Scali V (1971) Immaginal diapause and gonadal maturation of *Maniola jurtina* (Lepidoptera: Satyridae) from Tuscany. *Journal of Animal Ecology* **40**, 435-440.

Scali V, Masetti M (1973) The population structure of *Maniola jurtina* (Lepidoptera: Satyridae): the sex-ratio control. *Journal of Animal Ecology* **42**, 773-778.

Selvi F (1998) Floral biometrics of the *Anchusa undulata* L. group (Boraginaceae) from the central-eastern Mediterranean. *Botanical Journal of the Linnean Society* **128**, 251-270.

Schnittler M, Ludwig G (1996) Zur Methodik der Erstellung Roter Listen. *Schriftenreihe für Vegetationskunde* **28**, 709-739.

Schüle W (1993) Mammals, vegetation and the initial human settlement of the Mediterranean islands – a paleoecological approach. *Journal of Biogeography* **20**, 399-412.

Speranza F, Villa IM, Sagnotti L, Florindo F, Cosentino D, Cipollari P, Mattei M (2002) Age of the Corsica-Sardinian rotation and Liguro-Provencal Basin spreading: new paleomagnetic and Ar/Ar evidence. *Tectonophysics* **347**, 231-251.

Steininger FF, Rögl F (1984) Paleogeography and palinspastic reconstruction of the Neogene of the Mediterranean and Paratethys. In: *The Geological Evolution of the Eastern Mediterranean* (eds. Dixon, JE, Robertson AHF), pp. 659-668. Geological Society, London

Thomson G (1987) *Genetic variation in the genus* Maniola. PhD Thesis, London.

Tolman T, Lewington R (1997) *Butterflies of Britain and Europe*. Harper Collins, London.

Van der Voo R (1993) *Paleomagnetism of the Atlantic, Thethys and Iapetus Oceans*. Cambridge University Press, Cambridge.

Verlaque R, Médail F, Quézel P, Babinot JF (1997) Endémism végétal et paléogéographique dans le bassin méditerranéen. *Geobios Mémoire Spécial* **21**, 159-166.

Vigliotti L, Alvarez W, McWilliams M (1990). No relative motion detected between Corsica and Sardinia. *Earth Planetary Science Letters* **98**, 313-318.

Vigne JD (1992) Zooarcheology and the biogeographical history of the mammals of Corsica and Sardinia since the last ice age. *Mammal Review* **22**, 87-96.

Whittaker RJ (1997) *Island Biogeography: Ecology, Evolution and Conservation*. Oxford University Press, Oxford

Chapter 11

Are Macaronesian islands refugia of relict plant lineages?: a molecular survey

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Abstract

From a historical biogeographical perspective, Macaronesia has been considered as an island system where relict plants formerly distributed in Europe and northern Africa found appropriate ecological conditions for survival. In this context, and using previously published data on the Macaronesian flora, three basic relictualism concepts (geographic, taxonomic, and lineage relicts) are evaluated. A significant number of the 88 critically endangered species on the Canary Islands may stem from ongoing range reductions on each island (geographic relicts). Macrofossil evidence supports the occurrence of subtropical angiosperms in Europe in the Tertiary and subsequent extinction of genera and species on the continent, whereas they were sheltered in Macaronesia (taxonomic relicts). Using parsimony-based reconstructions, thirty angiosperm and two gymnosperm phylogenies are used to determine the number of stem-based versus crown-based lineages. In 18 plant groups, 16 unequivocal stem-based lineages (lineage relicts) were established in Macaronesia earlier than closely-related lineages differentiated on the mainland. In contrast, 13 lineages display a relatively more recent differentiation in Macaronesia as shown by unequivocal sister-group relationships with respect to their continental siblings. When introducing a time scale, some of these 13 crown-based lineages are found to have evolved in Macaronesia since the Tertiary. Relict status of ancient versus stem-based lineages is discussed and a fourth concept of lineage relictualism (ancient lineage) considered in terms of absolute timing. All stem-based lineages plus some crownbased lineages predate the Quaternary and are thus considered to be ancient lineages. Association between endozoochory and multiple colonizations is observed. Eight plant groups, out of a total of 29 properly sampled, underwent multiple colonizations, of which four tree groups (*Hedera, Ilex, Juniperus* sect. *Juniperus, Olea*) display endozoochorous syndromes. Endozoochory may have been crucial to recurrent long-distance dispersal of fleshy-fruited plants to Macaronesia and the establishment of ancient lineages in the Tertiary.

Keywords: Macaronesia, vascular plants, molecular phylogenetics, taxonomic relicts, geographic relicts, lineage relicts, ancient relicts, ancient dispersal

Introduction

Oceanic islands represent a 'natural laboratory' for the study of evolutionary processes and population dynamics in a known spatio-temporal framework.

Macaronesia consists of five volcanic archipelagos (Azores, Madeira, Selvagens, Canaries, Cape Verde) separated from the mainland by distances between circa 100 km (the Canarian island of Lanzarote to Africa) and circa 1500 km (the Azorean island of Santa María to Europe). Distinctive floral elements define the five archipelagos as a biogeographic unit (Médail & Quézel 1999), which is clearly recognized as the Macaronesian region, even though some authors consider it as a subregion of the Mediterranean region (Rivas-Martínez et al. 1993). Individual islands vary widely in their age, ranging from circa 21 million years for the Canary Islands (Fuerteventura) to 300 000 years for Azores (Pico) (Rothe 1996). Around 3100 species of flowering plants have been identified in Macaronesia (Hansen & Sundig 1993). This high diversity is a consequence of the pronounced ecological heterogeneity of the region, stemming from large differences in altitude (0-3710 m), latitude 40° N -15° N, annual precipitation means (270-1500 mm), and soil composition (Hobohm 2000). The above characteristics result in a high proportion of endemics, estimated independently by Humphries (1979) and Sundig (1979): Canary Islands (25.5/28%), Cape Verde Islands (15/14%), Madeira Islands (8.16/11%), Azores (5.2/5%) and Selvagem Islands (2.2/1%).

Only a few plant groups have successfully colonized and persisted on these islands after long-distance dispersal. At early stages, successful establishment may have occurred straightforward in a free-to-colonize land (preemption concept), followed by increasing competition and decreasing niche availability over time (Emerson 2002). Isolation from the continents, long distances between archipelagos, relatively young island age, and a limited land surface (circa 14 500 km^2) may have hindered Macaronesia's acquisition and distribution of a greater number of species from the mainland (McArthur & Wilson 1967). In contrast, the diverse abiotic conditions have promoted within-island speciation and endemism in habitats of the five extant vegetation zones (Bramwell 1975): sub-Alpine scrub, pine savanna, evergreen forest, transition zone, and semidesert. Over the last geological periods, a buffered climate has been generated in the five archipelagos due to the influence of the Atlantic Ocean (Fernandopullé 1976). This climate has bestowed Macaronesia with suitable characteristics for sheltering subtropical biota, in contrast to mainland dryness and cold during the late Tertiary and the Quaternary glaciations (Hewitt 2000).

Taking into account the above characteristics we may ask whether Macaronesia has played a significant role as a refugium for plant groups that went extinct on the continents after Tertiary and Quaternary climatic deterioration. In other words, how many plant groups can be considered relicts of Macaronesia?

To address the above questions, I have considered three existing concepts that can be used to classify relicts (Cronk 1992, 1997; Carlquist 1995): geographic, taxonomic, and lineage relicts. The three concepts are repostulated in the context of oceanic islands as follows.

1) *Geographic relict*: the surviving populations of an endemic species on oceanic islands resulting from range reduction of once more widely distributed populations.

2) *Taxonomic relict*: a group of one or more taxa diminished dramatically in number on the continent, but still occurring on islands.

3) *Lineage relict*: stem-based evolutionary sequence of ancestor-descendent species or populations (lineages) exclusive to islands.

This latter concept (lineage relict) is evaluated for the first time for Macaronesian plant groups using molecular evidence. A fourth concept (ancient lineage) is defined in terms of absolute times based on paleoclimatologic periods.

Materials and methods

Accumulation of previous data offers the opportunity of indirectly assessing geographic, taxonomic, and lineage relictualism in Macaronesia. Island characteristics on pollen remains do not allow a conventional survey of geographic and taxonomic relicts. Nevertheless, phylogenetic approaches are used in this study to evaluate the relict status of particular taxa from a considerable number (34) of molecular phylogenies.

The application of the geographic relict concept in Macaronesia is difficult because of the absence of pollen records deposited in stable sites. Accordingly, we are not able to properly evaluate the range reduction of island species that once were more widely distributed. A different approach based on the threatened status of species in the Canarian flora is used to infer range reduction. Critically endangered species present in a range < 100 km^2 and/or represented by two or three known populations with < 250 individuals (IUCN criteria, www.iucn.org) are evaluated to quantify population extinction and, hence, range reduction on islands. I hypothesize that more recently formed species represent a low proportion of threatened species, whereas recurrent extinction of established populations on oceanic islands (MacArthur & Wilson 1967) is interpreted as the major cause of species decline and thus involves a high proportion of the critically endangered species. A comprehensive list of Canarian endangered species (VV. AA. 2000) has been used for this approach.

The taxonomic relict concept is applied according to Cronk (1997): "*a species whose taxonomic isolation is due to* ex situ *extinction (of ancestral taxa and continental taxa descended from these) rather than in situ evolution (rapid evolution of traits associated with an island)*". In this study, *ex situ* extinction is referred to continental elimination and island persistence. As in previous publications (Saporta 1889; Depape 1922), I consider well-known macrofossils of genera and species extinct on the mainland that, surprisingly,

form part of Macaronesia's present-day flora. Extant Macaronesian species of former European floristic elements are summarized by Bramwell (1976) and Sundig (1979).

Parsimony analyses are used to evaluate i) natural groups of Macaronesian species and continental siblings to infer the number of introductions; ii) relictualism as inferred by stem-based lineages, i.e. by means of sister-group relationships of Macaronesian lineages in phylogenetic trees with respect to their continental siblings; and iii) shifts of ancestral syndromes related to long-distance dispersal to islands. Only a reliable sample and well-supported clades of monophyletic groups are considered to determine the number of lineages involved in different colonization events (Emerson 2002). The lineage relict concept is accepted herein and applied to assess the relevance of Macaronesia in harboring early branching lineages. Based on molecular phylogenies, tree-based inference is analyzed in which stem-based and crown-based clades refer to early and subsequent differentiation, respectively (Hennig 1966). Relict-ualism inferred by assessment of stem-based groups has been previously discussed in a phylogenetic context (Doyle & Donoghue 1993; Carlquist 1995; Baldwin *et al.* 1998). As every single lineage on oceanic (volcanic) islands originated from mainland lineages, we expect to describe three major patterns of island colonization (Figure 1): A) a single crown-base lineage as a consequence of recent establishment in Macaronesia relative to the continental lineages; B) a single stem-based lineage established early on the islands, whereas further speciation of siblings on the mainland occurs; and C) both crown-based and stem-based lineages resulting from multiple dipersal and establishment at different times.
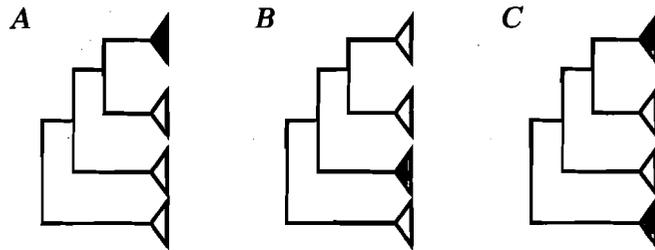

Figure 1. *The three major cases of phylogenetic relationships of natural groups including Macaronesian taxa. Groups of one or more taxa are represented by full (Macaronesia) and empty (mainland) triangles. A) Crown-based lineage as a result of a more recent establishment and evolution in Macaronesia relative to mainland; B) Stem-based lineage for a Macaronesian group, with speciation of sibling taxa occurring on the mainland. C) Both crown-based and stem-based lineages are the result of multiple dispersal events and establishment at different times (see text for discussion about relative times).*

Table 1. Plant groups, families, approximate number of Macaronesian species, and literature references of the 34 molecular phylogenetic reconstructions used in the present study.

Plant group	Family	Taxa number	References
Aeonium alliance	Crassulaceae	c. 70	Mes *et al. 1996;* Van Ham & Hart 1998; Mort *et al.* 2001
Arbutus	Ericaceae	1	Hileman *et al.* 2001
Argyranthemum	Compositae	c. 25	Francisco-Ortega *et al.* 1995, 1997
Armeria	Plumbaginaceae	1	Fuertes & Nieto 2003
Asteriscus	Compositae	c.10	Francisco-Ortega *et al.* 1999b; Goertzen *et al.* 2003
Bellis	Compositae	1	Fiz *et al.* 2002
Bencomia alliance	Rosaceae	c. 9	Helfgott *et al.* 2000
Chamaecytisus	Leguminosae	2	Badr *et al.* 1994; Käss & Wink 1995
Cheirolophus	Compositae	c.15	Susanna *et al.* 1999
Crambe	Cruciferae	c. 11	Francisco-Ortega *et al.* 1999a
Echium	Boraginaceae	c. 33	Böhle *et al.* 1996
Euphorbia	Euphorbiaceae	c. 16	Molero *et al.* 2002
Geranium	Geraniaceae	4	Vargas *et al.*, unpublished
Gonospermum	Compositae	c. 10	Francisco-Ortega *et al.* 2001a
Hedera	Araliaceae	3	Valcárcel *et al.* 2003; Vargas *et al.* 1999a
Ilex	Aquifoliaceae	2	Cuénoud *et al.* 2000
Ixanthus	Gentianaceae	1	Thiv *et al.* 1999
Juniperus	Cupressaceae	2	Martínez & Vargas 2002
Lavatera	Malvaceae	2	Ray 1995; Fuertes *et al.* 2002
Limonium	Plumbaginaceae	c. 20	Lledó *et al.* 1998
Lolium	Graminae	2	Charmet *et al.* 1997
Olea	Oleaceae	2	Hess *et al.* 2000
Pericallis	Compositae	14	Panero *et al.* 1999
Pinus	Pinaceae	1	Krupkin *et al.* 1996
Pulicaria	Compositae	2	Francisco-Ortega *et al.* 2001b
Sambucus	Caprifoliacae	5	Eriksson & Donoghue 1997
Saxifraga	Saxifragaceae	2	Vargas *et al.* 1999b
Sedum	Crassulaceae	9	Van Ham & Hart 1998
Sideritis	Labiatae	24	Barber *et al.* 2002
Sinapidendron	Cruciferae	6	Warwick & Black 1993
Solanum	Solanaceae	c. 31	Bohs & Olmstead 1997
Sonchus alliance	Compositae	c. 40	Kim *et al.* 1996
Teline	Compositae	c. 12	Käs & Wink 1995; Percy & Cronk 2002
Tolpis	Compositae	13	Moore *et al.* 2002; Park *et al.* 2001

Table 1 summarizes plant groups studied using parsimony-based analyses from which the relative placement of insular species is inferred. Ancestry of dispersal syndromes is evaluated by means of sister-group reconstructions and morphological characteristics.

Irrespective of the actual mode of dispersal, inference of ancestral characters (plesiomorphies) serves to quantify whether early dispersal syndromes were favored in the successful colonization of islands. The finding of no character shifts simplifies interpretation of a most likely syndrome of island founders, which would have developed dispersal characteristics analogous to those of extant relatives. Classification of these syndromes into five major dispersal-mechanism types (endozoochory, epizoochory, anemochory, hydrochory, unassisted) follows Ridley (1930) and van der Pijl (1979).

Results and discussion

Geographic relicts

The number of threatened species (88) on the Canary Islands varies from island to island, as compiled by Bañares and collaborators (VV. AA. 2000). The largest islands with high numbers of species have more critically endangered species (Figure 2). The ratio of total species to endangered species for each island is as follows: Tenerife (1400/25), Gran Canaria (1300/24), La Gomera (850/12), La Palma (850/8), Fuerteventura (600/9), Lanzarote (650/7), and El Hierro (600/3).
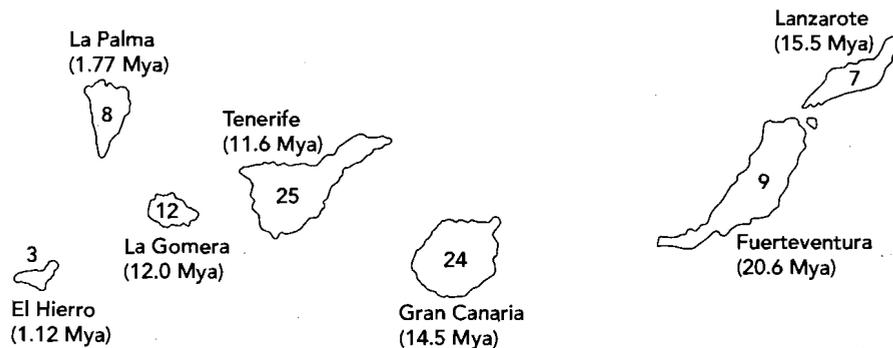

Figure 2. *Number of critically endangered species (88) in the seven Canary Islands, of which a significant number are interpreted as geographic relicts following range reduction. Island ages as in Carracedo (2002).*

Limited distribution of species in oceanic islands may be the result of two evolutionary processes: i) first stages of range expansion after recent speciation events; ii) last extinction stages of ancient species due to population demise. Accordingly, the 88 critically endangered species may represent either range reduction or early stages of range expansion. Analyses of character evolution of plant morphologies are misleading when trying to determine incipient versus decline stages with respect to continental levels of morphological differentiation. Extensive morphological differentiation, but low genetic variation, is common for island taxa including Macaronesian plants (see Table 1 for references). Disparate morphologies arise rapidly because species undergo 'character release' but they display similar or identical DNA sequences (Givnish 1997). For instance, woodiness is surprisingly frequent in genera of different Macaronesian families (*Argyranthemum, Sonchus, Echium, Isoplexis*) and has been considered a relictual character (Bramwell 1976). In contrast to previous considerations, molecular evidence indicates that the woody condition is an acquired character evolving from herbaceous Mediterranean ancestors as a result of *in situ* insular evolution (Panero *et al.* 1999). In contrast, continental species of the same natural group display similar morphologies but remarkable divergent sequences (Böhle *et al.* 1996; Baldwin *et al.* 1998).

Phylogenetic analysis of molecular data allows inference of historical processes in oceanic islands. A search for patterns of ancestor-descendant relationships of critically endangered species in molecular phylogenies (Table 1) reveals that six species with distinctive morphological characters have a relatively most recent origin, as suggested by low molecular divergence and crown-based placement (*Aeonium mascaensis, Bencomia brachystachya, Echium handiense, Isoplexis chalcantha, Pericallis hadrosoma, Sideritis discolor*). Two more species of *Globularia* (*G. ascanii, G. sarcophylla*) appear to fit into this pattern of crown-based lineages (P. Comes, pers. comm.). However, the rarity of four species (*Argyranthemum sudingii, Cheirolophus arboreus, Cheirolophus junonianus, Bencomia sphaerocarpa*) appears to be the result of decline instead of recent species formation as inferred from a stem-based phylogenetic position. Whether a significant number of the 88 critically endangered species are the result of range contraction should be addressed in future investigations by means of evaluating phylogenetic relationships, population genetics, and historical records. Human and naturally caused catastrophies on oceanic islands lead us to interpret that limited distribution, at least for taxa forming ancestral lineages, is the result of population demise, thus resulting in the formation of geographic relicts.

Taxonomic relicts

Eighteen extinct taxa of vascular plants documented from European macrofossils (Miocene and Pliocene) have living counterparts in Macaronesia

(Bramwell 1976). Among them, at least nine were identified as extant Macaronesian species, seven had closely related siblings, and two were not properly identified (Bramwell 1976; Sundig 1979). Therefore, some living fossils support the refugium status of Macaronesia as it harbors taxonomic relicts not found on the continents. As more fossils on the mainland are uncovered, the number of relicts is expected to increase.

Lineage relicts

Relative placement of Macaronesian plant lineages in molecular phylogenies reveals that 13 lineages from 14 plant groups are unequivocally crown-based (Table 2) and 16 lineages from 18 plant groups are unequivocally stem-based (Table 3). Parsimony-based reconstructions allow recognition of the 16 stem-based lineages as lineage relicts, i.e. descended from a common ancestor with an origin that predated differentiation of closely related species on the mainland. This summary of stem-based and crown-based groups gives phylogenetic support for lineage relictualism in Macaronesia. The relative primary position of almost half of the 32 plant groups analyzed to date reveals the importance of plant dispersal and establishment in early times. Colonization has been successful since island formation (starting circa 21 mya) and the establishment of new lineages likely decreased over time (Carlquist 1965; MacArthur & Wilson 1967; Simberloff 1974; Johnson et al. 2000). A scenario has been envisaged in which many plant groups succeeded in colonizing immediately after island formation, but increasing competition slowed down the rate of new colonizations. Early colonization may have been dependent on subtropical habitats that were more abundant in the Pliocene and the Miocene. Therefore, a significant number of the 16 relict-inferred lineages may have been present in Macaronesia since the Tertiary, when subtropical vegetation vanished in Europe (Bramwell 1976). The extant plants considered of subtropical origin and forming part of the Macaronesian laurisilva seem to be surviving representatives of a once more widely distributed Tethyan-Tertiary flora (Bramwell 1976; Mai 1995). Both macrofossil and molecular evidence unequivocally support that Macaronesia is indeed a refugium island system.

Additionally, vegetation zones described for Macaronesia include dry habitats and offered new opportunities to Mediterranean plants for colonization during the Quaternary. A new, highly-competitive flora originated 3.2 mya as aridity increased in the Mediterranean basin (Suc 1984). The Mediterranean region has been the main floristic source for dispersal and spawning of new evolutionary lineages in Macaronesian islands. Although 13 crown-based lineages are considered relatively recent lineages, estimates of divergence times are necessary to interpret actual timing of island colonization (Figure 3).

Table 2. List of 14 Macaronesian plant groups with only crown-based lineages, as interpreted from molecular phylogenies (see Table 1). Approximate number of taxa (Hansen & Sundig 1993), studied taxa (in brackets) if very different from total number, inferred number of introductions, particular relict status, and ancestral dispersal syndromes are also indicated.

Plant group	Taxa number	Number of introductions	Relict status: stem-based vs. crown-based lineages	Ancestral dispersal syndrome
Aeonium alliance	c. 70	1	Crown	unassisted
Asteriscus	c.10	1	Crown	uncertain
Bencomia alliance	c. 9	1	Crown	unassisted
Cheirolophus	c. 15	1	Crown	anemochory
Echium	c. 33	1	Crown	epizoochory
Euphorbia (tabaibas)	c. 16	2	2 crown	hydrochory
Geranium	4	1 (?)	Crown	epizoochory
Gonospermum	c. 10	1	Crown (?)	unassisted
Limonium	c. 20 (3)	?	1 crown	hydrochory
Pericallis	14	1	Crown	epizoochory
Sambucus	5 (1)	?	1 crown	endozoochory
Saxifraga	2	1	Crown	unassisted
Sideritis	24	1	Crown	epizoochory (?)
Sonchus alliance	c. 40	1	Crown (?)	hydrochory

Table 3. List of 18 Macaronesian plant groups with at least one stem-based lineage, as interpreted from molecular phylogenies (see Table 1). Approximate number of taxa (Hansen & Sundig 1993), studied taxa (in brackets) if very different from total number, inferred number of introductions, particular relict status, and ancestral dispersal syndromes are also indicated.

| Plant group	Taxa number	Number of introductions	Relict status: stem-based vs. crown-based lineages	Ancestral dispersal syndrome
Arbutus	1	1	Stem	endozoochory
Argyranthemum	c.25	1	Stem	uncertain
Armeria	1	1	Stem	anemochory
Bellis	1	1	Stem	uncertain
Chamaecytisus	2	1	Stem	endozoochory
Crambe	c. 11	1	Stem (?)	unassisted
Hedera	3	3	2 stem / 1 crown	endozoochory
Ilex	2	2	1 stem(?) / 1 crown	endozoochory
Ixanthus	1	1	Stem (?)	unassisted
Juniperus sect. *Juniperus*	2	2	1 stem / 1 crown	endozoochory
Lavatera	2	2	2 stem	hydrochory
Lolium	2 (1)	?	1 stem	endozoochory
Olea	2	2	1 stem / 1 crown	endozoochory
Pinus	1	1	Stem	uncertain
Sedum	9 (2)	1	1 stem	unassisted
Sinapidendron	6 (2)	1	Stem	unassisted
Solanum	c. 31 (3)	3 (native?)	1 stem / 2 crown (?)	endozoochory
Tolpis	13	1	Stem (?)	anemochory
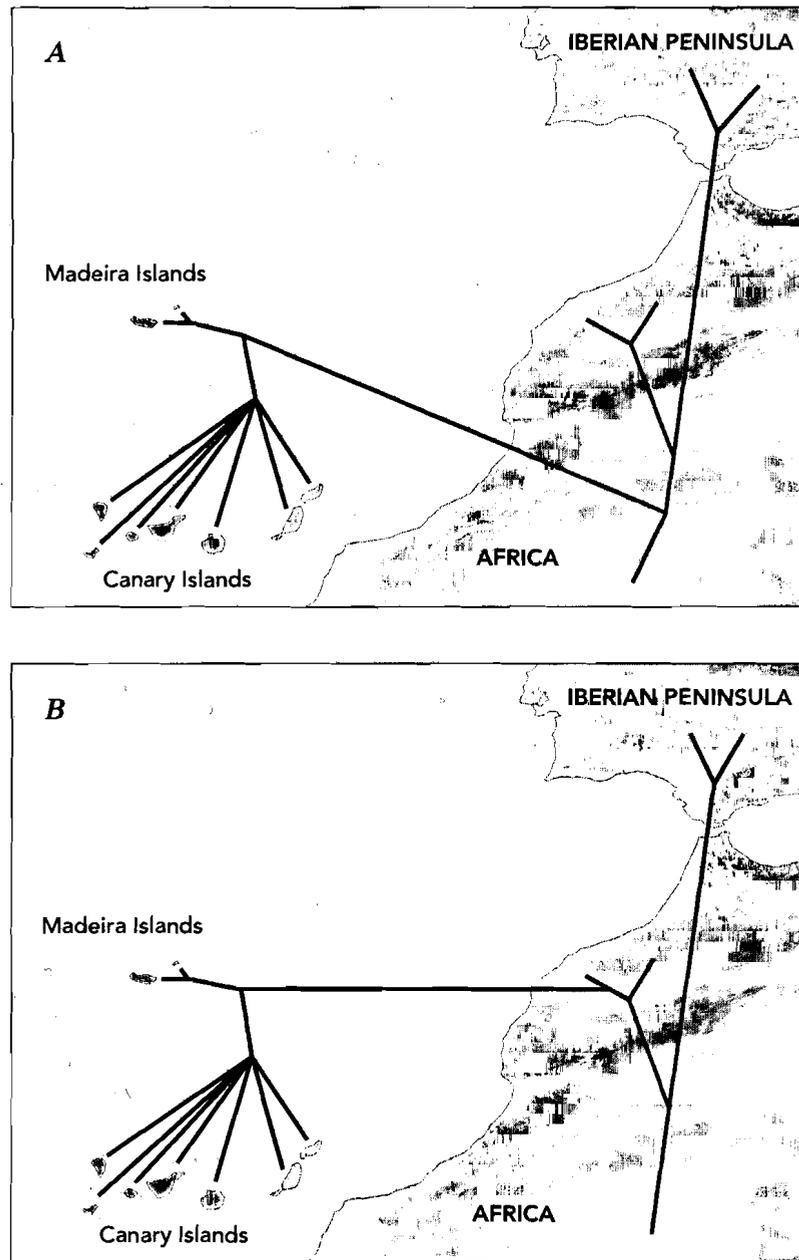
Figure 3. Hypothetical patterns of historical biogeography (see also Cronk 1997) in the Canary Islands as inferred from A) stem-based lineages and B) crown-based lineages.

Ancient versus stem-based lineages: ancient relicts

A higher number of old lineages is expected when applying a time scale. Recognition of a stem-based group indicates relative phylogenetic position whereas ancient refers to absolute times of origin (Wulff 1943). I consider stem-based lineages as ancient lineages that succeeded in Macaronesia in early times while continental differentiation necessarily postdated events of island colonization (Doyle & Donoghue 1993; Johnson et al. 2000). On the other hand, crown-based lineages may contain some ancient lineages from early times, which are not recognized because of species-poor lineages on the continent as a result of extinction or absence of speciation. When should we consider crown-based lineages as ancient? What is the time limit to include all stem-based and some crown-based groups into ancient lineages?

A criterion to designate ancient lineages in a flora generated primarily in the last 21 myr is herein reformulated (Cronk 1992). The time threshold between the Tertiary and the Quaternary (circa 1.7 mya, www.iugs.org/iugs/pubs/intstratchart.htm) is proposed because after this time limit i) a summer drought was already established (2.8 mya) in the Mediterranean climate zone (Suc 1984); ii) speciation in plants from oceanic islands can occur (Baldwin et al. 1998), and iii) island formation was already accomplished in Macaronesia, except for El Hierro (Figure 2). Macaronesia may have been profoundly influenced by the emerging Mediterranean flora in the late Pliocene, as adaptations to seasonal aridity have occurred on the continents since then. The ancient (Miocene) and subancient (Miocene-Pliocene) relicts defined by Cronk (1992) are herein merged into ancient (pre-Quaternary) relicts, i.e. those existing for over 1.7 million years.

Why should all stem-based lineages on islands be considered ancient lineages? Because they have, by definition, sister-relationships to groups of two or more continental species. Assuming previous speciation estimates of two and three mya in the mainland (Levin & Wilson 1976; Niklas et al. 1983; Vargas 2003), these lineages may have been present earlier than the Tertiary-Quaternary threshold (1.7 mya). Based on this assumption, any stem-based lineage is herein regarded as an ancient (Tertiary) lineage in the geological context of Macaronesian islands. Accordingly, the term paleoendemism (ancient), as proposed by Baldwin et al. (1998), includes all stem-based lineages, which are also interpreted herein as ancient lineages.

Lack of differentiation (speciation) or high extinction rates on the mainland may obscure cases of ancient lineages of Macaronesian plants identified as crown-based lineages because of their relative placement. Apart from the 16 stem-based lineages (Table 3), I suggest that some of the 13 crown-based lineages (Table 2) have originated in the Tertiary and, therefore, should be also considered as ancient lineages. How can we detect ancient lineages when we find a crown placement of any plant group in a particular phylogeny? Estimates of minimum colonization times can be established by the use of molecular clocks.

Four examples of the 13 crown-based lineages (Table 2) clearly illustrate the concept of ancient versus stem-based lineages. The *Aeonium* alliance includes four genera (*Aeonium, Aichryson, Greenovia, Monanthes,*) with a crown position in the Crassulaceae phylogeny (Van Ham & Hart 1998; Mort *et al.* 2001). We should consider the *Aeonium* alliance as an ancient lineage because its ancestor's arrival, establishment, evolution, and differentiation likely occurred in ancient times, manifested by remarkable morphological differentiation (four genera). Calibration of a molecular clock in Crassulaceae is necessary to assess divergence times; however, the high molecular variation found among the four genera – in comparison to other genera of Macaronesian angiosperms – indicates that differentiation of genera may have predated the Tertiary-Quaternary limit. Two more plant groups do not have a clear stem position and their molecular clocks indicate a Tertiary origin: *Sonchus* subgenus *Dendrosonchus* (Kim *et al.* 1996) and *Crambe* (Francisco-Ortega 1999a). Further molecular-clock estimates are necessary to quantify the number of crown-based lineages that may be considered ancient lineages, i.e. lineages established in Macaronesia between the onset of island formation (circa 21 mya) and the late Pliocene (> 1.7 mya).

Success of fleshy-fruited plants and ancient lineages

Colonization of islands involves multiple factors that influence dispersal and establishment such as geography, ecological interactions, inherent biological properties of the plant, and stochastic events (Ackerley 2003). While assuming multiple factors have been crucial for colonization, I focus only on the first step for a successful establishment: dispersal. Direct observations of the arrival of present-day diaspores are difficult and inference on specific arrivals of early Macaronesian founders is a speculative exercise. Availability of molecular phylogenetic data for a wide diversity of insular plants allows scholars to evaluate whether any particular diaspore syndrome may have favored dispersal to oceanic islands through time. Dispersal syndromes are arranged into five major groups (anemochory, hydrochory, endozoochory, epizoochory, and mechanisms not associated with long-distance dispersal or unassisted). Historical reconstructions based on 31 phylogenies from studies of different plant lineages (Table 1, excluding *Pulicaria, Solanum,* and *Teline*) indicate 38 introductions and no syndrome shift following colonization. Bramwell (1985) estimated that 186 founders generated the extant flora of the Canary Islands, of which 63 were endozoochorous (34%), 35 epizoochorous (19%), 48 anemochorous (26%), 8 hydrochorous (4.3%), and 32 uncertain (17%). The use of molecular phylogenies and character-evolution reconstruction of diaspore types of the 38 well-documented introductions (Tables 2 & 3, excluding *Solanum*) reveals the following preliminary results of ancestral syndromes for first colonizers to Macaronesia: 13 endozoochorous (34%), 4 epizoo-

chorous (10.5%), 3 anemochorous (8%), 6 hydrochorous (16%), 8 unassisted (21%), and 4 uncertain (10.5%). Although our dispersal syndrome classification includes one more type (unassisted) than that of Bramwell (1985), independent estimates reveal similar figures for success of endozoochory.

An additional argument for the success of endozoochory is based on a number of plant introductions. Multiple dispersals of a natural group to Macaronesia is not a common pattern in the colonization history of these archipelagos (Valcárcel *et al.* 2003). Twenty-two plant groups originated unequivocally from 22 single colonizers, whereas only eight had two or more dispersal origins (*Euphorbia, Hedera, Ilex, Juniperus* sect. *Juniperus, Lavatera, Olea, Pulicaria, Teline,* excluding *Solanum*)(Tables 1-3). Among them, four trees with endozoochorous fruits may have been favored not only for early dispersal (ancient lineages) but also for recurrent colonization of Macaronesia in different periods. Obviously, only a small subset (32 phylogenies) of the Macaronesian flora lends itself to this analysis. In any case, we infer that endozoochory accounts for a third of dispersal to these islands since their formation, and thus the establishment of ancient lineages. Association between the number of fleshy-fruited trees and their establishment in a particular habitat is not observed. Of those genera containing ancient lineages, three occur in the laurisilva (*Arbutus, Hedera, Ilex*) – the habitat more similar to former subtropical formations (Takhtajan 1969; Bramwell 1976) – while three others are adapted to mesic habitats (*Juniperus, Olea, Pinus*) (Table 3).

In summary, these results lead us to conclude that endozoochory has played a crucial role in successful dispersal to Macaronesia (Valcárcel *et al.* 2003), as it has been determined for the Hawaiian islands (Carlquist 1969; Vargas & Baldwin, unpublished data). Moreover, the importance of endozoochory is reflected in the proportion of diaspore syndromes favored in multiple introductions to Macaronesia. They include at least one stem-based lineage each, which indicate early colonizations and the formation of lineage relicts. It seems likely, therefore, that fleshy-fruited trees not only had higher probabilities for multiple dispersal to Macaronesia, but also a more active dissemination since ancient Tertiary times, thus fostering the origin and establishment of ancient lineages.

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References

Ackerley DD (2003) Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences* **164** (Supplement), S165-S184.

Badr A, Martin W, Jensen U (1994) Chloroplast DNA restriction site polymorphism in *Genisteae* (Leguminosae) suggests a common origin for European and American lupines. *Plant Systematics and Evolution* **193**, 95-106

Baldwin BG, Crawford DJ, Francisco-Ortega J, Kim, SC, Sang T, Stuessy TF (1998) Molecular phylogenetic insights on the origin and evolution of oceanic island plants. In: *Molecular Systematics of Plants. II DNA sequencing* (eds. Soltis D, Soltis P, Doyle JJ), pp. 410-441. Kluwer Academic Publishers, Boston.

Barber JC, Francisco-Ortega J, Santos-Guerra A, Turner KG, Jansen RK (2002) Origin of Macaronesian *Sideritis* L. (Lamioideae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. *Molecular Phylogenetics and Evolution* **23**, 293-306.

Böhle UR, Hilger HH, Martin WF (1996) Island colonization and evolution of the insular woody habitat in *Echium* L. (Boraginaceae). *Proceedings of the National Academy of Sciences, USA* **93**, 11740-11745.

Bohs L, Olmstead RG (1997) Phylogenetic relationships in *Solanum* (Solanaceae) based on ndhF sequences. *Systematic Botany*, **22**, 5-17.

Bramwell D (1975) Some morphological aspects of the adaptive radiation of Canary Islands *Echium* species. *Anales del Jardín Botánico de Madrid* **32**, 241-254.

Bramwell D (1976) The endemic flora of the Canary Islands: distribution, relationships and phytogeography. In: *Biogeography and Ecology in the Canary Islands* (ed. Kunkel G), pp. 207-240. Dr. W. Junk, The Hague.

Bramwell D (1985) Contribución a la biogeografía de las Islas Canarias. *Botanica Macaronesica* **14**, 3-34.

Carlquist S (1969) The biota of the long-distance dispersal. V. Plant dispersal to Pacific Islands. *Bulletin of the Torrey Botanical Club* **94**, 129-162.

Carlquist S (1974) *Island Biology*. Columbia University Press, Columbia.

Carlquist S (1995) Introduction. In: *Hawaiian Biogeography. Evolution on a hot spot archipelago* (eds. Wagner WL, Funk VA), pp. 1-13. Smithsonian Institution Press, Washington.

Carracedo JC, Pérez FJ, Ancochea E, meco J, Hernán F, Cubas CR, Casillas R, Rodríguez E, Ahijado A (2002) Cenozoic volcanism II: the Canary Islands. In: *The Geology of Spain* (eds. Gibbons W, Moreno T), pp. 439-472. The Geological Society of London, London.

Charmet G, Ravel C, Balfourier F (1997) Phylogenetic analysis in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. *Theoretical and Applied Genetics* **94**, 1038-1046.

Cronk QCB (1992) Relict floras of Atlantic islands: patterns assessed. *Biological Journal of the Linnean Society* **46**, 91-103.

Cronk QCB (1997) Islands: stability, diversity, conservation. *Biodiversity and Conservation* **6**, 477-493.

Cuénoud P, Martínez MP, Loizeau P, Spichiger R, Andrews S, Manen J (2000). Molecular phylogeny and biogeography of the genus *Ilex* L. (Aquifoliaceae). *Annals of Botany (Oxford)* **85**, 111-122.

Depape G (1922) Recherches sur la flore pliocêne de la Vallée du Rhône. *Annales des Sciences Naturelles, Botanique* **10**, 73-265.

Doyle JA, Donoghue MJ (1993) Phylogenies and angiosperm diversification, *Paleobiology* **19**, 141-167.

Emerson, BC (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* **11**, 951-966.

Eriksson T, Donoghue MJ (1997) Phylogenetic relationships of *Sambucus* and *Adoxa* (Adoxaceae) based on nuclear ribosomal ITS sequences and preliminar morphological data. *Systematic Botany* **22**, 555-573.

Fernandopullé D (1976) Climatic characteristics of the Canary islands. In: *Biogeography and Ecology in the Canary Islands* (ed. Kunkel G.), pp. 185-205. Dr. W. Junk, The Hague.

Fiz O, Valcárcel V, Vargas P (2002) Phylogenetic position of Mediterranean Astereae and character evolution of daisies (Bellis, Asteraceae) inferred from nrDNA ITS sequences. *Molecular Phylogenetics and Evolution* **25**, 157-171.

Francisco-Ortega J, Fuertes Aguilar J, Gómez-Campo C, Santos-Guerra A, Jansen RK (1999a) Internal transcribed spacer sequence phylogeny of *Crambe* L. (Brassicaceae): molecular data reveal two Old World disjunctions. *Molecular Phylogenetics and Evolution* **11**, 361-380.

Francisco-Ortega J, Goertzen LR, Santos-Guerra A, Benabid A, Jansen RK (1999b) Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) I: evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Systematic Botany* **24**, 249-266.

Francisco-Ortega J, Barber JC, Santos-Guerra A, Febles-Hernández R, Jansen RK (2001a) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. *American Journal of Botany* **88**, 161-169.

Francisco-Ortega J, Park SJ, Santos-Guerra A, Benabid A, Jansen RK (2001b) Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Biological Journal of the Linnean Society* **72**, 77-97.

Francisco-Ortega J, Fuertes-Aguilar J, Kim SC, Santos-Guerra A, Crawford DJ, Jansen RK (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. *American Journal of Botany* **89**, 1984-1990.

Fuertes-Aguilar J, Nieto Feliner G (2003) Additive polymorphisms and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). *Molecular Phylogenetics and Evolution* **28**, 430-447.

Fuertes-Aguilar J, Ray MF, Francisco-Ortega J, Santos-Guerra A, Jansen RK (2002) Mole-cular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. *Systematic Botany* **27**, 74-83.

Givnish TJ (1997) Adaptive radiation and molecular systematics: issues and approaches. In: *Molecular Evolution and Adaptive Radiation* (eds. Givnish TJ, Sytsma KJ), pp. 1-54. Cambridge University Press, Cambridge.

Goertzen LR, Francisco-Ortega J, Santos-Guerra A, Mower JP, Linder CR, Jansen RK (2002) Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) II: combines nuclear and chloroplast data. *Systematic Botany* **27**, 815-823.

Hansen A, Sundig P (1993) Flora de Macaronesia. Checklist of vascular plants. 4th revised edition. *Sommerfeltia* **17**, 1-298.

Helfgott DM, Francisco-Ortega J, Santos-Guerra A, Jansen RK, Simpson BB (2000) Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. *Systematic Botany* 25, 82-97.

Hennig W (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana, Illinois.

Hess J, Kadereit JW, Vargas P (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSR). *Molecular Ecology* 9, 857-868.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages, *Nature* 405, 907-913.

Hileman LC, Vasey MC, Parker VT (2001) Phylogeny and biogeography of the Arbutoideae (Ericaceae): implications for the Madreah-Tethyan Hypothesis. *Systematic Botany* 26, 131-143.

Hobohm C (2000) Plant species diversity and endemism on islands and archipelagos, with special reference to the Macaronesian Islands. *Flora* 195, 9-24.

Humphries CJ (1979) Endemism and evolution in Macaronesia. In: *Plants and Islands* (ed. Bramwell D), pp. 171-200. Academic Press, New York.

Johnson KP, Adler FR, Cherry JL (2000). Genetic and phylogenetic consequences of island biogeography. *Evolution* 54, 387-396.

Käss E, Wink M (1995) Molecular phylogeny of the Papilionoideae (Family Leguminosae): RbcL gene sequences versus chemical taxonomy. *Botanica Acta* 108, 149-162.

Kim SC, Crawford DJ, Francisco-Ortega J, Santos-Guerra A (1996) A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proceedings of the National Academy of Sciences, USA* 93, 7743-7748.

Krupkin AB, Liston A, Strauss SH (1996) Phylogenetic analysis of the hard pines (*Pinus* sugenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. *American Journal of Botany* 83, 489-498.

Levin DA, Wilson AC (1976) Rates of chromosomal evolution in seed plants: net increase in diversity of chromosome numbers and species numbers through time. *Proceedings of the National Academy of Sciences, USA* 73, 2086-2090.

Lledó MD, Crespo MB, Cameron KM, Fay MF, Chase M (1998) Systematics of Plumbagineaceae based upon cladistic analysis of *rbc*L sequence data. *Systematic Botany* 23, 21-29.

MacArthur RH, Wilson EO (1967) *The Theory of Island Biogeography*. Princeton University Press, New Jersey.

Mai DH (1995) *Tertiäre Vegetationsgeschichte Europas*. Gustav Fischer, Jena, Stuttgart, New York.

Martínez J, Vargas P (2002) Chloroplast differentiation and active dispersal of *Juniperus oxycedrus* in the Mediterranean basin based on trnL-trnF sequences. Paper held at the I International Symposium of Phylogeography in Southern European Refugia. 11–15 March, Vairão, Portugal.

Médail F, Quézel P (1999) The phytogeographical significance of S. W. Morocco compared to the Canary Islands. *Plant Ecology* 140, 221-244.

Mes THM, van Brederode J, Hart H. (1996) Origin of the woody Macaronesian *Sempervivoideae* and the phylogenetic position of the East African species of *Aeonium*. *Botanica Acta* 109, 477-491.

Molero J, Garnatje T, Rovira A, Garcia-Jacas N, Susanna A (2002) Karyological evolution and molecular phylogeny in Macaronesian dendroid spurges (Euphorbia subsect. Pachycladae). *Plant Systematics and Evolution* 231, 109-132.

Moore MJ, Francisco-Ortega J, Santos-Guerra A, Jansen RK (2002) Chloroplast DNA evidence for the roles of island colonization and extinction in *Tolpis* (Asteraceae: Lactuceae). *American Journal of Botany* 89, 518-526.

Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A (2001) Phylogenetic relationships and evolution of Crassulaceae inferred from matK sequence data. *American Journal of Botany* 88, 76-91.

Niklas KJ, Tiffney BH, Knoll AJ (1983) Patterns in vascular land plant differentiation. *Nature* 303, 614-616.

Panero JL, Francisco-Ortega J, Jansen RK, Santos-Guerra A (1999) Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian island endemic *Pericallis* (Asteraceae: Senecioneae). *Proceedings of the National Academy of Sciences, USA* 96, 13886-13891.

Park S, Korompai EJ, Francisco-Ortega J, Santos-Guerra A, Jansen RK (2001) Phylogenetic relationships of *Tolpis* (Asteraceae: Lactuceae) based on *ndh*F sequence data. *Plant Systematics and Evolution* 226, 23-33.

Percy DM, Cronk QCB (2002) Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista*, and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. *American Journal of Botany* 89, 854-864.

Ray MF (1995) Systematics of *Lavatera* and *Malva* (Malvaceae, Malveae) - a new perspective. *Plant Systematics and Evolution* 198, 29-53.

Ridley, HN (1930) *The Dispersal of Plants throughout the World*. L. Reeve & Co., Kent.

Rivas-Martínez S., Wildpret W., Arco-Aguilar M., Rodríguez O., Perez-de-Paz PL, García-Gallo A, Acebes JR, Díaz TE, Fernandez-Gonzalez F (1993). Las comunidades vegetales de la Isla de Tenerife (Islas Canarias). *Itinera Geobotanica* 7, 169-374.

Rothe P (1996) *Kanarische Inseln*. 2nd edition, Sammlung geologischer Führer, Band 81, Berlin, Stuttgart.

Saporta MG (1889) Dernières adjonctions a la Flore fossile d'Aix-en-Provence. Description des espèces, 2ª partie. *Annales des Sciences Naturelles, Botanique* 7, 1-192.

Simberloff DS (1974) Equilibrium theory of islands biogeography and ecology. *Annual Review of Ecology and Systematics* 5, 161-182.

Suc JP (1984) Origin and evolution of the Mediterranean vegetation and climate in Europe. *Nature* 307, 429-432.

Sundig P (1979) Origins of the Macaronesian Flora. In: *Plants and Islands* (ed. Bramwell D), pp. 13-40. Academic Press, New York.

Susanna A, Garnatje T, García-Jacas N (1999) Molecular phylogeny of *Cheirolophus* (Asteraceae: Cardueae-Centaureinae) based on ITS sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 214, 147-160.

Takhtajan A (1969) *Flowering Plants: Origin and Dispersal*. Smithsonian Institute, Washington.

Thiv M, Struwe L, Kadereit JW (1999) The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb. (Gentianaceae): evidence from matK and ITS sequences, and floral morphology and anatomy. *Plant Systematics and Evolution* 218, 299-317.

Valcárcel V, Fiz O, Vargas P (2003) Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean basin. *Molecular Phylogenetic and Evolution* **26**, 1-20.

Van Ham RCH, Hart H (1998) Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restriction-site variation. *American Journal of Botany* **85**, 123-134.

Van der Pijl, L (1979) *Principles of Dispersal in Higher Plants*. Springer Verlag, New York.

Vargas P (2003) Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon* **52**, 463-476.

Vargas P, McAllister HA, Morton CM, Jury SL, Wilkinson MJ (1999a) Polyploid speciation in *Hedera* L.: phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. *Plant Systematics and Evolution* **219**, 165-179.

Vargas P, Morton CM, Jury SL (1999b) Biogeographic patterns in Mediterranean and Macaronesian species of *Saxifraga* (Saxifragaceae) inferred from phylogenetic analyses of ITS sequences. *American Journal of Botany* **86**, 724-734.

VV. AA. (2000) Lista roja de flora vascular Española (valoración según categorías UICN). *Conservación Vegetal* **6** (extra), 11-38.

Warwick SI, Black LD (1993) Molecular relationships in subtribe Brassicinae (Cruciferae, tribe Brassiceae). *Canadian Journal of Botany* **71**, 906-918.

Wulff EV (1943) *An Introduction to Historical Plant Geography*. Chronica Botanica, Waltham, Massachusetts.

Chapter 12

Nested clade phylogeographical analysis of barbel (*Barbus barbus*) mitochondrial DNA variation

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Abstract

We applied nested clade phylogeographical analysis (NCPA) to the mitochondrial DNA phylogeographical data of the barbel *Barbus barbus* to assess the historical biogeography scenario suggested for this species by a traditional phylogeographical approach. Major previously inferred historical events received support from the NCPA: i) twofold range fragmentation, an ancient one between the central European and Balkan/Anatolian populations and a more recent one ascribed to the survival of the central European lineage in two refugia during the latest glacial, and ii) contiguous range expansion from the Danubian refuge into the more northwestern river basins. Likely due to insufficient genetic variation, the hypothesized dispersion from the more western central European refuge was not detected by the NCPA as was not the hypothesized expansion throughout the Balkans and Anatolia. The NCPA interpretation of the significant pattern within the Danube river basin as reflecting a recurrent gene flow restricted through isolation by distance should be taken with caution. Similar patterns can reflect non-equilibrium conditions, such as population growth, which seems a plausible alternative interpretation given the star-like genealogy of the Danubian population, and its presumably short period of demographic stability.

Keywords: isolation by distance, mtDNA, phylogeography, Pleistocene, range fragmentation, range expansion

Introduction

Phylogeography is a standard tool for interpreting geographical patterns of genetic variation from a genealogical perspective. For a decade, phylogeographical studies relied on the visual examination of how estimated gene trees overlay upon the mapped geographical sources of genetic data, and the deduction of evolutionary processes compatible with the observed patterns (Avise *et al.* 1987; Avise 2000). Several caveats related to this intuitive approach have been appreciated. The population genetic structure can be the result of a combination of contemporary processes as well as historical events, and the traditional phylogeographical approach may not fully allow for the estima-