is the Florida Panther (*Puma concolor coryi* [Bangs 1899]) (Pimm et al., 2006). To save the dwindling, inbred population of panthers in Florida, individuals from Texas were introduced into the population, resulting in successful hybrids that have renewed the population.

For plants, many studies set the stage for genetic rescue in the wild, although they stop short of actually conducting a rescue attempt. For example, several studies have shown that outcrosses in garden trials or controlled pollinations in the wild are successful, though few attempts at rescuing natural populations in situ have been published (Bossuyt, 2007, Finger et al., 2011, Marsden et al., 2013, Pickup et al., 2013, Willi et al., 2007). One successful example was in the Illinois Lakeside Daisy (*Hymenoxys acaulis* var. *glabra* [A. Gray] K.F. Parker), which was effectively extinct and had not produced seeds for 15 years (**Figure 2.2**) (Demauro, 1993). Crossing with plants from an Ohio population resulted in viable offspring that were fertile, and subsequently implemented into a recovery program to save the Illinois population (Demauro, 1993). The Australian Plains Yam (*Microseris scapigera* Sch.Bip.) was also the target for genetic rescue, since it exists in small, fragmented populations (**Figure 2.2**) (Bainbridge, 2017). Plants were translocated from different locations and survival of the seeds was assessed, however, no other measures of fitness and genetic information have yet been reported (Bainbridge, 2017).

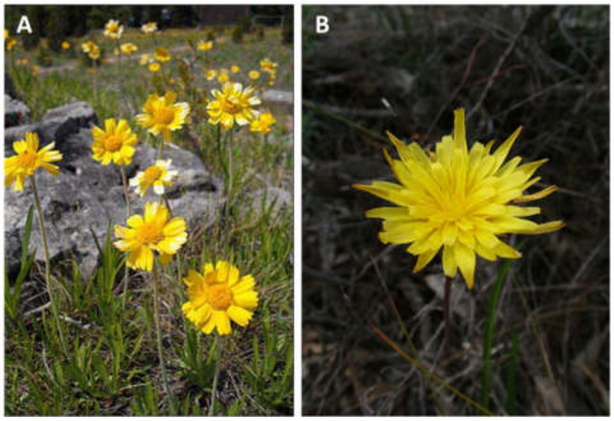


Figure 2.2. Two plants for which genetic rescue has been used for conservation purposes in the wild: A) Illinois Lakeside Daisy and B) Australian Plains Yam. Image credits: A) United States Fish and Wildlife Service, B) Mike Bayly CC BY-SA 3.0.

Review of the Evidence for Genetic Rescue with an Emphasis on Plant Studies

Genetic Rescue Reviews

There have been at least 20 published reviews discussing genetic rescue since the concept was first introduced, in addition to opinion pieces, policy papers, and a recent book on the subject (Frankham, 2015, Gewin, 2017, Hedrick and Garcia-Dorado, 2016, Ingvarsson, 2001, Ralls et al., 2018, Tallmon, 2017, Tallmon et al., 2004, Whiteley et al., 2015, Bell et al., 2019, Frankham et al., 2017). The following discussion focuses on reviews within the past ten years to look at the most recent analyses that took a quantitative approach and made data on their included studies available. The evidence specifically relating to plants has been extracted from the overall datasets where possible and presented below. In general, the reviews found a beneficial effect for genetic rescue across taxa, as well as when considering plants separately. Although some of the studies in the analyses were for conservation purposes using threatened species, not all of them were directly relevant to conservation.

Whiteley et al., (2015) conducted a literature search to update an earlier review led by Tallmon et al. (2004). They surveyed the literature for genetic rescue studies from 2004 to 2014, and recorded relative and absolute fitness data as positive, negative, neutral, or a combination of effects for each of the resulting 138 studies (Whiteley et al., 2015). Most of the included studies only assessed F1 hybrids, which is an issue as it only illustrates the often-positive impacts of heterosis in this generation, and may mask potentially deleterious effects that would not show up until later generations. Of the 18 studies that addressed absolute fitness effects of outcrossing or immigration, 78% showed either positive or a mix of positive and neutral effects. The review included 65 plant studies. Fitness in 40 studies was measured in a lab setting, 22 in the wild, and three using a combination of settings. Measures of relative fitness included growth and reproductive measures, and 23 studies reported positive effects, seven reported negative, nine reported neutral, and 26 reported mixed effects based on different measures or generations (Whiteley et al., 2015). Only two studies reported absolute fitness measures in plants, one measuring population growth rate in *Jacquemontia reclinata* House and the other measuring population size and flowering in *Leucochrysum albicans* (A. Cunn.) (Maschinski et al., 2013, Morgan et al., 2013). Both studies showed a mix of positive and neutral results (Whiteley et al., 2015).

Frankham (2015) published a meta-analysis of the magnitude of effect of outcrossing on fitness for small, inbred populations of invertebrates, plants, and vertebrates. He defined successful genetic rescue as a net beneficial effect of a composite outcome consisting of fitness and reproductive success in populations with a low risk for outbreeding depression (Frankham et al., 2011). Genetic rescue effect size was measured as a ratio of the mean fitness for the outcrossed population divided by the mean fitness for the inbred population. In total, there were 145 beneficial, nine harmful, and two equivocal studies on the impact of gene flow across taxa (Frankham, 2015). The analysis included 49 studies on plants (44 species), all angiosperms, from a variety of families ranging from Poaceae to Asteraceae. The median genetic rescue ratio for plant data was 1.591, and the calculated genetic rescue ratios in plant studies ranged from 0.891 in *Echinacea angustifolia* D.C. to 154.569 in *Maianthemum canadense* Desf (**Figure 2.3**). *Ziziphus celata* Judd & D.W. Hall, the Florida ziziphus, had an infinite genetic rescue ratio, since the inbred population was self-incompatible and only able to reproduce when outcrossed (Frankham, 2015, Menges et al., 2016). The results from plants are consistent with the analyses from invertebrates (GR, 1.584) and vertebrates (GR, 1.942) (Frankham, 2015). Parsing the data according to stressful or benign conditions showed a substantial increase in the median increase in composite fitness for outcrossing across taxa (148% vs. 45%).

Figure 2.3. Plants included in the genetic rescue meta-analysis, spanning the effect size of genetic rescue ratios from a negative effect in *Echinacea angustifolia* (A) up to 155 in *Maianthemum canadense* (B). As a completely self-incompatible plant, *Ziziphus celata* experienced the greatest benefit with outcrossing and had an infinite genetic rescue ratio (Frankham, 2015). Image credits: A) Tony Fischer CC BY 2.0, B) R. Bowman, C) downloaded from https://extension.umaine.edu/blueberries/weed-images/herbaceous-broadleaf-weeds/white-flowers/canada-mayflower/

A follow-up meta-analysis of the dataset focused on persistence of genetic rescue in F2 and F3 generations and concluded that beneficial effects were the same in F1 through F3 generations (Frankham, 2016). However, much of the data in the analysis was derived from the model system, *Drosophila melanogaster* (Meigen, 1830) in laboratory experiments, and only three plant taxa were included in the F2 analysis (*Diodia teres* Walt., *Ranunculus reptans* L., and *Rutidosis leptorrhynchoides* F.Muell), and only one for the F3 (*Chamaecrista fasciculata* (Michx.) Greene) (Frankham, 2016, Willi et al., 2007, Fenster and Galloway, 2000a, Fenster and Galloway, 2000b, Hereford, 2009, Pickup and Young, 2008).

Derry et al., (2019) compared the effect of various conservation strategies, including genetic, demographic, and evolutionary rescue, on population fitness in the context of the adaptation/maladaptation continuum, and whether the strategies target the adaptive state or the adaptive process. Using a meta-analysis, they assessed the outcome of these strategies on fitness over the short and long term. The authors included studies that measured fitness, included a control or pre-intervention measures, had measurements from at least two different times post-intervention, and included sample size and variance metrics, resulting in 15 studies on 15 species (Derry et al., 2019). Only two studies were in plants, and only one study used genetic rescue, for *R. reptans*, (Derry et al., 2019, Willi et al., 2007). The overall mean effect size of relative or absolute fitness for genetic rescue for all of the studies was 0.341 immediately after the intervention, and 0.387 at the latest time point tested, showing a very modest increase and persistence in the beneficial effect (Derry et al., 2019).

Recent Studies in Plants

The end search date for the two most recent comprehensive literature reviews on genetic rescue, which made available their data on included individual studies, was 2014 (Frankham, 2015, Whiteley et al., 2015). I conducted a search from 2014 to May 2020 as an update to assess the most recent literature, specifically in plants by searching Web of Science (May 2020) and SCOPUS (March 2020) databases using keywords: “genetic rescue”, “outbreeding depression”, “inbreeding depression”, “assisted gene flow” AND “conservation”. Irrelevant journals, such as those pertaining to medicine or animal conservation, were filtered out of the Web of Science search manually and by using database filters as an initial broad filter. I also searched references in review articles and a book on genetic rescue (Frankham et al., 2017), as well as searching for references that cited key recent reviews. A search on Google Scholar (March 2020) using: intitle:"genetic rescue", yielded 209 results, all of which were identified in the other databases.

Combined, 1587 articles were imported into Endnote v.X9 for screening, many of which were in replicate from the several search strategies. Reviews or related articles were retained for further reading and reference mining. References were initially filtered by title, then by abstract, and finally to full text to select primary studies. Inclusion criteria were: publication date 2014 to present, studies in plant species, crosses of two or more different populations, and reporting of at least one measure of reproductive or survival fitness. Studies that only reported genetic diversity measures (e.g. using microsatellite data) without any measures of reproductive or survival fitness or population growth were excluded, as this level of evidence is considered weak (Bell et al., 2019). Filtering the studies resulted in a total of 15 studies for further analysis.

The results of the 15 included studies are summarized in **Table 2.1** and include 15 different species in 14 plant families. The majority of studies (12/15) assessed fitness only of F1 hybrids, while three studies included results of the F2 generation, and one study included the F3. No studies directly assessed the application of genetic rescue to conserve endangered species in the wild, though several studies conducted experimental crosses to potentially inform future conservation strategies. Nine out of 16 studies were on endangered or otherwise dwindling and rare species that have important conservation implications.

Following Whiteley’s approach, the overall outcome for the effect of outcrossing on fitness in each study is summarized as positive, negative, neutral, or mixed results in **Table 2.1** (Whiteley et al., 2015). Consistent with past evidence, overall, the majority of work shows that outbreeding has positive impacts on fitness (12 out of 16 studies with overall positive or mixed positive and neutral results, with six studies each). One study reported neutral or negative results and found evidence of outbreeding depression when crossing between populations of

Phlox hirsuta E.E.Nelson (Ruane et al., 2015). However, the population studied was large, and would not be a likely candidate for genetic rescue strategies, which target small, isolated populations.

Two studies included polyploid plants (Schmidt-Lebuhn et al., 2018, Volis et al., 2016). Polyploid species are expected to benefit as much or potentially less from genetic rescue compared to outbreeding diploid species (Frankham, 2015). A prime concern is mixing populations with different ploidy, which often results in sterile offspring. One study in the polyploid *Rutidosis lanata* A.E. Holland, found higher seed set for outcrossing in all but one population, and demonstrated the importance of mixing populations with the same number of genomes (Schmidt-Lebuhn et al., 2018). Another study in the allopolyploid, *Triticum dicoccoides* (Asch. & Graebn.) Schweinf., carried out analysis to the F3 generation and showed mixed results that varied according to populations (Volis et al., 2016). However, the peripheral population benefited from outcrossing, and showed heterosis in the F1 that did not decline through F3, suggesting no hybrid breakdown in this species.

Overall, the studies add to the literature base for the published reviews and support their conclusions that genetic rescue and outcrossing can have beneficial effects on small, isolated populations. However, there were no groundbreaking new studies including high-quality evidence, and there are still many unanswered questions (see section below). There remains caution of the potential risk for outbreeding depression in certain situations, although deleterious effects tended to happen in studies using larger populations. As with previously published studies, most only assessed fitness in F1 hybrids, so the long-term effects of genetic rescue in plants remain unknown, and this is an important limitation.

The Ideal Study

Despite several genetic rescue attempts and substantial research, there is not yet an ideal published study. Such a study would take a considerable amount of time and follow the plants over many generations, assessing not only fitness measures, but the population growth rate, and ideally include various species from disparate habitats. Substantial analyses would need to be done on the remnant population before any intervention, including genetic analyses and calculations of population genetics measures, including inbreeding depression, heterozygosity, private alleles, and others. Additionally, extensive study of the current traits of the remnant population would be needed if not already available, as well as monitoring if and how they change over time with the augmentation, since it is important not only to increase population numbers, but also to preserve the unique character of the original population. This can be monitored both phenotypically as well as genetically, to ensure that private alleles are maintained and that the genetic integrity of the original population is not entirely diluted.

Table 2.1: Plant studies addressing genetic rescue or the effect of outcrossing on fitness published from 2014 to May 2020.

Species	Family	Crosses	Setting	Gen	Conservation	Fitness measure	Fitness effect	Overall*	Ref
Rutidosis lanata	Asteraceae	Selfing and between population crosses	Hand pollinations in field	F1	Yes, rare endemic with fragmented population	Seed set	Higher seed set for crossing between populations (all but 1 population)	+	Schmidt-Lebuhn et al., 2018
Brassica nigra	Brassicaceae	Inbred, within population, between population outcrosses	Common garden trial with drought stress	F1	No	Plant height, number of inflorescences, aboveground biomass	Height: Outbred taller than within population crosses, both taller than inbred; similar height for outbred and within population, both taller than inbred under drought stress Biomass: reduced for between population crosses but not within population compared to normal watering Inflorescence: Higher number for outbred vs. within population, but not under drought stress	+/N	Prill et al., 2014
Sinocalycanthus chinensis	Calycanthaceae	Crosses between populations compared to open-pollinated	Controlled pollination in wild populations	F1	Yes, species has protected status	Seed number per fruit, seed weight, seed size, total germination rate, seedling emergence rate and biomass, seed nutrient content, seedling photosynthetic rate, and morphological characteristics	F1 progeny from crosses had higher fitness components and performance than open-pollinated plants. Crosses with one population were clearly superior to the other.	+	Li et al., 2020
Lobelia siphilitica	Campanulaceae	Within and between population crosses	Greenhouse	F1	No	Seeds per fruit, seed size, germination success, final aboveground biomass	Seed size was larger for between population crosses, but not for all pairs. Other outcomes were no different comparing within or between crosses.	N/+	Caruso et al., 2015
Dianthus guliae Janka	Caryophyllaceae	Intra- and inter-population crosses of small peripheral and	Greenhouse	F1, F2	Yes, rare endemic	F0: no. stems per plant, and the no. of flowers per stem F1: % seed germination,	Improved fitness for F1, F2 of outbred small population (plant size, survival); F1 fitness improved for outbred large population, but F2 fitness declined	+/-	Gargano et al., 2015

Species	Family	Crosses	Setting	Gen	Conservation	Fitness measure	Fitness effect	Overall*	Ref
		large central populations				cumulative survival, plant size F2: % seed germination, cumulative survival			
Schiedea kaalae	Caryophyllaceae	Selfing, within, and between population crosses	Hand pollination of ex situ plants, progeny sown in common gardens	F1	Yes, endangered species	Cumulative fitness = mean number of seeds per pollination x proportion of plants surviving in the field x total number of seeds per field plant	Cumulative fitness higher for between population crosses compared to selfing or within population crosses	+	Weisenberger et al., 2014
Trifolium virginicum	Fabaceae	Selfing, outcrosses of nearby and far populations, open-pollinated control	Hand pollination of plants in the field	F1	No	Fruit set	Higher fruit set with outcrosses with far population compared to selfing or near population crosses	+	Frye and Neel, 2017
Iris lortetii	Iridaceae	Crosses within or between populations from different environments	Re-introduction into similar sites	F1	Yes, endangered species	Fruit and seed set	Higher fruit and seed set for between population crosses compared to within population crosses	+	Shemesh et al., 2018
Lythrum salicaria	Lythraceae	Intrapopulation, interpopulation, and interregional crosses	Crosses done in greenhouse then planted in garden trials in different regions	F1, F2	No	Biomass, plant height, fruit production	Outcomes varied by site; either benefit with interpopulation cross or no difference No overall difference between F1 and F2 traits	N/+	Shi et al., 2018
Himantoglossum adriaticum	Orchidaceae	Crosses between large and small, isolated populations, between small, not isolated populations, and	Hand pollination in wild populations, fruit collected and seeds	F1	Yes, European endemic of priority interest	Germination percentage, fruit set	Crossing between small-sized and less isolated population increased germination, crossing largest population to smaller populations increased total germination in 1 out of 2 crosses; fruit/flower ratio similar for all crosses	+/N	Del Vecchio et al., 2019

Species	Family	Crosses	Setting	Gen	Conservation	Fitness measure	Fitness effect	Overall*	Ref
		within populations	germinated in lab						
Pinus torreyana	Pinaceae	Cross 2 different populations, compare fitness to parents	Common garden trial	F1	Yes, very rare pine	Seed weight, the number of days to germination, cotyledon, height accumulation	Increased average fitness in F1 compared to parents (height and fecundity)	+/N	Hamilton et al., 2017
Triticum turgidum L. ssp. dicoccoides	Poaceae	Cross between different populations, habitats		F1, F2, F3	No	Total weight of produced spikelets, days to awning, and individual spikelet weight	Relative fitness varied depending on parents, with hybrids fitter than one (8/12 crosses) or both parents (1/12); effects persist through F2 suggesting no hybrid breakdown	+/N	Volis et al., 2016
Phlox hirsuta	Polemoniaceae	Within and between population crosses of differing distances	Hand pollinations in field, germinations in lab	F1	Yes, endangered plant	Seed set, germination, progeny growth	No effect of distance of cross on seed characteristics, negative effect on cotyledons	N/-	Ruane et al., 2015
Primula vulgaris	Primulaceae	Crosses between populations compared to selfed, backcrosses of hybrid to parents	Seeds from controlled pollination in the wild grown in garden trial	F1, F2	Yes, rare plant in fragmented populations in study country	Fruit and seed set, seed weight, cumulative fitness (fruit set x seed set x mean seed weight)	Cumulative fitness highest for F1 hybrid, F2 hybrid experienced outbreeding depression	+/-	Barmentlo et al., 2018
Pyrus bourgaeana	Rosaceae	Selfing, nearby donor, distant donor, open-pollinated control	Hand pollination in field	F1	No	Fruit production, fruit and seed weight, proportion of mature seeds per fruit, and seed germination	Pollination with distant donors led to increased fruit initiation and development, seed viability (but not weight), fruit weight, and seed germination	+	Castilla et al., 2019

The overall assessment is marked + if outbreeding had positive impacts on fitness outcomes, N if there was no significant difference between outbreeding and controls, and – if outbreeding resulted in negative consequences. Studies with mixed results are given two marks, with the first being the more predominant effect in the study. The conservation column indicates whether or not the studied species has an important impact on conservation, for example, it is an endangered species and research could be used to inform conservation strategies. None of the included studies performed genetic rescue in the wild as a conservation strategy to save a threatened population.

Source Plant Material for Genetic Rescue

The question of where to source plant material for translocations, augmentations, and genetic rescue has received considerable attention. In addition to using plants from another natural population of the species, potential sources for translocations include ex-situ cultivations in botanic gardens and elsewhere, seedbanks, and even potentially herbarium material (Godefroid et al., 2011, Abeli et al., 2019). Consideration of local adaption is thought to be important to ensure that species remain fit in their current environment, and therefore the most common paradigm is using local source populations for augmentations. Concern has been expressed that this view may be too narrow and dilute the importance of genetic variation, preventing attempts to rescue threatened populations when there are no local populations to draw from (Weeks et al., 2011). Additionally, maximizing evolutionary potential for plants to adapt to changing conditions, as will be the case for impending climate change, is important, and can be achieved by mixing sources (Broadhurst et al., 2008). Individual studies have shown mixed results with various source populations. One study showed that mixed source populations of *Jacquemontia reclinata* had superior survival and predicted growth rate compared to local-only populations, even in the face of hurricanes (Maschinski et al., 2013). Another study concluded that crossing small, inbred populations with individuals from large, genetically diverse populations yields the best results (Pickup et al., 2013). Evidence from a meta-analysis suggests that only large populations of more than 1000 flowering individuals develop local adaptation (Leimu and Fischer, 2008). Therefore, translocating plants from one small population to rescue another may be spared from the effects of introducing maladaptation.

Published Recommendations and Assessment Tools for Genetic Rescue

Since there is a need for genetic rescue strategies to be incorporated into conservation plans, as well as some uncertainty pertaining to the approach itself, several researchers proposed guidelines and assessment tools to aid conservationists in selecting and applying genetic rescue when it is appropriate (Bell et al., 2019, Edmands, 2007, Frankham, 2015, Frankham et al., 2017, Hedrick and Fredrickson, 2009, Ottewell et al., 2016, Weeks et al., 2011). Although there are many potential benefits of genetic rescue, all recommendations suggest proceeding with caution and with careful assessment of the target and source populations. There are still many unknowns about the long-term effects of genetic rescue in the wild, and there are potential risks for negative outcomes (Hedrick et al., 2019, Kyriazis et al., 2019). Ultimately, the decision to pursue genetic rescue is a balance of the potential benefits and potential risks involved for the particular population. The following selected guidelines, frameworks, and decision tools for restoring genetic diversity to populations are presented in chronological order.

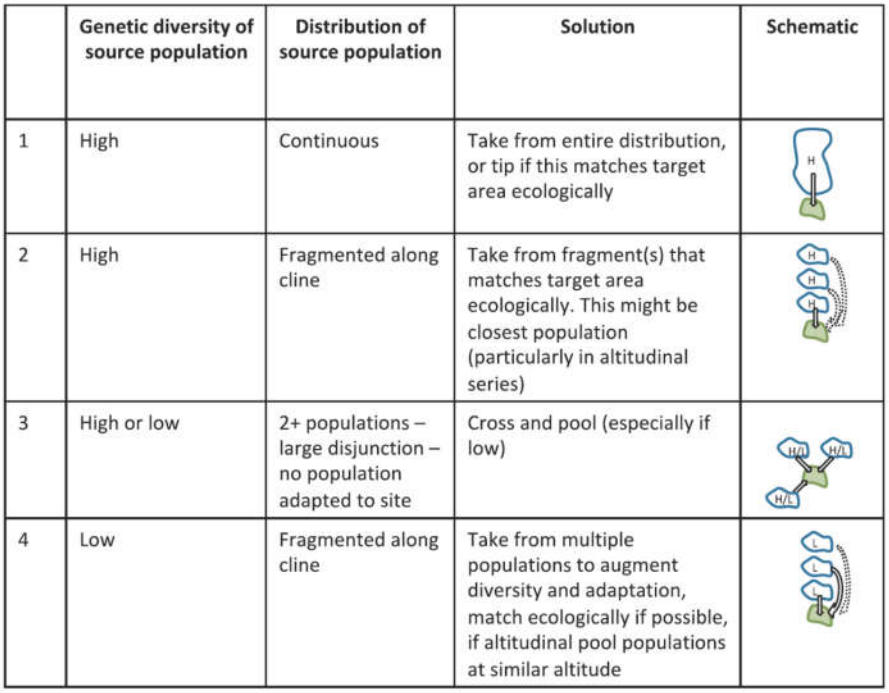
Edmands (2007) conducted a review on inbreeding versus outbreeding depression studies and offered several recommendations for crossing populations. She suggests the following guidelines (Edmands, 2007):

1. Only proceeding with crossing populations when there is clear evidence of inbreeding depression.
2. Selecting rescue sources that are as similar to the target population as possible both genetically and adaptively
3. Testing the fitness of the crosses for at least two generations, and emphasizing backcrosses, before implementing in the wild.

Hedrick and Fredrickson issued ten guidelines for genetic rescue for conservation purposes in 2009 and illustrated their points with the examples of Mexican wolves and Florida panthers. Although the guidelines were developed for animal conservation, many of these principles would be relevant to plants as well. The guidelines cover decisions on whether genetic rescue is appropriate, implementation and management, and potential negative consequences (Hedrick and Fredrickson, 2009). They stress that a substantial amount of information about the species under consideration should be known, including the fitness. The authors concluded that genetic rescue is only a temporary fix, and although it can buy time, issues that rendered the species endangered, such as habitat loss or other environmental factors, need to be fixed for long-term success (Hedrick and Fredrickson, 2009).

Weeks et al., (2011) developed a decision tree and risk assessment framework for translocations which include considerations of genetic diversity. They present various situations and the ensuing consequences if translocations occurred or are abandoned, and supplement their framework with examples of endangered plants (Weeks et al., 2011). Their discussion includes consideration of adaptive and evolutionary potential. They outline different potential population structures and suggest management based on the genetic diversity (**Figure 2.4**) (Weeks et al., 2011).

Frankham et al., have published several papers, and more recently, a book on genetic rescue (Frankham, 2010, Frankham, 2015, Frankham et al., 2017, Frankham et al., 2011, Frankham et al., 2014). Since outbreeding depression is a primary concern for a negative consequence of genetic rescue, Frankham developed a decision tree to assess the risk for outbreeding depression in crossing populations (Frankham et al., 2011). The first question on the tool is whether or not the taxonomy for the species is resolved. Ensuring correct taxonomy is crucial for conservation efforts in general, and should always be carefully considered before proceeding (Thomson et al., 2018). Other questions address chromosomal differences, whether or not there has been gene flow within the last 500 years, environmental differences, and whether or not populations have been separated for more than 20 generations (Frankham et al., 2011). The author has internally validated this tool in the original and in a subsequent publication (Frankham, 2015, Frankham et al., 2011). Frankham also published guidelines and decision trees for when to use genetic rescue that incorporate some concepts from the inbreeding depression assessment tool (Frankham, 2015, Frankham et al., 2017) (**Figure 2.5**). Frankham emphasizes that genetic rescue strategies should be pursued more aggressively in the face of rising extinctions.



	Genetic diversity of source population	Distribution of source population	Solution	Schematic
1	High	Continuous	Take from entire distribution, or tip if this matches target area ecologically	H
2	High	Fragmented along cline	Take from fragment(s) that matches target area ecologically. This might be closest population (particularly in altitudinal series)	H H H
3	High or low	2+ populations – large disjunction – no population adapted to site	Cross and pool (especially if low)	H/L H/L H/L
4	Low	Fragmented along cline	Take from multiple populations to augment diversity and adaptation, match ecologically if possible, if altitudinal pool populations at similar altitude	L L L

Figure 2.4. Genetic considerations in establishing populations outside the current or historical distribution of a species. Reproduced from Weeks et al., 2011.

Ottewell et al., (2016) developed a genetic conservation plan specifically for plants, with an eye toward bridging the gap between researchers and conservationists. Their decision tree to determine which strategy to pursue looks at genetic differentiation between populations, genetic diversity within populations, and inbreeding within populations, determined as either high or low (**Appendix Table 1**) (Ottewell et al., 2016). Similar to Frankham's decision tree, the starting point is whether or not the taxonomy for the population is clearly resolved, and if it is not, they recommend not proceeding. They offer eight strategies for managing the population, ranging from maintaining the habitat for healthy populations to translocating plants to increase the genetic diversity (**Appendix Table 1**) (Ottewell et al., 2016).

All authors stress the importance of genetic rescue as a tool for conservation after carefully considering if the population is a good fit. A recurring theme for all of these guidelines is that a significant amount of information must be known about the target and source populations before proceeding with genetic rescue. This may be difficult in conservation settings, particularly for populations with very low numbers in a severely threatened habitat. Further, as species move more rapidly towards extinction, the time spent obtaining all of the required information might be better utilized saving the genetic diversity of the population, which may further deteriorate over time.

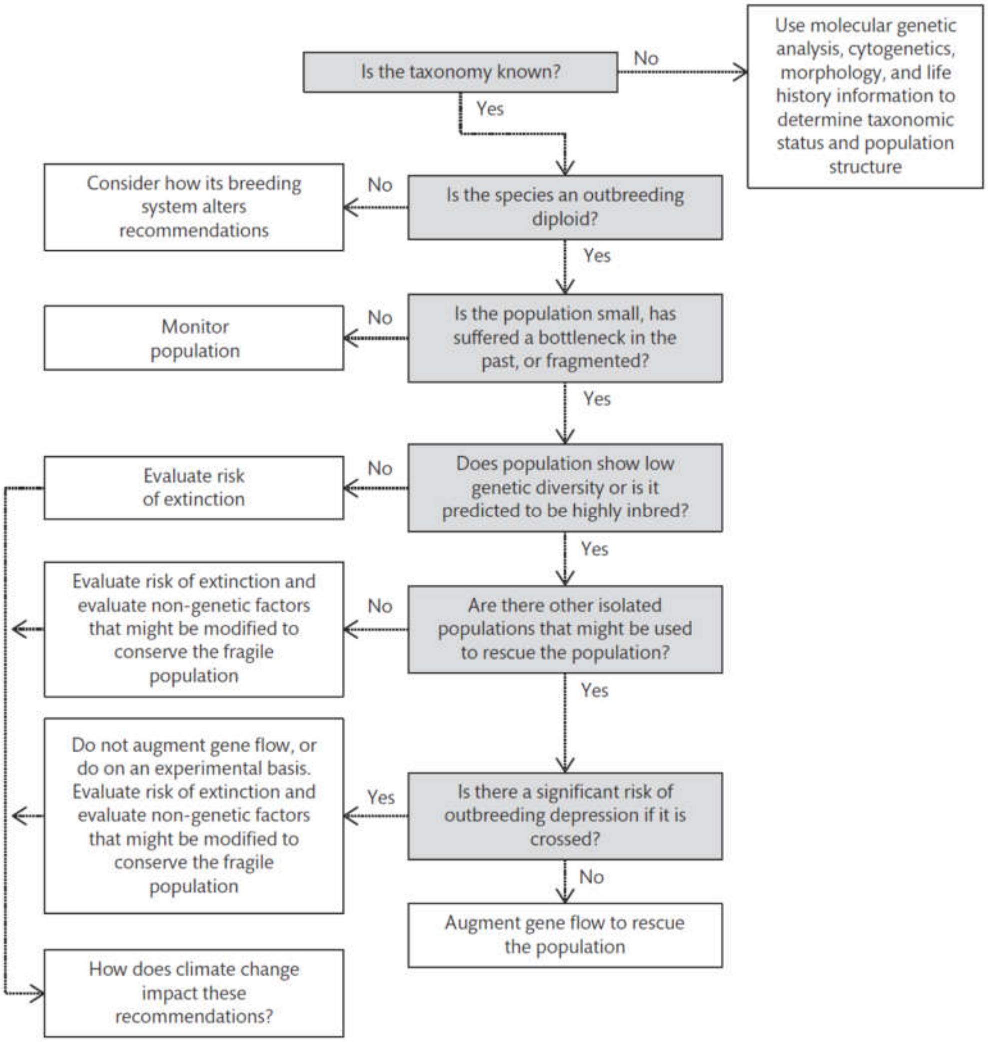


Figure 2.5. Decision tree with the main questions that need to be asked when genetically managing fragmented populations. Reproduced from Frankham et al., 2017.

Barriers to Implementing Genetic Rescue

Although there have been many calls to increase the use of genetic rescue for recovering threatened species, there remain few studies pursuing this strategy (Ralls et al., 2018, Tallmon, 2017, Whiteley et al., 2015). A study on if and how genetic factors are incorporated into conservation plans for threatened species in the United States, Europe, and Australia showed a low level of incorporation of genetic information, which was even lower for plants compared to animals (Pierson et al., 2016). Genetic factors relating to fitness, such as the level of inbreeding, inbreeding and outbreeding depression, and hybridization, all of which are important considerations for genetic rescue, were very rarely included in species recovery plans. The study found that only 7% of the plans they assessed mentioned the concept of inbreeding (Pierson et al., 2016). The authors suggest that the potential benefit of including this information is likely underestimated due in part to practitioners' lack of understanding about the underlying processes. A survey of conservation practitioners in New Zealand to address why the "conservation genetics gap" exists highlights that conservationists report lacking both funding and genetics expertise, which contribute to them not implementing genetics in conservation, despite their interest in doing so (Taylor et al., 2017). The authors propose increasing communication between conservation practitioners and researchers and facilitating expertise and collaboration as ways to overcome barriers, ideas also supported other studies (Cook and Sgrò, 2017, Taylor et al., 2017).

Barriers to implementation specifically of genetic rescue include scientific, cultural, financial, legal, and political issues (Cook and Sgrò, 2017, Frankham, 2015, Love Stowell et al., 2017, Taylor et al., 2017). Concerns related to biology, including outbreeding depression, maladaptation, and potentially spreading diseases and pests are prevalent (Frankham, 2015, Love Stowell et al., 2017). Cultural barriers also play an important role in the lack of uptake for genetic rescue (Love Stowell et al., 2017). For example, the perception of "naturalness" and a desire to ensure the purity of the local population is an impediment, as genetic rescue can be perceived as diluting the purity of a population (Frankham, 2015).

Remaining Questions and Research Gaps

There are several remaining questions and important research gaps concerning genetic rescue. As discussed, the ideal long-term study has not yet been published, but such work would contribute greatly to our understanding of this strategy. A big question is for how long do the beneficial effects of genetic rescue last (Bell et al., 2019)? Heterosis in the F1 generation is well known, but few studies assess fitness of outcrosses in later generations. Several researchers have suggested that genetic rescue really just buys time for threatened populations while conservation practitioners can address habitat issues, but how much time does it buy (Gewin, 2017, Hedrick and Fredrickson, 2009)? Additional questions pertain to the biology and mechanisms behind genetic rescue, such as how can one avoid introducing deleterious alleles into the population? How can one ensure that the phenotypic and genetic qualities unique to the threatened population, as well as local adaptations are maintained and avoid genetic swamping (Bell et al., 2019)? The case of the Isle Royale wolves, initially "rescued" by a migrant wolf, but then deteriorating even further is a prime example of deleterious effects in the long run (Hedrick et al., 2019). This example raises questions and potential issues that are relevant for any organism, such as: why did the species become nearly extinct in the first place, and how can the issues it face be remedied? There also remains a gap between research and implementation, which could be bridged by more connections between researchers and those implementing conservation programs, and with feedback loops between the two in place (Ottewell et al., 2016). Publication of conservation efforts is often lacking, and it is important for publication of not only successes but also failures.

Chapter 3: Prospects for Genetic Rescue of St. Helena's Endemic Flora

Status of St. Helena's Endemic Flora

As an island, St. Helena boasts a rich and diverse endemic flora. However, these species have experienced substantial habitat destruction due to logging, farming, soil erosion, and the introduction of grazing animals and foreign or invasive species since the discovery of the island by humans in the 1500s (Cronk, 2000, Lambdon, 2012). Six plant species are known to have gone extinct since 1771, and two are currently extinct in the wild (Lambdon and Cronk, 2020). Predictions suggest that another four of the island's extant endemic plants will become extinct within the next 50 years (Lambdon and Cronk, 2020).

Out of the remaining 46 extant endemic plant species on St. Helena, the majority (35 out of 46, 76%) are under threat. The IUCN Red List classifies 19 as Critically Endangered, five as endangered, nine as vulnerable, and two near threatened (IUCN, 2020). A recent study on extinction dynamics in St. Helena's flora has also classified an additional two ferns as critically endangered and one as endangered according to IUCN criteria, although they do not yet appear on the official ICUN Red List (Lambdon and Cronk, 2020, IUCN, 2020). The predicted extinction half-life for the critically endangered angiosperms ranges from a mere 20 years for *Commidendrum spurium* DC. and *Wahlenbergia linifolia* (Roxb.) A. DC. up through 1450 years for *Frankenia portulacifolia* (Roxb.) Spreng., with 10 out of 16 species having a half-life of less than 100 years (Lambdon and Cronk, 2020) (**Figure 3.1** and **Table 3.1** for select species). Some of the endemics have been reduced to very small population numbers, the most extreme of which is *Commidendrum rotundifolium* (Roxb.) DC., which has only one known remaining mature plant in the wild (Lambdon and Cronk, 2020). Many plant populations are also highly fragmented into several locations throughout the island (Lambdon, 2012, IUCN, 2020). Because of the high extinction threat and that the small, isolated populations may suffer from elevated inbreeding depression and lower genetic diversity, the St. Helena endemic flora are likely be good candidates for genetic rescue (**Table 3.1**).

Figure 3.1. Some of the critically endangered St. Helena endemic plants that are predicted to have extinction half-lives of less than 50 years: A) *Withania begoniifolia* (Roxb.) Hunz. & Barboza, 30 years B) *Nesohedyotis arborea* (Roxb.) Bremek., 30 years C) *Kewa acida* (Hook.f.) Christenh., 43 years. Image credits: © P. Lambdon, 2008.

Assessment of St. Helena Plant Species as Candidates for Genetic Rescue

Genetic rescue may be a useful tool to save some of St. Helena's endemic plant species from the brink of extinction. However, before attempting genetic rescue, care must be taken to ensure that the species are good candidates for this conservation strategy. Although ferns make up an important component of St. Helena's endemic flora with nine species appearing on the IUCN Red List, evidence is lacking for the role of genetic rescue in ferns. Potential reasons that genetic rescue may be less effective in these plants include a higher degree of asexual reproduction and that ferns are often polyploid. Therefore, the discussion will focus on St. Helena's critically endangered endemic angiosperms, since studies on angiosperms form the evidence base for genetic rescue in plants (**Chapter 2**). Of the 16 critically engendered endemic angiosperms, 12 species have severely fragmented populations that are found in at least two different locations (IUCN, 2020) (**Table 3.1**). The remaining four species have under ten mature individuals (ranging from one to six) in a single location in the wild (IUCN, 2020).

Table 3.1. All critically endangered St. Helena endemic angiosperms with severely fragmented populations of more than ten mature individuals in more than one location. These species are potential candidates for genetic rescue, and likely meet the criteria for outbreeding depression if crossed between their subpopulations.

Species Name Common Name	No. Mature Individuals	No. Locations	Environment	Current Threats	Extinction Half Life* (years)	Other Considerations	Seed Availability	Reference
Plantago robusta St. Helena Plantain	2740	7	Misty cliffs and waterfalls	Grazing, invasive plants	104	High seed set and can be grown easily from seed.	Seed from several populations collected and available on St. Helena and in Millennium seed bank.	(Lambdon and Ellick, 2016j, Lambdon, 2012)
Euphorbia heleniana French Grass	210	5-6	Dry, rocky, near sea, lower elevation	Grazing, potential for human disturbance for populations on footpaths	1350	Annual plant that can die out in harsh conditions and requires recolonization from other populations. Probably self-pollinated. Low productivity.	Small seed collections available, but not widely sampled.	(Lambdon and Ellick, 2016d)
Eragrostis episcopulus Cliff Hair Grass	3190	5	Exposed cliffs	Invasive plants, grazing	42	Northern populations have darker pigmentation, but unknown whether environmental or genetic. Caution should be taken before mixing these different populations.	Seeds collected and cultivated by St. Helena Government's Environmental Conservation Section.	(Lambdon and Ellick, 2016c)
Kewa (Hypertelis) acida Salad Plant	3190	4-7	Open, dry hillsides	Grazing, invasive plants	43	Fluctuating numbers by year, seeds may be able to remain dormant for a long time. Limited dispersal ability.	Seeds collected and cultivated by St. Helena Government's Environmental Conservation Section.	(Lambdon and Ellick, 2016f)
Frankenia portulacifolia St. Helena Tea Plant	3530	4-6	Dry, rocky, usually near sea	Grazing, low recruitment rates, invasive plants	1450	Endemic *Agdistis* species moth feeds only on this plant at Man and Horse, thus important for endemic invertebrate species. Variation in	Seeds collected but difficult to cultivate.	(Lambdon and Ellick, 2016e)

Species Name Common Name	No. Mature Individuals	No. Locations	Environment	Current Threats	Extinction Half Life* (years)	Other Considerations	Seed Availability	Reference
						morphology in different populations may be due to genetic variation.		
Bulbostylis neglecta Neglected Tuft Sedge	20300	4-10	Hillside, pine forest, mid-altitude	Invasive plants	450	Scattered locations, so it was likely widespread before being eradicated. Ephemeral with poor dispersal.	Seeds collected and cultivated by St Helena Government's Environmental Conservation Section.	(Lambdon and Ellick, 2016a)
Phylica polifolia St. Helena Rosemary	35	3	Rocky, dry areas	Invasive plants, severely limited dispersal and gene flow	60	Highly fragmented, very small populations with no gene flow.	Seeds collected and there are established cultivated populations and seed orchards on St. Helena.	(Lambdon and Ellick, 2016h)
Pelargonium cotyledonis Old Father Live Forever	1640	2-6	Cliffs and slopes	Grazing, invasive plants and pests	1350	Very small numbers in populations in the wild. Wind-dispersal of seeds likely limited in current population locations. Used in cultivation.	Seeds collected and cultivated on island and at Kew.	(Lambdon and Ellick, 2016g)
Pladaroxylon leucadendron He Cabbage Tree	55	2	Diana's Peak tree thicket, some at High Peak	Invasive species, lack of suitable habitat for colonization	60	Often produces many infertile seeds, suggesting outcrossing could be beneficial. Short lifespan suggests high levels of recruitment needed. Seed collection difficult due to inaccessible location and limited chance for collection.	Seeds collected and small numbers cultivated, though difficult to grow.	(Lambdon and Ellick, 2016i)
Nesohedyotis arborea St. Helena Dogwood	47	2	Tree fern thicket, open stands of He Cabbage	Habitat quality	30	Complex mating system with protandrous and protogynous flowers.	Seeds collected, and efforts to restore population by planting seedlings undertaken	(Ellick and Lambdon, 2016b)

Species Name Common Name	No. Mature Individuals	No. Locations	Environment	Current Threats	Extinction Half Life* (years)	Other Considerations	Seed Availability	Reference
			trees, Diana's Peak and High Peak			Suggest planting in clusters for cross pollination, which increases seed production. Many successfully replanted in Diana's Peak park.		
Commidendrum robustum Scrubwood	678	2	Very few, only at Peak Dale and Deep Valley	Damage from rats, seedlings difficult to establish due to weeds and cattle, pests	700	Most planted recently, older species mainly from cultivation, only a few wild trees remain.	Seeds collected, and efforts to restore population by planting seedlings undertaken, though competition from invasive plants leads to poor survival.	(Ellick and Lambdon, 2016a)
Wahlenbergia linifolia Large Bellflower	40	1-2	Ledges, cliff faces	Hybridization, land slips, invasive species	20	Main population has <50 at High Peak. Hybrids with *W. angustifolia* are known and make obtaining pure specimens challenging.	Seedlings difficult to establish, but a priority to retain genetically diverse plants.	(Lambdon and Ellick, 2016l)

*Extinction half-life data taken from Lambdon and Cronk, 2020.

Ensuring that the taxonomy is resolved is one of the important considerations for any conservation strategy (Thomson et al., 2018). This is the first question on an outbreeding assessment tool, a genetic conservation decision tree, and a decision framework for managing genetic conservation in plants (Frankham et al., 2011, Ottewell et al., 2016, Frankham et al., 2017). Some of St. Helena's endemic plant taxonomy remains uncertain, such as for *Hydrodea cryptantha* (Hook.f.) N.E.Br. (Lambdon, 2012). However, the species listed in **Table 3.1** have no reported taxonomic issues and genetic conservation strategies can proceed from this perspective. One important consideration is the presence of hybrids between related species, which are known to occur in some endemics, such as *Commidendrum* and *Wahlenbergia* (Gray et al., 2016, Lambdon, 2012). Care must be taken to ensure that selected plants are indeed the correct species if attempting genetic rescue or any other conservation strategy.

Outbreeding Depression Risk Assessment

Since outbreeding depression is one of the primary risks associated with genetic rescue, assessing the potential for outbreeding depression is an important component of the decision to use genetic rescue (Frankham et al., 2011, Ralls et al., 2018). In addition to ensuring that taxonomy is fully resolved, criteria for suspecting a high risk of outbreeding depression between two populations include fixed chromosomal differences, gene flow between populations more than 500 years ago, and substantial environmental differences in populations separated by more than 20 generations, for which additional assessment is needed (Frankham et al., 2011). Fixed chromosomal differences in plants can be due to different ploidy levels, which can be assessed using flow cytometry to estimate the nuclear DNA content, and confirmed by cytology if required (Dolezel et al., 2007, Schmidt-Lebuhn et al., 2018). Conducting such a test would be a valuable safeguard to ensure that offspring from crossing populations are not infertile due to differences in chromosome numbers. Since St. Helena was discovered approximately 500 years ago, and human activities have primarily caused the population fragmentation through habitat destruction or introduction of herbivores, it is likely that gene flow between the fragmented populations has occurred within the last 500 years, and thus the threated species pass this criterion. The final criterion for a potentially high risk for outbreeding depression, which pertains to the habitats in which the populations live, must be done on a case-by-case basis and is summarized in **Table 3.1**. Generally, fragmented populations of individual species in St. Helena have been restricted to similar areas, for example a species may be scattered across mountaintops on Diana's Peak and High Peak. In these cases there are few major disparities between the habitats of the subpopulations. However, this is not always the case, and some morphological differences have been noted between populations in different locations. For example, morphological differences and variation in growth form have been observed in different populations of *F. portulacifolia*, and it is unknown whether these differences are due to phenotypic plasticity and response to the local environment or if they are due to genetics (Lambdon and Ellick, 2016e). Conducting common garden trials and molecular analyses may be useful before mixing populations from different environments, especially those that show morphological differences. Altogether, St. Helena's fragmented populations are likely at a low risk for experiencing outbreeding depression from outcrossing between them, according to established criteria (Frankham et al., 2011).

Genetic Rescue Assessment

The initial question on a tool for determining whether to attempt genetic rescue asks whether the populations are inbred (Frankham et al., 2017, Ralls et al., 2018). The authors suggest a minimum inbreeding coefficient of 0.1, or at least a 10% loss of its genetic diversity (whether known or inferred) to proceed with genetic rescue. The level of inbreeding can be measured several ways, including analysis of pedigrees and the use of genetics or genomics. Although small, isolated populations frequently experience inbreeding depression, some primarily self-fertilizing species do not suffer substantial negative consequences in the absence of outcrossing. The species in **Table 3.1** more than likely experience inbreeding depression, however, confirmation and quantification of this phenomenon would be advised before proceeding with genetic rescue. The following two questions on the tool address the suitability of populations for crosses, and essentially gauge the risk of outbreeding depression, which was addressed above using the outbreeding depression risk assessment tool. The final question asks if crossing the populations will result in worthwhile genetic rescue. This is the most difficult to predict, although the authors of the assessment tool suggest that the magnitude of the effect of genetic rescue is usually large (Ralls et al., 2018).

Additional Considerations

Many of St. Helena's threatened species face competition from invasive plants and grazing from herbivores **(Table 3.1)** (Lambdon, 2012). It is crucial that any genetic rescue program is coupled with efforts to control environmental factors, such as these, which could thwart the success of the initiative. For example, fencing may need to be erected to ward of herbivores, and has proven successful in translocation attempts (Fenu et al., 2016, Bainbridge, 2017). Much of the grazing in St.

Helena today is due to introduced rabbits, and control of these animals is important for protecting both any translocated plants and the remnant populations. Likewise, since nearly all of the critically endangered plants are threatened by invasive plants, adequate control of these invasive species, not only within the area where plants are translocated, but in surrounding areas, is crucial for successful growth of the target population. The establishment of previous translocations of *C. robustum* was only successful when coupled with intensive management of invasive species (Ellick and Lambdon, 2016a).

When there are multiple available subpopulations, crosses with one population may outperform others, as shown in many studies (Li et al., 2020, Marsden et al., 2013, Volis et al., 2016). Some researchers recommend performing test crosses ex situ over at least two generations to ensure that outcomes from the crosses are favorable before proceeding in the wild (Edmands, 2007). This can be a safeguard, but comes at the expense of additional time. Others disagree that this step is necessary, arguing that evidence for outcrossing inbred populations is overwhelmingly positive, and highlighting the need to act swiftly (Frankham, 2015). In situations where genetic rescue is inappropriate or not possible, various other conservation methods can be considered (Ottewell et al., 2016, Weeks et al., 2011).

Potential Sources of Plants for Genetic Rescue Attempts

The endemic populations split into two or more subpopulations in different locations can provide material to move from one location to another. A meta-analysis of plant translocation studies comparing local or foreign sources found that the “local is best” paradigm held true for large populations, but not small ones (Leimu and Fischer, 2008). They showed that local adaptation was very uncommon in populations with less than 1000 flowering individuals. A recent modeling study also predicted that translocations from small populations could mitigate extinction risk, whereas translocations from large populations might augment the risk (Kyriazis et al., 2019). This has implications for several of St. Helena’s threatened species. Eight out of 16 critically endangered angiosperms are estimated to have very small populations totaling less than 1000 mature individuals. Additionally, species with more than 1000 individuals total are fragmented into smaller populations. Evidence from this study suggests that these small St. Helena populations may not have experienced local adaptations, and translocation of individuals from different populations for genetic rescue may not present with negative consequences of maladaptation. However, since St. Helena has a diverse topology and vastly different environments and microclimates, there may be added selective pressure to adapt depending on the location, and this is an important consideration for mixing populations. The presence of local adaptation in the populations can be tested using common garden trials and supplemented with molecular studies.

In addition to the populations themselves, other potential sources for plants to use for genetic rescue include the seed banks stored both on St. Helena and in Kew's Millennium Seed Bank (**Table 3.1**). Conservation agencies on St. Helena have also been working on growing endemic species, and many have been planted in the Millennium Forest (**Figure 3.2**). Some specimens are also grown ex situ in the collections of botanic gardens, such as Kew. However, there are concerns about the use ex situ cultivated plants and risk of maladaptation or loss of alleles that are important for local adaptation. For these potentially higher risk translocations, the use in garden trials in local conditions would be preferable to assess outcomes before proceeding in the field.

Figure 3.2. St. Helena's Millennium Forest, planted in 2000, consists mostly of *C. robustum,* and can serve as one potential source of plants for genetic rescue. Image credit: Alan Gray.

Additional Research Needed

Although St. Helena has been known to humans for just over 500 years, there is a surprising lack of information about the island's flora (Cronk, 2000, Lambdon, 2012). Relatively few specimens are stored in herbaria, and those that are available are generally from the 1800s or earlier. Modern-day specimens are rarely available, and some, such as many in Quentin Cronk's collection, are not yet registered in the herbarium for viewing. Modern specimens would be helpful for study of the current flora and for making comparisons to the older specimens to understand population changes over time, especially losses in variation. Additional research in genetics and genomics would be invaluable, as little is known about the population genetics of most of St. Helena's endemic plant species, or relationships with related continental taxa. In order to most effectively apply conservation strategies, and in particular genetic rescue, it is crucial to understand population structures and dynamics, especially the levels of inbreeding in the endemic flora. Detecting the presence of private alleles in isolated populations could aid in preserving the unique genetic heritage of these populations. Likewise, genomics can be used to find loci important for adaptation and ensure that they are retained, and to determine whether differences between related populations are genetic. For example, there are morphological differences between subpopulations of plants such as *Frankenia portulacifolia* and *Eragrostis episcopulus* Lambdon, Darlow, Clubbe & Cope, though it is not confirmed that these differences are due to genetics or simply to environmental variation (Lambdon and Ellick, 2016c, Lambdon and Ellick, 2016e). Additionally, since several of the plants on the IUCN Red List are known to form hybrids with closely related species, a more complete understanding of the full breadth of hybridization in wild populations, and the ability to identify pure or hybrid specimens using molecular markers or genomics, is important. For example, what was believed to be a seed orchard of *C. rotundifolium* actually contained hybrids with *C. spurium* (Gray et al., 2016). Finally, further research into the role of native pollinators and the health of their populations would help to understand the connectivity of fragmented entomophilous species and ensure their prosperity, as few studies have addressed this issue (Paajanen and Cronk, 2020). The genetic diversity of species that are pollinated by animals as opposed to the wind relies on those organisms to connect populations through pollen transfer, a concept especially critical for self-incompatible plants (Melen et al., 2016).

Conclusion

Genetic rescue may be a promising tool for saving small, isolated plant populations, such as the important endemics of St. Helena. However, introducing genetic variation into the population alone is not sufficient to bring it back from the brink of extinction. The approach needs to be holistic and consider not only increasing genetic heterogeneity, but also ensure that suitable habitat remains for the population to grow, and that invasive species are kept in check. Ensuring viable connections in the landscape to maintain adequate gene flow between populations is also an important consideration. Additional research in genetic rescue and long-term studies that incorporate both genetics as well as population growth would be invaluable for conservation practitioners in general, as well as those in St. Helena. Several of the island's critically endangered endemic plants would potentially be good candidates for genetic rescue, as many exist only in small, fragmented populations. Although additional research would better inform conservation decisions for these species, they are rapidly heading towards extinction (Lambdon and Cronk, 2020). Therefore, a balance needs to be struck between the potential harms of genetic rescue attempts, such as outbreeding depression, and the complete loss of a species due to extinction. Time is limited for St. Helena’s valuable endemics, some of which are predicted to become extinct in the next 50 years. Inaction will lead to worse, or at best, equivalent outcomes as attempts to rescue these plants, which has the potential for substantial benefit. During the current extinction crisis, letting perfect be the enemy of the good is a risky approach.

Chapter 4: Morphometrics to Assess Genetic Variation in Endemic Plant Species from St. Helena

Introduction

Changing environmental conditions put pressure on species to adapt in order to survive and thrive. Faced with climate change, many species will encounter additional challenges in the future, such as extreme weather events, increased temperatures, and changes in precipitation levels (Intergovernmental Panel on Climate Change, 2015). These stresses will exacerbate ongoing issues such as deforestation, pollution, and the pressure to produce more food and amenities for the expanding human population, leading to habitat destruction, removal of native vegetation, and increases in problems like invasive species, disease, and erosion (Wheeler and Von Braun, 2013, Anderson et al., 2004, Hellmann et al., 2008). Species will therefore need the ability to react by effectively adapting to these stresses (Jump and Peñuelas, 2005). Populations with higher levels of genetic variation harbor a greater ability to respond to environmental changes and to adapt accordingly (Anderson et al., 2011). In contrast, small, fragmented populations suffering from inbreeding depression and lower heterogeneity are less resilient and less able to cope with such changes (Frankham et al., 2017). Genetic variation within a population can be assessed using various methods, including common garden trials and molecular studies, such as next-generation sequencing. Morphometrics can be used to quantify differences in plant shape and size to assess historical levels morphological variation in herbarium specimens, which may be due to genetic or environmental factors. Morphometric analyses can also be used to compare historical (possibly extinct) populations with modern populations to gauge if historical levels of genetic variation are intact or have dwindled in the species over time.

The endemic plants of St. Helena, now largely restricted to small, remnant populations on the island, have faced various ongoing threats since the island's discovery over 500 years ago. Despite conservation efforts and the establishment of protected areas, these plants continue to face environmental challenges, including the persistence of invasive species and the potential negative impacts of climate change. Understanding the genetic variation within the endemic plant populations is important for conservation decisions on the island. For this study, three St. Helena endemic genera were selected for morphometrics to gauge levels of variation in the populations: *Carex* L., *Phylica* L., and *Wahlenbergia* Schrad. ex Roth. These genera were selected because digitized herbarium specimens were readily available and because each is associated with an underlying hypothesis that there may be morphological differences in subpopulations (see **Aims**).

Carex dianae

Carex dianae Steud., in the Cyperaceae, is known as Diana's Peak Grass, and is the only endemic *Carex* species on St. Helena. It grows as dense tussocks with long flowering culms in glades and next to paths in the cloud forest area, and on cliff ledges with other vegetation (**Figure 4.1**) (Lambdon, 2012). *Carex dianae* is categorized as a species of least concern on the IUCN Red List, with an estimated stable population of 32,916 plants, mostly along Diana's Peak Ridge (Lambdon and Ellick, 2016b). Interestingly, Melliss (1875) described the species as "somewhat scarce", growing along the ridge between Diana's Peak and High Peak. Today's populations are largely concentrated at Diana's Peak and High Peak, with occasional smaller subpopulations, including only one at a lower elevation, which is located at the Barn (Lambdon and Ellick, 2016b).

Figure 4.1. (A) *Carex dianae*, (B) close-up of reproductive spikes, and (C) habit of *C. dianae* growing at Central Peaks. Image credits: A, B) © P. Lambdon, 2010; C) A. Gray.

There is some debate as to whether the different morphological variations of *C. dianae* at Diana's Peak and High Peak are indeed different species, different varieties, or one species with widely ranging morphology (Cronk, 2000, Lambdon, 2012). Burchell identified two species of *Carex* on St. Helena in the 1800s, *C. elata* on Diana's Peak (Burchell 19), and *C. longifolia* at High Peak (Burchell 20). These were later specified as holotypes for *C. praealta* Boott and *C. aequabilis* Boott, respectively. Cronk (2000) also considered the two forms to be different species, *C. praealta* and *C. dianae*. The currently accepted taxonomy is that there are two varieties of *C. dianae*: *C. dianae* var. *aequabilis* at High Peak, and *C. dianae* var. *dianae* on Diana's Peak ridge and Cuckhold Point, although it is acknowledged that there is a need for further taxonomic work, including molecular studies to confirm the status of the species delineation (Lambdon, 2012, Lambdon and Ellick, 2016b).

The key differences between the varieties are largely in reproductive characters, as summarized in **Table 4.1.** The morphological differences in *C. dianae* may be due to limited gene flow between the partly isolated populations and self-fertilization, therefore genetically derived, or differences could potentially be attributed to adaptation to the local environment (Lambdon, 2012). The IUCN Red List does not acknowledge the different varieties in its assessment (Lambdon and Ellick, 2016b). However, if taxonomic revision determines that they are two different species, it would have implications for potential reclassification for the species with lower population numbers to a more protected status.

Table 4.1: Key differences between *C. dianae* var. *dianae* and *C. dianae* var. *aequabilis* as described in Lambdon, 2012.

Character	***Carex dianae* var. *aequabilis*.**	***Carex dianae* var. *dianae***
Flowering culms	<1.2 m long, often shorter than leaves	Up to 1.5 m long, much longer than leaves
Middle bisexual spikes	Generally male at both top and bottom of spike	Generally male at top of spike
Lowest spike	Upright	Nodding
Female spikes (in fruit)	~4 mm wide, nutlets not widely spreading	6-7 mm wide, nutlets spreading at 45°
Nutlet glands	Present	Absent
Location	High Peak, more common in shaded, sheltered locations and cliff ledges	Diana's Peak ridge, eastern slopes of Cuckhold's Point

Phylica polifolia

Phylica polifolia (Vahl) Pillans, or St. Helena Rosemary, is an endemic shrub with leathery, linear leaves in the Rhamnaceae family (**Figure 4.2**). The species is considered critically endangered with an estimated 35 individuals remaining in three very isolated populations at High Hill, Lot, and Ebony Point (one plant only) (Lambdon and Ellick, 2016h). It was rare and already declining by the mid 1800's, with reportedly only 100 plants on the island at that time (Melliss, 1875). Historically, there were descriptions of populations with broader leaves, although the species occurs in a more ericoid form today (Richardson et al., 2001).

Phylogenetic analysis suggests that *P. polifolia* shares a common ancestor with other island species in the genus, and that it arrived on St. Helena approximately one to two million years ago (Richardson et al., 2001). In addition to leaf tomentum, the morphological differences between the island species are in reproductive characters, such as inflorescence form and sepal size (Richardson et al., 2003). An additional molecular study included two *P. polifolia* populations from St. Helena: one at High Hill, which has an upright growth form, and another at Lot, which has a more prostrate growth form (Richardson et al., 2003). The study proposed that these two populations are genetically distinct, which suggests limited gene flow between them (Richardson et al., 2003). However, the molecular results have been interpreted as equivocal elsewhere (Lambdon, 2012). It is possible that the differences in growth form are due to the environment, but this would need to be confirmed in common garden trials.

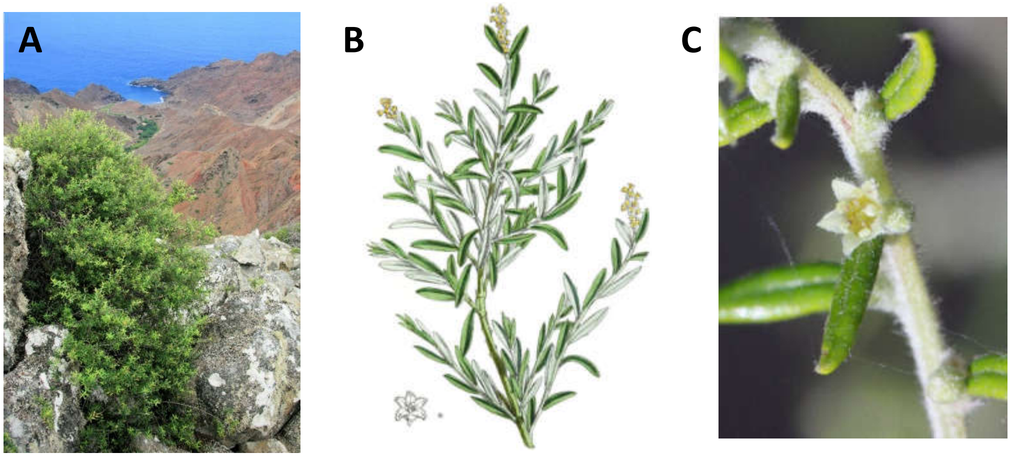


Figure 4.2. *Phylica polifolia* habit (A), drawing (B), and close-up of leaves, hairy stem, and flower (C). Image credits: A) © P. Lambdon, 2008 B) Melliss, 1875, C) © P. Lambdon, 2010.

Wahlenbergia Species

Wahlenbergia angustifolia (Roxb.) A. DC. (small bellflower) and *W. linifolia* (large bellflower) are the two extant species on St. Helena of this genus of the Campanulaceae (**Figure 4.3**). *Wahlenbergia angustifolia* is classified as vulnerable on the IUCN Red List, with an estimated decreasing population of 8,803 individuals in several locations, including Devil's Cap Ridge, Wild Ram Spring, and at various points along the Central Ridge (Lambdon and Ellick, 2016k). *Wahlenbergia linifolia* is present in much smaller numbers, with an estimated 49 to 53 individuals in a severely fragmented, decreasing population found at Red Rock, The Depot, and High Peak (Lambdon and Ellick, 2016k). This species was reported to be present in much higher numbers in the past, and even higher than *W. angustifolia*, which was more scarce at the time (Melliss, 1875). An additional two endemic species, *W. burchelllii* A. DC. and *W. roxburghii* A. DC. are now extinct (Cronk, 2000). Both extant species produce white bell-shaped flowers, though compared to *W. linifolia*, the flowers of *W. angustifolia* are smaller in size and yellowish at the base. Hybrids between the two species have been described, and appear as an intermediate between the parents species for several characters (**Table 4.2**) (Lambdon, 2012).

Figure 4.3. Growth form and flowers of *Wahlenbergia angustifolia* (A, B) and *W. linifolia* (C, D). Image credits: A) P. Lambdon, 2008, B), C) P. Lambdon, 2010, D) P. Lambdon, 2012.

Table 4.2. Key differences between *W. angustifolia* and *W. linifolia*, and features of the hybrid between the two species, as described in Cronk, 2000, and Lambdon, 2012.

Character	***W. angustifolia***	***W. linifolia***	**Hybrid**
Lower stem texture	Woody	Woody with petiole scars resembling pine bark	Ridged in distal part, with peg-like lips below petioles
Leaf width	4 mm	9 mm	4-9 mm
Leaf margin	Subserrate margin moderately fringed with small, glandular, blunt, peg-like teeth	Serrulate margin with small, blunt, peg-like teeth	Small, peg-like teeth
Peduncle	Up to 20 cm	0-4 cm	6-9 cm
Calyx length	4 mm	Up 5 mm	Not specified
Calyx shape	Triangular, untoothed	Lanceolate, weakly toothed	Sparsely serrate to almost toothed
Corolla width	12 mm	20 mm	15-20 mm
Corolla color	White, slightly yellow at base	White	White, slightly yellow at base

Despite their similarities, phylogenetic analyses have shown that *W. angustifolia* and *W. linifolia* are not sister taxa, and these species likely colonized St. Helena independently from different sources in the Southern Hemisphere (Crowl et al., 2014, Haberle et al., 2009). *Wahlenbergia angustifolia* is most closely related to *W. berteroi* Hook. & Arn. from the Juan Fernandez Islands, while the closest relative to *W. angustifolia* is *W. gloriosa* Lothian from Australia (Crowl et al., 2014, Haberle et al., 2009).

Aims

The aim of this study was to assess the morphological variation in historical St. Helena endemic plant specimens and to develop character sets for future morphological study of these species. The following species-specific questions were addressed:

- **Are there substantial morphological differences between the suspected varieties in the *C. dianae* population of St. Helena?**
- **Are there morphological differences in the *P. polifolia* population from historical specimens, and do these differ according to location?**
- **Can a set of characters be developed to readily identify *W. angustifolia* and *W. linifolia* using morphometrics, and are there hybrids between *W. angustifolia* and *W. linifolia* in the historical specimens?**

Materials and Methods

Specimens

This study was limited to digitized specimens available on herbarium websites and on JSTOR's Global Plants in May of 2020. Herbaria catalogues were selected and searched largely according to a list of collectors on St. Helena and where they deposited specimens from the island, and included: Royal Botanic Gardens of Edinburgh and Kew, British National History Museum, Paris, Geneva, Smithsonian, Berlin, Oxford and Trinity (Cronk, 2000). Global Plants was also used to identify additional specimens and types. Specimens used for the analysis are listed in **Appendix Table 2**. Type specimens were available for all species. For the *C. dianae* analysis, an outgroup from South Africa, *C. subinflata* Nelmes, was selected to more readily differentiate between the two St. Helena varieties.

Selected Characters and Specimen Measurements

Characters for morphometrics were selected according to descriptions in the protologues and modern descriptions of St. Helena's flora (Cronk, 2000, Lambdon, 2012). Characters were selected according to what could be readily measured on the available digital specimens. When multiple plants or parts of plants were included on one herbarium sheet, they were considered and measured individually. ImageJ (https://imagej.nih.gov/ij/) was used to measure the majority of specimens (Schneider et al., 2012). Type specimens that were available on the Global Plants site were measured using its image viewer tool due to availability of higher resolution images on the site. Up to ten measurements were taken per quantitative character when possible.

For *C. dianae*, characters were mainly reproductive, as they are generally what distinguishes the two varieties (**Table 4.1**) (Cronk, 2000, Lambdon, 2012). Reproductive characters included male, female, and bisexual spike number, length, and width, and whether the top three spikes were male; and nutlet length, width, and beak length. Spike width was measured in the middle of the spike from the tips of the nutlets, and the bisexual spike measurements were included with the female spike measurements due to difficulty in differentiating between them on the digital specimens. Flowering culm length and whether or not it was substantially longer than the leaves, as well as the length of the stem for the lowest spike and the distance from the lowest spike to the main inflorescence, were also scored, since these are distinguishing characters between the two varieties (**Table 4.1**) (Cronk, 2000, Lambdon, 2012). Leaf length and width were scored for as many specimens as possible. For *P. polifolia*, few characters could be measured based on the size and quality of specimens, and reproductive characters were unavailable. Scored characters included leaf length and width, petiole length, and how revolute the leaf margin was (scaled from 1, slightly, to 3, very revolute). For the *Wahlenbergia* species, characters were selected based on the differences between *W. angustifolia* and *W. linifolia*, as described (**Table 4.2**) (Cronk, 2000, Lambdon, 2012). Quantitative and qualitative characters scored included leaf length, width, margin (gently serrated and toothed or clearly serrated), and tip (mucronate or acute); lower stem texture (rough or smooth), inflorescence stem and individual flower peduncle length, corolla lobe length, entire corolla width, calyx lobe length, and calyx tube width.

Statistical Analyses

Multivariate data were analyzed using PAST v4.03 (Hammer et al., 2001). Principal Coordinate Analysis (PCoA) was used to gauge variation across all variables using the Gower distance measure, since this method allows for the inclusion of both quantitative and qualitative data in the analysis (Gower, 1971). Each set of measurements (each row) was considered a data point, therefore each specimen was represented multiple times, allowing for visualization not only between groups, but within individuals. For rows with missing values, the mean measurement for that specimen was calculated and included in the analysis. Principal Component Analysis (PCA) was used with a variance-covariance matrix to identify which quantitative characters contribute most to the variation. For *Carex*, the St. Helena specimens were grouped according to hypothetical species of *C. aequabilis* or *C. dianae*, as indicated by the collector or according to Cronk (2000). For *P. polifolia*, specimens were grouped according to leaf size. For *Wahlenbergia*, specimens were grouped according to their species determination as either *W. angustifolia* or *W. linifolia*. Statistical significance between groups was calculated using one-way permutational multivariate analysis of variance (PERMANOVA) using a Gower distance measure for the combination of quantitative and qualitative variables and Euclidean distance for quantitative data (Anderson, 2001). Probability values for pairwise comparisons of the multivariate data were adjusted using the conservative Bonferroni correction to avoid false-positive results. Two-sample t-tests were used to determine statistical significance of differences in mean measurements of individual quantitative characters between groups in a genus. Graphs for summary statistics were generated using Minitab 18.

Results

Carex dianae

A total of ten St. Helena *Carex* specimens, and two outgroup *C. subinflata* specimens were included in the analysis. Half of the St. Helena specimens were collected in the 1800s, and the other half were collected in the 1980s. Nine specimens indicated a location, which included on and around Diana's Peak, High Peak, and near a path between Diana's Peak and Cuckhold Point. One specimen indicated that the plant was collected at 2700 ft, the altitude at Diana's Peak. Scoring of spike details was not possible for one specimen, which was not in fruit, and thus difficult to differentiate with certainty whether the spikes were male, female, or bisexual. Spikes were clearly missing for another specimen. Differentiating between entirely female spikes and those with small male sections at the apex was difficult. Leaf length was often not possible to measure (data missing for four out of ten specimens) and typically only one to three leaves per specimen could be measured in full. Urticle characters were the most widely available measurement per specimen (up to ten each), though they could not be measured for three specimens.

Figure 4.4 summarizes the variability in the quantitative measures according to hypothetical St. Helena *Carex* species and includes the South African *C. subinflata* outgroup for comparison. Female spike characters, urticle width, and leaf length clearly differentiate the St. Helena species from the outgroup (**Figure 4.4**). Male spike length ranged widely for both St. Helena groups, largely due to the typical presence of one very long male spike surrounded by several smaller ones, all of which were included in the measurements. Female spike length, which included bisexual spikes, showed similar median values, with more variability in the *C. aequabilis* group. Female spikes were wider in *C. dianae* compared to *C. aequabilis* ($p < .001$), a known differentiating feature between the two varieties. Combining female and bisexual spike measurements as in **Figure 4.4** or keeping them separate had no substantial impact on the analysis. Urticle beak length varied little between individual measurements, hypothetical species, or the St. Helena and South African species, and thus is not a good taxonomic character (**Figure 4.4**). The most variability in the urticle characters measured was in the length, which was the only urticle character statistically significantly different between *C. dianae* and *C. aequabilis* ($p = .005$), however, this may be an artefact due to the difficulty in measuring the length on the digital herbarium specimens (**Figure 4.4**). Besides female spike width and urticle length, no other measures were statistically significantly different between the *C. dianae* and *C. aequabilis* groups.

The PCoA, which included all of the quantitative and qualitative variables showed a separation of the *C. aequabilis, C. dianae*, and *C. subinflata* groups, and differences in these groups as calculated by PERMANOVA were statistically significant ($p < .001$) (**Figure 4.5**). While there was clear separation between the outgroup and the St. Helena groups, there was considerable overlap between the *C. dianae* and *C. aequabilis* groups (**Figure 4.5**). A PCA with only quantitative variables measured in centimeters showed that leaf length and flowering culm length accounted for the most variability between the specimens, although these variables frequently had few measurements (**Figure 4.6**). The first component accounted for 66% of the variance, while the second component accounted for 20%. Including only quantitative characters in a PERMANOVA increased the *p*-value and suggests that qualitative characters contributed substantially to differentiating between the groups ($p = .0167$).

Phylica polifolia

A total of ten individual plants were included in the analysis. There were no modern, digitized specimens of *P. polifolia* available, with all specimens collected from 1775 to 1821. Only one indicated a specific St. Helena location for the collection, which was given as Longwood, therefore it was not possible to test the hypothesis that plants from different locations would exhibit morphological differences. Several of the specimens collected by Forster state that they were collected in New Zealand. This is believed to be an error, questioned even in the protologue for the species, as the plant is endemic to and only occurs on St. Helena (Vahl and Forsskål, 1790, Cronk, 2000). **Figure 4.7** shows the summary statistics and variation within the quantitative measures, of which leaf length is the most variable.

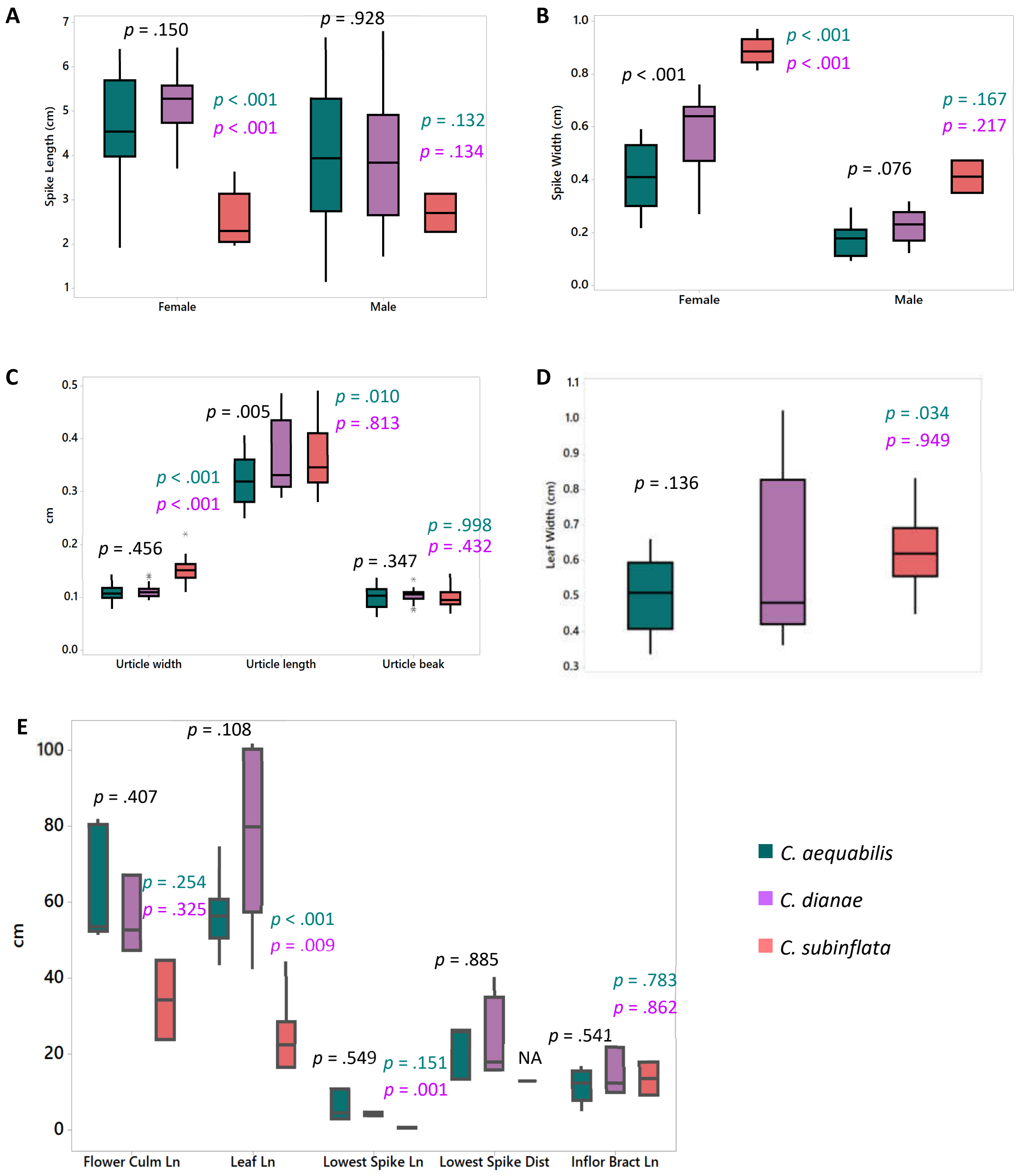


Figure 4.4. Boxplots for quantitative measures of *Carex* specimens included in this study. A) Female and male spike length, B) female and male spike width, C) urticle width, length, and beak length, D) leaf width, E) flowering culm length, leaf length, lowest spike peduncle length, distance from the lowest spike to the base of the terminal inflorescence, and terminal inflorescence bract length. Probability values for t-tests comparing the mean values for each of the groups are shown near each set of boxplots, and *p*-values for comparisons to *C. subinfalta* are color-coded as in the legend. NA, not available due to only one measurement for *C. subinfalta;* Ln, length; Dist, distance.

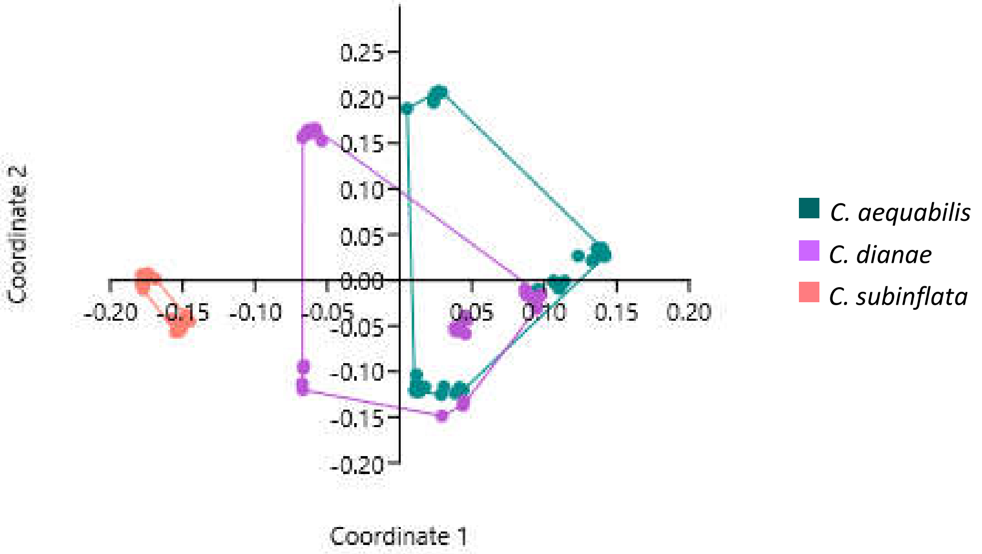


Figure 4.5. Principal coordinate analysis of both quantitative and qualitative characters for the St. Helena *Carex* groups (teal and pink) and the outgroup (salmon). A PERMANOVA test with Bonferroni correction showed that the groups were statistically significantly different from one another ($p < .001$). All specimens had multiple measurements and are represented by multiple points on the graph.

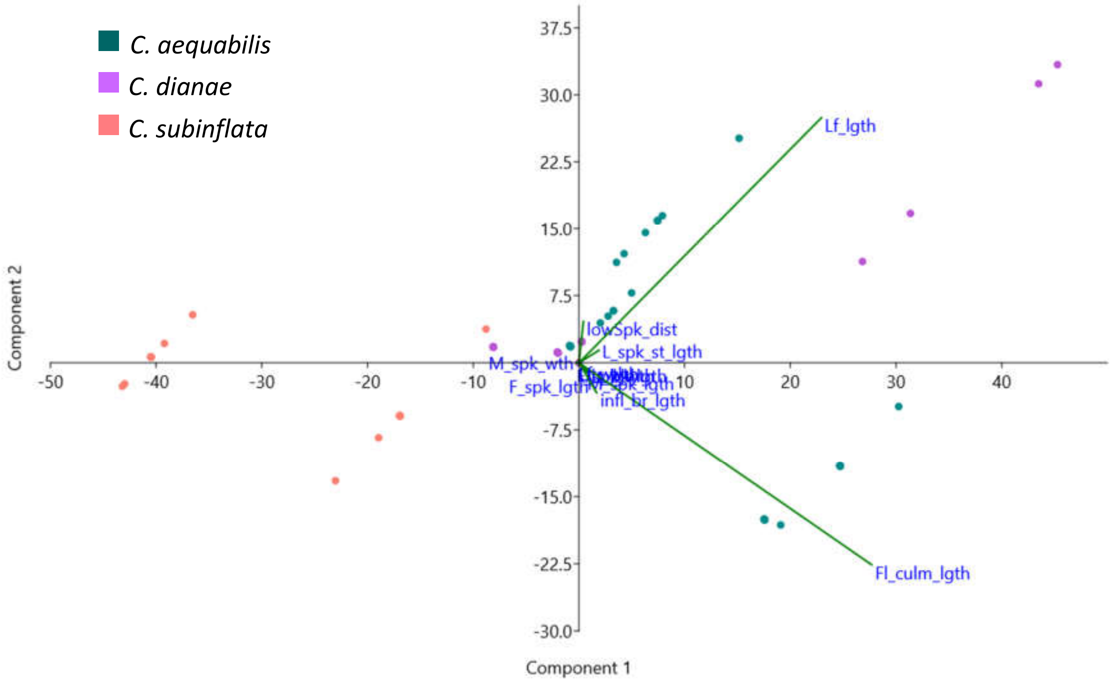


Figure 4.6. Principal component analysis of quantitative characters for the St. Helena *Carex* groups (teal and pink) and the outgroup (salmon color). A PERMANOVA test with Bonferroni correction showed that the St. Helena groups were statistically significantly different from the outgroup ($p = .003$) and compared to one another ($p = .0165$).

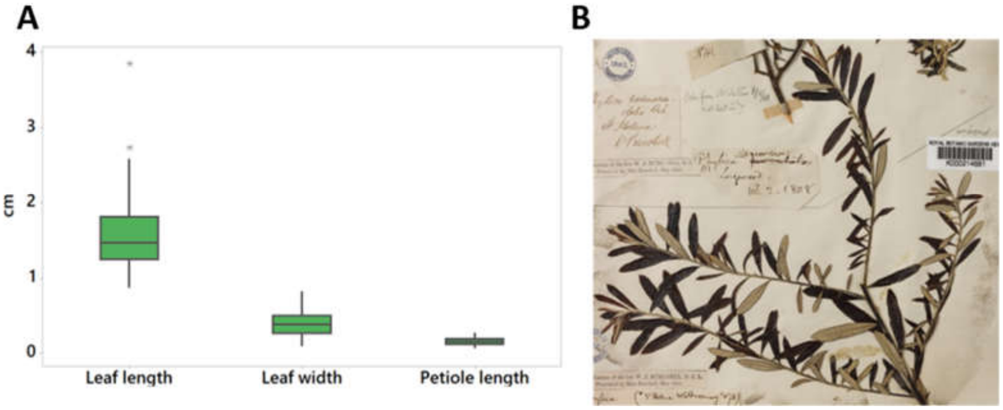


Figure 4.7. A) Box plot showing variation in *P. polifolia* quantitative measures. B) Specimen showing wide variation in leaf length and width on a single plant (Burchell s.n., Kew).

The PCoA showed wide variability among the specimens measured, and the analysis suggests two or three statistically significant different groupings (**Figure 4.8**). These groups are readily visible on the herbarium sheets and can be divided into narrow and ericoid; wide and less revolute; and intermediate-sized leaf forms (**Figure 4.8**). The intermediate group represents only one specimen. Inclusion of this group with either flanking group, or keeping it separate is arbitrary, as all three scenarios produce statistically significant *p*-values. The highest level of significance ($p < .001$) is achieved when including this specimen with the narrow group. Location information was unavailable for the specimens, so grouping according to locality was not possible.

Wahlenbergia Species

A total of nine *W. angustifolia*, and 20 *W. linifolia* specimens were included in the analysis, collected from 1773 to 1890. Locations on St. Helena were specified for three specimens of *W. angustifolia*, including Diana's Peak, High Peak, and Longwood. For *W. linifolia*, Diana's Peak was specified as the location for seven specimens, where it was believed to be prevalent in the past, although the species does not grow there in the present day. Floral characters were unavailable for most specimens and could only be measured in four out of nine *W. angustifolia* and eight out of 20 *W. linifolia* specimens. When present, very few corollas were intact on the specimens, although since both *Wahlenbergia* species have a persistent calyx, calyx lobe length was more readily measurable.

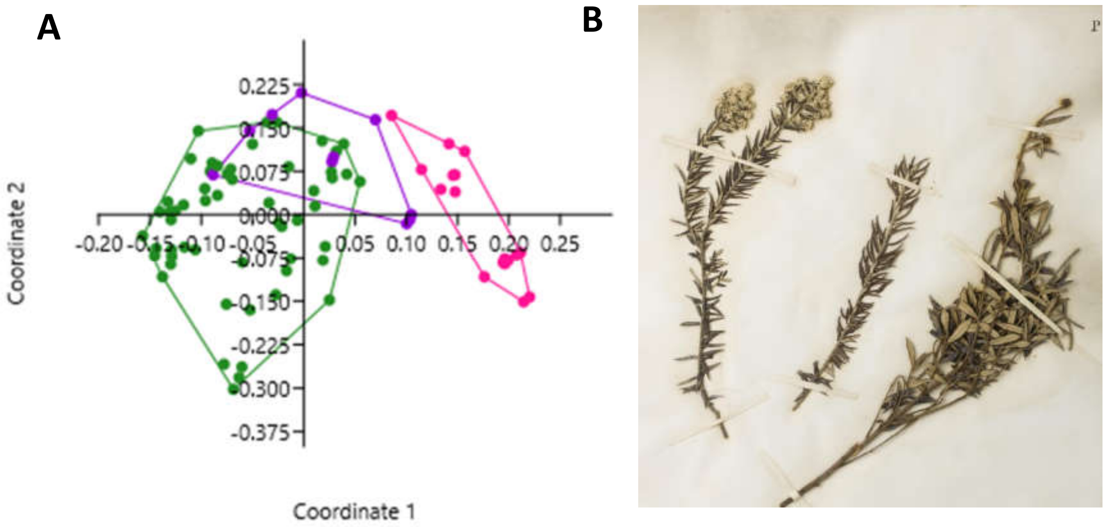


Figure 4.8. A) Principal coordinate analysis of both quantitative and qualitative characters for *P. polifolia* specimens. The middle group in purple is represented by only one specimen, the pink group represents the narrow-leaved form and had two specimens. The green is the wide group, represented by seven specimens in the analysis. All specimens had multiple measurements and are represented by multiple points on the graph. All groups are statistically significantly different from one another in PERMANOVA testing using Bonferroni correction for *p*-value calculations ($p < .001$). B) The narrow-leaved and wide-leaved forms of *P. polifolia* are evident from Hooker s.n. (E).

For *W. angustifolia*, individual flower peduncle length and inflorescence stem length were the most variable quantitative characters (**Figure 4.9**). For *W. linifolia*, the most variability was seen for leaf length and individual peduncle length, although only five measurements were taken for the peduncle (**Figure 4.9**). Comparing the summary statistics for each species, *W. linifolia* has statistically significantly shorter peduncles and flower stems and larger corollas and calyxes compared to *W. angustifolia*, which is consistent with species descriptions, and leaves were longer and narrower in *W. linifolia* compared to *W. angustifolia* ($p < .001$) (**Figure 4.9, Table 4.2**).

The PCoA showed a very pronounced separation of the two *Wahlenbergia* species according to species determination ($p < .001$) (**Figure 4.10**). One specimen (Haughton s.n. from Paris) appears to be incorrectly labeled as *W. linifolia* (**Figure 4.10**). This was evident upon visual inspection, since it lacks the scarred, pine-like lower stem and had lengthy flower stems. The specimen clearly grouped with *W. angustifolia* in the PCoA and was hence labeled as *W. angustifolia* for summary statistics and other analyses. There were no clear intermediates between the species in the analysis, suggesting that there are no hybrids in the historical collections. The PCoA also revealed a cluster of two larger specimens with longer and wider leaves that form a statistically significant group separate from the rest of the *W. linifolia* specimens (**Figure 4.10**).

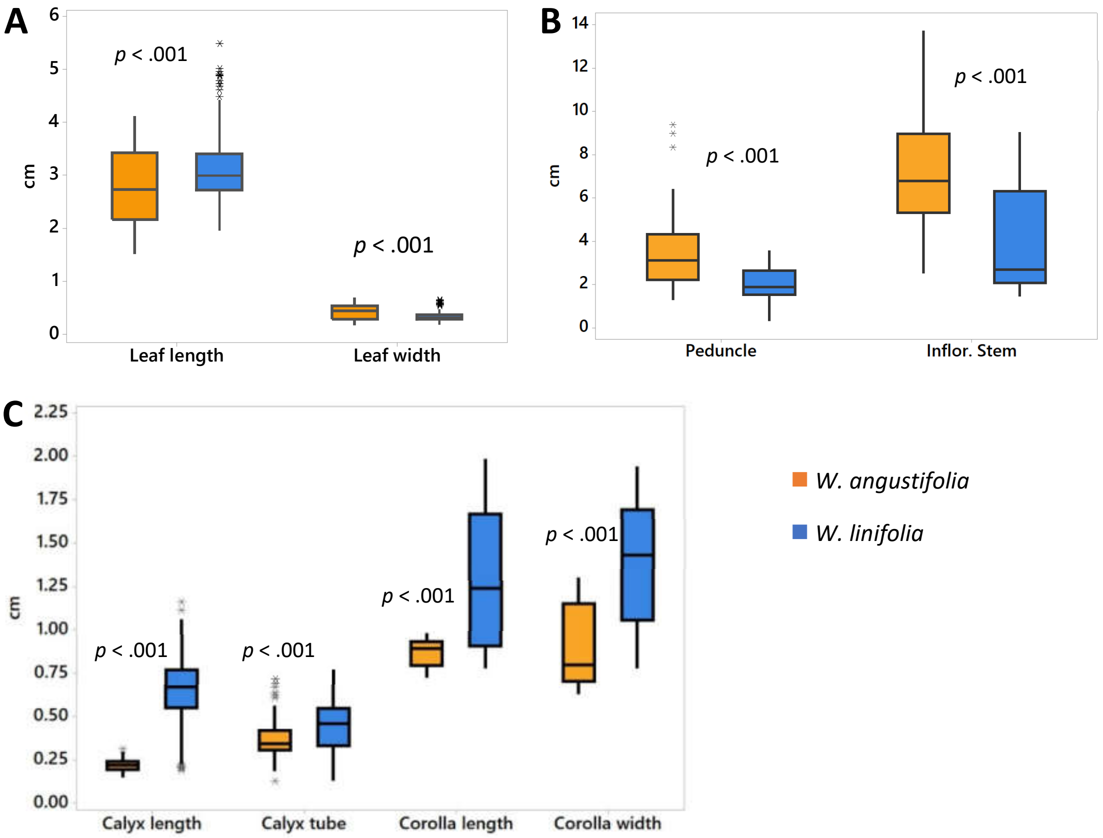


Figure 4.9. Boxplots showing the variability within and between extant St. Helena *Wahlenbergia* species in A) leaf length and width, B) individual flower peduncle and inflorescence stem length, and C) flower characters. Probability values for t-tests comparing the mean values for the two species are shown near each set of boxplots.

A PCA was used to determine which quantitative characters contributed the most to separating out the two species. The first component accounted for 77% of the variance, while the second component accounted for 15%. Not surprisingly, inflorescence stem and individual flower peduncle length were the two characters with the most influence (**Figure 4.11**). The PCA also suggests the importance of the qualitative characters in clearly dividing the species, as the separation was not as clear cut as with the PCoA, which included both quantitative and qualitative variables. The two species were 100% split according to the qualitative characters, with all of the *W. linifolia*, and none of the *W. angustifolia* specimens having rough stem texture, serrated leaf margins, and acute leaf tips.

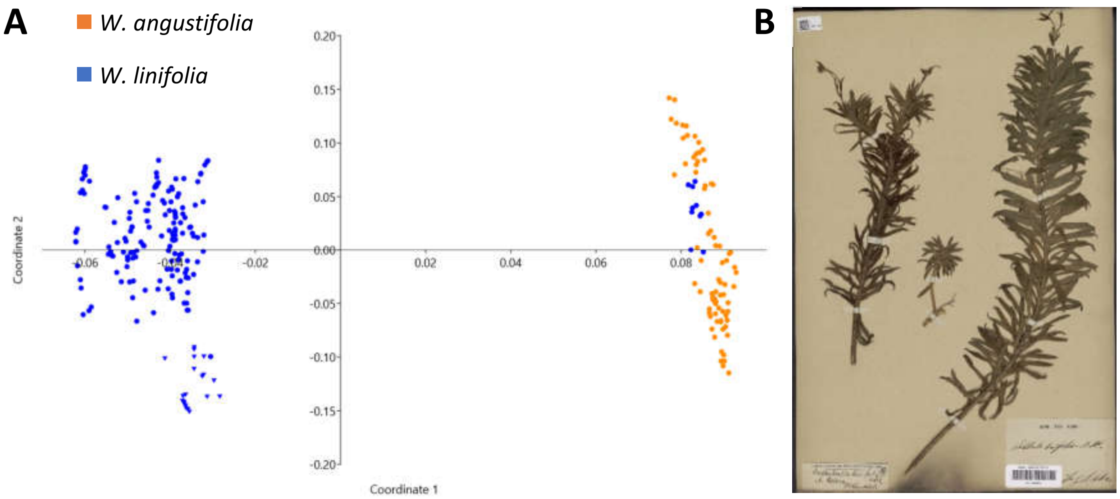


Figure 4.10. Principal coordinate analysis of both quantitative and qualitative characters of *W. linifolia* (blue) and *W. angustifolia* (orange). A PERMANOVA test with Bonferroni correction showed that the two groups were statistically significantly different from one another ($p < .001$). A specimen misidentified as *W. linifolia* (blue) is evident in the *W. angustifolia* group (orange). The cluster of blue inverted triangles at the bottom left represents two larger specimens of *W. linifolia* with longer and broader leaves, which as a group is statistically significantly different from the rest of the *W. linifolia* specimens according to a PERMANOA test ($p < .001$). B) A specimen of the larger-leaved form of *W. linifolia* is evident from Hooker s.n. from the Royal Botanic Garden Edinburgh.

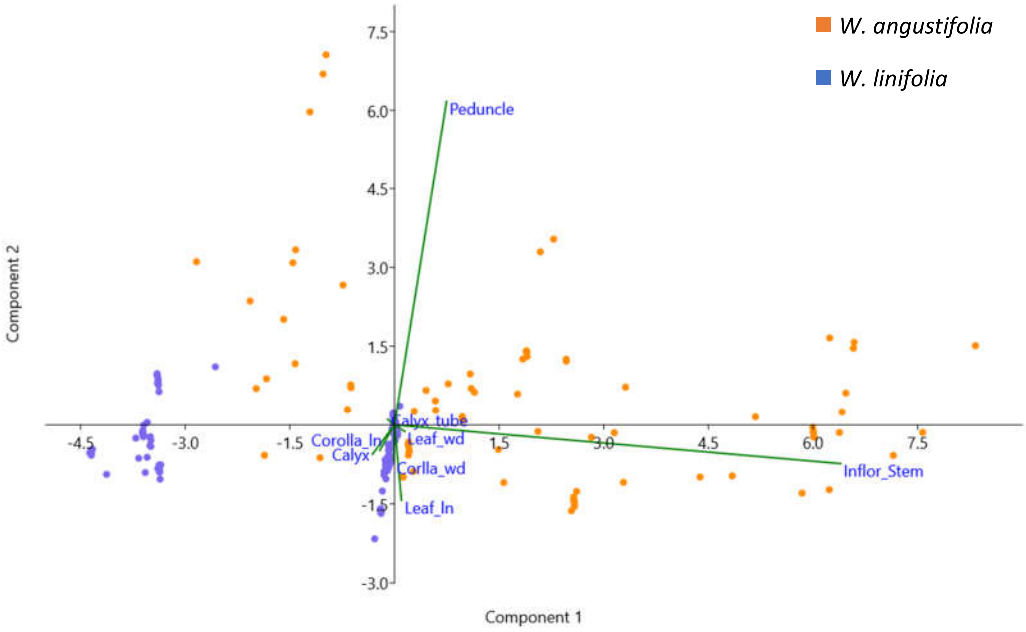


Figure 4.11. Principal component analysis of quantitative characters for *W. angustifolia* (orange) and *W. linifolia* (blue). A PERMANOVA test with Bonferroni correction showed that the two groups were statistically significantly different from one another ($p < .001$).

Discussion

The current morphometrics study developed a set of characters using digitized herbarium specimens that can differentiate between species (*Wahlenbergia*) or different forms of three endemic genera on St. Helena. Since the use of common garden trials was unavailable during this time, herbarium specimens were used to gauge the variation in historical specimens and to establish a character set for future study. These characters can subsequently be used to assess variation in common garden trials from seeds collected from wild populations, and changes from historical specimens can be recorded and assessed.

Carex dianae

Several characters that define the two *C. dianae* varieties were difficult or impossible to measure on the available digital specimens (**Table 4.1**). For example, one defining characteristic is whether the lowest spike is nodding or upright, which cannot be determined from a pressed specimen. Another important defining characteristic is whether the flowering culm is longer than the leaves, which was difficult to judge with certainty, as leaves were frequently missing or trimmed on the specimens. These characteristics would be much more readily visible in living populations, a limitation of using only herbarium specimens. Female spike width, a defining character for the varieties, was indeed wider for *C. dianae* var. *dianae* compared to *C. dianae* var. *aequabilis*, as expected (**Figure 4.4**).

The PCoA showed a clear differentiation between the outgroup species, *C. subinflata,* and the St. Helena endemic *Carex* specimens, though there was some overlap between the two St. Helena groups (**Figure 4.5**). Differences between all groups were statistically significant. However, if the two varieties of *C. dianae* represent two distinct *Carex* species, one would expect a clearer differentiation without overlap between them, as seen with the *C. subinflata* outgroup compared to the St. Helena *Carex*, as well as with the *Wahlenbergia* species (**Figure 4.10**). One word of caution is about the missing data for several specimens, which has been shown to bias results (Borges et al., 2020). Lambdon (2012) has suggested that the morphological differences expressed by *C. dianae* var. *aequabilis* may be in response to the environment in which it is found, as it tends to be in shadier and more sheltered places on High Peak, compared to *C. dianae* var. *dianae* growing along Diana's Peak. Only one specimen was collected from High Peak, whereas the other putative *C. aequabilis* specimens were collected at various places along a path between Diana's Peak and Cuckhold Point. It is possible that specimens from High Peak would more clearly separate out from those collected around Diana's Peak. However, it is also possible that *C. dianae* exists along a gradient of forms rather than two individual species or varieties, and the morphological differences represent natural variation within the population.

Although the analysis in this study can be interpreted that *C. dianae* represents either one morphologically variable species, or at most two varieties as currently accepted, additional research is needed to confirm these findings with certainty. Conducting morphometrics on present-day plants in the field would reduce the collection bias of specimens available in herbaria, which may not be entirely representative of the populations. Seeds from the two different populations can be grown in common garden trials to rule out the effect of environmental conditions and see if differences in morphology persist and are consistent. Molecular studies can also be used to confirm findings from the garden trials and further assess genetic variation in the populations and status of variants or species. Whether or not *C. dianae* represents two different species, variants, or one diverse species has important implications for conservation on the island. If it is one species, the morphological variation suggests that the population has a healthy level of genetic variation, supporting its IUCN Red List status as a species of least concern. Additionally, it means that if subpopulations become cut off in the future, seeds from any plants can be used to connect them, since there are no concerns for local adaptation issues or maintaining unique variants. Conversely, if *C. dianae* is found to be two different *Carex* species, current populations numbers need to be recalculated for each species and IUCN Red List status re-evaluated to protect the species with the smaller population.

No specimens from the subpopulation of *C. dianae* at the Barn were identified in the herbaria searched. Since most of the population of *C. dianae* grows at a higher elevation along the Central Ridge in the middle of the island, the population at the Barn would be interesting to study in detail. The Barn is a hill on the north coast with a maximum altitude of 616 m, compared to the ridge, which is 800 m and above. It is separated from the main population by approximately 11 km, likely limiting genetic exchange between the populations, especially given that neither seeds nor pollen are predicted to travel long distances (Lambdon, 2012). It would seem more likely that the population at the Barn is morphologically, and potentially genetically different than the populations at Diana’s Peak or High Peak, both due to the isolation of the population and the coastal, lower-altitude environment. Future studies to determine whether or not there are morphological differences between the populations, confirmed with common garden trials and genetic studies, would result in interesting and valuable insights into the *Carex* population on the island. If the subpopulation does show substantial differences, conservation efforts should be undertaken to protect it as distinct from the *C. dianae* population at large.

Phylica polifolia

Analysis of the herbarium specimens, confirmed by PCoA, revealed two to three different forms of *P. polifolia*, suggesting variation within the population historically. This would be an interesting subject for a paleogenomics study to determine if there are genetic differences underlying the morphological differences observed in the specimens (Kistler et al., 2020). As no modern specimens were available for the analysis, it is unknown what the variation in today's populations is like. However, the modern population more closely resembles the more narrow-leaved form, with a leaf length of up to 3 cm (Lambdon, 2012). Currently *P. polifolia* is limited to three populations, located at Lot, High Hill, and Ebony Point (Lambdon and Ellick, 2016h). The herbarium specimens did not provide the collection location or detailed notes about the specimens, although it is possible that the specimens with different morphology were found in different locations. However, as both narrow and wide-leaved forms were found on the same herbarium sheet by the same collector, it is also possible that they were collected in the same location and represent the different morphological forms observed in the area where they were collected. One study suggested that the populations at Lot and High Hill have genetic differences, and described differences in growth forms from prostrate to upright (Richardson et al., 2003). Common garden trials with seeds from these two populations can determine whether or not the difference in growth form is genetic or due to environmental influence. As only small portions of the plants were preserved on the herbarium specimens and no notes on habit were recorded, it is not possible to speculate whether or not the different forms identified represent either of these modern populations.

The modern population of *P. polifolia* is in much need of intensive conservation efforts. Since the species is present in such limited numbers and in highly fragmented populations, it is a good candidate for genetic rescue. It has been suggested that the population at Lot is uniform, and may represent a single colonist to the area (Lambdon, 2012). Lot and High Hill are separated by approximately 11 km, so it is likely that gene flow between these populations is limited. If this is the case, the introduction of genetic variation into both populations is crucial to ensure the resilience and survival of the species.

Wahlenbergia Species

Both PCoA using a mixture of qualitative and quantitative measures as well as PCA with only the quantitative measures show a robust set of characters for differentiating between *W. angustifolia* and *W. linifolia*. These characters should therefore be able to identify hybrids between the two species, which would appear as intermediates (**Table 4.2**). The current analysis of herbarium specimens from the 1800s or earlier did not find any evidence of hybrids between *W. angustifolia* and *W. linifolia*, despite collections of both species from the same location (Diana's Peak). This does not entirely rule out the presence of hybrids in the past, only that none were collected among the specimens analyzed. However, hybridization between the two species may not have been prevalent in the past, because population levels of *W. linifolia* were estimated to be much higher than they are now, and suitable pollen donors of the same species were likely readily available. Although the current population of *W. angustifolia* numbers in the thousands, there are estimated to be less than 50 individual *W. linifolia* plants left in the wild (Lambdon and Ellick, 2016l, Lambdon and Ellick, 2016k). Additionally, several members of the *Wahlenbergia* genus are known to be self-incompatible, including species in South Africa (Welsford and Johnson, 2012, Lloyd and Jocelyn, 1982). Although self-incompatibility status is unknown for the St. Helena *Wahlenbergia* species, the anthers of *W. angustifolia* dehisce in bud, restricting pollen access to the stigma (Lambdon, 2012). This phenomenon is likely present in *W. linifolia*, which has similar flowers. The very small population number coupled with the possibility that the flowers are self-incompatible likely greatly increase the possibility of *W. linifolia* hybridizing with the related *W. angustifolia* today.

There are several possible explanations for the two larger *W. linifolia* specimens collected from Diana's Peak. One is that they may simply represent variation within the population at the time. It is known that *Wahlenbergia* had varying morphotypes historically, exemplified by the now-extinct, but dubious *W. roxburghii*, which Burchell named to divide variants of the also extinct, *W. burchellii* (Lambdon, 2012). The larger specimens in the collection may have been growing under more favorable conditions, thus responding to light, nutrients, or lack of competition, and may have stood out to the collectors. An additional possibility is that these specimens may be hybrids with *W. burchellii*, which reportedly had larger leaves and was also found on Diana's Peak during the time period when the collections were made.

As the selected characters clearly separated out the two *Wahlenbergia* species, they can potentially be used to check for hybrids in present-day populations. Hybrid status could be confirmed with molecular tools, such as Random Amplified Polymorphic DNA (RAPD) or genome sequencing, and cytology. The very small, fragmented population and the suspicion that *W. linifolia* may either be self-incompatible or have low reproductive success with self-pollination, make it a good candidate and a priority for genetic rescue. Studying the reproductive success of self-fertilization and the potential that the species is self-incompatible would add valuable knowledge about the biology of the species and avenues for conservation. Self-incompatible plants derive the most benefit from genetic rescue, as they are unable to reproduce without compatible pollen donors, except potentially through hybridization (Frankham, 2015). Before embarking on genetic rescue attempts for this plant, it is particularly important to verify that pollen donor plants are indeed *W. linifolia*, and not a hybrid of the two species.

Study Limitations

The study was restricted to available digitized herbarium specimens, which had inherent constraints, including a limited ability to fully appreciate the dimensionality of the plant or to move structures that obscure features for more accurate measurements, and no access seed packets. Obscured features and low-resolution images are the most common limitations with using digitized specimens, however, measurements have been shown to be as precise as when directly measuring specimens, especially for larger features (Borges et al., 2020). The quality of some of the specimens was deteriorated and some were missing components, limiting their use. Additionally, the process of pressing the plant may have distorted some features, particularly the thick *Carex* spikes, and would not have captured the nodding versus upright form of the lower spikes. Measuring the long leaf blades of *Carex* was difficult and sometimes not possible, as they were often cut off, and in some cases deteriorated. Certain distinguishing features, such as yellow coloration on the corollas of *W. angustifolia*, and glands on nutlets of *C. dianae* var. *aequabilis* could not be used with the digitized dried specimens, although some additional detail for the nutlets might be possible with a dissecting scope on herbarium specimens in person. The collections frequently lacked details describing the habit or habitat of the plant, and often included only portions of the plant which would easily fit on the herbarium sheet. The specimens also lacked information on locations, so although one can speculate that different forms were collected in different areas, it is impossible to know for certain. All of the *Phylica* and *Wahlenbergia* specimens were from the 1800s or earlier, and the *Carex* specimens were either from one collector in the 1980s, or from the previous century, so analysis of variation in current populations was not possible in this study. Collection bias may also be reflected in the specimens, as plants that stood out (for example, the larger *Wahlenbergia* or narrower *Phylica*) or those that were easy to collect may have been preferred by the collectors. Today many of St. Helena's endemics are restricted to difficult-to-access areas on cliff faces, and even by the 1800s, most of St. Helena's endemics were restricted to the central ridge on the island, likely with populations in inaccessible areas (Melliss, 1875). Finally, morphometrics with herbarium specimens represents only a snapshot of a particular time and specific growing conditions of when those specimens were collected. Common garden trials can confirm whether morphological differences are due to genetics or differences in environment.

Future Directions and General Implications for Conservation on St. Helena

The quantification of variation in historical specimens via morphometrics can be used to compare to modern populations and serve as a baseline for variation within the population. This can help to answer the question of whether or not the inherent variation within a population has been maintained or lost over time. In the approximately 200 years since the analyzed specimens were collected, the habitats for many endemic plants on St. Helena have further deteriorated, and population numbers have been drastically reduced. Many of the island's current plant populations are now present only in small numbers, and they have become severely fragmented. Of the plants studied for the current analysis, this is especially true for *P. polifolia* and *W. linifolia*. These species, and many others on the island have likely experienced a loss of genetic variation through genetic drift and inbreeding depression. Erosion of genetic variability can result in a lower ability for adaptation, putting already vulnerable species even more at risk. Thus conservation efforts are especially critical, and can be directed towards increasing genetic diversity in the populations. This can be accomplished via improving gene flow by connecting populations with supplemental plantings or through genetic rescue attempts. Intensifying existing conservation efforts to protect the endemic flora, such as keeping invasive plants in check, is also an important step for preserving the future of these unique species.

Chapter 5: Climate Change and the Future of St. Helena's Babies' Toes and Lowland Desert Habitat

Introduction

With the threat of climate change looming large over the planet, islands are believed to face a potentially greater share of the negative impacts from it, especially considering the relatively large number of endemics found on islands (Kier et al., 2009). When island endemic species become extinct, it is not only a loss for the island, but a global loss as well, since these species were found nowhere else on the planet. The disproportionate impact of climate change on small islands is also heightened considering the relatively minor contribution of small island developing states towards the problem compared to larger and more economically advanced countries, as well as the potentially devastating effects of severe weather events on their economies (Nurse et al., 2014, Thomas et al., 2020). Although they are important systems for study, modeling future climate change scenarios on small islands remains problematic (Foley, 2018, Fernandes and Pinho, 2017). The General Circulation Models typically have a resolution too large to account for small islands, which appear as more ocean in the models (Foley, 2018). Although regional climate models accounting for the local island topology can be used for downscaling, they still rely on the larger models which lack adequate detail, and statistical downscaling misses important island features (Foley, 2018). All climate models are ultimately hypotheses, however, they can provide a clue about possible changes and challenges in the future.

As a location with many unique and endangered endemic plants and invertebrates, St. Helena is a prime example of a small island susceptible to detrimental effects from climate change. At a size of only 122 km^2, with steep and rugged terrain and extremes in annual precipitation ranging from 175 mm in the dry areas to 1050 mm on the peaks, the island is too small to be modeled with the global climate data, which do not account for these varying microclimates in such a small land mass. In addition to climate change, St. Helena's endemic species will also be contending with increased globalization and tourism in the future. Although humans have been accessing the island for more than 500 years, the opening of an airport to commercial flight in 2017 offers considerably easier access to St. Helena, and will bring its own set of economic benefits for the island in addition to environmental challenges.

Much of the conservation focus on St. Helena has revolved around the remnant cloud forests, home to the majority of the island's endemics and an important fresh water source due to mist capture at the high elevation. Other major efforts have focused on preserving the island's iconic *Commidendrum* Burch. ex DC. species, the primary constituent of the planted Millennium

Forest. However, the lower altitude desert areas also contain important endemic plants, such as *Hydrodea cryptantha* and *Pelargonium cotyledonis* (L.) L'Hér., in addition to many endemic lichens and invertebrates that may suffer negative consequences due to climate change and increased tourism.

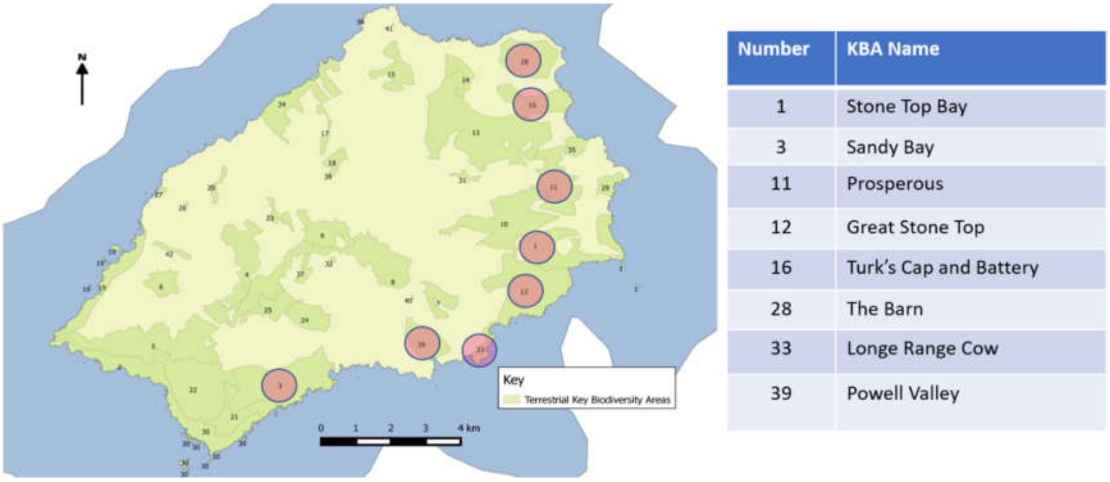
The aims of this chapter are to outline the potential impact of climate change and increased tourism on *H. cryptantha* and other species in St. Helena's lowland arid habitats; and to propose actions to address issues related to climate change and tourism on the island, as well as areas for further research.

St. Helena's Babies' Toes: *Hydrodea cryptantha*

Hydrodea cryptantha, or "babies' toes" as it is referred to in St. Helena, is a member of the Aizoaceae and is considered to be endemic to the island (**Figure 5.1**) (Cronk, 2000, Lambdon, 2012). A charismatic species, it has appeared on St. Helena stamps, phone cards, and book covers. Melliss (1875) described it as being "so very succulent that it will not support its own weight" and that "water is seen to drop from it when simply carried in the hand without any pressure". The seasonal annual plant appears after winter rainfall and turns from bright green to yellow as it dries out and ages in the heat of the summer. Numbers of the plant vary each year, thought to be correlated with the amount of rain during the winter (Lambdon, 2012). As of writing, it is one of the island's few endemic plants not considered threatened on the IUCN Red List.

Figure 5.1. *Hydrodea cryptantha*, known on St. Helena as babies' toes. Image credits: Left, St. Helena Tourism (image cropped to show detail), Right, Melliss, 1875.

As with other members of the Aizoaceae, *H. cryptantha* can withstand high salt levels, and thus grows in the otherwise barren, arid areas of the island. It is located along the southern coast of St. Helena, spanning from Turk's Cap down to Devil's Hole, generally at an altitude of 200 m and below, though it does grow at higher elevations in some locations (Lambdon, 2012). *Hydrodea cryptantha* is found in nine recently delineated Key Biodiversity Areas (KBAs), two of which are considered high priority, and five of which are wholly protected (**Figure 5.2**) (Taylor, 2016). The other endemic plants commonly found in KBAs with *H. cryptantha,* such as *Commidendrum rugosum* (Aiton) DC., *Kewa acida* (Hook.f.) Christenh., and *Frankenia portulacifolia* (Roxb.) Spreng. are listed in **Table 5.1**. Other species sharing some of the KBAs with *H. cryptantha* include St. Helena's only extant endemic bird, *Charadrius sanctaehelena* (St. Helena Plover), known locally as the Wirebird, and many endemic invertebrates (Gray et al., 2019, Mcculloch, 2009).



Number	KBA Name
1	Stone Top Bay
3	Sandy Bay
11	Prosperous
12	Great Stone Top
16	Turk's Cap and Battery
28	The Barn
33	Longe Range Cow
39	Powell Valley

Figure 5.2. Key Biodiversity Areas on St. Helena where *H. cryptantha* grows. Image and data from Taylor, 2016.

Taxonomic Issues

Although *H. cryptantha* is considered endemic to St. Helena by individuals who study the island's flora, a taxonomic revision in the 1960's combined all members of the *Hydrodea* genus into one species in the *Mesembryanthemum* genus, thus the currently accepted name is *M. cyrptanthum* (Cronk, 1987, Lambdon, 2012, Lambdon and Cronk, 2020). However, authors of recent floras for St. Helena retain the *Hydrodea* genus to distinguish the St. Helena species from the African species, a convention retained here (Cronk, 1987, Cronk, 2000, Lambdon, 2012). The African species include *H. bossiana* Dinter from Namibia and *M. forskahlii* Hochst. ex Boiss. from Egypt. As currently delimited, *M. cyrptanthum* has a native range across northern and southern

Africa, including Algeria, Angola, Cape Provinces, Egypt, Gulf States, Libya, Namibia, Palestine, Saudi Arabia, Sinai, and St. Helena (POWO, 2019). The circumscription of the *Mesembryanthemum* genus itself has been problematic, and phylogenetic analyses have been contentious (Klak and Bruyns, 2013, Gerbaulet, 2012). However, no recent revisions of *Mesembryanthemum* or phylogenetic analyses of the Aizoaceae have directly considered or sampled the species from St. Helena.

Table 5.1. Other endemic plant species that are found in more than one Key Biodiversity Area with *H. cryptantha.*

Species	Common Name	Number of KBAs shared with *H. cryptantha*	IUCN Red List Category
Commidendrum rugosum	St. Helena scrubwood	6	VU
Kewa acida	Salad plant	5	CR
Ceterach haughtonii	Barn Fern	4	CR
Eragrostis episcopulus	Cliff Hair Grass	4	CR
Osteospermum sanctae-helenae	St Helena Boneseed	4	LC
Panicum joshuae	Rock Millet	4	VU
Bulbostylis lichtensteiniana	St Helena Tufted sedge	3	LC
Chenopodium helenense	St Helena Goosefoot	3	VU
Euphorbia heleniana	St Helena spurge	3	CR
Frankenia portulacifolia	St Helena Tea Plant	3	CR
Pelargonium cotyledonis	Old Father Live Forever	3	CR

KBA, Key Biodiversity Area; VU, vulnerable; CR, critically endangered; LC, least concern

Current Threats

Hydrodea cryptantha and its co-inhabitants historically faced and continue to face several threats on St. Helena, including habitat destruction and competition from invasive species. The new airport on the island was recently built in an area where *H. cryptantha* grew. Although an online government report describes transplanting some of these plants, as well as preserving some lichen and biocrusts, there was no available follow-up report on the success of the translocations (St. Helena Landscape & Ecological Mitigation Programme, 2015). A major current threat comes from competition from another Aizoaceae, *Carpobrotus edulis* (L.) N.E.Br., known in St. Helena as creeper. This highly invasive species forms dense mats that exclude any other plants from colonizing the area, and the arid, eroded regions of St. Helena covered in *C. edulis* mats are referred to as "creeper waste" (Cronk, 1989). Additionally, this species concentrates salinity and alters the soil pH to render it more alkaline, further prohibiting other less-tolerant species from growing near it, a problem for succession to more diverse vegetation cover (Lambdon, 2012).

The Potential Impact of Climate Change on St. Helena

Climate Change Scenarios for St. Helena

Studies on climate change modeling in the South Atlantic are lacking. No studies have comprehensively addressed climate change scenarios on St. Helena, accounting for the island's diverse topology and varying microclimates. A report on UK Overseas Territories by the Joint Nature Conservation Committee suggested that St. Helena will experience warmer temperatures, a small decrease in rainfall, and possible periods of drought, flooding, altitudinal shifts in vegetation, and further erosion (Brown, 2008). Another preliminary report on climate change in UK Overseas Territories reported a relative decrease in precipitation of 4% to 7% by 2035-2065 compared to 1961-1990, according to a mean of model ensemble for RCP2.6 or RCP8.5, respectively (Wade et al., 2015). A study compiling approximately 100 years of climate data from St. Helena from 1893 to 1999, normalized to the altitude at Bottoms Woods (400 m), reported trends of precipitation decreasing by 10 mm/100 year, air temperature increasing by 0.9°C/100 year, and air pressure decreasing by 0.6 hPa/100 year (Feistel et al., 2003).

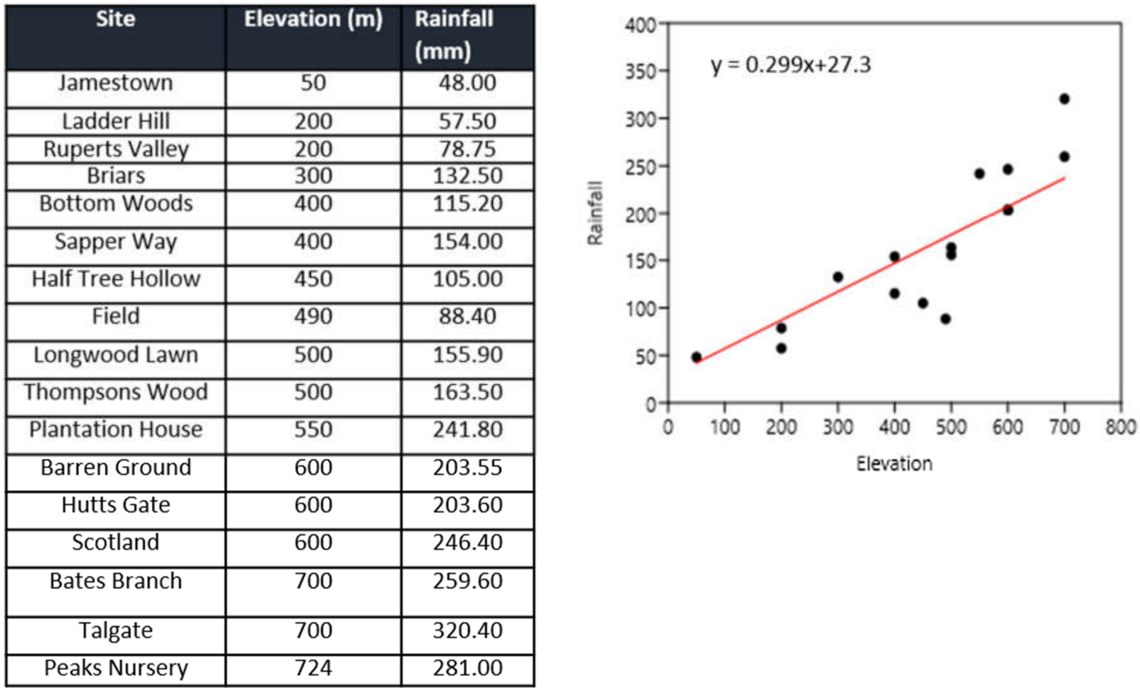
One study, acknowledging the limited resolution of climate models for small islands, calculated the predicted change in aridity for islands around the world (Karnauskas et al., 2016). The aridity change index considers not only precipitation, but also accounts for evaporative potential, and was defined as the ratio of the fractional change in potential evaporation to the fractional change in precipitation (>1 is drier). For St. Helena, the aridity change index was predicted to be 1.06 in 2050, increasing to 1.2 in 2090, suggesting an increasingly drier environment on the island in the future (Karnauskas et al., 2016). Another island-focused study looked at island vulnerability to rising sea levels, rainfall, and temperatures (Veron et al., 2019). The study did not include St. Helena, and the closest island that could be used to extrapolate to St. Helena is Ascension Island, which lies 1200 km northwest of St. Helena. Ascension Island was predicted to have low vulnerability for sea level rise and temperature, and moderate vulnerability for rainfall (Veron et al., 2019). Arid regions are predicted to be the most vulnerable to climate change and increase in desertification, a potential issue for the vast arid regions of St. Helena (Huang et al., 2016). While limited, altogether the available projections suggest that St. Helena on the whole will face a warmer and drier future with climate change.

Due to its diverse topology and interactions with the trade winds, St. Helena experiences multiple microclimates. The mist-shrouded peaks are wet, while the low-lying areas get very little rainfall. Rainfall data collected in various locations on the island in January of 2017 show a fairly linear relationship between precipitation and elevation, as determined by ordinary least squares linear regression (**Figure 5.3**) (Sansom et al., 2019). However, compared to historical weather data from Bottom Woods, this particular dataset showed more than average rainfall during the summer when it was collected. Thus the resulting equation cannot be used to estimate the monthly rainfall at lower altitudes from the historical precipitation data from Bottom Woods (400 m elevation) (Feistel et al., 2003). Additionally, although the relationship between altitude and precipitation is fairly linear for this limited dataset, as Cronk (2000) pointed out, there are noted discrepancies for rainfall observations on the island, such as less than expected precipitation at the Barn and more at Upper Sandy Bay. Further, mist collection is an important component of precipitation on the island, with endemic plants such as *C. robustum* effectively capturing low mist in its circular canopy in areas with less frequent rainfall (Cronk, 1989, Sansom et al., 2019).

Using the currently available data of average monthly precipitation from 1961 to 1990 from Bottom Woods and applying a 4 to 7% decrease in precipitation as estimated by Wade et al. (2015), shows the potential rainfall amounts predicted for 2035-2065 at approximately 400 m altitude (**Table 5.2**) (Feistel et al., 2003). Presumably, these amounts will decrease further when moving down in elevation toward sea level, as suggested by **Figure 5.3** (Sansom et al., 2019). Major remaining questions concerning precipitation on St. Helena in the future include how the rainfall will be distributed over the year, and the frequency and severity of severe weather, such as droughts, storms, and floods. Additional rainfall measurements and mist collection, preferably collected over a span of years from various altitudes and locations, would be useful for future modeling of precipitation on the island at different elevations.

Potential Impact on H. cryptantha

Lower amounts of rainfall or droughts, especially during the winter, would likely impact numbers of *H. cryptantha* the following spring, as there are generally larger flushes of the plant with wetter winters (Lambdon, 2012). Seeds of members of the Aizoaceae family typically harbor germination inhibitors that require enough rainfall to wash them out and allow the seeds to germinate (Fearn, 1981). Extended periods of winter drought may therefore result in little to no *H. cryptantha* germination. As more severe weather extremes are predicted with climate change in the future, extended periods of drought are an increasingly real possibility. Likewise, if winter rainfall were to be distributed as a quickly-evaporating drizzle, adequate moisture to wash away the germination inhibitors in the seeds may not be available.



Site	Elevation (m)	Rainfall (mm)
Jamestown	50	48.00
Ladder Hill	200	57.50
Ruperts Valley	200	78.75
Briars	300	132.50
Bottom Woods	400	115.20
Sapper Way	400	154.00
Half Tree Hollow	450	105.00
Field	490	88.40
Longwood Lawn	500	155.90
Thompsons Wood	500	163.50
Plantation House	550	241.80
Barren Ground	600	203.55
Hutts Gate	600	203.60
Scotland	600	246.40
Bates Branch	700	259.60
Talgate	700	320.40
Peaks Nursery	724	281.00

Figure 5.3. Rainfall measured at different locations on the island shows a linear relationship with elevation Sansom et al., 2019.

Table 5.2. Average monthly rainfall (mm) from 1961-1990 (baseline) at Bottom Woods, and a predicted 4% and 7% decrease by years 2035-2065 (Feistel et al., 2003, Wade et al., 2015).

	Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Baseline	29.4	58.2	44.1	43.6	55.6	58.0	45.2	32.7	22.0	16.5	18.3
4% Decrease	28.2	55.9	42.4	41.8	53.4	55.7	43.4	31.3	21.1	15.8	17.5
7% Decrease	27.3	54.2	41.0	40.5	51.7	53.9	42.1	30.4	20.5	15.3	17.0

In the face of possible long-lasting droughts, an important question pertains to the length of time for which the *H. cryptantha* seedbank would remain viable in the soil. Information from the species formerly known as *H. bossiana* in southwestern Africa, now grouped with the other *Hydrodea* as *M. cryptanthum,* may provide a clue for St. Helena's babies' toes, especially as the two are now considered taxonomically to be the same species. A winter of heavy rains in 1934 brought large numbers of *H. bossiana* to the otherwise barren Namib plain, where local people could not remember previously having ever seen the plant (Fearn, 1981). As Fearn (1981) put it, "seeds of this plant would appear to hold the record for longevity amongst the succulents", a very promising prospect for St. Helena's *H. cryptantha* if faced with long periods of drought.

Potential Impacts on Other Species and Habitat in General

Of the 11 endemic plant species sharing at least one KBA with *H. cryptantha*, six are critically endangered and two are listed as vulnerable on the IUCN Red List (**Table 5.1**) (IUCN, 2020). Two species, *K. acida* and *E. episcopulus* are predicted to have extinction half-lives of less than 50 years (**Figure 5.4**) (Lambdon and Cronk, 2020). These species may be especially vulnerable to impacts from climate change, the effects of which could be catastrophic given their imminent predicted extinction. The endemic flora is projected to experience altitudinal shifts with climate change as species migrate to higher altitudes for more access to water (Brown, 2008). This may result in loss of plants as competition for viable habitat increases, as well as causing additional fragmentation of many already-divided species populations. Fragmentation can lead to decreased gene flow and therefore decreased genetic variation, potentially further reducing species' ability to adapt to changing conditions. Additionally, while there are many protected areas on St. Helena, and the delineation of KBAs may lead to more protected land, these areas are scattered and not directly connected to one another.

Figure 5.4. *Kewa acida*, known as Salad Plant (A), and *Eragrostis episcopulus*, known as Cliff Hair Grass (B), grow in arid regions and are in danger of becoming extinct. Image credits: A) © P. Lambdon, 2008 B) © P. Lambdon, 2013

In addition to plants, the effects of climate change will impact other organisms inhabiting the arid lowland habitat. There are over 200 species of lichen on St. Helena, and the semi-desert area is home to many of the endemic lichen species on the island (Aptroot, 2008). For example, there are estimated to be at least four endemic *Ramalina* species, which are found in Prosperous Bay Plain, Little Stone Top, and the Barn (Aptroot, 2008). There are also many unique and endangered invertebrates on St. Helena, some of which, such as the St. Helena Mole Spider, have not yet been formally described, and thus cannot be protected on the IUCN Red List (Gray et al., 2019). Climate change is also expected to impact the soil microbiome, which plays important roles in nutrient cycling as well as maintaining complex relationships with plants (Jansson and Hofmockel, 2020). The resilience of the soil microbiome to periods of drought and rewetting, both in terms of composition and chemical activity, and the subsequent consequences on plants and other higher organisms within the ecosystem is unknown (Jansson and Hofmockel, 2020).

Potential Impacts and Challenges Stemming from Tourism and Increased Globalization

With the construction of the first airport on the island in Prosperous Bay Plain, St. Helena widened its doors to the outside world as a remote tourist destination. While the role of ecotourism can be controversial, if managed properly it can be overall beneficial (Stronza et al., 2019, Buckley, 2011). For St. Helena, increased tourism could play a key role in contributing to the development of a much-needed self-sustaining economy on the island, as the island currently subsists on financial aid from the UK. However, complete economic reliance on tourism can be risky when faced with global disasters, such as pandemics, as 2020 has shown (Rashid et al., 2020). In addition to boosting the local economy, ecotourism can also positively impact residents' values on their involvement in conservation (Stronza, 2007). However, it can also lead to a faster rate of decline in some species in some circumstances, and over-tourism can result in further environmental degradation (Buckley et al., 2016). A large and sustained increase in tourism may also lead to the construction of more amenities catering to the tourism business, such as hotels, restaurants, new roads, or even potentially an expansion of the airport. Where and how these amenities are constructed and considerations for conservation in their planning can have substantial negative impacts on the local flora and fauna.

Arid regions are often covered by delicate biological soil crusts consisting of cyanobacteria, lichens, and other microorganisms which provide various useful functions in the local environment, such as soil stability and nutrient access (Belnap, 2003). Biocrusts are susceptible to climate change, particularly as precipitation levels drop and heat rises (Belnap, 2003). The semi-desert region of St. Helena is known to have lichen-rich crusts (Aptroot, 2008). The leading cause of destruction of these microenvironments is trampling by humans and animal hooves, which could be a problem in St. Helena with increased tourist footfall (Belnap, 2003). This may be especially relevant as individuals seek to get a glimpse the endemic Wirebird, which lives not only in pastures on the island, but also in semi-arid areas, such as at

Stone Top and Prosperous Bay. Since these crusts provide valuable services to vascular plants, their destruction could have add-on negative impacts on endemic plants in the region.

The ongoing problem of erosion will likely be exacerbated by both climate change and expanded tourism. Erosion on the island was severely worsened by removal of the native vegetation by goats and other grazing herbivores as well as by humans removing the endemic trees for timber. Historically, erosion is believed to have occurred at a large scale during major storms, rather than gradually with winter rains (Cronk, 1989). Therefore a predicted increase of severe weather with climate change can worsen erosion, which is severe when rare thunderstorms occur on the island (Cronk, 1989). Increased tourism may also worsen the problem with the need for more infrastructure and amenities to be constructed. Increased erosion may also invite more competition from *C. edulis,* which is very salt-tolerant but has fairy shallow roots and excludes any other plants from colonizing and providing soil stabilizing functions. A mixture of plants with different root types has been shown to be the most effective for preventing erosion and landslides, therefore a monoculture of *C. edulis* will likely contribute further to the erosion problem (Ghestem et al., 2014).

Increased globalization will inevitably lead to an increase in invasive and non-native species entering the country, and tourism has been linked to increases in alien species (Anderson et al., 2015). Climate change will also complicate the problem, for example, as the range for invasive species shifts (Hulme, 2017). Despite biosecurity controls at points of entry, an increase in invasive alien species inadvertently arriving on St. Helena from traffic coming from all over the world is likely unavoidable. Additionally, predicting the identity of invasive alien species is challenging, as relying on information from historical invasions alone is insufficient, with the increasing arrival of different species from various new sources worldwide (Seebens et al., 2018). Island endemics are especially susceptible to detrimental effects from invasive species, and remote oceanic islands such as St. Helena are even more at risk (Moser et al., 2018, Russell et al., 2017). There is already an ongoing battle against introduced species, such as non-native plants that outcompete the native species on the island, as well as insects and mammals. For example, an exotic scale infestation severely threatened the remaining 2500 *C. robustum* trees on St. Helena until it was eliminated using biological control with a natural predator (Fowler, 2004). Horizon scanning to predict the potential alien invasive species that would pose the biggest threat to an island has been carried out elsewhere and may be a useful approach for St. Helena (Peyton et al., 2019).

Suggested Conservation Actions and Areas for Future Research

Suggested Conservation Actions

A lack of data on climate modeling means that in the meantime, policy makers and conservationists must make decisions in the face of considerable and inevitable uncertainty (Foley, 2018). The St. Helena government released a climate change policy for the island in 2019, which outlined objectives for increasing the island's sustainable practices and decreasing emissions (Environmental Management Division, 2019). While it alluded to the importance of the cloud forest vegetation for maintaining fresh water on the island, it did not address specific plant conservation practices for this area or elsewhere on island (Environmental Management Division, 2019). Considering a future of changed climate and increased tourism, the following conservation actions are suggested:

1. Careful monitoring of existing endemic species population numbers and locations.

   It is crucial to know the baseline status of plants, lichens, and invertebrates in order to track changes associated with pressures from climate change and increased traffic to the island in the future. Monitoring population numbers and health is imperative to watch for declines or problematic pests in order to take appropriate action in a timely manner. Additionally, establishing baseline genetic diversity of populations, while costly and time consuming, would provide valuable insights for potential future genetic erosion (Watson-Jones et al., 2006). Engaging citizen scientists may be an effective strategy for accomplishing such a monitoring program on the island (Brown and Williams, 2019, Silvertown, 2009).

2. Intensive control of invasive alien species and naturalized problem species.

   Invasive species already introduced to the island, such as *C. edulis*, have been problematic (Cronk, 2000). It is expected that more non-native and potentially invasive plants and pests will be arriving with increased traffic to the island, which will need to be monitored closely and kept in check.

3. Erosion control by planting with endemics.

   The problem of erosion on the island is centuries old, with the destruction of the native vegetation and introduction of goats. Replacing invasive species or monocultures, such as *C. edulis*, with native plants may help to keep the problem at bay (Cronk, 2000). For example, when *Commidendrum rugosum* establishes, it helps to limit erosion issues, and ensuring that plants with different types of roots are preset is more effective for erosion control (Cronk, 1989, Ghestem et al., 2014).

4. Mitigation and contingencies for potential negative impacts of increased tourism.

   The construction of new amenities should be built with consideration of the local environment, for example, not fragmenting populations, providing continuity in landscapes, and avoiding areas with small numbers of highly threatened flora and fauna.

5. Protected area management: expand, connect, and future-proof protected areas.

   Species distributions on St. Helena may change in the future with climate change, and protected areas may need to be reassessed and redrawn to ensure that vulnerable species remain protected (Ferreira et al., 2019). Climate change is predicted to bring altitudinal shifts as species find more suitable habitat higher on the island, and the development of protected corridors could benefit these shifts (Harter et al., 2015). Maintaining or developing habitat connectivity has been shown to be substantially beneficial (Damschen et al., 2019). Further, the fate of land surrounding protected areas tends to predict what happens within the protected areas themselves, so care of surrounding areas and buffer zones warrants attention (Laurance et al., 2012).

6. Genetic rescue for endemic species in highly fragmented and small populations.

   As discussed in Chapters 2 and 3, genetic rescue can be a promising strategy for saving fragmented populations with low genetic diversity (Frankham, 2015). Several endemics growing in the arid regions of the island, such as *P. cotyledonis*, *F. portulacifolia, K. acida,* and *Euphorbia heleniana* Thell. & Stapf are critically endangered and would be good candidates for genetic rescue attempts. Increasing genetic diversity will be even more crucial with impending climate change and ecotourism, giving species the best chance to adapt to changes and pressures.

7. Increase conservation efforts and focus on less charismatic species, such as lichens, invertebrates, and less iconic plants, as well as habitats as a whole.

   Focusing on charismatic species is much easier than directing efforts towards others that are less so, however, it can come at the detriment of organisms in more dire need of conservation (Le Saout et al., 2013). With no native mammals on St. Helena, the charismatic species could be considered to be the Wirebird and some of the more iconic plants, such as the woody Asteraceae. However, lichens, invertebrates, and small plants such as *E. heleniana* in the arid regions are equally worthy of conservation efforts. Approaching conservation from the community or habitat level would also be beneficial for all of the resident species, from the microscopic to iconic macroscopic organisms. Additionally, conservation of even small, disconnected patches of habitat is important for maintaining biodiversity (Wintle et al., 2019).

8. Establish a novel funding mechanism linked to tourism to support monitoring and ongoing conservation efforts on St. Helena.

   Linking tourism to conservation funding through a potential tax, surcharge, or fees can help to offset some of the negative impacts that tourism may bring, as well as provide a much needed revenue source for conservation efforts on St. Helena (Buckley, 2011). Models such as volunteer tourism have proven successful, and provide not only funding, but also the labor needed to follow through with conservation projects (Brightsmith et al., 2008). Potential projects for St. Helena could involve managing invasive species and monitoring endemic species population numbers and health.

Future Research Areas

There is still much to be learned about St. Helena's flora, and there are many unknowns associated with the potential impacts of climate change and increased tourism. The following are important areas for future research:

1. Model climate change scenarios on St. Helena, accounting for local weather patterns, mist collection, and island topography.

   As described, St. Helena is too small and too topologically diverse with its own microclimates to model climate change scenarios using the standard datasets, therefore additional, island-specific work needs to be undertaken. This is a common issue with other small islands with diverse terrains and biodiversity, such as Ascension Island and islands in the Caribbean. Additionally, understanding future species distributions, especially potential altitudinal movements associated with organisms seeking wetter environments, is important for planning and managing protected areas on St. Helena.

2. Resolve the taxonomy of *H. cryptantha* to confirm or refute the status as an endemic species of St. Helena, and include it on the IUCN Red List if warranted.

   Ensuring correct taxonomy is crucial for conservation (Thomson et al., 2018). Currently, St. Helena's *H. cryptantha* is grouped together with a more widely distributed species, thus diluting its status as a species needing protection. However, if the plant is indeed endemic to St. Helena, it may warrant inclusion on the ICUN Red List. The South African Aizoaceae, the family in which *H. cryptantha* is classified, represents a relatively young lineage that diversified quickly, resulting in various circumscriptions of the family (Gerbaulet, 2012, Klak and Bruyns, 2013, Klak et al., 2007, Klak et al., 2004). A combination of morphological and molecular studies would aid in resolving the status of *H. cryptantha* as an endemic of the island.

3. Understand how endemic species respond to and tolerate extended periods of drought.

   Although there is much uncertainty in the predictions, and modeling specific to St. Helena needs to be produced, the consensus from existing research is that the island will become more arid with climate change and may experience periods of drought. It is therefore crucial to understand how endemic species, particularly those in areas that already get very little rain, will respond to extended drought. Such analyses, for example, would involve assessing seedbank longevity for annual plants such as *H. cryptantha*, as well as assessing the impact of drought on other species in greenhouse experiments.

4. Expand knowledge on the lesser known, microscopic inhabitants of the island, including the biological soil crusts and soil microbiome of the arid regions.

   Biocrusts play important roles in arid regions, and very little is known about St. Helena's biocrusts. Additionally, research on the fungal partners with plants in oceanic islands is lacking, with the first study on fungal communities in St. Helena being published in 2020 (Detheridge et al., 2020). These under-explored areas on the island offer much potential for research opportunities.

Conclusion

Typical of islands, St. Helena's flora is characterized by high numbers of endemic species. Habitat loss and degradation since the island's discovery have left many plant species fragmented into small subpopulations, and many are critically endangered and threatened with extinction. Current and future pressures, including climate change to a potentially drier environment with more erratic weather, increased tourism, invasive species, and erosion of both the land and the genetic architecture of populations, will continue to challenge the endemics' ability to adapt to their changing environments. This study focused on the impact of these pressures on the lowland desert habitat and species such as *Hydrodea cryptantha*, and proposed important areas for conservation and future research.

Genetic variation within species populations provides them with a toolkit for adaptability, enabling them to survive and thrive with shifting environmental conditions. Historical levels of variation, as assessed here using morphometric analysis of herbarium specimens, can be used as a baseline for assessing variation within modern populations, ideally coupled with common garden trials and molecular studies. Species with small, highly fragmented populations, which represent many of St. Helena's endemic species, such as *Frankenia portulacifolia, Phylica polifolia*, and *Pelargonium cotyledonis,* will need assistance in building back healthy levels of genetic variation lost from severe population fragmentation. This review suggests that genetic rescue can prove invaluable for restoring population viability when time is limited for species on the brink of extinction, though additional research and long-term studies would give conservationists more confidence in pursuing this strategy.

Regardless of levels of genetic variation within populations, all of the endemic flora will need intensive conservation efforts to stem rampant invasive non-native species, which will likely increase in number and variety, since global access to St. Helena has become easier. Connecting fragmented populations through the use of protected corridors or supplemental planting and ensuring that even small patches of remnant endemic populations remain protected will be important conservation approaches for the island. Additionally, as confirmed here, the taxonomy of several species, such as *C. dianae* and *H. cryptantha*, needs to be resolved to ensure that they are appropriately classified and protected on the IUCN Red List.

Despite its small size and discovery hundreds of years ago, there remain many unanswered questions about St. Helena's flora. Combined with gaps in knowledge about climate change modelling for the island as well as its microscopic inhabitants, there are many avenues for future research. Additional research to inform evidence-based conservation decisions will be vital for preserving the uniqueness and resilience of St. Helena's splendid endemic plant life.

References

Abeli, T., Dalrymple, S., Godefroid, S., Mondoni, A., Muller, J. V., Rossi, G. & Orsenigo, S. 2019. Ex situ collections and their potential for the restoration of extinct plants. *Conservation Biology*, 34, 303-313.

Akesson, M., Liberg, O., Sand, H., Wabakken, P., Bensch, S. & Flagstad, O. 2016. Genetic rescue in a severely inbred wolf population. *Molecular Ecology,* 25**,** 4745-4756.

Anderson, J. T., Willis, J. H. & Mitchell-Olds, T. 2011. Evolutionary genetics of plant adaptation. *Trends in Genetics:* 27**,** 258-266.

Anderson, L. G., Rocliffe, S., Haddaway, N. R. & Dunn, A. M. 2015. The role of tourism and recreation in the spread of non-native species: A systematic review and meta-analysis. *PLOS ONE,* 10**,** e0140833.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* 26**,** 32-46.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R. & Daszak, P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution,* 19**,** 535-544.

Aptroot, A. 2008. Lichens of St Helena and Ascension Island. *Botanical Journal of the Linnean Society,* 158**,** 147-171.

Arunkumar, R., Ness, R. W., Wright, S. I. & Barrett, S. C. H. 2015. The evolution of selfing is accompanied by reduced efficacy of selection and purging of deleterious mutations. *Genetics,* 199**,** 817-829.

Bainbridge, B. 2017. Genetic rescue of the Plains Yam Daisy ('*Microseris scapigera*') on the Merri Creek. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation,* 25:4**,** 9-11.

Barmentlo, S. H., Meirmans, P. G., Luijten, S. H., Triest, L. & Oostermeijer, J. G. B. 2018. Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: consequences for genetic rescue. *Conservation Genetetics,* 19**,** 545-554.

Bell, D. A., Robinson, Z. L., Funk, W. C., Fitzpatrick, S. W., Allendorf, F. W., Tallmon, D. A. & Whiteley, A. R. 2019. The exciting potential and remaining uncertainties of genetic rescue. *Trends in Ecology and Evolution,* 34**,** 1070-1079.

Belnap, J. 2003. The world at your feet: desert biological soil crusts. *Frontiers in Ecology and the Environment,* 1**,** 181-189.

Bijlsma, R., Westerhof, M. D. D., Roekx, L. P. & Pen, I. 2010. Dynamics of genetic rescue in inbred *Drosophila melanogaster* populations. *Conservation Genetics,* 11**,** 449-462.

Borges, L. M., Reis, V. C. & Izbicki, R. 2020. Schrödinger's phenotypes: herbarium specimens show two-dimensional images are both good and (not so) bad sources of morphological data. *Methods in Ecology and Evolution,* Accepted Author Manuscript.

Bossuyt, B. 2007. Genetic rescue in an isolated metapopulation of a naturally fragmented plant species, *Parnassia palustris*. *Conservation Biology,* 21**,** 832-41.

Brightsmith, D. J., Stronza, A. & Holle, K. 2008. Ecotourism, conservation biology, and volunteer tourism: A mutually beneficial triumvirate. *Biological Conservation,* 141**,** 2832-2842.

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., Mcdonald, M., Vesk, P. A. & Yates, C. 2008. Seed supply for broadscale restoration: Maximizing evolutionary potential. *Evolutionary Applications,* 1**,** 587-597.

Brown, E. D. & Williams, B. K. 2019. The potential for citizen science to produce reliable and useful information in ecology. *Conservation Biology,* 33**,** 561-569.

Brown, J. H. & Kodric-Brown, A. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology,* 58**,** 445-449.

Brown, N. 2008. *Climate change in the UK Overseas Territories: An overview of the science, policy and you.* Peterborough, UK: Joint Nature Conservation Committee.

Buckley, R. 2011. Tourism and environment. *Annual Review of Environment and Resources,* 36**,** 397-416.

Buckley, R. C., Morrison, C. & Castley, J. G. 2016. Net Effects of ecotourism on threatened species survival. *PLOS ONE,* 11**,** e0147988.

Caruso, C. M., Benscoter, A. M., Gale, N. V., Seifert, E. K., Mills, E. R. & Case, A. L. 2015. Effects of crossing distance on performance of the native wildflower *Lobelia siphilitica*: Implications for ecological restoration. *The Journal of the Torrey Botanical Society,* 142**,** 140-151.

Castilla, A. R., Garrote, P. J., Żywiec, M., Calvo, G., Suárez-Esteban, A., Delibes, M., Godoy, J. A., Picó, F. X. & Fedriani, J. M. 2019. Genetic rescue by distant trees mitigates qualitative pollen limitation imposed by fine-scale spatial genetic structure. *Molecular Ecology,* 28**,** 4363-4374.

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., Garcia, A., Pringle, R. M. & Palmer, T. M. 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances,* 1**,** e1400253.

Cook, C. N. & Sgrò, C. M. 2017. Aligning science and policy to achieve evolutionarily enlightened conservation. *Conservation Biology,* 31**,** 501-512.

Cronk, Q. 2016. Plant extinctions take time. *Science,* 353**,** 446-447.

Cronk, Q. C. B. 1987. The history of endemic flora of St Helena: A relictual series. *New Phytologist,* 105**,** 509-520.

Cronk, Q. C. B. 1989. The past and present vegetation of St Helena. *Journal of Biogeography,* 16**,** 47-64.

Cronk, Q. C. B. 2000. *The Endemic Flora of St Helena*, A. Nelson.

Crowl, A. A., Mavrodiev, E., Mansion, G., Haberle, R., Pistarino, A., Kamari, G., Phitos, D., Borsch, T. & Cellinese, N. 2014. Phylogeny of Campanuloideae (Campanulaceae) with emphasis on the utility of nuclear pentatricopeptide repeat (PPR) genes. *PLOS ONE,* 9**,** e94199.

Damschen, E. I., Brudvig, L. A., Burt, M. A., Fletcher, R. J., Haddad, N. M., Levey, D. J., Orrock, J. L., Resasco, J. & Tewksbury, J. J. 2019. Ongoing accumulation of plant diversity through habitat connectivity in an 18-year experiment. *Science,* 365**,** 1478-1480.

Del Vecchio, S., Pierce, S., Fantinato, E. & Buffa, G. 2019. Increasing the germination percentage of a declining native orchid (*Himantoglossum adriaticum*) by pollen transfer and outbreeding between populations. *Plant Biology,* 21**,** 935-941.

Demauro, M. M. 1993. Relationship of Breeding system to rarity in the Lakeside Daisy (*Hymenoxys acaulis* var. glabra). *Conservation Biology,* 7**,** 542-550.

Derry, A. M., Fraser, D. J., Brady, S. P., Astorg, L., Lawrence, E. R., Martin, G. K., Matte, J. M., Negrin Dastis, J. O., Paccard, A., Barrett, R. D. H., Chapman, L. J., Lane, J. E., Ballas, C. G., Close, M. & Crispo, E. 2019. Conservation through the lens of (mal)adaptation: Concepts and meta-analysis. *Evolutionary Applications,* 12**,** 1287-1304.

Detheridge, A. P., Cherrett, S., Clasen, L. A., Medcalf, K., Pike, S., Griffith, G. W. & Scullion, J. 2020. Depauperate soil fungal populations from the St. Helena endemic *Commidendrum robustum* are dominated by Capnodiales. *Fungal Ecology,* 45**,** 100911.

Díez-Del-Molino, D., Sánchez-Barreiro, F., Barnes, I., Gilbert, M. T. P. & Dalén, L. 2018. Quantifying temporal genomic erosion in endangered species. *Trends in Ecology & Evolution,* 33**,** 176-185.

Dolezel, J., Greilhuber, J. & Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols,* 2**,** 2233-44.

Edmands, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology,* 16**,** 463-75.

Ellick, S. & Lambdon, P. W. 2016a. *Commidendrum robustum*. The IUCN Red List of Threatened Species 2016: e.T37583A67370371. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T37583A67370371.en.

Ellick, S. & Lambdon, P. W. 2016b. *Nesohedyotis arborea*. The IUCN Red List of Threatened Species 2016: e.T30378A67379194. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T30378A67379194.en.

Environmental Management Division. 2019. *A climate change policy for St Helena*. St. Helena Government.

Fearn, B. 1981. Seed germination: the modern approach. *The Cactus and Succulent Journal of Great Britain,* 43**,** 13-16.

Feistel, R., Hagen, E. & Grant, K. 2003. Climatic changes in the subtropical Southeast Atlantic: the St. Helena Island Climate Index (1893–1999). *Progress in Oceanography,* 59**,** 321-337.

Fenster, C. B. & Galloway, L. F. 2000a. Inbreeding and Outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). *Conservation Biology,* 14**,** 1406-1412.

Fenster, C. B. & Galloway, L. F. 2000b. Population differentiation in an annual legume: Genetic architecture. *Evolution,* 54**,** 1157-72.

Fenu, G., Cogoni, D. & Bacchetta, G. 2016. The role of fencing in the success of threatened plant species translocation. *Plant Ecology,* 217**,** 207-217.

Fernandes, R. & Pinho, P. 2017. The distinctive nature of spatial development on small islands. *Progress in Planning,* 112**,** 1-18.

Ferreira, M. T., Cardoso, P., Borges, P. a. V., Gabriel, R., De Azevedo, E. B. & Elias, R. B. 2019. Implications of climate change to the design of protected areas: The case study of small islands (Azores). *PLOS ONE,* 14**,** e0218168.

Finger, A., Kettle, C. J., Kaiser-Bunbury, C. N., Valentin, T., Doudee, D., Matatiken, D. & Ghazoul, J. 2011. Back from the brink: potential for genetic rescue in a critically endangered tree. *Molecular Ecology,* 20**,** 3773-84.

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N. & Hoban, S. 2018. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications,* 11**,** 1035-1052.

Foley, A. M. 2018. Climate impact assessment and "islandness": Challenges and opportunities of knowledge production and decision-making for small island developing states. *International Journal of Climate Change Strategies and Management,* 10**,** 289-302.

Fowler, S. V. 2004. Biological control of an exotic scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree, *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the island of St. Helena. *Biological Control,* 29**,** 367-374.

Frankham, R. 2010. Challenges and opportunities of genetic approaches to biological conservation. *Biological Conservation,* 143**,** 1919-1927.

Frankham, R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology,* 24**,** 2610-8.

Frankham, R. 2016. Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biological Conservation,* 195**,** 33-36.

Frankham, R., Ballou, J., Ralls, K., Eldridge, M., Dudash, M., Fenster, C., Lacy, R. & Sunnucks, P. 2017. *Genetic Management of Fragmented Animal and Plant Populations,* Oxford UK, Oxford University Press.

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R. & Fenster, C. B. 2011. Predicting the Probability of Outbreeding Depression. *Conservation Biology,* 25**,** 465-475.

Frankham, R., Bradshaw, C. J. A. & Brook, B. W. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation,* 170**,** 56-63.

Frye, C. T. & Neel, M. C. 2017. Benefits of gene flow are mediated by individual variability in self-compatibility in small isolated populations of an endemic plant species. *Evolutionary Applications,* 10**,** 551-562.

Gargano, D., Pellegrino, G. & Bernardo, L. 2015. Genetic and fitness consequences of interpopulation mating in *Dianthus guliae* Janka: Conservation implications for severely depleted and isolated plant populations. *Conservation Genetics,* 16**,** 1127-1138.

Gerbaulet, M. 2012. One or many genera in Mesembryanthemoideae (Aizoaceae)? Discussion of a conflict in genus perception. *Bradleya,* 30**,** 187-198.

Gewin, V. 2017. Genetic rescue isn't a one-shot solution. *Frontiers in Ecology and the Environment,* 15**,** 178-178.

Ghestem, M., Cao, K., Ma, W., Rowe, N., Leclerc, R., Gadenne, C. & Stokes, A. 2014. A framework for identifying plant species to be used as 'ecological engineers' for fixing soil on unstable slopes. *PloS ONE,* 9**,** e95876-e95876.

Global Invasive Species Database. 2020. Available: http://www.iucngisd.org/gisd/search.php [Accessed August 24 2020].

Godefroid, S., Van De Vyver, A., Stoffelen, P., Robbrecht, E. & Vanderborght, T. 2011. Testing the viability of seeds from old herbarium specimens for conservation purposes. *Taxon,* 60**,** 565-569.

Gosse, P. 1938. *St. Helena, 1502-1938,* London, Cassell and Company Limited.

Gower, J. C. 1971. A General Coefficient of Similarity and Some of Its Properties. *Biometrics,* 27**,** 857-871.

Gray, A. 2018. The ecology of plant extinction: rates, traits and island comparisons. *Oryx,* 53**,** 424-428.

Gray, A., Perry, A., Cavers, S., Eastwood, A., Biermann, M., Darlow, A., Thomas, V. & Lambdon, P. 2016. Hybrid plants preserve unique genetic variation in the St Helena endemic trees *Commidendrum rotundifolium* DC Roxb. and *C. spurium* (G.Forst.) DC. *Conservation Genetics,* 18**,** 241-246.

Gray, A., Wilkins, V., Pryce, D., Fowler, L., Key, R. S., Mendel, H., Jervois, M., Hochkirch, A., Cairns-Wicks, R., Dutton, A.-J. & Malan, L. 2019. The status of the invertebrate fauna on the South Atlantic island of St Helena: problems, analysis, and recommendations. *Biodiversity and Conservation,* 28**,** 275-296.

Grove, R. 1993. Conserving Eden: The (European) East India Companies and their environmental policies on St. Helena, Mauritius and in Western India, 1660 to 1854. *Comparative Studies in Society and History,* 35**,** 318-351.

Haberle, R., Dang, A., Lee, T., Peñaflor, C., Cortes-Burns, H., Oestreich, A., Raubeson, L., Cellinese, N., Edwards, E., Kim, S.-T., Eddie, W. & Jansen, R. 2009. Taxonomic and biogeographic implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. *Taxon,* 58**,** 715-734.

Hamilton, J. A., Royaute, R., Wright, J. W., Hodgskiss, P. & Ledig, F. T. 2017. Genetic conservation and management of the California endemic, Torrey pine (*Pinus torreyana* Parry): Implications of genetic rescue in a genetically depauperate species. *Ecology and Evolution,* 7**,** 7370-7381.

Hammer, O., Harper, D. & Ryan, P. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica,* 4**,** 1-9.

Harrisson, K. A., Pavlova, A., Telonis-Scott, M. & Sunnucks, P. 2014. Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications,* 7**,** 1008-25.

Harter, D. E. V., Irl, S. D. H., Seo, B., Steinbauer, M. J., Gillespie, R., Triantis, K. A., Fernández-Palacios, J.-M. & Beierkuhnlein, C. 2015. Impacts of global climate change on the floras of oceanic islands – Projections, implications and current knowledge. *Perspectives in Plant Ecology, Evolution and Systematics,* 17**,** 160-183.

Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B. & Briskie, J. 2013. The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. *Proceedings of the Royal Society B-Biological Sciences,* 280.

Hedrick, P. W., Adams, J. R. & Vucetich, J. A. 2011. Reevaluating and broadening the definition of genetic rescue. *Conservation Biology,* 25**,** 1069-70.

Hedrick, P. W. & Fredrickson, R. 2009. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conservation Genetics,* 11**,** 615-626.

Hedrick, P. W. & Garcia-Dorado, A. 2016. Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology & Evolution,* 31**,** 940-952.

Hedrick, P. W. & Kalinowski, S. T. 2000. Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics,* 31**,** 139-162.

Hedrick, P. W., Robinson, J. A., Peterson, R. O. & Vucetich, J. A. 2019. Genetics and extinction and the example of Isle Royale wolves. *Animal Conservation,* 22**,** 302-309.

Hellmann, J. J., Byers, J. E., Bierwagen, B. G. & Dukes, J. S. 2008. Five potential consequences of climate change for invasive species. *Conservation Biology,* 22**,** 534-543.

Hereford, J. 2009. Postmating/prezygotic isolation, heterosis, and outbreeding depression in crosses within and between populations of *Diodia teres* (Rubiaceae) Walt. *International Journal of Plant Sciences,* 170**,** 301-310.

Huang, J., Yu, H., Guan, X., Wang, G. & Guo, R. 2016. Accelerated dryland expansion under climate change. *Nature Climate Change,* 6**,** 166-171.

Hufbauer, R. A., Szucs, M., Kasyon, E., Youngberg, C., Koontz, M. J., Richards, C., Tuff, T. & Melbourne, B. A. 2015. Three types of rescue can avert extinction in a changing environment. *Proceedings of the National Academy of Sciences,* 112**,** 10557-62.

Hulme, P. E. 2017. Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews,* 92**,** 1297-1313.

Humphreys, A. M., Govaerts, R., Ficinski, S. Z., Nic Lughadha, E. & Vorontsova, M. S. 2019. Global dataset shows geography and life form predict modern plant extinction and rediscovery. *Nature Ecology & Evolution,* 3**,** 1043-1047.

Ingvarsson, P. K. 2001. Restoration of genetic variation lost – the genetic rescue hypothesis. *Trends in Ecology & Evolution,* 16**,** 62-63.

Intergovernmental Panel on Climate Change 2015. *Climate Change 2014: Mitigation of Climate Change: Working Group III Contribution to the IPCC Fifth Assessment Report*.

IUCN. 2020. *The IUCN Red List of Threatened Species. Version 2020-1* [Online]. Available: https://www.iucnredlist.org [Accessed May 11 2020].

Jansson, J. K. & Hofmockel, K. S. 2020. Soil microbiomes and climate change. *Nature Reviews Microbiology,* 18**,** 35-46.

Jump, A. S. & Peñuelas, J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters,* 8**,** 1010-1020.

Kardos, M., Taylor, H. R., Ellegren, H., Luikart, G. & Allendorf, F. W. 2016. Genomics advances the study of inbreeding depression in the wild. *Evolutionary Applications,* 9**,** 1205-1218.

Karnauskas, K. B., Donnelly, J. P. & Anchukaitis, K. J. 2016. Future freshwater stress for island populations. *Nature Climate Change,* 6**,** 720-725.

Kier, G., Kreft, H., Lee, T. M., Jetz, W., Ibisch, P. L., Nowicki, C., Mutke, J. & Barthlott, W. 2009. A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Sciences,* 106**,** 9322-9327.

Kistler, L., Bieker, V. C., Martin, M. D., Pedersen, M. W., Madrigal, J. R. & Wales, N. 2020. Ancient plant genomics in archaeology, herbaria, and the environment. *Annual Review of Plant Biology,* 71**,** 605-629.

Klak, C. & Bruyns, P. 2013. A new infrageneric classification for *Mesembryanthemum* (Aizoaceae: Mesembryanthemoideae). *Bothalia,* 43.

Klak, C., Bruyns, P. V. & Hedderson, T. a. J. 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon,* 56**,** 737-756.

Klak, C., Reeves, G. & Hedderson, T. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature,* 427**,** 63-65.

Kyriazis, C. C., Wayne, R. K. & Lohmueller, K. E. 2019. High genetic diversity can contribute to extinction in small populations. *bioRxiv***,** 678524.

Lambdon, P. 2012. *Flowering Plants & Ferns of St Helena*, Pisces Publications for St. Helena Nature Conservation Group.

Lambdon, P. & Cronk, Q. 2020. Extinction dynamics under extreme conservation threat: the flora of St Helena. *Frontiers in Ecology and Evolution,* 8.

Lambdon, P. W. & Ellick, S. 2016a. *Bulbostylis neglecta.* The IUCN Red List of Threatened Species 2016: e.T67374400A67374414. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67374400A67374414.en.

Lambdon, P. W. & Ellick, S. 2016b. *Carex dianae.* The IUCN Red List of Threatened Species 2016: e.T67374433A67376301. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67374433A67376301.en.

Lambdon, P. W. & Ellick, S. 2016c. *Eragrostis episcopulus*. The IUCN Red List of Threatened Species 2016: e.T67376345A67376362. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67376345A67376362.en.

Lambdon, P. W. & Ellick, S. 2016d. *Euphorbia heleniana*. The IUCN Red List of Threatened Species 2016: e.T67379098A67379104. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67379098A67379104.en.

Lambdon, P. W. & Ellick, S. 2016e. *Frankenia portulacifolia.* The IUCN Red List of Threatened Species 2016: e.T43989A67372537. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T43989A67372537.en.

Lambdon, P. W. & Ellick, S. 2016f. *Kewa acida*. The IUCN Red List of Threatened Species 2016: e.T67379066A67379073. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67379066A67379073.en.

Lambdon, P. W. & Ellick, S. 2016g. *Pelargonium cotyledonis*. The IUCN Red List of Threatened Species 2016: e.T67379110A67379117. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67379110A67379117.en.

Lambdon, P. W. & Ellick, S. 2016h. *Phylica polifolia.* The IUCN Red List of Threatened Species 2016: e.T30377A67372313. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T30377A67372313.en.

Lambdon, P. W. & Ellick, S. 2016i. *Pladaroxylon leucadendron.* The IUCN Red List of Threatened Species 2016: e.T37596A67371569. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T37596A67371569.en.

Lambdon, P. W. & Ellick, S. 2016j. *Plantago robusta*. The IUCN Red List of Threatened Species 2016: e.T67374369A67374372. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T67374369A67374372.en.

Lambdon, P. W. & Ellick, S. 2016k. *Wahlenbergia angustifolia*. The IUCN Red List of Threatened Species 2016: e.T43988A67371447. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T43988A67371447.en.

Lambdon, P. W. & Ellick, S. 2016l. *Wahlenbergia linifolia.* The IUCN Red List of Threatened Species 2016: e.T44010A67371678. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T44010A67371678.en.

Laurance, W. F., Carolina Useche, D., Rendeiro, J., Kalka, M., Bradshaw, C. J. A., Sloan, S. P., Laurance, S. G., Campbell, M., Abernethy, K., Alvarez, P., Arroyo-Rodriguez, V., Ashton, P., Benítez-Malvido, J., Blom, A., Bobo, K. S., Cannon, C. H., Cao, M., Carroll, R., Chapman, C., Coates, R., Cords, M., Danielsen, F., De Dijn, B., Dinerstein, E., Donnelly, M. A., Edwards, D., Edwards, F., Farwig, N., Fashing, P., Forget, P.-M., Foster, M., Gale, G., Harris, D., Harrison, R., Hart, J., Karpanty, S., John Kress, W., Krishnaswamy, J., Logsdon, W., Lovett, J., Magnusson, W., Maisels, F., Marshall, A. R., Mcclearn, D., Mudappa, D., Nielsen, M. R., Pearson, R., Pitman, N., Van Der Ploeg, J., Plumptre, A., Poulsen, J., Quesada, M., Rainey, H., Robinson, D., Roetgers, C., Rovero, F., Scatena, F., Schulze, C., Sheil, D., Struhsaker, T., Terborgh, J., Thomas, D., Timm, R., Nicolas Urbina-Cardona, J., Vasudevan, K., Joseph Wright, S., Carlos Arias-G, J., Arroyo, L., Ashton, M., Auzel, P., Babaasa, D., Babweteera, F., Baker, P., Banki, O., Bass, M., Bila-Isia, I., Blake, S., Brockelman, W., Brokaw, N., Brühl, C. A., Bunyavejchewin, S., Chao, J.-T., Chave, J., Chellam, R., Clark, C. J., Clavijo, J., Congdon, R., Corlett, R., Dattaraja, H. S., Dave, C., Davies, G., De Mello Beisiegel, B., De Nazaré Paes Da Silva, R., Di Fiore, A., Diesmos, A., Dirzo, R., Doran-Sheehy, D., Eaton, M., Emmons, L., Estrada, A., et al. 2012. Averting biodiversity collapse in tropical forest protected areas. *Nature,* 489**,** 290-294.

Le Saout, S., Hoffmann, M., Shi, Y., Hughes, A., Bernard, C., Brooks, T. M., Bertzky, B., Butchart, S. H. M., Stuart, S. N., Badman, T. & Rodrigues, A. S. L. 2013. Protected areas and effective biodiversity conservation. *Science,* 342**,** 803-805.

Leimu, R. & Fischer, M. 2008. A meta-analysis of local adaptation in plants. *PLoS ONE,* 3**,** e4010.

Li, J., Qi, C., Gu, J. & Jin, Z. 2020. Effect of sire population on the genetic diversity and fitness of F1 progeny in the endangered Chinese endemic *Sinocalycanthus chinensis. Ecology and Evolution,* 10**,** 4091-4103.

Lloyd, D. G. & Jocelyn, M. a. Y. 1982. Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). *Evolution,* 36**,** 903-913.

Love Stowell, S. M., Pinzone, C. A. & Martin, A. P. 2017. Overcoming barriers to active interventions for genetic diversity. *Biodiversity and Conservation,* 26**,** 1753-1765.

Marsden, B. W., Engelhardt, K. a. M. & Neel, M. C. 2013. Genetic rescue versus outbreeding depression in *Vallisneria americana*: Implications for mixing seed sources for restoration. *Biological Conservation,* 167**,** 203-214.

Maschinski, J., Wright, S. J., Koptur, S. & Pinto-Torres, E. C. 2013. When is local the best paradigm? Breeding history influences conservation reintroduction survival and population trajectories in times of extreme climate events. *Biological Conservation,* 159**,** 277-284.

Mcculloch, N. 2009. Recent decline of the St Helena Wirebird *Charadrius sanctaehelenae. Bird Conservation International,* 19**,** 33-48.

Melen, M. K., Herman, J. A., Lucas, J., O'malley, R. E., Parker, I. M., Thom, A. M. & Whittall, J. B. 2016. Reproductive success through high pollinator visitation rates despite self incompatibility in an endangered wallflower. *American Journal of Botany,* 103**,** 1979-1989.

Melliss, J. C. 1875. *St. Helena : a physical, historical, and topographical description of the island, including it geology, fauna, flora and meteorology,* London, L. Reeve & Co.

Menges, E. S., Smith, S. A. & Weekley, C. W. 2016. Adaptive introductions: How multiple experiments and comparisons to wild populations provide insights into requirements for long-term introduction success of an endangered shrub. *Plant Diversity,* 38**,** 238-246.

Miller, S. M., Druce, D. J., Dalton, D. L., Harper, C. K., Kotze, A., Packer, C., Slotow, R. & Bloomer, P. 2020. Genetic rescue of an isolated African lion population. *Conservation Genetics,* 21**,** 41-53.

Morgan, J. W., Meyer, M. J., Young, A. G. & Editor: Michele, R. D. 2013. Severe habitat fragmentation leads to declines in genetic variation, mate availability, and reproductive success in small populations of a once-common Australian grassland daisy. *International Journal of Plant Sciences,* 174**,** 1209-1218.

Moser, D., Lenzner, B., Weigelt, P., Dawson, W., Kreft, H., Pergl, J., Pyšek, P., Van Kleunen, M., Winter, M., Capinha, C., Cassey, P., Dullinger, S., Economo, E. P., García-Díaz, P., Guénard, B., Hofhansl, F., Mang, T., Seebens, H. & Essl, F. 2018. Remoteness promotes biological invasions on islands worldwide. *Proceedings of the National Academy of Sciences,* 115**,** 9270-9275.

Nurse, L., Mclean, R., Agard, J., Briguglio, L., Duvat, V., Pelesikoti, N. & Tompkins, E. 2014. *Chapter 29: Small islands. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Ottewell, K. M., Bickerton, D. C., Byrne, M., Lowe, A. J. & Burridge, C. 2016. Bridging the gap: a genetic assessment framework for population-level threatened plant conservation prioritization and decision-making. *Diversity and Distributions,* 22**,** 174-188.

Paajanen, M. P. T. & Cronk, Q. 2020. Moth versus fly: a preliminary study of the pollination mode of two species of endemic Asteraceae from St Helena (*Commidendrum robustum* and *C. rugosum*) and its conservation implications. *Biodiversity Data Journal,* 8.

Pennisi, E. 2019. Genomics guides help for dwindling species. *Science,* 365**,** 210.

Peyton, J., Martinou, A. F., Pescott, O. L., Demetriou, M., Adriaens, T., Arianoutsou, M., Bazos, I., Bean, C. W., Booy, O., Botham, M., Britton, J. R., Cervia, J. L., Charilaou, P., Chartosia, N., Dean, H. J., Delipetrou, P., Dimitriou, A. C., Dörflinger, G., Fawcett, J., Fyttis, G., Galanidis, A., Galil, B., Hadjikyriakou, T., Hadjistylli, M., Ieronymidou, C., Jimenez, C., Karachle, P., Kassinis, N., Kerametsidis, G., Kirschel, A. N. G., Kleitou, P., Kleitou, D., Manolaki, P., Michailidis, N., Mountford, J. O., Nikolaou, C., Papatheodoulou, A., Payiatas, G., Ribeiro, F., Rorke, S. L., Samuel, Y., Savvides, P., Schafer, S. M., Tarkan, A. S., Silva-Rocha, I., Top, N., Tricarico, E., Turvey, K., Tziortzis, I., Tzirkalli, E., Verreycken, H., Winfield, I. J., Zenetos, A. & Roy, H. E. 2019. Horizon scanning for invasive alien species with the potential to threaten biodiversity and human health on a Mediterranean island. *Biological Invasions,* 21**,** 2107-2125.

Pickup, M., Field, D. L., Rowell, D. M. & Young, A. G. 2013. Source population characteristics affect heterosis following genetic rescue of fragmented plant populations. *Proceedings of the Royal Society B: Biological Sciences,* 280**,** 20122058.

Pickup, M. & Young, A. G. 2008. Population size, self-incompatibility and genetic rescue in diploid and tetraploid races of *Rutidosis leptorrhynchoides* (Asteraceae). *Heredity (Edinb),* 100**,** 268-74.

Pierson, J. C., Coates, D. J., Oostermeijer, J. G. B., Beissinger, S. R., Bragg, J. G., Sunnucks, P., Schumaker, N. H. & Young, A. G. 2016. Genetic factors in threatened species recovery plans on three continents. *Frontiers in Ecology and the Environment,* 14**,** 433-440.

Pike, S., Medcalf, K., Scullion, J. & Detheridge, A. P. 2018. *DPLUS052: Mapping St Helena's Biodiversity and Natural Environment. Final report*.

Pimm, S. L., Dollar, L. & Bass Jr, O. L. 2006. The genetic rescue of the Florida panther. *Animal Conservation,* 9**,** 115-122.

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M. & Sexton, J. O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science,* 344**,** 1246752.

POWO. 2019. *Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew.* [Online]. Available: http://www.plantsoftheworldonline.org/ [Accessed July 16 2020].

Prill, N., Bullock, J. M., Van Dam, N. M. & Leimu, R. 2014. Loss of heterosis and family-dependent inbreeding depression in plant performance and resistance against multiple herbivores under drought stress. *Journal of Ecology,* 102**,** 1497-1505.

Quinn, C. B., Alden, P. B. & Sacks, B. N. 2019. Noninvasive sampling reveals short-term genetic rescue in an insular red fox population. *Journal of Heredity,* 110**,** 559-576.

Ralls, K., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C., Sunnucks, P. & Frankham, R. 2018. Call for a paradigm shift in the genetic management of fragmented populations. *Conservation Letters,* 11.

Rashid, H., Ng, P. L. & Cheng, H. W. J. 2020. *United Nations Department of Economic and Social Affairs Policy Brief #64: The COVID-19 pandemic puts Small Island Developing economies in dire straits* [Online]. Available: https://www.un.org/development/desa/dpad/publication/un-desa-policy-brief-64-the-covid-19-pandemic-puts-small-island-developing-economies-in-dire-straits/ [Accessed August 12 2020].

Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *American Naturalist,* 155**,** 383-394.

Richardson, J. E., Fay, M. F., Cronk, Q. C. B. & Chase, M. W. 2003. Species delimitation and the origin of populations in island representatives of *Phylica* (Rhamnaceae). *Evolution,* 57**,** 816-827.

Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001. Phylogenetic analysis of *Phylica* L. (Rhamnaceae) with an emphasis on island species: evidence from plastid trnL-F and nuclear internal transcribed spacer (ribosomal) DNA sequences. *Taxon,* 50**,** 405-427.

Robinson, Z. L., Coombs, J. A., Hudy, M., Nislow, K. H., Letcher, B. H. & Whiteley, A. R. 2017. Experimental test of genetic rescue in isolated populations of brook trout. *Molecular Ecology,* 26**,** 4418-4433.

Ruane, L. G., Dickens, M. E. & Wall, M. E. 2015. Fitness consequences of short- and long-distance pollinations in *Phlox hirsuta*, an endangered species. *American Journal of Botany,* 102**,** 1659-1665.

Russell, J. C., Meyer, J.-Y., Holmes, N. D. & Pagad, S. 2017. Invasive alien species on islands: impacts, distribution, interactions and management. *Environmental Conservation,* 44**,** 359-370.

Sansom, B., Gray, A., Jervois, M., Cherrett, S. & Connect Saint Helena. 2019*. DPLUS051: Water Security and Sustainable Cloud Forest Restoration on St Helena. Final report.*

Schmidt-Lebuhn, A. N., Marshall, D. J., Dreis, B. & Young, A. G. 2018. Genetic rescue in a plant polyploid complex: Case study on the importance of genetic and trait data for conservation management. *Ecology and Evolution,* 8**,** 5153-5163.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* 9**,** 671-675.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., Causton, C. E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E. P., Fuentes, N., Guénard, B., Jäger, H., Kartesz, J., Kenis, M., Kühn, I., Lenzner, B., Liebhold, A. M., Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H. E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Walker, K., Ward, D. F., Yamanaka, T. & Essl, F. 2018. Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences,* 115**,** E2264-e2273.

Shafer, A. B., Wolf, J. B., Alves, P. C., Bergstrom, L., Bruford, M. W., Brannstrom, I., Colling, G., Dalen, L., De Meester, L., Ekblom, R., Fawcett, K. D., Fior, S., Hajibabaei, M., Hill, J. A., Hoezel, A. R., Hoglund, J., Jensen, E. L., Krause, J., Kristensen, T. N., Krutzen, M., Mckay, J.

K., Norman, A. J., Ogden, R., Osterling, E. M., Ouborg, N. J., Piccolo, J., Popovic, D., Primmer, C. R., Reed, F. A., Roumet, M., Salmona, J., Schenekar, T., Schwartz, M. K., Segelbacher, G., Senn, H., Thaulow, J., Valtonen, M., Veale, A., Vergeer, P., Vijay, N., Vila, C., Weissensteiner, M., Wennerstrom, L., Wheat, C. W. & Zielinski, P. 2015. Genomics and the challenging translation into conservation practice. *Trends in Ecology & Evolution,* 30**,** 78-87.

Shemesh, H., Shani, G., Carmel, Y., Kent, R. & Sapir, Y. 2018. To mix or not to mix the sources of relocated plants? The case of the endangered *Iris lortetii*. *Journal for Nature Conservation,* 45**,** 41-47.

Shi, J., Joshi, J., Tielbörger, K., Verhoeven, K. J. F. & Macel, M. 2018. Costs and benefits of admixture between foreign genotypes and local populations in the field. *Ecology and Evolution,* 8**,** 3675-3684.

Silvertown, J. 2009. A new dawn for citizen science. *Trends in Ecology & Evolution,* 24**,** 467-471.

St. Helena Landscape & Ecological Mitigation Programme. 2015. *Annual Report No. 02. April 2014 – March 2015* [Online]. Available: https://www.sainthelena.gov.sh/wp-content/uploads/2018/06/LEMP-Annual-Report2_Apr-14_Mar-15.pdf [Accessed July 20 2020].

Stronza, A. 2007. The economic promise of ecotourism for conservation. *Journal of Ecotourism,* 6**,** 210-230.

Stronza, A. L., Hunt, C. A. & Fitzgerald, L. A. 2019. Ecotourism for conservation? *Annual Review of Environment and Resources,* 44**,** 229-253.

Supple, M. A. & Shapiro, B. 2018. Conservation of biodiversity in the genomics era. *Genome Biology,* 19**,** 131.

Tallmon, D. A. 2017. Get a move on: the value of rescue. *Animal Conservation,* 20**,** 16-17.

Tallmon, D. A., Luikart, G. & Waples, R. S. 2004. The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution,* 19**,** 489-96.

Taylor, H. R., Dussex, N. & Van heezik, Y. 2017. Bridging the conservation genetics gap by identifying barriers to implementation for conservation practitioners. *Global Ecology and Conservation,* 10**,** 231-242.

Taylor, M., Pelembe, T., & Brickle, P. 2016. *Regional ecosystem profile: South Atlantic*. EU Outermost Regions and Overseas Countries and Territories. BEST, Service contract 07.0307.2013/666363/SER/B2, European Commission.

Thomas, A., Baptiste, A., Martyr-Koller, R., Pringle, P. & Rhiney, K. 2020. Climate Change and Small Island Developing States. *Annual Review of Environment and Resources,* 45.

Thompson, V. 2008. St. Helena's Millennium Forest: A Symbol of the FIght to Defend Fragile Ecosystems. *Wirebird,* 36**,** 11-15.

Thomson, S. A., Pyle, R. L., Ahyong, S. T., Alonso-Zarazaga, M., Ammirati, J., Araya, J. F., Ascher, J. S., Audisio, T. L., Azevedo-Santos, V. M., Bailly, N., Baker, W. J., Balke, M., Barclay, M. V. L., Barrett, R. L., Benine, R. C., Bickerstaff, J. R. M., Bouchard, P., Bour, R., Bourgoin, T., Boyko, C. B., Breure, A. S. H., Brothers, D. J., Byng, J. W., Campbell, D., Ceriaco, L. M. P., Cernak, I., Cerretti, P., Chang, C. H., Cho, S., Copus, J. M., Costello, M. J., Cseh, A., Csuzdi, C., Culham, A., D'elia, G., D'udekem D'acoz, C., Daneliya, M. E., Dekker, R., Dickinson, E. C., Dickinson, T.

A., Van Dijk, P. P., Dijkstra, K. B., Dima, B., Dmitriev, D. A., Duistermaat, L., Dumbacher, J. P., Eiserhardt, W. L., Ekrem, T., Evenhuis, N. L., Faille, A., Fernandez-Triana, J. L., Fiesler, E., Fishbein, M., Fordham, B. G., Freitas, A. V. L., Friol, N. R., Fritz, U., Froslev, T., Funk, V. A., Gaimari, S. D., Garbino, G. S. T., Garraffoni, A. R. S., Geml, J., Gill, A. C., Gray, A., Grazziotin, F. G., Greenslade, P., Gutierrez, E. E., Harvey, M. S., Hazevoet, C. J., He, K., He, X., Helfer, S., Helgen, K. M., Van Heteren, A. H., Hita Garcia, F., Holstein, N., Horvath, M. K., Hovenkamp, P. H., Hwang, W. S., Hyvonen, J., Islam, M. B., Iverson, J. B., Ivie, M. A., Jaafar, Z., Jackson, M. D., Jayat, J. P., Johnson, N. F., Kaiser, H., Klitgard, B. B., Knapp, D. G., Kojima, J. I., Koljalg, U., Kontschan, J., Krell, F. T., Krisai-Greilhuber, I., Kullander, S., Latella, L., Lattke, J. E., Lencioni, V., et al. 2018. Taxonomy based on science is necessary for global conservation. *PLoS Biology,* 16**,** e2005075.

Vahl, M. & Forsskål, P. 1790. *Symbolae botanicae, sive plantarum, tam earum, quas in itinere, imprimis orientali,* Hauniae, imprensis auctoris, excudebant N. Möller et filius.

Vandepitte, K., Honnay, O., Jacquemyn, H. & Roldan-Ruiz, I. 2010. Effects of outcrossing in fragmented populations of the primarily selfing forest herb *Geum urbanum*. *Evolutionary Ecology,* 24**,** 1353-1364.

Veron, S., Mouchet, M., Govaerts, R., Haevermans, T. & Pellens, R. 2019. Vulnerability to climate change of islands worldwide and its impact on the tree of life. *Scientific Reports,* 9**,** 14471.

Volis, S., Ormanbekova, D., Yermekbayev, K., Abugalieva, S., Turuspekov, Y. & Shulgina, I. 2016. Genetic architecture of adaptation to novel environmental conditions in a predominantly selfing allopolyploid plant. *Heredity,* 116**,** 485-490.

Wade, S., Leonard-Williams, A. & Salmon, K. 2015. *Assessing climate change and its likely impact on selected UK Overseas Territories: Inception Report*. Evidence on Demand, UK.

Watson-Jones, S. J., Maxted, N. & Ford-Lloyd, B. V. 2006. Population baseline data for monitoring genetic diversity loss for 2010: A case study for *Brassica* species in the UK. *Biological Conservation,* 132**,** 490-499.

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., Byrne, M., Coates, D. J., Eldridge, M. D. B., Sunnucks, P., Breed, M. F., James, E. A. & Hoffmann, A. A. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* 4**,** 709-725.

Weisenberger, L. A., Weller, S. G. & Sakai, A. K. 2014. Remnants of populations provide effective source material for reintroduction of an endangered Hawaiian plant, *Schiedea kaalae* (Caryophyllaceae). *American Journal of Botany,* 101**,** 1954-1962.

Welsford, M. R. & Johnson, S. D. 2012. Solitary and social bees as pollinators of *Wahlenbergia* (Campanulaceae): single-visit effectiveness, overnight sheltering and responses to flower colour. *Arthropod-Plant Interactions,* 6**,** 1-14.

Wheeler, T. & Von Braun, J. 2013. Climate change impacts on global food security. *Science,* 341**,** 508-513.

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C. & Tallmon, D. A. 2015. Genetic rescue to the rescue. *Trends in Ecology & Evolution,* 30**,** 42-9.

Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K. & Triantis, K. A. 2017. Island biogeography: Taking the long view of nature's laboratories. *Science,* 357**,** eaam8326.

Willi, Y., Van Kleunen, M., Dietrich, S. & Fischer, M. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proceedings of the Royal Society B: Biological Sciences,* 274**,** 2357-64.

Wintle, B. A., Kujala, H., Whitehead, A., Cameron, A., Veloz, S., Kukkala, A., Moilanen, A., Gordon, A., Lentini, P. E., Cadenhead, N. C. R. & Bekessy, S. A. 2019. Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. *Proceedings of the National Academy of Sciences,* 116**,** 909-914.

Appendix Table 1. Genetic management strategies according to levels of genetic differentiation, diversity, and inbreeding. Adapted from Table 1 in Ottewell, 2016.

Genetic differentiation: Low
Populations have historically been connected and currently maintain gene flow
Translocation between populations is a viable option to boost population sizes
Local adaptation/outbreeding depression is likely to be minimal unless there are strong environmental gradients

	Diversity	**Inbreeding**	**Description**	**Management**
1	High	Low	Populations are genetically healthy and maintain high levels of gene flow, potentially acting as metapopulations (source/sink)	Manage species-level ecological and demographic threats to maintain population sizes and gene flow (e.g. corridors) Maintain/facilitate metapopulation dynamics
2	High	High	High diversity and gene flow, but risk of population declines due to inbreeding depression in the long term	Manage populations to reduce breeding between genetically related individuals, for example facilitate pollen/seed immigration; active translocations to introduce new genetic diversity. Continue to monitor outcomes. Manage pollinator/seed disperser populations where these have been perturbed
3	Low	Low	Mating patterns are maintained but erosion of diversity due to low population size, founder or bottleneck effects	Manage to increase recruitment and facilitate pollen/seed immigration, or actively translocate individuals to increase population size Investigate means to recover diversity in situ, for example manipulate disturbance regime to facilitate recovery from seedbank
4	Low	High	Inbreeding exacerbated by low diversity, but populations not significantly differentiated	Introduce new genetic material by facilitating migration, managing pollen and seed dispersal vectors or by active translocation (source recruits from genetically diverse populations elsewhere) Investigate means to recover diversity in situ if inbreeding is a recent phenomenon, for example from a persistent soil seedbank

Genetic differentiation: High
Populations have historically been isolated and have little contemporary gene flow
Translocations between populations could be risky due to potential outbreeding depression. Assess risks with further research or employ risk management strategies [e.g. ‘composite provenancing’ (Broadhurst et al. 2008)]

	Diversity	**Inbreeding**	**Description**	**Management**
5	High	Low	Populations are genetically healthy but are divergent, constituting individual management units	Manage habitat and ecological threats to maintain as many populations across the species range as possible and to maintain large size

6	High	High	Populations retain high diversity but are inbred and genetically divergent	Manage populations to increase breeding between genetically unrelated individuals, for example manage pollinators/seed dispersers Consider ex situ conservation measures at this stage before further loss of genetic diversity
7	Low	Low	Mating patterns are maintained but low gene flow and low diversity	Investigate means to recover diversity in situ (e.g. from soil seedbank) or increase recruitment to boost population sizes Consider ‘composite provenancing’ translocation strategy to introduce new diversity in the absence of information on outbreeding risks
8	Low	High	Populations are inbred, genetically depauperate, genetically divergent and are unlikely to be resilient to environmental change	Investigate means to recover diversity in situ (e.g. from seedbank); consider translocations if outbreeding depression risks managed Intensive and costly management (e.g. ongoing translocations) may be required to recover and sustain populations Invoke triage measures to assess whether the likelihood of recovery/persistence is sufficiently high to justify ongoing management expense

Appendix Table 2: Exsiccatae for the morphometric Analysis. The note indicates type specimens, and for *Carex*, which hypothetical species the specimen was grouped with for the analysis.

Species	Collector	Number	Herbarium	Note
Carex dianae	Cronk	291	E	Grouped as *C.* var. *aequabilis*
C. dianae	Cronk	292	E	Grouped as *C.* var. *aequabilis*
C. dianae	Cronk	293	E	Grouped as *C.* var. *aequabilis*
C. dianae	Cronk	294	E	Grouped as *C.* var. *aequabilis*
C. dianae	Cronk	329	E	Grouped as *C.* var. *dianae*
C. dianae	Melliss	s.n.	E	Grouped as *C.* var. *dianae*
C. dianae	Burchell	19	K	Grouped as *C.* var. *dianae*
C. dianae	Burchell	20	K	Type of *C. aequabilis*
C. dianae	Dumont d'Urville	s.n.	P	Type of *C. dianae*
C. dianae	Dumont d'Urville	s.n.	P	Type of *C. dianae*
C. subinflata	Hilliard & Burtt	16512	E	Outgroup from South Africa
C. subinflata	Hilliard & Burtt	16652	E	Outgroup from South Africa
Phylica polifolia	Hooker	s.n.	E	Contained 3 individual plants for separate measurements
P. polifolia	Burchell	81	K	Contained 2 individual plants for separate measurements. Neotype.
P. polifolia	Forster & Forster	33	BM	Contained 2 individual plants for separate measurements. Type.
P. polifolia	Forster & Forster	s.n.	FR	Isotype
P. polifolia	Forster & Forster	s.n.	B	Isotype
P. polifolia	Forster & Forster	s.n.	C	Holotype
Wahlenbergia angustifolia	Dumont d'Urville	s.n.	P	Contained 2 individual plants for separate measurements.
W. angustifolia	Dumont d'Urville	s.n.	P	
W. angustifolia	Burchell	67	K	Contained 2 individual plants for separate measurements.
W. angustifolia	Burchell	67-1	K	Contained 2 individual plants for separate measurements.
W. angustifolia	Robertson	s.n.	BM	Contained 2 individual plants for separate measurements. Type.
W. angustifolia	Wallich	s.n.	G	
Wahlenbergia linifolia	Burchell	s.n.	K	
W. linifolia	Brown & Brown	248	US	

W. linifolia	Melliss (?)	s.n.	BM	Contained 4 individual plants for separate measurements. Type.
W. linifolia	Unknown	s.n.	M	
W. linifolia	Royle	s.n.	G	
W. linifolia	Macrae	s.n.	G	
W. linifolia	Hooker	s.n.	G	
W. linifolia	Limminghe	s.n	P	
W. linifolia	Dumont d'Urville	s.n	P	Contained 2 individual plants for separate measurements.
W. linifolia	Dumont d'Urville	s.n	P	
W. linifolia	Haughton	s.n	P	Incorrectly labeled as *W. linifolia*.
W. linifolia	Burchell	s.n	P	Contained 2 individual plants for separate measurements.
W. linifolia	Savatier	1992	P	
W. linifolia	Balansa	s.n	P	
W. linifolia	Dumont d'Urville	s.n	P	

Herbarium abbreviations:

B: Botanischer Garten und Botanisches Museum Berlin
BM: The Natural History Museum
C: University of Copenhagen
E: Royal Botanic Garden Edinburgh
FR: Senckenberg Forschungsinstitut und Naturmuseum
G: Conservatoire et Jardin botaniques de la Ville de Genève
K: Royal Botanic Garden Kew
M: Botanische Staatssammlung München
P: Muséum National d'Histoire Naturelle
US: Smithsonian Institution