

PHYLOGENETICS AND BIOGEOGRAPHY OF *AICHRYSON*
INFERRED FROM CHLOROPLAST DNA SEQUENCES.

BY
T. RYAN O'LEARY

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CHAIR PERSON: _____

MARK MORT

COMMITTEE: _____

BRYAN FOSTER

KIRSTEN JENSEN

DANIEL CRAWFORD

DATE DEFENDED:

The Thesis Committee for Thomas Ryan O'Leary certifies that this is the approved version of the following thesis:

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CHAIR PERSON: _____

MARK MORT

ABSTRACT

Aichryson (Crassulaceae) is a genus of succulent herbs that are endemic to the islands of Macaronesia. The current study provides the best estimate of maternal phylogeny in *Aichryson* to date. Multiple accessions of various taxa were recovered in separate strongly supported clades, calling into question the monophyly of many widespread species, including *A. pachycaulon*, *A. pachycaulon* subsp. *gonzalez-hernandezii*, *A. parlatorei*, *A. punctatum* and *A. villosum*. The taxonomic difficulties in this genus may be complicated by processes such as convergent evolution and/or high levels of hybridization. The biogeography, morphological evolution and modes of speciation in *Aichryson* are discussed in the context of a dated chloroplast phylogeny. The need for further study and nuclear genomic sampling is discussed in light of our results and a plan for future investigation is outlined.

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CHAPTER 1
AN INTRODUCTION TO *AICHRYSON* AND
THE MACARONESIAN CRASSULACEAE

Crassulaceae are a cosmopolitan family of leaf and stem succulent herbs or shrubs that comprise approximately 1500 species in as many as 35 genera (Berger 1930, Mort *et al.* 2001, Eggli 2003, Thiede and Eggli 2007). Members of this lineage can be found in diverse habitats and are especially well adapted to seasonally dry areas with warm temperatures. Although a perennial habit is characteristic of the family, annuals have arisen in various genera, including *Sedum*, *Crassula* (including *Tillea*, Mort *et al.* 2009), *Aichryson* and *Monanthes*. Most taxa in the family produce radially symmetric flowers with a free, unfused calyx, corolla, androecium and gynoecium, although a few notable genera, such as *Kalanchoe*, *Cotyledon* and *Dudleya* share various degrees of connation. Most members of the family possess five-parted flowers, but polymeric flowers occur in several genera including *Sempervivum* and members of the Macaronesian Crassulaceae.

The genera *Aichryson*, *Monanthes* and *Aeonium* (including *Greenovia*) compose a monophyletic assemblage of taxa commonly referred to as the “Macaronesian Crassulaceae” or “*Aeonium* clade” (Ham and ‘t Hart 1998, Mort *et al.* 2001). The age of the most recent common ancestor of the Macaronesian Crassulaceae has been inferred to be 15.2 ± 1.95 Ma (Kim *et al.* 2008) and likely colonized the westernmost

Canary Islands from Morocco, where their closest continental ancestors now reside (Ham and 't Hart 1998, Mort *et al.* 2001). Previous phylogenetic analyses of a combined data set using the chloroplast DNA (cpDNA) regions *matK*, *psbA-trnH* and *trnL-trnF* and the nuclear ribosomal (nrDNA) internal transcribed spacer region (*ITS*) recovered *Aichryson* as monophyletic and sister to a paraphyletic *Monanthes* and monophyletic *Aeonium* (Mort *et al.* 2002, Figure 1.1).

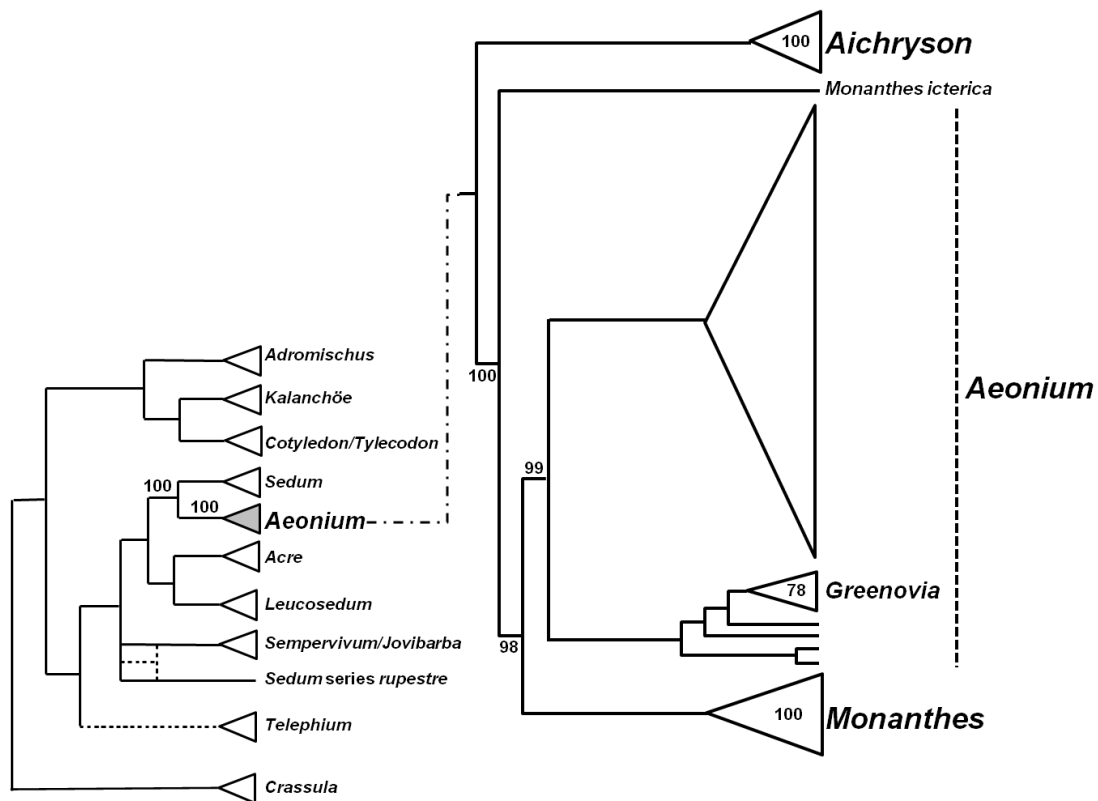


FIGURE 1.1: THE PHYLOGENETICS OF THE MACARONESIAN CLADE OF CRASSULACEAE. *Aichryson* is recovered as the sister genus to a paraphyletic *Monanthes* and a clade comprising *Aeonium* and *Greenovia* (Adapted from Mort *et al.* 2001, 2002).



FIGURE 1.2. REPRESENTATIVE DIVERSITY OF THE MACARONESIAN CRASSULACEAE. Clockwise from the top left: *Aichryson bollei* (T. Ryan O’Leary), *Aichryson bethencourtianum* (T. Ryan O’Leary), *Monanthes* sp. (Mark Mort), *Aeonium palmense* (Mark Mort), *Aichryson villosum* (Hanno Schaefer), *Aichryson laxum* (Mark Mort).

Aichryson contains 17 extant taxa, all of which are endemic to Macaronesia with one species in the Azores, three species in Madeira and nine species in the Canaries.

Aichryson is morphologically, cytologically and ecologically distinct from its closest relatives (Theide and Eggli 2007). Kim *et al.* (2008) inferred the age of the most recent common ancestors of *Aichryson*, *Monanthes* and *Aeonium* to be 8.67 Ma, 6.93 Ma, and 10.23 Ma, respectively. *Aichryson* comprises mostly annual to triennial

herbs, whereas *Monanthes* is composed of a single annual and nine perennial mat-forming shrublets, and *Aeonium* includes ~40 woody, perennials of various size and growth form. Moist rocky slopes and partially shaded areas among the laurel forests comprise the largest distribution of biomass in *Aichryson*, whereas *Monanthes* and *Aeonium* prefer exposed, semi-arid scrubland or gravelly mountain slopes. The perennial habit appears to have arisen once in *Aichryson*, in the most recent common ancestor of *Aichryson tortuosum* and *A. bethencourtianum*. These species are confined to the arid regions of Lanzarote and Fuerteventura, which are the two driest of the Canary Islands (Fairfield 2004). The ancestral base chromosome number in *Aichryson* is $x = 15$ (with the derived conditions of $x = 17, 30, 31, 32,$ and 34 ; Uhl 1961, Bramwell 1977). *Aeonium* and *Monanthes* share a base chromosome number of $x = 18$ (Uhl 1961, 1963).

Aichryson is one of the most difficult Macaronesian genera to understand taxonomically, phylogenetically or biogeographically (Santos-Guerra pers. com.). At least six taxa within *Aichryson* are polyploids (i.e. the five subspecies of *A. pachycaulon* from the Canary Islands and *A. villosum* from Madeira and the Azores). The most recent taxonomic treatments of *Aichryson* recognize 13 species and underscores the need for a thorough revision of the genus (Nyffeler 2003, Theide and Eggli 2007). There are few morphological characters that have been used to distinguish the taxa within *Aichryson* except in a geographic context, because most characters have been found to vary both within and between taxa. For example, the

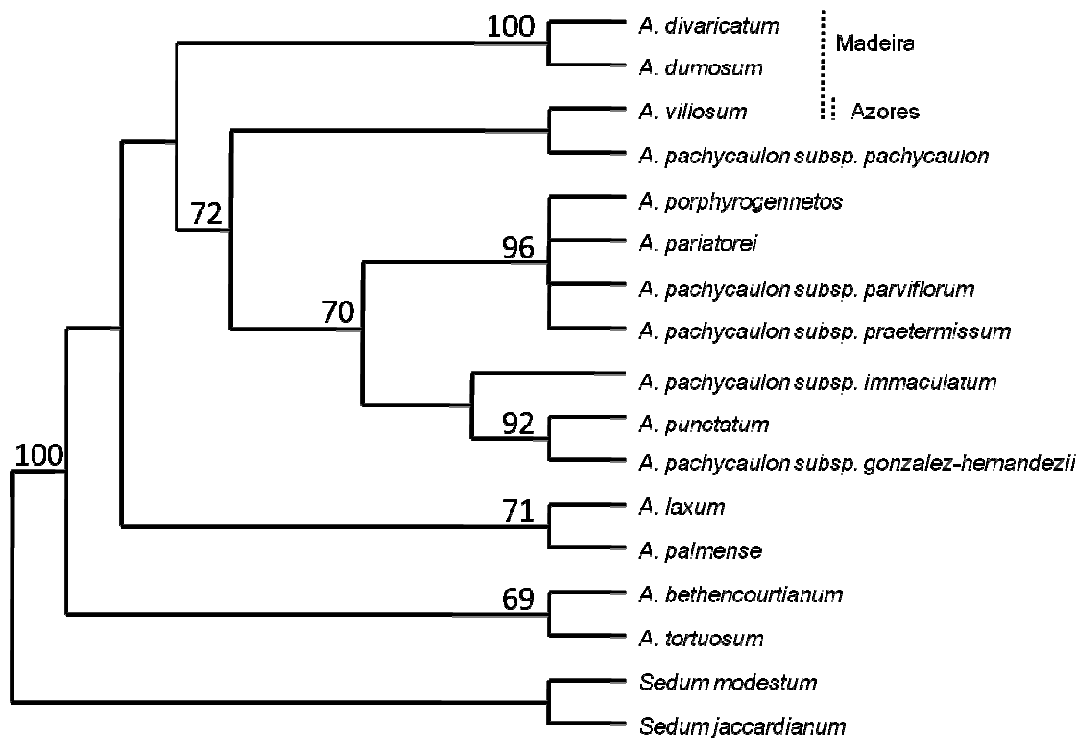


FIGURE 1.3. A PHYLOGENY OF *AICHRYSON*. The numbers before the nodes represent bootstrap support values from parsimony analyses. This analysis inferred at least two archipelagic dispersals in *Aichryson* and recovered the subspecies of *A. pachycaulon* as polyphyletic (Adapted from Fairfield *et al.* 2004).

polyploid subspecies of *A. pachycaulon* are distinguished from each other in the field primarily by the island of occurrence. Within each island, these subspecies are distinguished from a widespread, morphologically similar species, *Aichryson punctatum*, by the presence of punctate glands in the crenulations of leaf margins in *A. punctatum* (Bramwell 1977). However, the presence of punctate glands and crenulations is a variable character within and between populations of *A. pachycaulon* and *A. punctatum* and can vary with the age of the plant (O’Leary pers. obs.) or the condition of growth (Nyffeler 2003). Chapter 3 provides a more thorough examination of morphological variability in this genus.

A preliminary molecular phylogeny, including 15 of the 17 extant taxa within *Aichryson*, combined the cpDNA *trnL-trnF* region with the nrDNA *ITS* region and recovered the five subspecies of *A. pachycaulon* as polyphyletic (Fairfield *et al.* 2004). In the combined analysis, the two perennial species were resolved as sister to the herbaceous species (Figure 1.3), but this placement, as well as the next two branching lineages received low bootstrap support (< 50%). Stronger support (72%) existed for at least two dispersals of *Aichryson* from the Canary Islands to the northern archipelagos of Madeira and the Azores.

Subsequent work on *Aichryson* with complete taxon sampling recovered multiple strongly supported incongruences between the *ITS* and cpDNA phylogenies (O’Leary *et al.* 2008, Figure 1.4). Hybridization has been proposed as an important mode of speciation in *Aichryson*, especially allopolyploid speciation (Bramwell 1977), although no subsequent work has been done to confirm this. Common problems with the use of *ITS*, including gene duplication, the formation of pseudogenes and concerted evolution (Alvarez and Wendel 2003, Small *et al.* 2004), may be compounded in *Aichryson* due to possible genomic evolution following polyploidy in the genus. To approach the problem of phylogenetic inference in the genus, it was necessary to infer phylogeny using chloroplast DNA sequences, which are maternally inherited and non-recombining. However, molecular diversity in cpDNA regions is

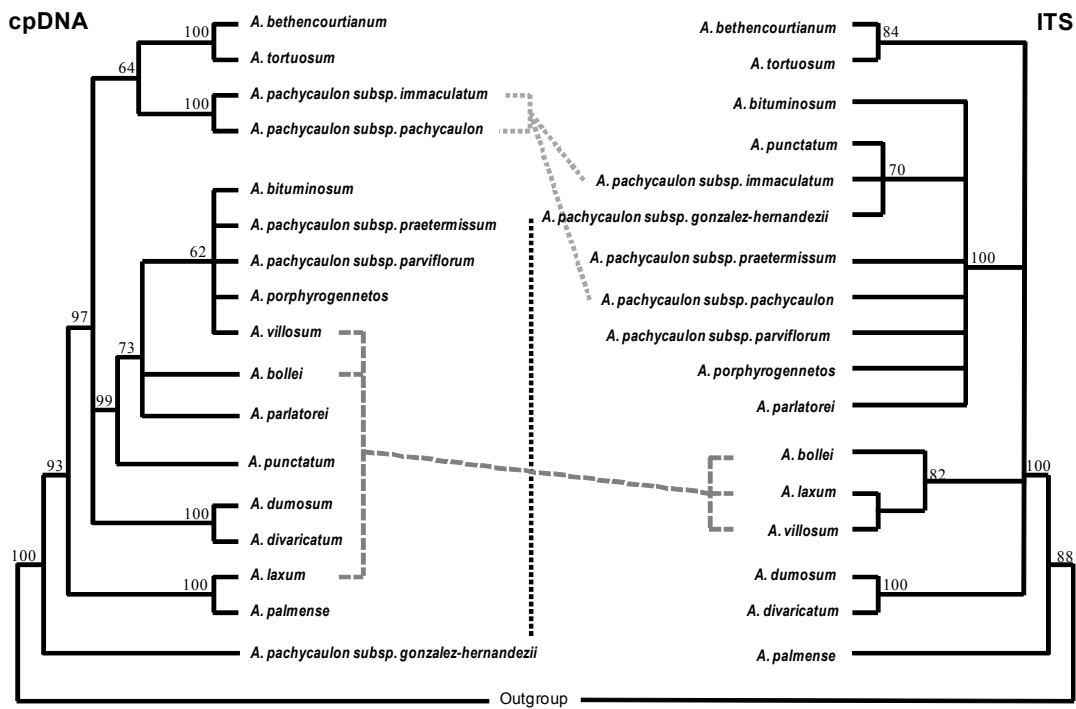


FIGURE 1.4. INCONGRUENCE BETWEEN NUCLEAR AND PLASTID PHYLOGENIES. Preliminary work on the genus has uncovered multiple well supported incongruences between *ITS* and cpDNA phylogenies (Adapted from O’Leary *et al.* 2008). The current study focuses on producing a well resolved and strongly supported cpDNA phylogeny.

often particularly low in insular taxa such as *Aichryson*, which requires screening a large number of regions for phylogenetic utility (Mort *et al.* 2007).

The current study provides the best estimate of maternal phylogeny in *Aichryson* to date, including multiple accessions of morphologically similar taxa and a broad sample of the chloroplast genome. Multiple accessions of various taxa were recovered in separate strongly supported clades, calling into question the monophyly of *A. pachycaulon*, *A. pachycaulon subsp. gonzalez-hernandezii*, *A. parlatorei*, *A. punctatum* and *A. villosum*. The taxonomic difficulties discussed above may be worse than previously suspected and may be complicated by processes such as

convergent evolution and high levels of hybridization. If hybridization has been common in *Aichryson* then both chloroplast capture and/or allopolyploidy may play a role in the phylogenetic incongruence. The biogeography, morphological evolution and modes of speciation in this genus are discussed in the context of a dated chloroplast phylogeny. The need for further study and nuclear genomic sampling is discussed in light of our results and a plan for future investigation is outlined.

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CHAPTER 2

*AICHRYSO*N AND THE GEOLOGY OF THE CANARY ISLANDS

INTRODUCTION

Islands provide natural laboratories for scientists to study biological phenomena. For evolutionary biologists, island systems are an indispensable and threatened natural resource. Indeed, Charles Darwin's collections on the Galapagos Islands and the nearby continent as well as Alfred Russell Wallace's subsequent visit to the Malay archipelagos provided the biological ingredients to complement Thomas Malthus' economic theories and result in two independent formulations of the Theory of Natural Selection.

Probably the most important and defining characteristic of island systems is their relative isolation. This isolation can provide island communities the space and time to develop insular floras and faunas without continual gene flow from ancestral populations. The theory of island biogeography (MacArthur and Wilson 1967) attempts to explain patterns of species richness on islands through an equilibrating process of invasion and extinction, where distance from the mainland is inversely proportional to the number of species that colonize an island. This theory provides a framework for establishing diversity on islands on geologically short timescales. Phylogeneticists, on the other hand, are interested in phenomena that occur over a

much longer timescale and so would add *speciation* to the list of factors important to island biogeography (Whittaker 1998). In particular, organisms that arrive on islands may be released from the competition they experience on the continent and so may diversify ecologically to a greater extent than natural selection formerly allowed. Spectacular island organisms, such as the Galapagos finches, the Hawaiian silversword alliance or the *Aeonium* alliance of Macaronesia, are all purported to be the result of adaptive radiations following colonization of oceanic islands and isolation from continental ancestors (Lems 1960, Baldwin 1997, Grant 1999, Jorgensen and Olesen 2001). Furthermore, one of the most strongly supported (although controversial) hypotheses of sympatric speciation comes from an island system (Savolainen *et al.* 2006). It is the extreme isolation and small size of Lord Howe Island that led researchers to hypothesize that two sister species of palm (*Howea belmoreana* and *H. forsteriana*) diverged in sympatry.

Another important aspect of island systems is that they are generally much younger than the nearest continental land masses. The emergence of islands can often be dated with a high degree of precision, so that the maximum ages of endemic taxa can be inferred (assuming the taxa have always been endemic to the island), which provides a calibration point for estimating rates of evolution and divergence times (e.g. Fleischer *et al.* 1998, Contreras-Díaz *et al.* 2007, Kim *et al.* 2008). Dated phylogenies can, in turn, be used to test biogeographic hypotheses attempting to

explain dispersal histories of organisms from the mainland to islands or between islands or archipelagos.

In this chapter, I will discuss the characteristics of the Canary Islands that make them an exciting yet complicated place to conduct phylogenetic research. These islands provide a living laboratory for researchers to explore evolutionary processes in a variety of relatively isolated environments that have persisted for as long as 23 million years or as short as a few hundred thousand years. Active volcanoes, combined with a history of landslides and erosion, have produced dynamic environmental gradients within and between islands. Some islands, such as Fuerteventura, Lanzarote and La Gomera have remained relatively stable for millions of years, while others, such as Tenerife, Gran Canaria, La Palma and El Hierro have undergone recent, massive changes (see below). The Canary Islands have also served as a source area for subsequent colonization of other archipelagos within Macaronesia. Efforts by geologists over the past three decades have provided dated evidence of major events on each island that are essential for the interpretation of the biogeographic history of life in the Macaronesian archipelagos.

THE CANARY ISLANDS

The Canary Islands comprise seven volcanic islands and a few islets in the Atlantic Ocean west of Morocco. The leading hypothesis for the formation of the Canaries

proposes that the volcanoes formed from hotspots in the crust that were caused by mantle plumes (Morgan 1971, Burke 1976, Geldmacher *et al.* 2005). However, data from the Macaronesian Islands are not easy to interpret using the mantle plumes hypothesis (when compared to data from the Hawaiian Islands, for example) so controversy exists as to how these volcanoes were formed (e.g. Anderson 2000, Smith 2003, Guillou *et al.* 2004). At their closest point on Fuerteventura, the Canary Islands are only ~100 km from the continent. The point of the Canaries most distant from the mainland is on the western side of La Palma at about 450 km. There are nearly 700 endemic plant species and subspecies, which account for nearly 50% of the native flora (Machado 2002). It is possible to roughly divide the Canary Islands into two groups: (1) the old, low islands in the east (i.e. Fuerteventura and Lanzarote) and (2) the younger, high islands in the west (i.e. Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro). These groups can be distinguished by age, height, habitat diversity and biodiversity (Figure 2.1).

Fuerteventura and Lanzarote are the oldest islands and although they are mountainous and volcanically active, their peaks are substantially lower than those of the western islands (Figure 2.1). The five western-most islands have higher habitat diversity than the older, lower islands due in part to their high elevation coupled with the moisture-laden northeast trade winds that are forced up steep mountain slopes, cooling and condensing in mid to high elevations. The eastern islands lack the elevation necessary to promote these substantial moisture gradients. The generally arid conditions combined with centuries of human exploitation have burdened the flora of

the eastern islands. In 1924, a prominent Canary Island botanist, R. Lloyd Praeger, noted, “On Fuerteventura the people think themselves lucky if they get good rain twice in the year, and this amount suffices to yield excellent crops.” Additionally, the two eastern islands are exposed to the scorching, dusty winds blowing west from the Sahara. Deposits of these dusts preserved under lava flows in Lanzarote have recently been used to reconstruct the paleoclimate of the Saharan region of Africa (von Suchodoletz *et al.* 2008).

TABLE 2.1. THE REPORTED OCCURRENCES OF *AICHRYSON* IN MACARONESIA.

SPECIES	DISTRIBUTION	ISLAND	# OF SPECIES
<i>A. bethencourtianum</i>	F	Fuerteventura	3 or 4*
<i>A. bituminosum</i>	GC	Lanzarote	1
<i>A. brevipetalum</i>	LP – extinct?	Gran Canaria	6
<i>A. bollei</i>	LP	Tenerife	4 or 5**
<i>A. divaricatum</i>	M	La Gomera	4
<i>A. dumosum</i>	M	La Palma	6 + 1 extinct
<i>A. laxum</i>	T, GC, LP, H, LG	El Hierro	3
<i>A. pachycaulon</i>		M:MADEIRA, AZ:AZORES, F:FUERTEVENTURE, L:LANZAROTE, GC: GRAN CANARIA, T:TENERIFE, LP:LA PALMA, H:EL HIERRO.	
<i>subsp. gonzalez-hernandezii</i>	LG		
<i>subsp. immaculatum</i>	T		
<i>subsp. pachycaulon</i>	F		
<i>subsp. parviflorum</i>	LP		
<i>subsp. praetermissum</i>	GC		
<i>A. palmense</i>	LP		
<i>A. parlatoresi</i>	T, GC, LP, LG, H, <u>F*</u>		
<i>A. porphyrogennetos</i>	GC, <u>T**</u>		
<i>A. punctatum</i>	T, GC, LP, H, LG		
<i>A. tortuosum</i>	F, L		
<i>A. villosum</i>	M, AZ		

*Fairfield *et al.* (2004) report that *A. parlatoresi* occurs on Fuerteventura but not on El Hierro, but this appears to be an error. *A. parlatoresi* has reported occurrences on El Hierro, but not Fuerteventure.

**Praeger (1928) and Bañares (2002) report the historical occurrence of *A. porphyrogennetos* on Tenerife as well as Gran Canaria, but Fairfield *et al.* (2004), Nyffeler (2003) and Bañares *et al.* (2008) report the taxon as a Gran Canarian endemic.

Compared to the western islands, biodiversity is low on Fuerteventura and Lanzarote. On these islands, species diversity is greatest on the northeast point of Lanzarote and southwestern Fuerteventure (Reyes-Betancort *et al.* 2008), two areas that coincide with the highest altitudes on each island, peaking at ~670 m on Lanzarote and ~800 m

on Fuerteventura. Four species of *Aichryson*, including both perennial subshrubs, occur in the eastern islands. Of the perennials, *A. bethencourtianum* is endemic to Fuerteventura and *A. tortuosum* occurs on both Fuerteventura and Lanzarote. *Aichryson pachycaulon subsp. pachycaulon* is restricted to the relatively moist, north-facing slopes of the Jandía peninsula of Fuerteventura. Although Fairfield *et al.* (2004) reported *A. parlatorei* to be present on Fuerteventura, this appears to be a typographical error that substituted Fuerteventura for El Hierro.

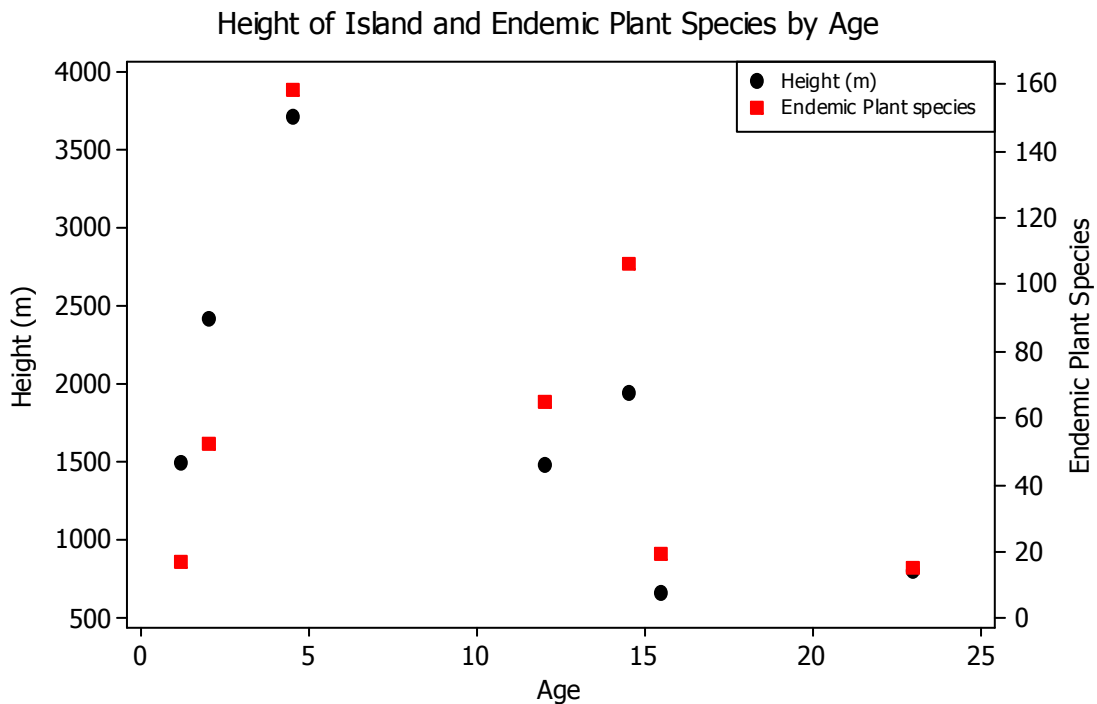


FIGURE 2.1. THE NUMBER OF ENDEMIC PLANT SPECIES AND HEIGHT OF AN ISLAND VS. ITS AGE. From left to right the points represent El Hierro, La Palma, Tenerife (central), La Gomera, Gran Canaria, Lanzarote and Fuerteventura. See text for a more thorough discussion of island formation and biodiversity. Data from Machado (2002) and Reyes-Betancort *et al.* (2008).

Gran Canaria can be considered a transition between the eastern and western islands. Although Gran Canaria is only 1-2 million years younger than Lanzarote, much of the surface of Gran Canaria is 3-5 Ma. Massive volcanic activity during this period added substantially to the mass of the island and may have destroyed most or all life on the island's surface (Araña and Carracedo 1980, Pérez Torrado *et al.* 1995, Marrero and Francisco-Ortega 2001). Multiple phylogenetic investigations are consistent with the hypothesis of widespread destruction of life on Gran Canaria (Emerson 2002 and citations within), because of the young age and close relatedness of the Gran Canarian taxa. *Aichryson* can serve as an additional test of the biogeographic hypothesis of a young age for Gran Canarian endemics. Despite the relatively short time since the scorching of the island's surface, Gran Canaria is a hotspot for diversity and endemism in *Aichryson*. Six species and subspecies occur on Gran Canaria (Table 2.1) of which three are endemic, including: *A. porphyrogenetos*, *A. pachycaulon subsp. praetermissum*, and *A. bituminosum*.

The highest point on the Canaries is Mount Tiede, a volcanic peak near the center of Tenerife that lies within Las Cañadas Caldera and abreast of multiple volcanoes that have been active over the past 200 thousand years (Carracedo *et al.* 2007). Tenerife may be the most geologically and biogeographically complex of all the islands. The now continuous island is a conglomeration of three shield volcanoes (Anaga, Teno and Roque del Conde), ranging in ages from 11.9 to 4.9 million years (Guillou *et al.* 2004). Phylogenetic evidence has pointed to the joining of Anaga and Teno less than

2 Ma as important for the phylogeography of many animal species including lizards, skinks, beetles, mites and cockroaches (Juan *et al.* 2000 and citations within). Four species and subspecies of *Aichryson* occur on Tenerife (Table 2.1), three of which are widespread across the Canary Islands. *Aichryson pachycaulon subsp. immaculatum* is the only taxon endemic to the island.

PERIOD	EPOCH	OLDEST SURFACE VULCANISM*		IMPORTANT EVENTS		
		Island	Date (Ma)	Island	Event	Date (Ma)
QUATERNARY	HOLOCENE			Tenerife	120-present Las Cañadas Caldera fills in ^{#,%,S}	0.12-0.00
				El Hierro	El Golfo collapses ⁺	0.13-0.25
			Tenerife	Las Cañadas Caldera formed ^{#,%,S}	0.22-0.16	
	PLEISTOCENE	El Hierro	< 1.12	Tenerife	La Orotava and Guimar valley landslides ^{#,%,S}	0.37-0.80
La Palma		< 2.0	El Hierro	El Julan collapse ⁺	~0.5-0.3	
La Gomera			La Palma	Cumbre Nueva embayment forms ⁺	0.56	
Gran Canaria			El Hierro	Tiñor collapse ⁺	~0.88	
				Gran Canaria	Explosive volcanism and scorching of island surface***	3.0-3.9
TERTIARY	PLIOCENE	Tenerife Las Cañadas volcanism between paleo-islands	4.5	Gran Canaria	Roque Nublo formation***~	3.0-5.6*** 3.5-4.5~
	MIOCENE	Tenerife Anaga@ Teno@	4.9 6.2	*Unless otherwise noted, dates are from Carracedo <i>et al.</i> 1998, 2002. ** Gurenko <i>et al.</i> 1991 *** Pèrez-Torrado <i>et al.</i> 1995 ~Funck and Schmincke 1998 + Carracedo <i>et al.</i> 1999 % Juan <i>et al.</i> 2000 # Hürlimann and Ledesma 2000 @ Guillou <i>et al.</i> 2004 ! Paris <i>et al.</i> 2005 S Carracedo <i>et al.</i> 2007		
		La Gomera'	9.43			
		Tenerife Roque del Conde@	11.9			
		Gran Canaria**	14.5			
		Lanzarote	15.5			
		Fuerteventura	23			

In recent times, La Gomera has been the most volcanically stable island. Volcanic activity on the island ceased around 3 Ma (Cerracedo 1998), so the last three million years can be characterized as a period of continual erosion. Even so, the highest point on La Gomera (1484 m) is over twice as high as that of Lanzarote (671 m). Despite its old age and height, La Gomera houses only four named species and subspecies of *Aichryson*, three of which are purportedly widespread on the western islands. The only named endemic is *A. pachycaulon subsp. gonzalez-hernandezii*.

La Palma and El Hierro are the youngest islands and consequently have changed dramatically in the past one million years. The approximate date of emergence for these islands is 2.0 and 1.12 Ma, respectively and they remain volcanically active. In the past century, two series of eruptions (1949 and 1971) were recorded on La Palma (Pararas-Carayannis 2002). Despite an increase in mass due to lava flows and uplift, massive landslides have left an indelible mark on these islands and reduced their surface area substantially. Nearly half a million years ago, La Palma lost 200 km³ of land during the formation of the Cumbre Nueva embayment, while El Hierro has lost 400-500 km³ in multiple landslides, leaving only 140 km³ currently (Carracedo *et al.* 1999). Like Gran Canaria, La Palma is a hotspot for diversity in *Aichryson* with six species and subspecies, three of which are endemic (*A. bollei*, *A. palmense* and *A. pachycaulon subsp. parviflorum*). There currently are no species of *Aichryson* endemic to El Hierro, but three of the widespread species (*A. laxum*, *A. punctatum* and *A. parlatorei*) occur there.

Three species of *Aichryson* occur on archipelagoes north and west of the Canary Islands. The islands of Madeira (5 to 18 million years old) set about 400 km north of La Palma, while the Azores (<1 to 8 million years old,) are located approximately 1100 km to the northwest (Abdel-Monem *et al.* 1975, Fernandez-Palacios and Martín-Esquivel 2001). Previous phylogenetic work recovered the two Madeiran endemics (*A. divaricatum* and *A. dumosum*) as monophyletic with 100% bootstrap support, while a single accession of *A. villosum* from the Azores (but also occurs on Madeira) was nested among a broadly distributed assemblage of Canary Island species (Figure 1.3).

The extensive geological work on the Canary Islands has made it possible to put upper limits on node ages for single island endemics. This is important because the fossil record is uninformative with respect to the Macaronesian Crassulaceae. This approach, however, can result in age inference throughout the phylogeny to be limited relative to the oldest age of a single island. If, due to a large degree of uncertainty, only the root of the phylogeny can be restricted to the origination of the archipelago, then ages of internal nodes may be erroneously pushed back in time until the estimate for the root bumps up against the age of the oldest island. In *Aichryson*, the presence of three taxa endemic to La Palma allows an upper limit of 2 Ma on the deepest of the three nodes to prevent drastic over-estimation of divergence times. In the next

chapter, the phylogenetics, morphological evolution and biogeography of *Aichryson* is discussed in reference to a dated phylogeny.

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CHAPTER 3

PHYLOGENETICS, TAXONOMY AND BIOGEOGRAPHY OF *AICHRYSON*

INTRODUCTION

Previous phylogenetic inference in *Aichryson* supports the clade as sister to the remaining Macaronesian Crassulaceae. The monophyly of *Aichryson* has been supported by all previous analyses, but the monophyly of its component taxa has yet to be tested extensively. Fairfield *et al.* (2004) recovered the subspecies of *A. pachycaulon* as polyphyletic, but only a single accession of each taxon was used; additionally, *A. bollei* and *A. bituminosum* were not included in those analyses. With subsequent, unpublished investigation in this genus, it has become increasingly obvious that the major obstacle to molecular phylogenetic inference within *Aichryson* is not taxon sampling, *per se*, but the more fundamental problem of taxon circumscription and identification. Few species are easily distinguished from morphologically similar species and many characters used to define species are variable within that taxon. Multiple efforts to refine the original treatment of *Aichryson* (Praeger 1932, Bramwell 1968, 1977, 1990, Bañaras 1997, 2002, Bañaras *et al.* 2008) have provided more precise taxon circumscription, which has aided species identification, but none have done so in a phylogenetic context.

The most morphologically distinct clade comprises the two woody subshrubs, *A. tortuosum* and *A. bethencourtianum*, that share a perennial habit and tortuous branching. They are distinguished from each other primarily by the excretion of a viscid substance from the dense hairs of *A. tortuosum*, which has sessile leaves, as opposed to *A. bethencourtianum*, which has indistinctly petiolate leaves and longer, non-glandular hairs. They occur sympatrically on Fuerteventura, but only *A. tortuosum* has reported occurrences on Lanzarote. Bañaras et al. (2008) proposed sinking the species *A. bethencourtianum* into the variety *A. tortuosum* var. *bethencourtianum* on the basis of their similar morphology that becomes less distinct within their sympatric distribution in central and northern Fuerteventura as opposed to the allopatric populations of *A. bethencourtianum* in southern Fuerteventura and *A. tortuosum* on Lanzarote.

The same sort of minor vegetative distinctions that vary over a species range separate various taxa from the widespread species *A. laxum*. *Aichryson laxum* is readily distinguished by its 9-12-meris flowers as opposed to 5-9-meris flowers common in the rest of the genus, although *A. laxum* var. *latipetalum* Bañaras and M. V. Marrero (Tenerife, Barranco de Añavingo) is distinguished from its conspecifics by sharing the more generic condition of 7-9-meris flowers. On La Palma, *A. palmense* is distinguished vegetatively from *A. laxum* and *A. bollei* by the presence of dense glandular hairs and young leaves that curl at the margins in *A. palmense* as opposed to dense non-glandular hairs and less curling of young leaf margins in the latter taxa.

Aichryson bollei is distinguished from *A. laxum* by shorter, often less dense hairs and the inconspicuous presence of dark, glandular spots (punctate glands) in the crenulations of the leaf margins that are lacking in *A. laxum*, although dark pigmentation may occur in an indentation at the leaf apex in *A. laxum*.

All species of *Aichryson* (except possibly *A. divaricatum*) have various levels of red to purplish pigmentation of the leaves and sepals, which can vary with the age and condition of growth. *Aichryson porphyrogenetos* is separated from *A. laxum* because it is generally more conspicuously red-pigmented throughout the leaves, stem and calyx, and more divaricately branching (branching at a wider angle) than the populations of *A. laxum* on Gran Canaria (Bañaras 2002, Nyffler 2003).

Due to its wide distribution and morphology, *A. punctatum*, like *A. laxum*, is a taxon that may be confused with many other members of the genus. *Aichryson punctatum* is named for the conspicuous punctate glands in crenulations along the leaf margins, and may be partially to sparsely hairy or entirely glabrous. *Aichryson bituminosum* as described by Bañares (2002) is also distinctly punctate, but the punctate glands are along the non-crenulate leaf margin, which may be papillose (or not). The glandular hairs of *A. bituminosum* exude a substance that smells of bitumen. The subspecies of *A. pachycaulon*, which are glabrous to subglabrous, can be particularly difficult to distinguish from various forms of *A. punctatum*. A generally thicker stem and lack of distinct punctation traditionally separates *A. pachycaulon* from *A. punctatum*, but like

most features used to separate taxa in this genus, these characters are variable.

Within *A. pachycaulon*, Bramwell (1977) stressed small differences in mean floral diameter as a way to distinguish populations of the subspecies, but island of occurrence is still the most consistent way to separate the taxa.

Aichryson parlatoresi is more or less defined as a dwarf version of *A. punctatum*, although *A. parlatoresi* may possess papillae along the leaf margins and at times can be densely hairy, but these characters are variable among the widespread populations of *A. parlatoresi* on the Canaries (Nyffler 2003). In addition, size at flowering is a highly variable condition in the genus that is strongly affected by microenvironmental factors (Nyffler 2003, O'Leary pers. ob.). The purportedly extinct dwarf species, *A. brevipetalum*, is distinguished from *A. parlatoresi* and other species by possessing petals that are shorter than sepals and glandular hairs. Bañares (2002) reports collections of *A. brevipetalum* in northeastern La Palma, but attempts to collect it are rarely successful.

Aichryson brevipetalum may be a taxon defined by a suit of rare characteristics that occur in various taxa under stressful conditions. The condition of a calyx that exceeds the corolla is present in an individual of *A. laxum* from El Hierro that was grown from two cuttings at the University of Kansas. The emergent inflorescence of these cuttings were composed of 1 to 3 flowers densely packed in the leaf axis. The flowers did not completely open, but produced seed, presumably through selfing.

Each cutting was in flower when taken and originally possessed the more common characteristic of a corolla that exceeded the calyx on a lax inflorescence.



Aichryson villosum is a scarcely studied taxon that occurs both on Madeira and the Azores. This taxon is the only member of the

FIGURE 3.1. *AICHRYSON LAXUM*. The condition of sepals that exceed the petals, although characteristic of the doubtful taxon *A. brevipetalum* from La Palma was found on single-flowered axial inflorescences in an accession of *A. laxum* from El Hierro, grown from a cutting.

genus known to occur naturally on the Azores, and is briefly described as a relatively short (8-18 cm tall) member of the genus, and may be nearly rosette-forming during early stages of life. Internodes lengthen during flowering and the flowers can be conspicuously large for the genus (5-15 mm as compared to a range of 3-8 mm for the rest of the genus), especially considering its diminutive size. However, the lower range for flower size and the upper range for overall height is typical of the genus.

Aichryson villosum is named for its soft and dense hairs, although these may be shed on the lower part of the plant, which can be sparsely to profusely red pigmented.

The previous species comparisons are a short illustration of how taxon identification can be a problem in this genus and that taxon definition is likely a larger problem.

Traditional taxonomy may not have the tools necessary to describe and categorize

diversity in this genus, because it relies on superficial similarities that are often variable and rarely diagnostic except in a geographical context. As this chapter will demonstrate, members of *Aichryson* are highly vagile. Consequently, morphologically identical taxa that occur on the same island may be polyphyletic, while morphologically distinct taxa that are geographically isolated may be close relatives. For *Aichryson*, molecular phylogenetics is necessary to provide a framework for the interpretation of morphological diversity and the identification of cryptic or hybrid species among all populations of *Aichryson* in Macaronesia. The completion of this project is far beyond the reasonable bounds of a master's thesis, but this thesis provides evidence that phylogenetic inference in *Aichryson* is likely to be as intricate a problem as taxonomic definition has been. In particular, the monophyly of many taxa is called into question, which underscores the need for more extensive study.

METHODS

Taxonomic sampling includes all extant taxa within the genus with the exception of the doubtful *A. brevipetalum*. Multiple accessions of *A. laxum*, *A. parlatoresi*, *A. punctatum*, *A. pachycaulon*, *A. pachycaulon subsp. gonzalez-hernandezii*, and *A. villosum* were included as a preliminary test of the monophyly of these taxa (Table 3.1). Leaf tissues or flowers and stems were collected from field locations on the Canary Islands. A single sample of *A. bethencourtianum* was taken from a cutting

TABLE 3.1. ACCESSION LOCALITY AND VOUCHER INFORMATION.

L:Lanzarote, F:Fuerteventura, GC:Gran Canaria, T:Tenerife, LP:La Palma, EH:El Heirro, SM:Santa Maria (Azores), M:Madeira, MO:Morocco.

TAXON	ISLAND	VOUCHER
<i>A. bethencourtianum</i> Bolle	F	HBG
	F	TRO s.n. (KANU)
<i>A. bituminosum</i> Bañares	GC	Santos s.n. (CI)
<i>A. bollei</i> Webb ex Bolle	LP	Mort 1904 (KANU)
<i>A. divaricatum</i> (Ait.) Praeger		Fontinha s.n. (KANU)
<i>A. dumosum</i> (Lowe) Praeger		Fontinha s.n. (KANU)
<i>A. laxum</i> (Haw.) Bramwell	LP	Santos s.n. (CI)
	EH	Mort 1494 (WS)
	EH	Mort 08.039 (KANU)
<i>A.cf. pachycaulon</i> Bolle	EH	Mort 08.040 (KANU)
<i>A. pachycaulon</i> Bolle	LG	KF21 (CI)
<i>subsp. gonzalez-hernandezii</i> (Kunk.) Bramw.	LG	KF19 (CI)
	LG	Mort 08.018 (KANU)
<i>subsp. immaculatum</i> (Webb ex Christ) Bramw.	T	Santos s.n. (CI)
<i>subsp. pachycaulon</i>	F	Santos s.n. (CI)
<i>subsp. parviflorum</i> (Bolle) Bramw.	LP	Santos s.n. (CI)
<i>subsp. praetermissum</i> Bramw.	GC	Santos s.n. (CI)
<i>A. palmense</i> Webb ex Bolle	LP	TRO s.n. (KANU)
	LP	Mort 1482 (WS)
<i>A. parlatoarei</i> Bolle	LP	Santos s.n. (CI)
	T	Mort 1838 (KANU)
<i>A. porphyrogennetos</i> Bolle	GC	Santos s.n.
	GC	Mort GC0306
	LG	Mort 08.029 (KANU)
	LG	Mort 1450 (WS)
<i>A. punctatum</i> (Chr. Sm. ex Buch) Webb et Berth.	LG	Santos s.n. (CI)
	LP	Santos s.n. (CI)
	T	Santos s.n. (CI)
<i>A. tortuosum</i> (Aiton) Webb and Berthel.	L	Santos s.n. (CI)
<i>A. villosum</i> (Ait.) Webb et Berth.	SM	Santos s.n. (CI)
	SM	Santos s.n. (CI)
<i>Aeonium longithrysum</i>		Mort 1498 (WS)
<i>Monanthes laxiflora</i> (DC.) Bolle		Mort 1417
<i>M. icterica</i> (Webb ex Bolle) Praeger		Mort s.n.
<i>Sedum jaccardianum</i> Maire and Wilczek	MO	32211 (UT)
<i>S. modestum</i> Ball	MO	33112 (UT)

grown in the University of Kansas greenhouses by T. Ryan O’Leary, and was found to have an *ITS* sequence identical to the accession used in Fairfield *et al.* (2004).

Genomic sampling included seven chloroplast regions (*matK*, *psbA-trnH*, *rpoB-trnC*, *rpoC*, *rps16*, *trnL-trnF*, *ycf6-psbM*; Table 3.2) resulting in 5741 aligned base pairs.

Geographic locality of each accession and voucher information are reported in Table 3.1.

The outgroup consists of two species previously shown to be the closest continental relatives of the Macaronesian Crassulaceae (*Sedum jaccardianum* and *S. modestum*) as well as *Aeonium longithrysum* (a member of the *A. canariense* (L.) Webb and Berth. species complex), *Monanthes laxiflora* and *M. ictERICA*. Various molecular analyses have recovered *Monanthes* as paraphyletic, despite a distinct floral morphology that seems to provide an obvious synapomorphy for a *Monanthes* clade. In particular, the only annual, *M. ictERICA*, has been recovered as sister to *Aichryson* (Mes *et al.* 1997) or sister to a clade composed of the an otherwise monophyletic *Monanthes* and *Aeonium* (including *Greenovia*, Mort *et al.* 2002). It is important to include *M. ictERICA* in phylogenetic inference in *Aichryson* not as a test of the monophyly of *Monanthes*, but because its affinities to *Aichryson* suggest that it is an appropriate outgroup for the inference of pleisiomorphic character states.

Genomic DNA was extracted from dried leaf samples using a modified CTAB method (Mort *et al.* 2001) and subsequently frozen in Tris-EDTA. All regions were

TABLE 3.2. PCR AMPLIFICATION OF SEVEN PLASTID REGIONS.
¹Johnson and Soltis (1994), ²Sang *et al.* (1997), ³Shaw *et al.* (2005), ⁴Chase *et al.* 2007, ⁵Shaw *et al.* 2005, ⁶Taberlet *et al.* (1991).

Region	Primer ID	Approximate Length	Primers μM dilution $\mu\text{L}/\text{rxn}$	Master Mix 1X	DMSO $\mu\text{L}/\text{rxn}$	DNA Template $\mu\text{L}/\text{rxn}$	Total Reaction μL
<i>matK</i>	<i>1470F-psbAR</i> ¹	1150	2 μM 3 $\mu\text{L}/\text{rxn}$	Bulls Eye	0.34	2.66	50
<i>psbA-trnH</i>	<i>psbAF-trnHR</i> ²	300	2 μM 2 $\mu\text{L}/\text{rxn}$	Bulls Eye	0.20	4.2	50
<i>rpoB-trnC</i>	<i>rpoB-trnC^{GCA}R</i> ³	1230	20 μM 1.5 $\mu\text{L}/\text{rxn}$	Biomix	0	2.0	50
<i>rpoC</i>	<i>rpoC2-rpoC4</i> ⁴	535	2 μM 1.5 $\mu\text{L}/\text{rxn}$	Bulls Eye	0	2.0	50
<i>rps16</i>	<i>rps16F-rsp16R</i> ⁵	825	20 μM 1.5 $\mu\text{L}/\text{rxn}$	Biomix	0	2.0	50
<i>trnL-trnF</i>	<i>C-F</i> ⁶	875	1 μM 1.5 $\mu\text{L}/\text{rxn}$	Biomix	0	3.0	50
<i>ycf6-psbM</i>	<i>ycf6F-psbMR</i> ⁵	890	20 μM 1.5 $\mu\text{L}/\text{rxn}$	Biomix	0	2.0	50

amplified from 10:1 dilutions of distilled water and DNA extract, respectively. The various regions required minor modifications of PCR protocols. Table 3.2 reports the parameters used for PCR reactions for each region. Sequencing reactions were accomplished either in house on a Beckman Coulter CEQ 8000 genetic analysis system or at MacroGen Corporation (Seoul, Korea).

Sequences were aligned manually using Se-AL v2.0a11 (© Andrew Rambaut 1996-2009) and exported in NEXUS format. The exported data sets were reexamined and edited using the text editor in PAUP* (Swofford 1998). Maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BI) methods of phylogenetic inference were employed using PAUP*, GARLI (Zwickl 2006) and BEAST (Drummond *et al.* 2007), respectively. For each method the NEXUS file was modified to meet the

requirements of the program (e.g. no interleaving in GARLI-GUI). For the Bayesian analyses, the program BEUti in the BEAST package was used to build an XML file from the NEXUS file.

For all MP analyses characters were equally weighted and unordered. Tree searches were conducted in PAUP* with 1000 random addition sequence repetitions, using TBR branch-swapping, collapsing branches with minimum length of zero and saving one tree at each step. Bootstrap support was estimated using 200 using the above settings except that 20 addition sequence replicates were conducted per bootstrap replicate. ML analyses were conducted in PAUP* by implementing the Modeltest block (Posada and Crandall 1998) and the best model was chosen using both the AIC and BIC criteria in Modeltest (Posada and Buckley 2004). Both criteria agreed on the same model (TVM+I+G: $-\ln L = 11917.2471$; $K = 9$; $BIC = 23912.3926$, $AICc = 23852.5254$; $A = 0.3368$, $C = 0.1548$, $G = 0.1611$, $T = 0.3473$; $A-C = 1.0025$, $A-G = 0.8943$, $A-T = 0.1957$, $C-G = 0.6162$, $C-T = 0.8943$, $G-T = 1.0000$; $P_{inv} = 0.5459$; $\alpha = 0.8622$).

ML analysis and parameter estimation was also conducted using GARLI, which implements a genetic algorithm to mutate parameters and tree topology over many generations until mutation no longer results in appreciable topological or model improvement. The general advantage of GARLI is the speed at which it can arrive at both a model and a tree (less than 20 minutes for *Aichryson*) as opposed to many

hours of computation time and the use of multiple programs using the ModelTest and PAUP* methods. This speed is especially effective during bootstrapping procedures. The model inferred by GARLI was similar to ModelTest (GTR+I+G: A = 0.3373, C = 0.1539, G = 0.1620, T = 0.3467; A-C = 1.0466, A-G = 0.8422, A-T = 0.2082, C-G = 0.5913, C-T = 0.9592, G-T = 1.0000; Pinv = 0.12314155; α = 0.2542932). And the best topology is likewise similar. Two hundred bootstrap replicates in GARLI were used to determine support values for ML analyses.

For BI analyses, the ingroup and three sister relationships were set to monophyletic [(*A. bethencourtianum*, *A. tortuosum*), (*A. dumosum*, *A. divaricatum*) and (*A. pachycaulon subsp. immaculatum*, *A. pachycaulon subsp. pachycaulon*)], because these sister relationships had been strongly supported in all MP and ML analyses as well as preliminary BI reconstruction. The GTR model was implemented with an uncorrelated, lognormal relaxed-clock (Drummond *et al.* 2006), a birth-death speciation process, and a starting tree constructed using UPGMA. All prior distributions of the parameters were provided by the default settings of the program. Trees were saved every 2500 chains over 10 million Markov Chain Monte Carlo repetitions. Posterior probabilities were calculated for the maximum clade credibility tree using TreeAnnotator in the BEAST package with a burn-in of 1000.

Molecular dating was conducted using the PAML/MULTIDIVTIME manual (Rutschmann 2005, Kishino *et al.* 2001, Thorne *et al.* 1998, Yang 1997). Parameters

for nucleotide frequencies, transition/transversion rate ratio, rate heterogeneity among sites and the gamma shape parameter were estimated using BASEML under the F84+G model. The program ESTBRANCHES provided maximum likelihood estimates for branch lengths and calculated a variance-covariance matrix through approximation of a likelihood surface with a multivariate normal distribution with means provided by the maximum likelihood estimates of the branch lengths (Thorne *et al.* 1998). Bayesian estimation for divergence times was provided by MULTIDIVTIME with upper limits on the most recent common ancestor (MRCA) of the Macaronesian Crassulaceae (21 Ma – the maximum age of the Canary Islands), the MRCA of *A. palmense* and *A. laxum* (2 Ma – the maximum age of La Palma), the MRCA of the *A. punctatum/A. pachycaulon subsp. gonzalez-hernandezii* group (12 Ma – the maximum age of La Gomera) and the MRCA of the La Palman/Gran Canarian group (2 Ma – the maximum age of La Palma). Simple Fitch parsimony reconstruction for the MRCA distribution, treating each island as a discrete, unordered character state, provided the framework for limiting the maximum age for each common ancestor. The node that restricted age estimates the most proved to be the 2 Ma limit on the *A. palmense/A. laxum* MRCA.

RESULTS AND DISCUSSION

Phylogenetics

Relationships between two major lineages within *Aichryson* were consistently resolved and well supported in all analyses (Figure 3.2), hereafter referred to as the Gomeran clade and the Pancanarian clade. Within the Gomeran clade a paraphyletic grade of La Gomeran taxa leads to a well supported clade of taxa from islands immediately surrounding La Gomera, hereafter referred to as the Metagomeran clade. Within the Pancanarian clade are four well supported lineages: the Laxum clade (including *A. palmense* and *A. laxum*), the Madeiran clade (including the Madeiran endemics), the Occidental clade (including primarily western island accessions), and the Oriental clade (including primarily eastern island accessions).

Parsimony analyses resulted in 480 trees of 643 steps (CI = 0.873, RI = 0.886). All of the clades circumscribed above were included in all 480 MP trees. These clades were also recovered in both ML analyses and the BI analyses. Disagreement between trees is confined to relationships within the well-supported clades. There is notable conflict over whether there is a hard polytomy between the Madeiran clade, the Occidental clade and the Oriental clade or a sister relationship between the Madeiran clade and the Oriental clade. A hard polytomy was recovered in all MP trees and in the ML tree recovered in PAUP* (not shown), but the ML tree from GARLI (Figure

3.2) and the BI tree from BEAST (not shown) recovered the sister relationship between the Madeirans and the Orientals, although with very low support (< 50% bootstrap and a posterior probability of 0.3865).

All taxa from which there were multiple accessions, except for *A. laxum*, were not recovered as monophyletic, but instead grouped in different well-supported clades. The Gomeran clade comprises a complex of *A. pachycaulon subsp. gonzalez-hernandezii* (La Gomera) and *A. punctatum* from Tenerife, La Palma and La Gomera, along with the accession of *A. parlatoresi* from Tenerife and one of the two accessions of *A. villosum* from Santa Maria (Azores). The Occidental clade within the Pancanarian clade contains the other accessions of *A. parlatoresi* (La Palma) and *A. villosum* as well as a single accession of *A. pachycaulon subsp. gonzalez-hernandezii* and an accession of *A. punctatum* from Gran Canaria.

The proposed sinking of *A. bethencourtianum* into *A. tortuosum* would result in a monophyletic species, but the molecular results suggest that *A. bethencourtianum* from Fuerteventura and *A. tortuosum* from Lanzarote have distinct maternal lineages that diverged before 13 other internal nodes in the tree (Figure 3.2), many of which connect more morphologically distinct lineages. Bañaras et al. (2008) admit that the convergence in trichome characteristics between particular populations of the two taxa is likely due to similar environmental pressures on population of *A. bethencourtianum* in northern Fuerteventura and *A. tortuosum* on Lanzarote.

Additionally, this decision was made without consideration for phylogeny and combining these two entities into a single species could complicate conservation efforts if levels of endemism are measured on a per species basis.

In general these results demonstrate that with regard to maternal phylogeny, the exclusive use of morphological characters can be misleading for identification and classification of phylogenetically related organisms. Indeed, hierarchical classification itself will likely prove insufficient to catalogue and describe the diversity in this genus. The current study uses maternally inherited, non-recombining DNA to construct phylogeny, which in the absence of horizontal gene flow or chloroplast capture, provides a relatively straight-forward interpretation of relatedness among the main chloroplast lineages in *Aichryson*. A detailed look into the nuclear genome will likely reveal a far more complex evolutionary history and network of relatedness among various populations.

Molecular Dating and Biogeography

Molecular dating results and the biogeography of *Aichryson* are treated together here because of the highly complementary results. Kim *et al.* (2008) proposed the MRCA for the Macaronesian Crassulaceae to be 15.2 ± 1.95 (standard deviation) Ma and this study recovered an estimate of 16.47 ± 1.61 Ma. Taxon sampling for *Aeonium* and *Monanthes* in the current study was not sufficient to have a high level of confidence in this estimate due to a positive correlation between taxon sampling and increased

error in estimation with increased distance from the point of calibration (Linder *et al.* 2005). The Kim *et al.* (2008) study contained a much more thorough taxon sampling across the Macaronesian Crassulaceae than this study and used a combined data set of both the nrDNA *ITS* region and the cpDNA *psbA-trnH* and *trnL-trnF* regions from Mort *et al.* (2002). The *ITS* and plastid data sets for members of the Macaronesian Crassulaceae have been shown to have substantially different rates of evolution (Mort *et al.* 2007), with the number of parsimony informative characters per site nearly 10 times greater in *ITS* than the plastid data. Additionally, *matK* data from Mort *et al.* (2002) was excluded because it was not “highly variable.” However, the results of the Kim *et al.* (2008) study are generally consistent with the results of the current study. Kim *et al.* (2008) estimated the age of the MRCA for the *Aichryson* clade at 8.67 Ma, which is similar to the 8.36 ± 1.61 Ma recovered in this study.

The current study has robust taxon sampling and a sufficiently large dataset for confident estimation of the rate of evolution for chloroplast regions within *Aichryson*. All age estimates should be treated as confidence intervals around a maximum date, because all of the calibration points are upper limits. The ages of many of the nodes may be younger than estimated. The MRCA of the Gomeran clade is estimated at 2.28 ± 1.15 Ma, the Metagomeran clade at 0.52 ± 0.44 Ma, the Pancanarian clade at 4.72 ± 1.13 Ma, the Oriental clade at 2.84 ± 0.94 Ma, the Occidental clade at 1.51 ± 0.33 Ma and the Madeiran clade at 0.78 ± 0.30 Ma.

The sister relationship between the Gomeran clade and the Pancanarian clade suggests a geographical partitioning of the genus early in its evolution between a lineage with its primary distribution on La Gomera, and a lineage that was probably more widespread on Lanzarote, Fuerteventura, Gran Canaria and the paleo-islands of Tenerife. La Gomera has been relatively stable since its formation, when compared to other islands. There are no documented cases of cataclysmic volcanic activity or massive landslides in the literature. The reduction of surface area on the island has occurred primarily through slow erosion and volcanism ceased on the island as late as 3 Ma. The lack of extraordinary disturbance seems to have led to moderate intra-island diversification of the Gomeran group with a long branch attaching it to the rest of the genus. Only in the last 1 million years has the group substantially invaded other islands and archipelagoes and these taxa appear to be each other's closest relatives. Considering the effect that increased taxon sampling has had for phylogenetic inference in *Aichryson*, this interpretation may change, especially when more populations from Tenerife are included in the analysis.

Considering the age of Gran Canaria, it is curious that taxa from this island are exclusively nested among taxa from La Palma. This is consistent with the hypothesis of widespread destruction of life on Gran Canaria, during a period of intense volcanism that ended approximately 3 million years ago (see previous chapter). The absence of taxa from Tenerife and the inclusion of only a single individual from La

Gomera in the Occidental clade are conspicuous, but may be an artifact of taxon sampling that will change with future study.

The sister relationship between the perennials and the subspecies of *A. pachycaulon* from Fuerteventura and Tenerife suggest a distinct old island lineage with a single, recent dispersal from Fuerteventura to Tenerife. The occurrence of ancestors of *A. pachycaulon subsp. pachycaulon* in the relatively high altitude regions of Fuerteventura may have facilitated dispersal to Tenerife by wind. Large dust storms frequently blow over the islands from the Sahara and could carry the minute seeds of *Aichryson* along with it. Ancestors or close relatives of these populations may also have occurred on Gran Canaria but gone extinct during the Roque Nublo volcanism. Again, greater population sampling on Tenerife and Gran Canaria is necessary to test these hypotheses.

The ancestral area of the Metagomeran clade is ambiguous and includes La Palma, El Heirro, Tenerife and/or the Azores. Any of the western islands may have been the source for the second and most recent dispersal of *Aichryson* to the Azores (possibly via Madeira, but this analysis does not include any accession of *A. villosum* from Madeira). The inferred ancestral area for the Occidental clade and the Laxum clade is La Palma. The analysis suggests long distance dispersal within the Occidental clade from either La Palma or Gran Canaria to Santa Maria in the Azores (possibly via Madeira also).

Dispersals between La Palma, Gran Canaria and El Hierro are both recent and frequent, suggesting a role for geological disturbance in dispersal and speciation within and among islands that have experienced substantial change in the last few million years. This begs the question whether extreme disturbances such as explosive volcanism or massive landslides have played a major role in the evolutionary history of *Aichryson*. Such events could simultaneously cause the extinction of a population on an island and the dispersal of that population to another island by the deposition of seeds. The seeds of *Aichryson* lack endosperm and could easily disperse long distances by winds blowing dust clouds ejected into the atmosphere by crumbling mountains or erupting volcanoes. The younger, or recently reformed islands also provide greater habitat heterogeneity, increasing the likelihood that seeds dispersed to those islands will find a suitable habitat to grow. Extraordinarily destructive events can also cause vicariance within islands, such that widespread, contiguous populations are effectively cut into two diverging populations. Both La Palma and Gran Canaria provide ideal natural laboratories for studies at the population level within *Aichryson* that might reveal such phenomena among the endemics there. For La Palma, the endemics and native taxa comprise several species in distinct plastid lineages, while within Gran Canaria there seems to have been extensive *in situ* diversification.

It appears that there was a single dispersal of *Aichryson* into Madeira that resulted in the Madeiran endemics, *A. divaricatum* and *A. dumosum*. Due to taxonomic uncertainty in the position of this clade, the date of dispersal to Madeira may have been as early as 6 Ma or as recent as 2 Ma. In either case, these dates exclude the islands of La Palma and El Hierro as possible source areas for the dispersal of the ancestor of this clade into Madeira. If massive disturbance does play a role in the dispersal of *Aichryson*, then the intense volcanism on Gran Canaria 3-5 Ma may have been important. In addition to the Roque Nublo volcanism, there was a debris avalanche on the south of the island about 3.5 Ma that also could have facilitated the long distance movement of minute seeds (Funck and Schmincke 1998). The extinction of the ancestral lineages of the Madeiran endemics on Gran Canaria could also help explain the phylogenetic uncertainty in the placement of the Madeiran taxa. During the same period, active volcanism on Tenerife was connecting the three paleo islands (Carracedo *et al.* 1998), and may have played a role in dispersal of *Aichryson* within the Canarian Archipelago. More thorough population sampling including multiple accessions of the Madeiran endemics and of *A. villosum* from Madeira may help to resolve this phylogenetic uncertainty and place a smaller interval on the probable date of dispersal.

A PLAN FOR FUTURE STUDY AND CONCLUSION

Aichryson are highly vagile organisms that are especially diverse and widespread on young islands with dynamic landscapes. More thorough taxon and genomic sampling is necessary to infer important dates in the evolutionary history of this genus, but the current study makes clear that many of the organisms, though quite young, are either morphologically distinct and geographically distant from their closest relatives or morphologically similar too and geographically close to more distant relatives. The failure of general morphological features to distinguish among separate lineages of *Aichryson* is a problem that should have taxonomists working closely with molecular systematists to infer the relatedness of all extant populations and uncover morphological similarities that might prove more useful for identification.

The problem of apparent morphological atavism or convergence in this genus may be related to hybridization. The morphological incoherence of the taxa in *Aichryson* is indicative of a hybrid origin for many members or continual hybridization between populations of various species. The subspecies of *A. pachycaulon* as well as the species *A. villosum* have been shown to be polyploids (Uhl 1961, Bramwell 1977), although no work to date can confirm whether their origin was autopolyploidy or allopolyploidy and no work has been done in the past 30 years to confirm these results across all known populations of *Aichryson*.

An exhaustive analysis of this genus will require sampling of all extant populations, including voucher specimens annotated with precise geographic coordinates, chromosome counts and DNA sequence data. Given the advances in PCR technologies, it should soon become common place to have genomic chloroplast sequences available for phylogenetic analysis, as well as large nrDNA data sets comprising multiple, cloned low-copy nuclear genes. There is little doubt that signal in the nrDNA will continue to conflict with the plastid data (Chapter 1), especially if hybridization is common or has been common in the past. *Aichryson* can provide a researcher the opportunity to use and develop new methods to infer phylogeny under these circumstances, including maximum likelihood and Bayesian estimation, in order to construct and interpret the nuclear and plastid histories of this genus.

When general phylogenies or familial networks of the genus are resolved and the taxonomic circumscription is improved, studies of the population biology of *Aichryson* can commence. Due to its high variability, widespread distribution and annual to triennial habit, *Aichryson* may prove to be an important organism for long term biogeographical, ecological and genetical studies within and among populations in Macaronesia. These studies are essential for the consilience of the currently disparate fields of ecology, phylogenetics, geography and conservation biology and just as the Galapagos finches helped Darwin to merge the fields of animal husbandry,

taxonomy, geology and economics into the Theory of Natural Selection, *Aichryson* may prove similarly useful to contemporary researchers.

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