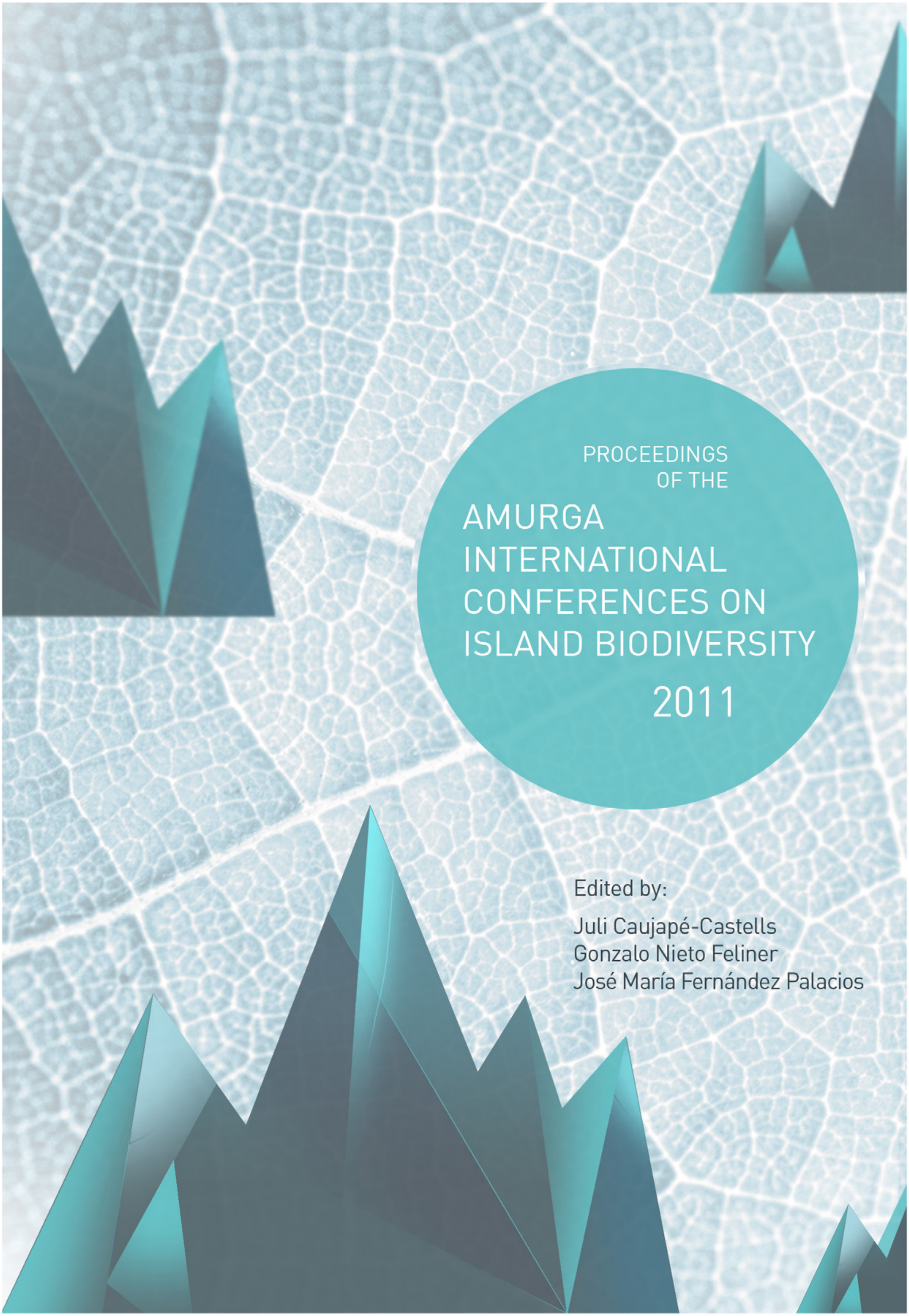
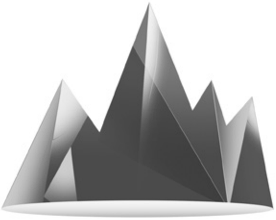


PROCEEDINGS OF THE AMURGA INTERNATIONAL CONFERENCES ON ISLAND BIODIVERSITY 2011

Edited by:

Juli Caujapé-Castells
Gonzalo Nieto Feliner
José María Fernández Palacios



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Cabildo de Gran Canaria
Gran Canaria Reserva de la Biosfera
CSIC
Fundación Amurga Familia del Castillo

PROCEEDINGS OF THE AMURGA INTERNATIONAL CONFERENCES ON ISLAND BIODIVERSITY 2011

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SUMMARY

1. Island Biogeography

2. Genetic Diversity and Ecology In The Canaries

3. Evolutionary Processes

4. Plant Life Out of Macaronesia

5. Macaronesian Floras

Introduction

Under the overarching idea that preserving biodiversity is only possible through its scientific understanding, the Fundación Canaria Amurga Maspalomas and the Jardín Botánico Canario "Viera y Clavijo-Unidad Asociada CSIC of the Cabildo Insular de Gran Canaria organised on March 2011 the first edition of the "Fundación Amurga International Conferences on Island Biodiversity".

The objective of this unprecedented meeting was to bring together some of the internationally most renowned researchers from around the world to share existing and developing knowledge on the evolution, diversity and conservation of the lush and unique (but also extremely fragile, and increasingly threatened) biota of oceanic islands.

As a consequence of the keen acceptance to attend of most selected scientists, a wide number of diverse topics was covered, ranging from classical and molecular taxonomy to reproductive biology, phylogenetic and genetic diversity, phylogeny, biogeography, cytogenetics, and the applications of all these fields to understand evolution on islands, or to design informed conservation and management strategies.

Sharing diverse worldviews of the past, present and future of insular plant biodiversity throughout a week inevitably triggered new and productive multi-disciplinary interaction, so we believe that the meeting's purpose was largely overachieved thanks to the general enthusiasm that it created.

The objective of this proceedings volume is simply to gather the ideas that were discussed during that week just as they were transmitted by the speakers, so we explicitly discouraged the authors from attempting to update the contents of the discussions in 2011. For similar reasons (and because of multiple heterogeneous commitments in our respective institutions), we have not reviewed the idiomatic contents of the contributions. As the scientific editors of this volume, our mission has restrained to make a consistent whole out of all the submitted texts, to oversee their scientific contents and, in some cases, to suggest a minimum number of formal changes. In all probability, this book will not have a high SCI impact factor, so that the fact that most such distinguished scientists made an effort to submit their full papers in time is perhaps another indicator of the meeting's success.

The specialist reader will notice that some contributions are slightly outdated, because their authors have already published refined versions of these ideas, in some cases thanks to the open and friendly discussions that all the attendees (speakers or not) furnished. For the non-specialists, this volume is a rare opportunity to catch up with ongoing progresses on the investigation of oceanic island floras, in some cases hand in hand with top-notch researchers in different fields of expertise.

It is thus with sheer pleasure that we thank the support of the Fundación Canaria Amurga Maspalomas and the Cabildo Insular de Gran Canaria in the organisation of this meeting. We hope that these two institutions from Gran Canaria continue showing that Macaronesia is today a remarkable hotspot of ideas about the evolution of life on islands, and a land of opportunity for new research endeavors.

Las Palmas de Gran Canaria, Madrid and La Laguna,
October 2013

Juli Caujapé-Castells

Gonzalo Nieto Feliner

José María Fernández Palacios

Preface

If we only considered its presently emerged area, many people would agree that Gran Canaria is a small island. However, its sheer geographical ruggedness and lush biodiversity do turn it into one of the biggest islands of its size in the world.

Indeed, the levels of biodiversity that Gran Canaria harbours (with about 81 exclusive plant endemic species) are among the highest in the Canarian archipelago, one of the most remarkable biodiversity hotspots on the planet.

Both important gaps affecting biodiversity knowledge, and the fast global changes that especially threaten island biota, impose upon us challenging management tasks. In such a demanding context, my Ministry is convinced that sensible conservation strategies can only emerge from the application of reliable, up-to-date information. Likewise, a hopeful future for our common natural legacy is contingent upon fostering constructive discussion among different specialists.

These basic tenets underlie the full support we give to projects related to the generation of knowledge that may assist us in the management of our biodiversity. Precisely, this is one of the driving missions of the Jardín Botánico Canario "Viera y Clavijo", the Cabildo's flagship in the provision of scientific guidelines for the conservation of Gran Canaria's terrestrial flora.

An internationally renowned institution almost since its foundation in 1952, our Botanic Garden is devoted to three closely interrelated areas of activity: scientific-technical research, environmental education, and ex situ conservation, boasting the most important living collections of Canarian endemic plants in the world.

Summoned by the research staff at this institution, many important specialists shared their worldviews of the past, present and future of insular plant biodiversity in the first edition of the "Amurga conferences of Island Plant biodiversity", celebrated during a week in March 2011.

Although I hadn't taken office yet at that time, the positive consequences of those discussions are delivering today on my team's task: many of our most valuable liaisons with other institutions were strengthened during that week, and other collaborations important for Gran Canaria's plant diversity started thanks to the meeting.

Bringing together such a panel of distinguished scientists throughout a week would not have been possible without the generous patronage of the Del Castillo family through the "Fundación Canaria Amurga-Maspalomas", which also funded the publication of this special volume.

I'm thus glad to thank the Fundación Canaria Amurga-Maspalomas for its unrestrained support to activities with positive impacts on the development of the Canary Islands, and also the organizers of this international meeting, all the attendees, and the scientific editors of this volume. Through their relentless compromise with the conservation of the world's island plants, they all give us great help in preserving the unique Canarian biodiversity.

María del Mar Arévalo Araya

Minister of Environment and Emergencies
Cabildo de Gran Canaria

Preface

For the Amurga Maspalomas Canary Foundation it has been a great honor to have actively participated in an event as significant as was the Amurga International Conferences on Insular Biodiversity, of which we now present the main conclusions in this work.

Our organization was founded with the aim of contributing to the promotion of research and education in the Canaries. That is our main objective, and we especially focus on promoting conservation and care of the environment. However, we cannot preserve what we do not know. Therefore, a critical first step before the study was a knowledge of our natural environment in order to implement appropriate and efficient conservation techniques.

Thanks to the collaboration established with the Local Ministry of Environment and Emergencies of the Coucil of Gran Canaria, and the Canarian “Viera y Clavijo” Botanical Garden - Unit associated to the Spanish National Research Council (CSIC) – it was possible to organise an event of this level, with the presence of leading national and international researchers. I would also like to thank the Councillor Maria del Mar Arévalo, for the opportunity provided for the successful event.

Pedro Agustín del Castillo

President
Amurga Maspalomas Canary Foundation

1

Island biogeography

The importance of windows of opportunity for long-distance dispersal to or from oceanic islands: examples from the Macaronesian archipelagos

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The current species composition of entire oceanic islands or specific insular ecosystems is the result of a complex scenario where several historical and ecological processes, including long-distance dispersal (LDD), subsequent colonization, speciation (within or among islands) and extinction may have played an important role.

Recently, one of us (Carine, 2005) coined the concept of colonization window, referred to the colonization opportunities in some islands or archipelagos that emerged for vagile continental taxa when either specific geological events (such as volcanic eruptions that destroy entire communities and ecosystems, creating open space) or ecological events (e.g., the availability of vacant resources in the insular ecosystems due to species impoverishment or disharmony), create the conditions for those species to establish.

In a parallel approach, here we vindicate the concept of window of opportunity for a long-distance dispersal (LDD) event, that could be defined as the time interval throughout which the different factors controlling LDD become simultaneously functional or available, in reference to the opportunities that appeared for vagile taxa to arrive to destinations usually locked to them due to the prevailing wind or marine current regimes. Usually, the occurrence of LDD windows of opportunity is analysed in retrospect; for instance, when palaeo-biogeographers try to reconstruct the past events that may have enabled a present bizarre (fossil or extant) species distribution.

Among the items that have to be checked for hypothesizing the existence of such window(s) we should consider:

A) The existence in the proper historical moment of continental ecosystems where the focal taxa thrived and may have later persisted *in situ* or not. This may have been, for instance, the case for the Iberian and North African Tethyan Palaeotropical Geoflora with respect to Macaronesia; its elements are not extant today, but they were present in the continental margins until the Pleistocene climate deterioration (Postigo-Mijarra *et al.*, 2010).

B) The existence in the proper historical moment of islands available to be reached and subsequently colonized, which may as well have later persisted or vanished under the sea, as was the case for several Palaeo-Macaronesian islands, that are today seamounts (Fernández Palacios *et al.*, 2011).

C) The historical occurrence of the proper dispersal facilitator agents, either physical (for instance, palaeo-sea-currents, palaeo-wind regimes, important rivers) or biological (birds, bats, etc.), which may be still active/extant today or not.

D) The availability of stepping-stones facilitating dispersal; for instance, through the emergence of non-subsided flat-topped seamounts (guyots) due to eustatic sea-level transgressions.

E) The existence of peak periods, i.e. the availability of discrete windows in time where the simultaneous occurrence of an array of downstream or leeward high islands enabled the transference (through island hoping) of species restricted to mountain and summit ecosystems in the decaying island before erosion and subsidence processes remove those ecosystems and their associated biota. For instance, Stillman (1999) and Acosta *et al.* (2003) argue that Fuerteventura may have reached 3,500 m of height in the past. Therefore, it is feasible that some of the species that, at present, only occur at high elevations in Tenerife originated in Palaeo-Fuerteventura and colonized Tenerife before losing their habitats in the former island.

Until very recently, it was considered that islands were evolutionary dead-ends of lineages, like no-way-out streets for taxa able to colonize them in proper moments, but unable to leave them, thus awaiting extinction (for instance, due to the taxon cycle). Nevertheless, increasing phylogenetic and phylogeographical evidence accumulated in the last years (from e.g., Bellemain & Ricklefs, 2008; Laenen *et al.*, 2010), challenges the traditional perspective of islands as dead-ends, and points to the putative role of oceanic islands as refugia for continental taxa, especially during Plio-Pleistocene Glaciations, when the climatic conditions were much milder on the islands (due to the buffer effect of the oceans and to the opportunity for altitudinal migration) than on the continent.

Thus, windows of opportunity for long-distance dispersal can be hypothesized as well for explaining these bizarre (at least under today's prevailing conditions) retro-colonization or back-colonization events from the islands to the mainland, i.e. the colonization of ecologically similar and available mainland ecosystems by insular (usually neoendemic) species, originally derived from mainland ancestors, when abrupt tectonic (for instance the closure of Gibraltar or Panamá straits) or climate changes (e.g., Plio-Pleistocene glaciations) have transformed in specific moments of the past history the marine-currents and wind-regimes nowadays prevailing in the zone.

In this context, recently published coupled-model simulations for the Last Glacial Maximum (LGM) evidence a substantial equatorward shift in the Westerlies position, 7° for a 3 K degree cooling and 11° for a 6 K degree cooling, in the Northern Hemisphere (Williams & Bryan, 2006) (Fig. 1). In the same line, Rognon & Coudé-Gausen (1996) asserted more than a decade ago that several geomorphological features of Canarian and Maghrebian natural landscapes point towards the dominance of the Westerlies wind regime across the Madeiran-Canarian latitudes (25-30° N) during the glacial periods in the Pleistocene. A shift of the Azores high southwards occurs due to the pressure exerted by the North Polar Front.

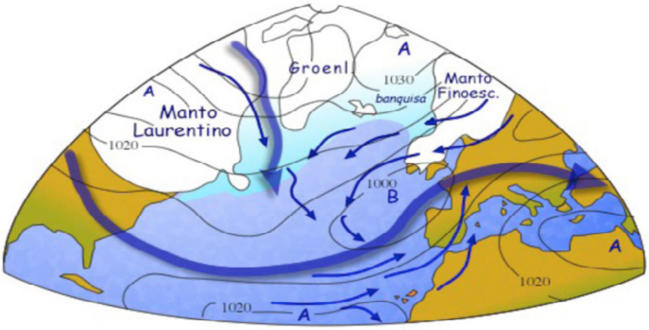

Figure 1. Hypothesized southwards shift of the Northern Hemisphere Westerlies during the LGM, affecting the Canarian latitude (Source: Uriarte, 2003).

These pieces of evidence include the existence of dune fields (*ergs*) in several Canary Islands (especially Esquinzo and Lajares dune fields in Fuerteventura) and Maghrebian areas (especially Agadir dune fields) that could not been formed under the influence of the present wind regime –the dune fields exhibit a triangle shape with the basis on the coast and the vertex pointing to the interior– (Fig. 2). The great amount of organogenic sands that have been deposited forming the dune fields that can only have been originated from deposits, today unavailable, located under the sea level, but available in the past due to the sea level transgression during the glaciations.

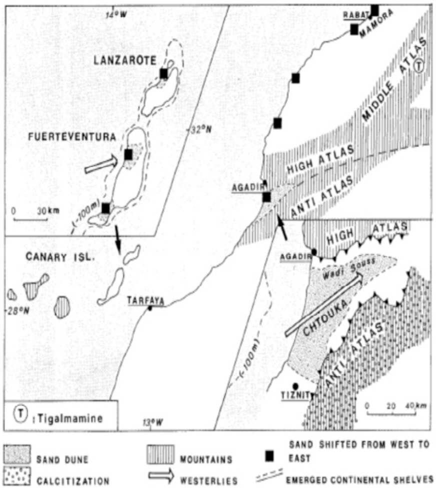

Figure 2. Recent ergs (dune fields) in the Eastern Canaries and Morocco formed due to the Westerlies activity during the LGM (Source: Rognon & Coudé-Gaussen, 1996).

Among other major climatic features (for instance, lower temperatures and precipitations), the Plio-Pleistocene glaciation climatic context on the Madeira–Canaries region implied: the existence of archipelagos larger (almost twice as large as today), higher (ca. 120 m higher) and less isolated from the African continent (just 60 km away) than during interglacial periods. Concomittantly, the reiterative emergence of an important array of seamounts feasibly facilitated the dispersal processes among archipelagos (Fig. 3; for instance Selvagens and Dacia for the Madeiran-Canarian connection, but also between the archipelagos and the Iberian and North African mainland (Conception, Seine, Ampere, Coral Patch, Ormonde, etc.) (Fernández Palacios *et al.*, 2011) (Fig. 3).

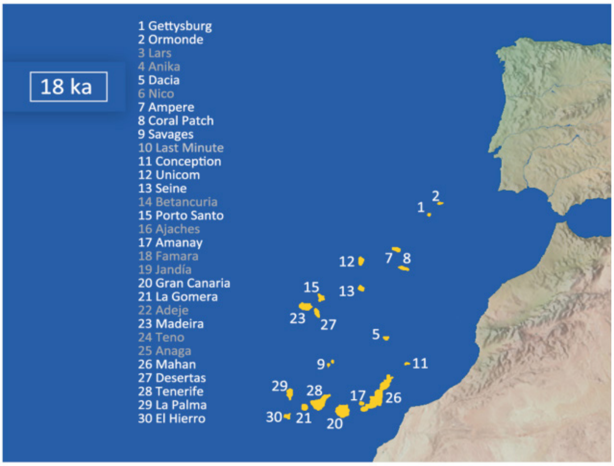

Figure 3. Reconstruction of Palaeo-Macaronesia ca. 18 Ky BP (Source: Fernández Palacios et al., 2011).

Today, it is considered that these climatic conditions have prevailed several times, perhaps as much as twenty, during the Late Pliocene and Pleistocene (Lisiecki & Raymo, 2007) coinciding with the different glacial maxima. Westerlies may have enhanced the retro-colonization from Macaronesian lineages to the African mainland. We also know that several Macaronesian lineages have representatives in the African and European continents (Table 1), and a number ofrecent molecular phylogenetic studies place Macaronesian taxa as basal to continental congeners (Table 1, Fig. 4). Retro-colonization events involving insular taxa have been termed *boomerangs* elsewhere (Caujapé-Castells, 2004, 2011).

Table 1. Some putative boomerangs suggested by molecular evidence for the Macaronesian flora (extracted from Caujapé-Castells 2011) and fauna. (A = Azores, CV = Cape Verde, E = Europe, EA = E-Asia, I = Iberia, Mac = Macaronesia, Ma = Madeira, Me = Mediterranean, Mu = Multiple, NEA = NE-Africa, NWA = NW-Africa, NW = New World, SA = S-Africa).

TAXON	FAMILY	ORIGIN	TARGET	REFERENCE
PLANTS				
Aeonium balsamiferum[E,S]	*Crassulaceae*	*Mac*[1]	*NEA (A. leucoblepharum)*	*Mort et al. 2002*
Andryala pinnatifida[S]	*Asteraceae*	*Ma*	*Me (A. ragusina, A. integrifolia)*	*Fehrer et al. 2007*
Arbutus canariensis[E,S]	*Arbutoideae*	*NW*	*Me (A. andrachne, A. unedo)*	*Hileman et al. 2001*
Convolvulus[S]	*Convolvulaceae*	*Mac*	*I (C. fernandezii)*	*Carine et al. 2004*
Helichrysum gossypinum[S,P]	*Asteraceae*	*Ma*[1]	*Me (ancestor of several spp.)*	*Galbany-Casals et al. 2009*
Ilex perado[E]	*Aquifoliaceae*	*Mu*	*EA (I. leucoclada, I. latifolia, I. rugosa)*	*Manen et al. 2002*
Kleinia neriifolia[S]	*Asteraceae*	*EA*	*NWA (K. anteuphorbium)*	*Pelser et al. 2007*
Lotus campylocladus[S]	*Fabaceae*	*NWA*	*NWA (L. assakensis)*	*Allan et al. 2004*
Matthiola bolleana[S]	*Brassicaceae*	*NWA*	*NWA (M. bolleana, M. longipetala ssp. viridis)*	*Jaén-Molina et al. 2009*
Sideroxylon mirmulano[E]	*Sapotaceae*	*EA*	*CV (S. marginatum), SA (S. inerme)*	*Smedmark et al. 2006*
Teline stenopetala (ssp. micropauci)[S]	*Fabaceae*	*NWA*	*NWA (T. monspessulana)*	*Percy & Cronk 2002*
Tinguarra montana [E]	*Apiaceae*	*NWA*	*NWA (T. sicula)*	*Downie et al. 2000*
Tolpis azorica[S]	*Asteraceae*	*A*[1]	*NWA (T. virgata, T. barbata)*	*Moore et al. 2002*
BIRDS				
Regulusregulusssp. ellenthareae	*Regulidae*	*E*	*E (R. regulus ssp. regulus)*	*Päckert et al. 2006*
Erythacus rubecula	*Muscicapidae*	*E*	*E (E. rubecula)*	*Dietzen et al. 2003*
Fringilla teydea	*Fringillidae*	*E*	*NWA, E (F. coelebs)*	*Marshall & Baker 1998*

[E] Possible major influence of extinction. [P] Phylogenetic tree poorly resolved (i.e., low support for the derived position of the mainland taxa, or polytomic topology combining mainland and island populations). [S] Sampling deficiencies, especially in mainland areas near the Canaries (i.e., the 'Macaronesian enclave' and its surroundings). [1] Canarian taxa with putative origin in Macaronesia have been included in this list on the grounds on their ultimate origin via a continental ancestor.

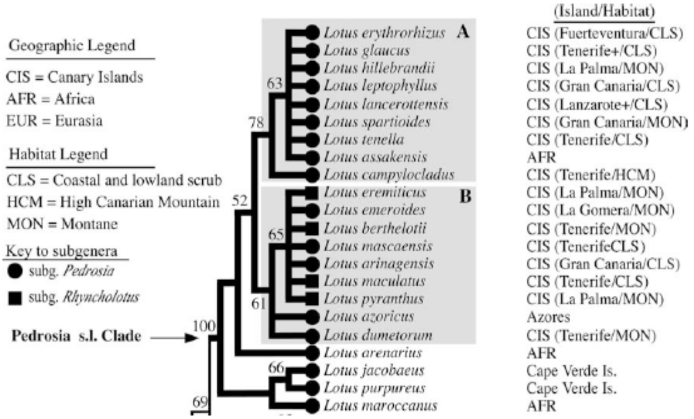

Figure4. Cladogram showing Lotus subgenus Pedrosia + L. subg. Rhyncholotus (= Pedrosia s.l. Clade), where L. assakensis is a possible example of a boomerang (Source: Allan et al., 2004).

If transient in nature, such a dispersal window will be followed by a period where the dispersal route is impossible or highly improbable, because the transportation means it ceased to exist. Of course, the existence of a certain dispersal window does not preclude a colonization event in exactly the opposite direction, but it makes it much unlikely. Thus, biodiversity boomerangs can't be older than the window onset, and several colonization events may be recognized within a specific taxon.

Dispersal windows may also disrupt the "normal" dispersal patterns within archipelagos. It has been postulated that in the Canaries the more common dispersal route within the archipelago is the so-called progression rule (Whittaker & Fernández Palacios, 2007), which implies a general propagule movement from the older, eastward Canaries to the younger, westward islands. Under Westerlies conditions the normal dispersal routes may have been substituted by other usually bizarre routes, for instance: from the western to the eastern islands within the Canaries, from Azores to Madeira or the Canaries or from Macaronesia to Iberia (Carine *et al*., 2004).

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José María Fernández Palacios

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Integrative phylogenetic evidence on the origin of island endemics in the Mediterranean region: Comparisons between oceanic and continental fragment islands.

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The unique life forms found on remote islands captured the keen observational talents and imagination of a young naturalist aboard a ship of the royal British navy. During his explorations of the Galapagos archipelago, he observed: *"Most of the organic productions are aboriginal creations, found nowhere else; there is even a difference between the inhabitants of the different islands; yet all show a marked relationship with those of America, …."* And *"Considering the small size of these islands, we feel the more astonished at the number of their aboriginal beings, and at their confined range" (*Darwin, 1839, p. 378). Islands thus played a seminal role in the development of the theory of evolution, which fundamentally changed the way we explain the origin and history of biological diversity. In "*The Origin of Species*", Darwin dedicated an entire chapter to islands (Chapter 12). Here, he remarked that his newly developed theory predicted the fact that islands would harbor species found nowhere else in the world. *"Although in oceanic islands the number of kinds of inhabitants is scanty, the proportion of endemic species (i.e., those found nowhere else in the world) is often extremely large"* And: *"This fact might have been expected on my theory, for… species occasionally arriving after long intervals in a new isolated district, and having to compete with new associates, will be eminently liable to modification, and will often produce groups of modified descendants" (*Darwin, 1859, pp. 381-382). Thus the existence of island endemics represented an important observation supporting Darwin's theory of evolution, while prompting him to lay the foundations for a theory of island biogeography.

Ever since Darwin's seminal studies on the Galapagos archipelago, islands have been viewed as ideal laboratories to study key evolutionary processes, including the origin of their endemic species, the relationships among species in nearby islands and the mainland, the relative roles of abiotic and biotic interactions in shaping community composition, and the vulnerability of islands to invasions. However, Darwin's observations on island life were based mainly on oceanic islands, which are formed de novo from the bottom of the ocean via volcanic/tectonic processes. Such islands emerge bare of life and biotic elements can colonize their empty ecological niches exclusively via long distance dispersal (LDD) over oceanic barriers (Darwin, 1859). Oceanic islands are often part of archipelagos where they were formed sequentially both in time and space, thus allowing for diversification on different islands (i.e., island radiations; Baldwin, 1997). Consequently, oceanic islands are often viewed as cradles of new species diversity, for they harbor many neoendemic species (Major, 1988).

Yet other kinds of islands exist that were once connected to continents and became separated from them either via tectonic fragmentation (continental fragment islands), via changes in sea level (continental shelf islands), or sometimes by both processes at different points in time (Whittaker and Fernandez Palacios, 2007). The colonization of continental islands was thus possible via a diversity of means, including the splitting of an ancestral broader range of distribution caused by plate fragmentation (classic vicariance), the expansion from the continent into an island via land bridges, followed by range fragmentation as the land bridge was submerged by water (Lieberman, 2003), or via LDD over oceanic barriers, as for oceanic islands. Owing to their geologic origin, continental islands are expected to harbor species that represent the ancestral continental biota (paleoendemics), but they can also provide suitable habitats for colonizers that reach them after island formation (neoendemics; Major, 1988). The diversity of means by which continental islands can be colonized hence complicates the task of reconstructing the sources, colonization routes, and community assembly of such islands, in comparison with oceanic islands (Yoder and Nowak, 2006).

Despite these challenges, comparing the evolutionary histories of species inhabiting oceanic and continental islands can illuminate aspects of the theory of island biogeography that remain unclear, focusing especially on how different geologic origins affect processes of island colonization and speciation, changes in biotic competition and niche filling through time, and the genetic diversity of island populations. We are now in a unique position to generate the integrative knowledge that is necessary to compare patterns and processes of speciation between oceanic and continental islands. Over the past few decades, the advent of new methodologies, including genetic analyses at the inter- and infra-specific levels (ie., phylogenetics and population genetics, respectively), the ability to link genetic relatedness with temporal and spatial frameworks at different time scales, and the development of sophisticated models connecting climatological data with species occurrences, now provide unprecedented opportunities to identify the evolutionary and ecological drivers that have shaped the current composition of island biota.

Until recently, my research in biogeography has mainly focused on using a phylogeny-based approach to test for congruence between speciation events and geologic/climatic events (Conti et al., 2002; Rutschmann et al. 2004, 2007). Phylogenies allow us to establish an evolutionary pattern for the origin of insular taxa by identifying their closest continental relatives, establishing whether individual island endemics form monophyletic groups, and assessing whether islands were colonized once or multiple times. By reconstructing ancestral areas of distribution for the common ancestors of extant species, phylogenies also allow us to infer which continental areas served as sources for island colonization. Finally, phylogenies allow us to establish a time frame for species origins (Fig. 1; Rutschmann et al., 2004). By combining phylogeny-based information with pre-existing knowledge on the geologic formation of different kinds of islands, their climate at the inferred time of species divergence, and ecological data on island species and niches we can attain a more integrative scenario for the origin of insular biota.

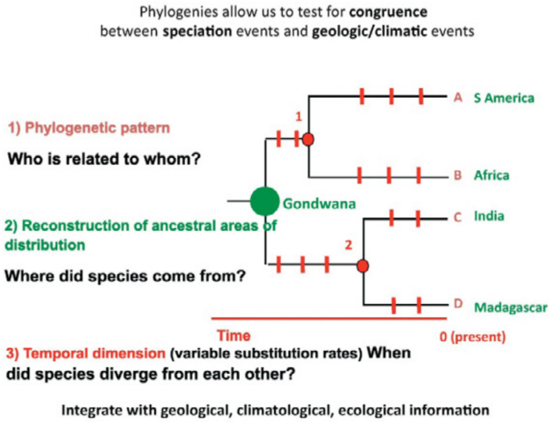


Fig.1

The optimal use of the phylogenetic approach to study the origin and evolution of insular lineages requires the identification of taxa that include both oceanic and continental island endemics, ideally occurring in a biogeographic region characterized by similar climatic conditions on both kinds of islands. The Mediterranean Region, comprising the Mediterranean basin and the Macaronesian archipelago, represents such an ideal study area, because it contains islands of different geologic origins (Quezel 1985, Medail and Quezel, 1997; Myers et al. 2000). I focused my research on plant genera with species that were endemic either to the oceanic Canary Islands or to the continental islands of Corsica and Sardinia. While the Canary Islands were formed in East-West progression starting with Fuerteventura around 20 Ma and ending with El Hierro around 1 Ma (Anguita and Hernan 2000), the Corso-Sardinian microplate split from the Hercynian block (located in the proto-Iberian peninsula) around 30 Ma (Rosenbaum et al., 2002), was temporarily connected with the Adria microplate (grossly corresponding to contemporary central Italy) in the Early-Middle Miocene (Cherchi and Montadert, 1982), and had episodic contacts with surrounding landmasses to the North and to the East during the Messinian Salinity Crisis of the Late Miocene (Krijgsman 2002) and the glacial maxima of the Pleistocene (Lambeck and Purcell 2005).

Within the geological contexts described above and by using an integrative phylogenetic approach, I addressed the following main questions on the origin of (a) oceanic and (b) continental islands endemics in the Mediterranean region: 1a) Were the Canary Islands colonized by a single LDD event, followed by diversification in the archipelago, or by multiple LDD events? The first scenario would be supported by the monophyly of the Canarian endemics, the second by their polyphyly; 2a) Did island colonization follow the sequence of island formation, as predicted by the stepping stone model (Sanmartin et al. 2008), or not? 3b) Did the geologic events of the Corso-Sardinian microplate (Oligocene plate splitting, Miocene and Pleistocene land corridors) drive the origin of its endemic plants or did the endemics reach the islands via LDD?

To investigate these questions, I selected plant groups that included: i) both Canarian and Corso-Sardinian endemics, to enable comparisons between evolutionary processes on different kinds of islands, ii) fossils that could be used to calibrate the phylogeny and estimate absolute rates of nucleotide substitution; iii) seeds and fruits that could not be easily dispersed by wind, thus reducing stochastic processes of dispersal to continental islands. After extensive screening, I selected the Araceae (Areae: *Helicodiceros, Arum, Biarum, Dracunculus*), Boraginaceae (*Borago, Anchusa, Echium*), and Rutaceae (*Ruta*). Phylogenies were inferred using chloroplast DNA (cpDNA) sequences from the following regions: the *trn*L-*trn*F intergenic spacer and the *mat*K gene in all three plant families; the *trn*L intron, part of the *trn*K intron, and the *rbc*L gene in Araceae and Boraginaceae; the *rps*16 intron in Araceae and Rutaceae; the *ndh*F gene in Boraginaceae. Phylogenetic topology, statistical support, and nodal ages were inferred in a Bayesian framework using Mr Bayes ver. 3.1. (Huelsenbeck and Ronquist 2001) and MULTIDIVTIME (Thorne et al. 1998) in all three groups, while BEAST (Drummond et al., 2009) was additionally used for dating analyses in Rutaceae; ancestral areas of distribution were estimated within a parsimony framework using DIVA (Ronquist 1996, 1997) in Areae and Boraginaceae and a maximum likelihood framework using Lagrange (Ree and Smith, 2008) in *Ruta* (see Mansion et al. 2008, 2009; Salvo et al. 2008, 2010 for methodological details).

The comparison of our molecular dating and ancestral area results with the geological events that formed different kinds of islands in the western Mediterranean Region allowed us to conclude that the origin of species endemic to the fragment islands of Corsica and Sardinia was driven by different geological and stochastic processes at different points in time, conforming with the complex geological history of this microplate. Such processes included: i) classic vicariance, caused by the splitting of an ancestral Hercynian mountain belt in proto-Iberia (*Helicodiceros muscivorus*); ii) colonization via land bridges with the Adria microplate from the East (*Arum pictum*), the North (ancestor of *Ruta corsica* and *R. lamarmoarae*), and the South (ancestor of *Borago pygmaea* and *B. morisiana*), followed by divergence driven by tectonic disconnection of the land corridor between the two microplates in the middle Miocene (about 16 Ma) for *Arum* and *Ruta* (Fig. 2) and flooding at the end of the Messinian Salinity Crisis (5-6 Ma) for *Borago*; iii) LDD in the Late Pliocene for the CS endemics of *Anchusa*. These results suggest that the persistence of ancient Hercynian elements (paleoendemics) in the Corso-Sardinian flora might be more rare than previously proposed (Cardona and Contandriopoulos, 1979), while the repeated episodes of land connection and disconnections between the CS microplate and surrounding landmasses in the Miocene offered numerous opportunities for range expansion and allopatric speciation driven by the severance of land corridors (Mansion et al., 2008, 2009; Salvo et al., 2010).

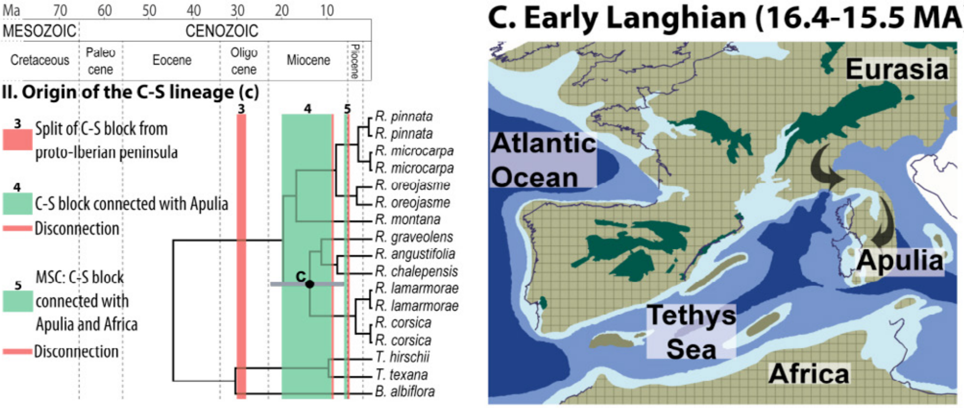


Fig 2. Origin of Corso-Sardinian endemics inferred from molecular dating and ancestral area reconstruction analyses of cpDNA sequences *(modified from: Salvo G., S. Ho, G. Rosenbaum, R. Ree, E. Conti. 2010. Tracing the temporal and spatial origins of island endemics in the Mediterranean region: A case study from the citrus family (Ruta L., Rutaceae).* Systematic Biology 59: 705-722)

Concerning the origin of oceanic island endemics, we were able to conclude that the Canarian *Dracunculus canariensis* diverged from its eastern Mediterranean sister *D. vulgaris* around 21 Ma, concomitantly with the emergence of the eastern islands in the Canarian archipelago. A possible explanation for the current absence of *D. canariensis* from Fuerteventura and Lanzarote might involve the disappearance of suitable habitats on these older islands, while the lack of diversification of *Dracunculus* in the archipelago remains to be explained. Confirming the results of other studies (for ex., Francisco Ortega et al., 2001; Boehle et al. 1996), our phylogenetic analyses of *Echium* and *Ruta* indicated that the Canary Islands were colonized once by the ancestors of the respective endemics, most likely from Africa, in the middle Miocene, following the formation of the oldest islands in the eastern part of the archipelago (Fuerteventura, 20 Ma). Our species sampling, including only eight out of 27 Macaronesian *Echium* species, was too limited to afford any conclusions on the model of inter-island colonization. Contrary to other studies, which, however, did not include molecular dating and ancestral area analyses (e.g. Thorpe et al., 1993; Besnard et al., 2009), our reconstruction of ancestral areas of distribution inferred Gomera as the first island colonized by *Ruta*, even though this island is younger and to the West of Gran Canaria, where *R. oreojasme* occurs. Our current results thus do not conform to a simple stepping stone model for island colonization, although uncertainty surrounding Lagrange reconstructions should be kept in mind (Fig. 3).

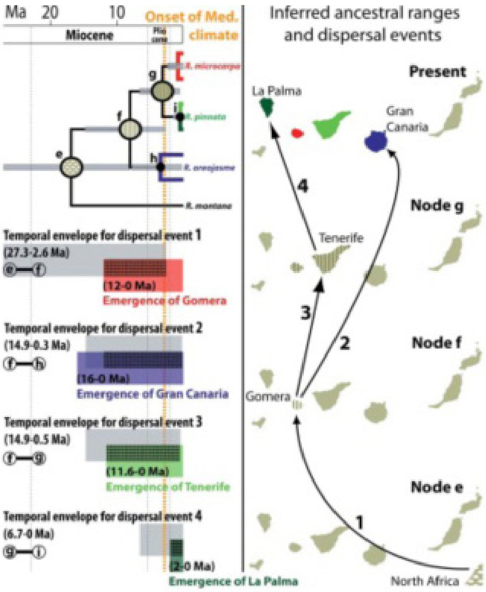
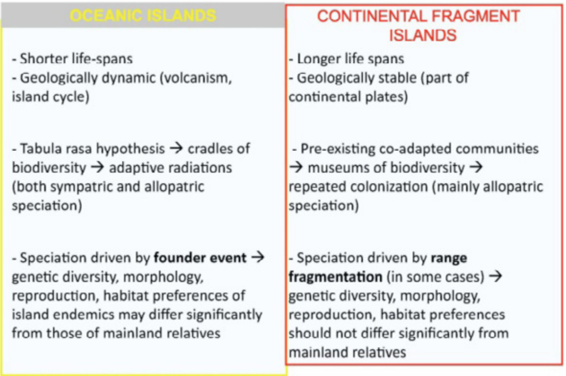


Fig 3. Origin of Canarian endemics inferred from molecular dating and ancestral area reconstruction analyses of cpDNA sequences (modified from: Salvo G., S. Ho, G. Rosenbaum, R. Ree, E. Conti. 2010. Tracing the temporal and spatial origins of island endemics in the Mediterranean region: A case study from the citrus family (*Ruta* L., Rutaceae). *Systematic Biology* 59: 705-722)

In all three families, both continental and oceanic island endemics originated well before the onset of the Mediterranean climate in the Late Pliocene, suggesting that the lineages had to adapt to changing climatological conditions in order to persist on the islands and underscoring the role of parallel filtering processes on different islands inhabited by closely related species. The studies performed so far highlight the importance of identifying suitable windows of opportunity for island colonization by finding the temporal overlap between the geologic origins of the islands, the confidence intervals around the stem and crown ages of island endemics, and the source areas that were available at the time of speciation, requiring detailed paleogeographic reconstructions.

I am now pursuing new questions stemming from the results and experience of my previous studies on Mediterranean island endemics. *Ruta* is a particularly suitable group to compare evolutionary patterns in oceanic and continental islands, because this strongly monophyletic genus of only nine species includes three Canarian endemics, two Corso-Sardinian endemics, and four widespread Mediterranean species, thus allowing for in-depth molecular and taxic sampling, which is necessary to address issues of monophyly and gene flow between island endemics. The first, obvious goal was to complement the results from cpDNA analyses (Salvo et al., 2008, 2010) with phylogenetic analyses of DNA sequences from the nuclear genome (nDNA) and increased infra-specific sampling to ask: i) Are the phylogenetic relationships and origins of island endemics inferred from cpDNA analyses confirmed by nDNA data? ii) Are individual island endemics monophyletic or not? If not, this result case might suggest gene flow between island endemics. We are currently addressing these questions by using ITS sequences, which were cloned to investigate infra-individual variation of haplotypes. The ITS phylogeny is partially congruent with the topology of the cpDNA tree, for it confirms the monophyly of both the Canarian and Corso-Sardinian endemic groups, respectively, and the sister relationship between the Canarian clade and the western-eastern Mediterranean disjunct *R. montana*. However, the relationships of the Corso-Sardinian clade differ between the cpDNA and nDNA trees, for the former supports a sister relationship with a clade formed by the widespread *R. chalepensis, R. angustifolia,* and *R. graveolens*, while the latter supports a sister relationship with *R. graveolens* only. Additionally, neither the two Corso-Sardinian nor the three Canarian endemics are reciprocally monophyletic in the ITS tree. This latter discrepancy between the cpDNA and nDNA trees suggests that, after the initial divergence between species tracked by the maternally inherited cpDNA, inter-island gene flow via pollen and/or seeds was possible in more recent times in both the oceanic and fragment island endemics, a signal detected by the nDNA sequences (Reid, Meloni, and Conti, *In preparation*). The ability of organellar and nuclear genomes to track older and younger evolutionary signals, respectively, has already been used to uncover superimposed patterns of initial species divergence followed by inter-specific gene flow (e.g., Eidesen et al., 2007; Palumbi et al., 2001; Casazza et al., 2012).

What are the open, remaining questions in island biogeography that can benefit from comparisons between oceanic and continental islands? The main examples can be drawn from investigating how the different geologic origins of such islands inform expectations on changes in genetic diversity, breeding systems, morphological traits, and ecological preferences between insular endemics and mainland relatives (Fig. 4). Speciation on oceanic islands is always driven by founder effect, where a few propagules establish a population that can then diverge into a new species. Consequently, we can expect that oceanic insular endemics, at least for a certain amount of time after colonization, will be characterized by significantly lower genetic diversity than their mainland counterparts. Furthermore, allogamous plants colonizing an oceanic island will most likely face a lack of suitable pollinators and con-specific mates. Therefore, autogamous phenotypes will have a better chance of establishment. According to Baker's law (Baker, 1955), island endemics will thus be generally more self-compatible than their mainland relatives. The genetic bottleneck associated with the establishment of oceanic island populations might also imply adaptation to abiotic ecological conditions that differ from those of the mainland. Alternatively, the new propagules might find either a lack of competitors or different competitors from those of the source population, a phenomenon that has been termed "ecological release" (Blondel 1985) and might allow for the invasion of a broader ecological niche on islands. Insular taxa have also been associated with increased woodiness, convincingly demonstrated especially for oceanic species (Carlquist 1974), and larger seed size (Cody and Overton, 1996).



OCEANIC ISLANDS	CONTINENTAL FRAGMENT ISLANDS
- Shorter life-spans - Geologically dynamic (volcanism, island cycle)	- Longer life spans - Geologically stable (part of continental plates)
- Tabula rasa hypothesis → cradles of biodiversity → adaptive radiations (both sympatric and allopatric speciation)	- Pre-existing co-adapted communities → museums of biodiversity → repeated colonization (mainly allopatric speciation)
- Speciation driven by **founder event** → genetic diversity, morphology, reproduction, habitat preferences of island endemics may differ significantly from those of mainland relatives	- Speciation driven by **range fragmentation** (in some cases) → genetic diversity, morphology, reproduction, habitat preferences should not differ significantly from mainland relatives

Fig 4. Comparisons between oceanic and continental islands

Conversely, the origin of fragment insular endemics may have been driven by range fragmentation due to plate splitting or land-bridge disconnection, as demonstrated, for example, for the origin of Corso-Sardinian endemics in *Ruta* (Salvo et al., 2010). In this case, the genetic diversity of the insular taxa may not differ significantly from that of their mainland relatives. Similarly, the breeding system and ecological preferences of fragment island endemics are not expected to significantly differ from those of the mainland relatives. Predictions would obviously differ under an inferred scenario of species origin via LDD for continental island endemics.

To address these different predictions, we are comparing the genetic diversity, breeding systems, and ecological preferences of *Ruta* species endemic to different kinds of islands and their mainland relatives. The similarity in the age of the Canarian (16 Ma) and Corso-Sardinian (15.19 Ma) clades makes the comparison especially informative, because it decreases the confounding effects of different ages. Within the Canarian clade, we have a series of species ages that might allow us to investigate the effect of time on the accumulation of genetic diversity, with *R. oreojasme* at 6.9 Ma and *R. pinnata*-*R. microcarpa* at 2.7 Ma. The age of the latter two species is similar to the age of the Corso-Sardinian *R. corsica* and *R. lamarmorae* (3.0 Ma; Salvo et al., 2010). We have already identified 11 microsatellite loci that are polymorphic in the three Canarian species and their *R. montana* sister and screened seven of these loci in four out of the five existing populations of *R. microcarpa* (La Gomera) and 8 out of the 11 populations of *R. oreojasme* (Gran Canaria). Our ultimate goal is to estimate the genetic diversity of insular and mainland taxa and perform comparisons within island systems, between island systems, and between islands and mainland (Meloni, Reid, Cajaupe-Castells, Fernandez Palacios, Conti, *In preparation; Meloni et al., 2013*).

Additionally, we are planning to explicitly compare the degree of woodiness between *Ruta* insular taxa and their mainland relatives. Available data indicate that *R. montana from the mainland* is a herbaceous species only 15-70 cm tall, while the three Canarian relatives range from small (*R. oreojasme*) to big shrubs (*R. microcarpa*) and small trees 1-2 m tall (*R. pinnata*).

Conversely, the two Corso-Sardinian endemics (15-50 cm) and their widespread relatives (14-75 cm) do not appear to significantly differ in size and, likely, degree of woodiness. We will also test the hypothesis of a shift in the ecological niche occupied by insular vs. widespread relatives. Preliminary observations on the presence of *R. microcarpa* on the northern, wetter part of La Gomera, *R. oreojasme* on the southern, drier part of Gran Canaria, and *R. pinnata* on both the wetter and drier sides of Tenerife are suggestive of a possible expansion of the ecological niche in the Canarian clade vs. *R. montana*. The formal comparison of ecological niches presents challenges linked with the low population number of the insular endemics. One possibility might be to project the ecological niche of the mainland species onto the island space, check for the presence or absence of similar niches, and compare them with the actual localities of insular taxa, but other, recently developed methods for the quantification of niche overlap will also be evaluated (Broennimann et al., in review)

We are planning to test the predictions of Baker's law (i.e., that insular taxa, and oceanic ones in particular, will be more self-compatible than mainland relatives) in Canarian and mainland relatives of *Limonium* (focusing on insular and Iberian populations of *L. sinuatum* and *L. lobatum*). This new project, already started by Dr. Ares Jimenez, will also investigate issues of monophyly and gene flow among Canarian *Limonium*, whether speciation occurred primarily allopatrically, sympatrically, or by a combination of both, and the prediction that the seeds of island species will be heavier than the seeds of mainland relatives (Jimenez, Fernandez Palacios, Cajaupe-Castells, Conti, In prep). *Limonium* represents an ideal genus to ask these questions because: i) it includes 18 species endemic to the Canarian archipelago and five additional species that occur both on the islands and mainland, thus allowing for multiple comparisons at different hierarchical and temporal levels; ii) a backbone phylogeny of the genus (including only single representatives of six insular endemics) is available (Lledo et al., 2005), thus guiding our planned, more intensive phylogenetic analyses; iii) variation of breeding systems and self-incompatibility has been documented in the genus, thus suggesting that shifts between mainland and island populations are possible (Baker, 1953).

In conclusion, comparisons between continental and oceanic islands endemics vs. mainland relatives that focus on the patterns, sources, and timing of species origins, inter-specific and inter-island gene flow, changes in genetic diversity, shifts of ecological preferences, reproductive strategies, and morphological characteristics (growth form, seed size, flower size) will bring new light on how the mode of island origin and initial colonization affect the evolution of island taxa. Such comparisons are especially informative if performed on genera that include taxa endemic to both kinds of islands in the same general climatological conditions (as, for example, in the same floristic region). Additionally, explicit knowledge of the age of the insular taxa and whether they reached the islands via classic vicariance, migration over land bridges, or long distance dispersal (for continental islands) is essential to account for the effects of time and colonization mode on changes in genetic diversity and ecological preferences. The integrative knowledge necessary to perform the discussed comparisons between oceanic and continental islands can be achieved only via extensive collaborations between island biogeographers, phylogeneticists, population geneticists, and ecological niche modelers.

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Elena Conti

Elena Conti's fundamental research goal is to understand the origin and evolution of plant diversity. To achieve it, she integrates different aspects of plant evolutionary biology into an explicit phylogenetic framework, addressing fundamental questions in biogeography and plant reproductive biology. In biogeography, she is using both phylogenetic/phylogeographic and population genetic approaches to investigate the origin of Alpine taxa, the link between secondary contact after glacial retreat and allopolyploid speciation, the role of geological events in shaping patterns of distribution in Mediterranean plants, and whether island colonization is linked with changes of genetic diversity and ecological preferences. She is investigating this last question by comparing oceanic (Macaronesian archipelago) and continental islands (Corsica and Sardinia). In plant reproductive biology, she is studying whether the evolution of heterostyly was the main driver of increased diversification rates in Primulaceae and whether the interaction between floral morphology and pollinators contributes to modulating gene flow between hybridizing species of Primula. She is also starting to explore whether island colonization is linked with switches from heterostyly to homostyly, bringing together her interests in reproductive biology and island biogeography.

Bayesian biogeography finds its roots*[1]: Statistical models in island biogeography

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Introduction

Biogeographic models and biogeographic scenarios:

When attempting to infer the biogeographic history of lineages or biotas, biogeographers usually focus on four different types of biogeographic scenarios. Each scenario is defined by states (i.e., geographic ranges or distributions) and a model that specifies the transition between states – i.e., changes in the geographic distribution of lineages – in terms of biogeographic processes. The first model corresponds to a continuous model in which individual lineages are modeled as occupying points in a 2D or 3D landscape, and biogeographic processes correspond to Brownian movements in this landscape. It is the domain of population genetics and phylogeography. The other three models correspond to discrete models in which geographic areas are defined *a priori* and bioeographic processes correspond to events that change the biogeographic state of the organism such as dispersal or extinction (Ronquist & Sanmartín, 2011).

Here, we will concentrate on the discrete models (Fig. 1) because these are the models most often used in historical biogeography (Ronquist & Sanmartín, 2011). There are three types of discrete models: hierarchical vicariance models (HVM), reticulate models, and island models.

A) Hierarchical-vicariance models (HVM) models (Fig. 1A): HVM models correspond to the classic vicariance model: the fragmentation of a contiguous ancient area by the successive appearance of dispersal barriers, followed by allopatric speciation or vicariance across the barrier. The best-known example of this type of scenario is the split-up of the southern Hemisphere landmasses from the Mesozoic onwards (Fig. 1A, left). This type of scenario has traditionally been the focus of cladistic biogeographic methods. The aim is to construct hierarchical branching structures or *area cladograms* in which areas of distribution are grouped based on shared endemic taxa. In the case of alternative groupings, those that imply the minimum number of changes (ad hoc

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[1] This title is taken from Lemey et al. (MBE 2009)'s paper "Bayesian phylogeography finds its roots", in which Peter Lemey and colleagues independently developed a BIB model for phylogeographic analysis

assumptions) are preferred (principle of parsimony). Although there is no explicit reference to the evolutionary processes that created the patterns, congruence between area cladograms is usually interpreted as evidence of vicariance (Fig. 1A, right). Other processes (e.g., dispersal) are inferred *a posteriori* to explain incongruence with the area cladogram.

B) Reticulate models (Fig. 1B): Contrary to the HVM model, in which an ancestral area is fragmented by successive splitting events, most regions conform to what is called a "reticulate"model, with alternate cycles of area splitting (vicariance) and area fusion events (Fig. 1B, right). For example, the collision between two continents previously separated by an ocean barrier can result in episodes of range expansion occurring simultaneously in different clades. This is termed "geodispersal". Probably the most studied example of reticulate scenario is the geological evolution of the Holarctic landmasses during the Mesozoic and Cenozoic (Fig. 1B, left).

Reticulate scenarios cannot be represented by a hierarchical branching pattern or area cladogram. They require a different type of methods, "event-based" (Ronquist, 2003), which are still based on the parsimony principle but use cost matrix approach to model transitions between states by assigning each process (i.e., dispersal, extinction, vicariance) a cost according to its likelihood. This allows reconstruction of both the common biogeographic patterns across taxa and the type of events that have generated those patterns.

Probably, the most popular event-based method is Dispersal-Vicariance Analysis (DIVA). Given an organism phylogeny and terminal distributions, DIVA finds the optimal, most parsimonious reconstruction that explains the observed biogeographic patterns by minimizing the number of extinction and dispersal events. Because the method allows all possible splits between areas as potential vicariance events, even those that do not conform to a hierarchical area cladogram, DIVA is very powerful to reconstruct "reticulate scenarios. Although originally conceived for taxon biogeography – inferring the biogeographic history of single clades – DIVA can also be used for extracting general biogeographic patterns by summarizing dispersal and vicariance frequencies across multiple co-distributed groups, or for analyzing variation of patterns over time by assigning events to time bins representing time classes (Sanmartín et al., 2001).

C) Island models (Fig. 1c): In island models, areas are considered as static, isolated discrete units between which organisms move or disperse. Volcanic archipelagos that arose directly from the ocean and were never connected to a continental landmass are a good example of this type of scenario (e.g., Hawaiian Archipelago). Because the focus is on migration between areas or "islands", the only process being considered is "jump dispersal", the rate of crossing across a geographic barrier. Hence, island biogeography has usually represented a challenge to biogeographic methods, more focused on vicariance and range division (Sanmartín et al., 2008).

Figure 1

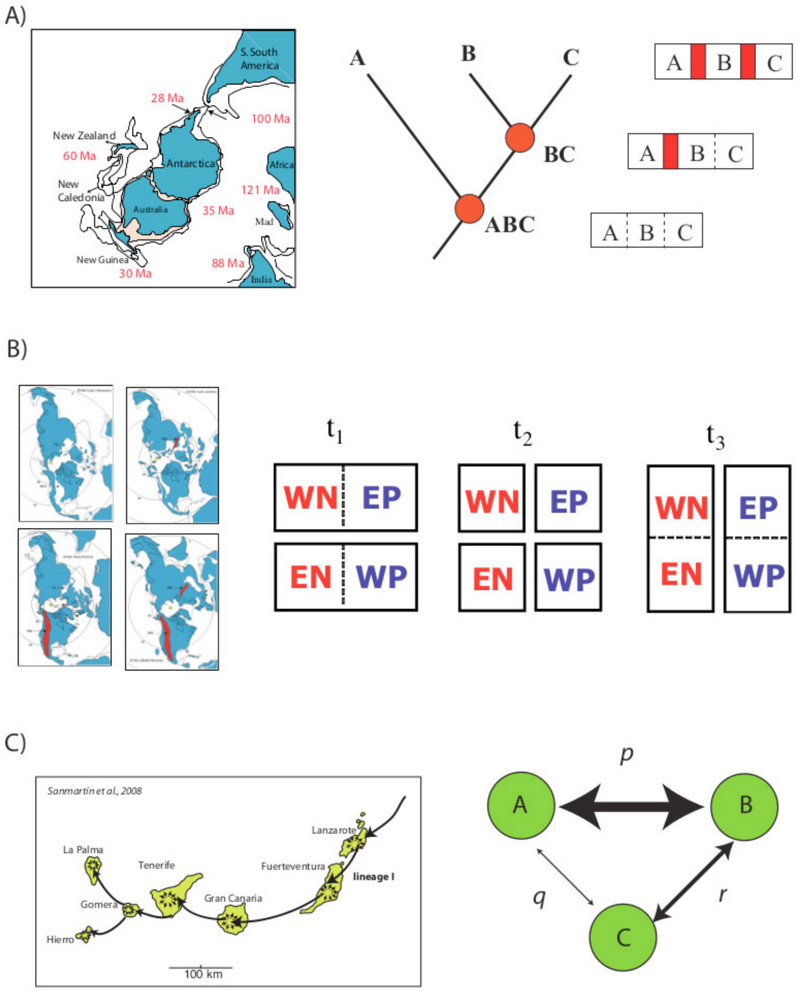


Figure 1. Discrete biogeographic models considered in historical biogeography: A) Hierarchical vicariance model (HVM): fragmentation of a contiguous ancestral area by successive geographic barriers leads to allopatric speciation (vicariance) of its native biota. The split up of the supercontinent of Gondwana is a classical example (left; adapted from Sanmartín 2002; red numbers give age of splitting event). B) Reticulate model: alternate cycles of area splitting (vicariance) and area fusion (geodispersal) leads to a non-hierarchical pattern of area relationships. An example of this type of biogeographic scenario is the geological history of the Northern Hemisphere (left). C) Island model: areas are considered as static, isolated discrete units between which organisms move or disperse. Volcanic archipelagos isolated from the continent, such as the Canary Islands, are a good example of this type of scenario.

Methods in island biogeography

Fitch Parsimony: Until recently, the most popular method to infer island colonization patterns was Fitch Parsimony. This method considers geographic range as a discrete character that is optimized ("mapped") onto a cladogram by minimizing the number of changes in the geographic state ("steps") required to explain the terminal distributions in the phylogeny. Fitch Parsimony assumes that ancestors are distributed in single areas. Although vicariance can be incorporated as a *polymorphic* character state in a step matrix, the number of steps would depend on the tree size, which makes it difficult to compare results across trees.

Limitations with Parsimony: There are several problems with the use of parsimony in island biogeography.

First, although parsimony can be used to model island scenarios of varying complexity – an unconstrained dispersal model (Fitch Parsimony) versus a model of sequential dispersal following the island chain (Wagner Parsimony) – one cannot statistically distinguish between these scenarios – the Fitch model will always be more parsimonious than the Wagner model. Parsimony lacks the statistical power of parametric methods (see below), in which observed patterns can be compared with null distributions generated under an explicit biogeographic model.

Second, parsimony methods cannot incorporate the uncertainty associated with the estimation of ancestral states ("mapping uncertainty"), since only the most parsimonious, minimum-cost reconstructions are considered (Sanmartín et al., 2008). It is also more difficult to accommodate phylogenetic uncertainty and dating information, two essential components in biogeographic analysis (Sanmartin et al., 2008). Phylogenetic uncertainty can be incorporated into parsimony methods by averaging parsimony-based reconstructions over a probability distribution of trees reflecting the relative confidence of the ancestral areas on the various clades in the phylogeny (Ronquist, 2003). Such distribution could be obtained through non-parametric bootstrapping of the original dataset or may be drawn from the posterior distribution of an MCMC Bayesian analysis. This is the basis of Bayes-DIVA, a Bayesian approach to dispersal-vicariance analysis that integrates DIVA biogeographic reconstructions over the posterior distribution of the tree topology (Nylander et al., 2008)

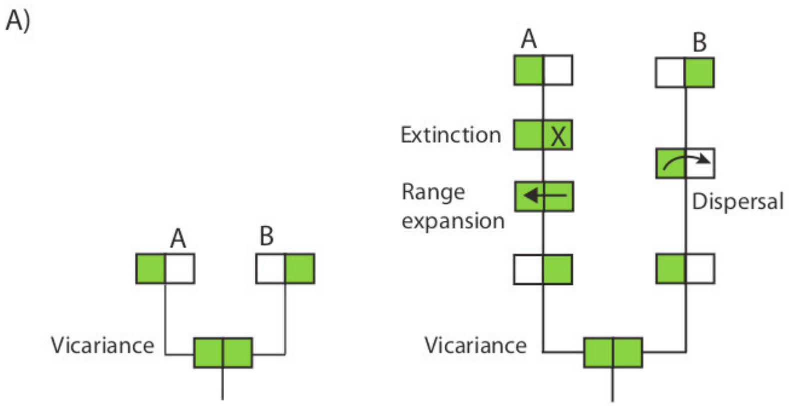
Third, the most important limitation of parsimony methods is probably their inability to account for differences in branch lengths or the time of evolutionary divergence between lineages (Donoghue & Moore, 2003; Fig. 2A). Thus, parsimony reconstructions cannot take account of the fact that the likelihood of biogeographical changes such as dispersal and extinction is higher along long branches than along shorter ones. Longer branch lengths imply higher opportunity for change in geographic distribution along the lineage, and also higher uncertainty in the inferred ancestral range (Fig. 2A).

Statistical models in biogeography

In recent years, new methods of biogeographic inference have been developed that are based on stochastic models of dispersal. These models are structured in the same way as event-based models, with states (geographic distributions) and transitions between states that are dependent on biogeographic processes such as dispersal and extinction. These rates are then used to estimate ancestral ranges using standard statistical methods such as Maximum likelihood and Bayesian inference (Ronquist & Sanmartín, 2011).

Statistical, parametric methods in historical biogeography model range evolution - i.e., the change in geographic range from ancestor to descendant - as a stochastic process with discrete states that evolve along the branches of the phylogeny according to a Continuous-Time Markov Chain (CTMC) model of the same type as those used for the evolution of molecular data in statistical phylogenetic inference (Fig. 2B). The states of the model are the geographic ranges or species distributions. Transitions between states are governed by an instantaneous rate matrix (Q), whose parameters are biogeographic processes such as dispersal or extinction determining the rate of range evolution (e.g., by range expansion or contraction) from ancestor to descendant as a function of time. Exponentiating this rate matrix as a function of branch length or time ($P = e^{Qt}$) gives the probability of geographic change from ancestor to descendants over the phylogeny (Fig. 2B).

Figure 2



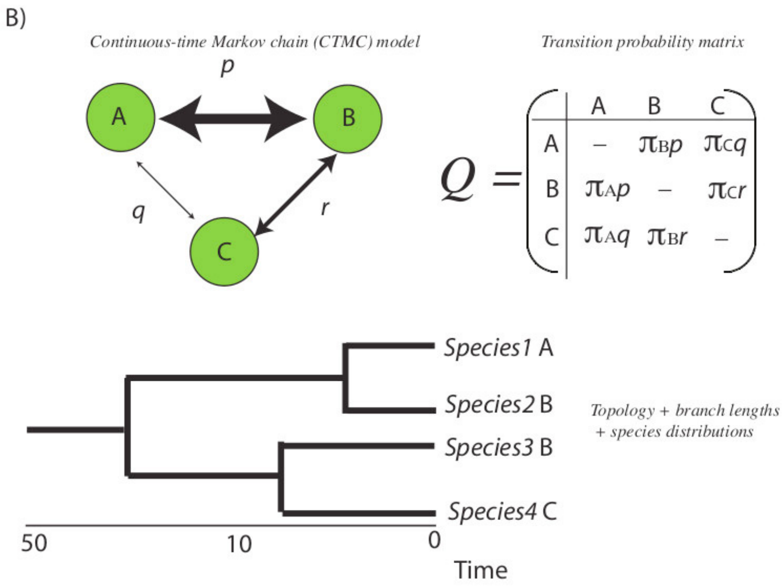


Figure 2. A) Influence of branch lengths on parametric biogeographic inference: the longer the branch length, the higher the opportunity for geographic change along the branch, and the higher the uncertainty in the inferred ancestral range. B) Parametric, model-based approach to island biogeography: range evolution is modelled as continuous-time Markov chain (CTMC) process that evolves along the branches of the phylogeny as a function of time. Transition between states is governed by an instantaneous rate matrix (Q), whose parameters are biogeographic processes (dispersal rates, carrying capacities). Exponentiating this rate matrix as a function of time gives the probability of geographic change from ancestor to descendants over the phylogeny ($P = e^{Qt}$).

In the first study to apply statistical methods in biogeography, Nepokroeff et al. (2003) used CTMC models and ML inference to reconstruct the biogeographic history of genus *Psychotria* in the Hawaiian Islands. They compared a Jukes-Cantor biogeographic model with a single rate of dispersal between islands with a stepping-stone model in which dispersal was constrained to follow the sequence of the island chain. By using an explicit CTMC model of range evolution, they were able to estimate the rate of migration between islands, while accounting for differences in branch lengths and the uncertainty in the inferred ancestral ranges. Since then, there has been an increasing interest in using CTMC models and ML inference in biogeographic analysis, usually to estimate rates of dispersal between areas within single organism groups. Unlike parsimony, ML inference allows to test alternative biogeographic models (e.g., symmetric vs. asymmetric dispersal) within a rigorous statistical framework (Pagel, 1999).

Dispersal-Extinction-Cladogenesis (the DEC model)

In a groundbreaking paper, Ree et al. (2005) developed what is probably the most complete probabilistic method of biogeographic reconstruction: Dispersal-Extinction-Cladogenesis (DEC), an ML version of DIVA that is implemented in the software Lagrange. Like in the latter method, there is an anagenetic component and a cladogenetic component. Range evolution along internodes (i.e., the branches leading to a splitting event) is modelled as a stochastic CTMC model with two parameters: dispersal ("range expansion") and extinction ("range contraction"). Dispersal and extinction rates can be the same across areas and over time (only one dispersal and extinction parameter) or they can differ between areas and vary across pre-defined time slices reflecting temporal constraints on area connectivity and dispersal routes (Buerki et al., 2011). Dispersal and extinction rates can also be constrained by using scalars according to geographic distance and area size .

The DEC cladogenetic component determines the inheritance of geographic ranges at speciation nodes. For ancestors distributed in one single area, this implies range division within the area, so both descendants inherit the entire, single-area ancestral range (Figure 3B). For widespread ancestors distributed in more than one area, speciation can take place via vicariance (allopatric) speciation (one descendant inherits one area and the other the rest of the ancestral range, Fig. 3A) or, unlike DIVA, via peripheral isolate speciation, in which one descendant inherits one area and the other the entire ancestral range. These three range inheritance scenarios are assigned equal likelihood in the model. Like DIVA, the DEC model does not allow widespread ancestral ranges to be inherited identically by the two descendants (considered biologically unrealistic, cf. Ronquist, 1997; Ree et al., 2005).

Ree & Smith (2008) used the DEC method to re-analyze Nepokroeff et al., 2003's dataset. They inferred the relative likelihood of ancestral areas at nodes and estimated the global rate of dispersal and extinction. Interestingly, they found that 4 out of 5 inferred dispersal events were associated with lineage divergence, and that simpler models (small contiguous 2-area ranges) and linear directional dispersal (dispersal to adjacent island following the direction of the Hawaiian chain) fit the data better than more general models with widespread states and flat dispersal rates. Clark et al. (2008) found a similar result in inferring the biogeographic history of the Hawaiian plant genus *Cyrtandra*. They concluded that Lagrange is not appropriate for analyzing island scenarios because of its tendency to infer widespread ancestors, whereas most insular species are endemic, occurring in single islands.

This difficulty of Lagrange in dealing with island lineages is directly related to its underlying biogeographic model (Figure 3):

A) Ancestral range evolutionary models" (Fig. 3A): In both Lagrange and DIVA the ancestral range can be altered by speciation, so that it is not always inherited identically by the two descendants. Widespread ancestral states (AB) are valid states in the model (*Q* matrix), being the direct outcome of dispersal events. They are divided at cladogenetic events by vicariance (DIVA) or alternatively by peripheral isolate speciation (DEC). Change to a new state (dispersal) takes place along the branch leading to the speciation/splitting event. Clark et al. 2008 termed this model as "vicariance-mediated allopatry", because moving from one area to another area requires going through a widespread state. It is more appropriate for continental scenarios in which areas are adjacent (share an edge), and gene flow is expected to be maintained for some time. Dispersal is equivalent to "range expansion" (Buerki et al., 2011).

B) "Character state evolutionary models" (Fig. 3B): Fitch Parsimony, CTMC-based ML inference, and the BIB model (Sanmartin et al., 2008; see below) belong to this type of model. The ancestral state is inherited identically by the two daughters following speciation, and change to a new state takes place along the descendant branch – i.e., equivalent to instantaneous extinction in the original state or area. Widespread ancestral states (AB) are not allowed in the Q matrix. This is equivalent to ignoring periods in which the ancestor is polymorphic. These models are more appropriate for island scenarios, in which as soon as one population colonizes an island it effectively becomes a new species (founder events). Dispersal is here equivalent to "jump dispersal" (Buerki et al., 2011).

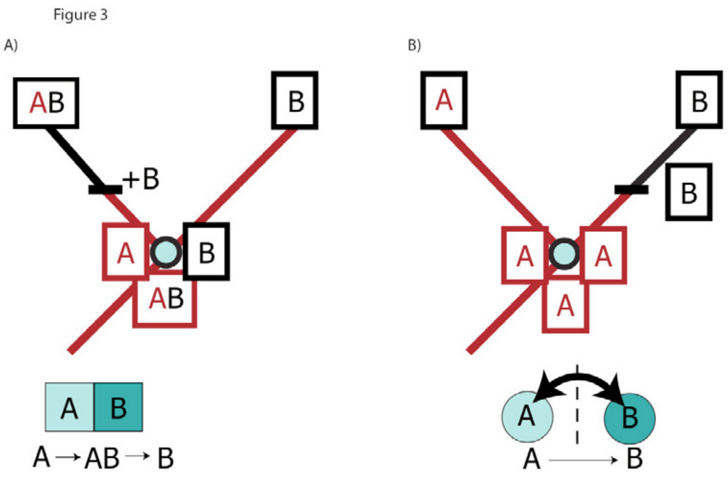


Figure 3. Two different biogeographic models underlying methods of biogeographic inference. A) Ancestral range inference models (e.g., DIVA, DEC): widespread ancestral states (AB) are allowed in the model and divided at cladogenetic events by allopatry; it is appropriate for continental scenarios in which areas are adjacent (share an edge) and gene flow is expected to be maintained; dispersal is equivalent to "range expansion". B) Character state inference models: ancestral state inherited identically by daughters following speciation; widespread ancestral ranges not allowed and change to a new state occurs along the descendant branch; it is more appropriate for island scenarios in which dispersal is equivalent to "jump dispersal".

Bayesian Island Biogeography (the BIB model)

In another pioneering study, Sanmartín et al (2008) extended the CTMC island model to infer general dispersal patterns of colonization across multiple co-distributed clades. They used a static island model in which the size and distance between islands were assumed to be constant through time, and islands were older than the organisms analyzed, i.e., the islands form the backdrop over which lineages move and disperse. Dispersal between islands was modelled as a stochastic CTMC model analogous to models in DNA evolution. There were two parameters in the model: a) the relative carrying capacity or the number of lineages in the island at equilibrium condition, analogous to the stationary state frequencies in a GTR molecular model; b) the relative dispersal rate between islands, analogous to the exchangeability substitution rates in the GTR model.

One advantage of the method is its flexibility. Different island models can be implemented by constraining parameters in the model. For example, one could constrain the rates in the model to be equal as in the F81 model, or make the rates between non-adjacent islands equal to zero as in the GTR "stepping-stone" model. Unlike previous CTMC models, the BIB model can be used to extract general patterns across multiple groups distributed in the same set of islands (macrobiogeography). Differences among groups in their age, molecular evolutionary rate, and dispersal abilities can be accounted for by using group-specific molecular and dispersal rate scalers (Sanmartín et al., 2008).

Some empirical applications

Canary Island biogeography

Sanmartin et al. (2008) applied the BIB model to analyze dispersal patterns in the Canary Islands across 13 plant and animal groups. They found that biotic exchange was primarily between adjacent islands, while exchange with the mainland was much lower than inter-island dispersal and not dependent on distance (Figure 4A).

Recently, we extended the Canary Island study by increasing the size of the phylogenetic dataset. Sanmartin et al.'s dataset was strongly biased towards animals (9 out of 13 groups). In collaboration with Javier Fuertes (Real Jardín Botánico) and Laura Martínez, an undergraduate student, we carried out a new analysis of 17 plant Canarian groups. One aspect we wanted to test was whether the pattern of colonization in Canarian plants fits the model of "inter-island colonization of similar ecological zones", in which island lineages move along the island chain colonizing similar habitats in different islands (Sanmartin et al., 2008). Results show a similar pattern to Sanmartin et al.'s study with the highest rates of dispersal between adjacent islands and the lowest exchange with the mainland. However, dispersal rates were in general higher than in Sanmartin et al. dataset, whereas island carrying capacities were lower.

To test whether these differences were associated to different patterns of colonization in plants versus animals, we reanalyzed Sanmartin et al.'s animal dataset but increasing the sample (23 animal groups; unpublished work by Raquel Martín). Preliminary results show a similar pattern to plants with two main differences: carrying capacities were considerably higher (one order of magnitude) and dispersal rates lower, except for dispersal with the mainland that was higher. If carrying capacities correspond to waiting times between dispersal events – the number and length of branches in the phylogeny that maintain the same state (i.e., area) – one can interpret carrying capacities as equivalent to "in-situ" or within-island diversification. Thus, higher carrying capacities in Canarian animals fit a colonization model in which lineages occasionally disperse between islands, usually following the island chain, but once in the island diversify rapidly, perhaps by colonizing new ecological habitats ("niche evolution"). In contrast, Canarian plants, with lower carrying capacities but higher dispersal rates, fit better a model of frequent inter-island colonization and rare within-island diversification. The latter is congruent with the "inter-island colonization of similar habitats" model (Sanmartín et al., 2008), in which plants follow their ecological niche as they proceed along the island chain (i.e., "niche conservatism"). In other words, plants would prefer to colonize a similar habitat in different islands rather than diversifying within the same island by colonizing new niches.

We have just started exploring other extensions of the BIB model such as using "distance-informed priors" to reflect higher probability of migration between closer (adjacent) islands. This seems to be a more realistic model than the very strict stepping-stone model, as evidenced by preliminary results showing higher model likelihood for the animal dataset. We are also exploring the possibility of modelling punctual (mass) extinction scenarios by using a compound-Poisson process (CPP) in which the stationary frequencies of the model (carrying capacities) are allowed to be different before and after a change-point in time. This "change-point" could be fixed or estimated from the data and could be correlated with geological events. One limitation of the BIB model and other CTMC models is that they assume that carrying capacities are at equilibrium at the offset of the colonization process. This does not seem very realistic in the case of islands recently colonized or when volcanic eruptions have decimated an island biota. It would be interesting to test this model in the Canary Islands because of their complex geological history and recent volcanic activity (e.g., Lanzarote, Sanmartín et al., 2008).

The "Rand Flora" continental pattern

Finally, we have explored the application of the BIB model to a continental setting: to disentangle the evolutionary origins of the Rand Flora, a disjunct continental pattern that evolutionarily relates isolated floras at both sides of the African continent (Sanmartín et al., 2010 , Fig. 4B). We gathered a large dataset of 13 plant groups and 753 species belonging to several angiosperm families (e.g., Campanulaceae, Geraniaceae, Euphorbiaceae, etc) distributed in disjunct subtropical regions along the margins of the African continent, such as Macaronesia, the Horn of Africa, Eastern Africa and South Africa. We estimated historical rates of dispersal and regional carrying capacities using the BIB model. Results show a Macaronesian flora with the lowest carrying capacity but highest rate of biotic exchange with nearby regions (e.g., Mediterranean, Horn of Africa-Southern Arabia), suggesting a flora formed mainly by immigration of new lineages. In contrast, the South African flora shows the highest carrying capacity but lowest rate of biotic exchange with other regions (except for Eastern Africa), suggesting a flora formed by in-situ diversification (Sanmartín et al., 2010 , Fig. 4B). A larger dataset and fossil information will be needed to test for asymmetry in the dispersal patterns and to study variation in rates of dispersal over time, which might be correlated to the geological and climatic history of Africa.

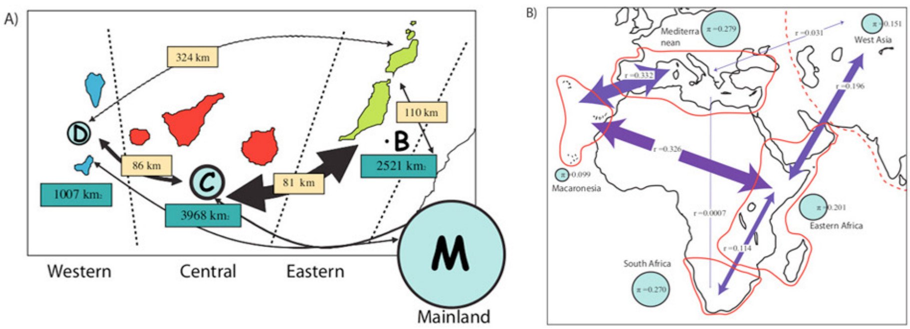


Figure 4. Two examples of the application of the Bayesian Island Biogeography (BIB) model to biogeographic analysis. A) Meta-analysis of 13 Canary Island plant and animal groups showing rates of dispersal between islands (arrows) and island relative carrying capacities (circles); geographic distances between islands and area size are shown for comparison (adapted from Sanmartín et al., 2008). B) A BIB analysis of the Rand Flora - a continental pattern that relates floras at both sides of Africa - suggests high levels of biotic exchange for the Macaronesian flora, but a South African flora formed mainly by in-situ diversification (adapted from Sanmartin et al., 2010).

Acknowledgments

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A comparison of bryophyte diversity in the Macaronesian Islands. Island versus habitat approach

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Introduction

Macaronesia has been recognized as a biogeographic region comprising the Azores, Madeira, Canary Islands (Engler, 1978) and Cape Verde Islands (e.g. Dansereau, 1961), from more than a century (Whittaker & Fernández Palacios, 2007). Despite the fact that there is considerable variation in abiotic conditions across these archipelagos, the Macaronesian concept has been widely accepted (e.g. Médail and Quézel, 1997), although some authors reject the wide concept of Macaronesia, excluding the Cape Verde islands for both, vascular plants (eg. Lobin 1982) and cryptogams (Vanderpoorten *et al.*, 2007). In addition, the Macaronesian concept may be different depending on the group of organisms. For instance, the relictualism theory of Macaronesia shows more consistency for ferns and liverworts than for mosses (Vanderpoorten *et al.*, 2007).

Nicolás *et al.* (1989) presented a study of relationships of the vascular floras within the Macaronesian region and highlighted the similarities between the Canarian and Madeiran floras and the distinctiveness of both the Azorean and Cape Verdean floras. These authors showed a close correlation between variation in floristic similarity and latitude, altitude and distance to the mainland. González-Mancebo *et al.* (2008a), shows that mosses respond to major environmental factors in a more complex way than liverworts. Sim-Sim *et al.* (2010a) by means of a cluster analysis revealed the affinities in the bryoflora composition of the driest islands of Madeira, namely Selvagens, Desertas and Porto Santo, and found a close proximity between Fuerteventura and Lanzarote in Canary Islands and the Selvagens bryoflora. The different patterns of richness and diversity for both liverworts and mosses of Madeira were due to the habitats' variability and environmental conditions of the different islands of Madeira Archipelago. Vanderpoorten *et al.* (2007), using parsimony and Bayesian analyses of floristic data sets, revealed for mosses the Canary Islands as sister to North Africa, while both liverwort and pteridophyte analyses suggested an Azorean-Madeiran-Canarian clade (hereafter Macaronesia s.s.). According to these authors, dynamic interchange of taxa with neighboring continental areas rather than relictualism best explains the relationships of the Cape Verde cryptogamic flora and the Canary Island moss flora.

Factors influencing these differences have also been included in studies on richness analyses; in fact, most factors that rule/condition inter-island similarity (area, altitude, age, geographical isolation) also explain richness pattern on islands (Rosenzweig, 1995; Whittaker & Fernández Palacios, 2007). In this context, biodiversity in the Macaronesian islands has been mostly related to age, area, isolation and habitat diversity (or its surrogate, maximum altitude) (Nicolás et al, 1989; Pereira *et al.*, 2007; Borges & Hortal, 2009). For bryophytes, González-Mancebo *et al.* (2008a) pointed out that altitude and island age are the most important factors influencing bryophyte richness in the Canary Islands.

Habitat diversity is a key factor explaining species richness (e.g. Kreft *et al.*, 2008) and similarity between islands (Nicolás *et al.*, 2008; González-Mancebo *et al.*, 2008; Borges *et al.*, 2011). Scientists have identified, beside the factor time, two major mechanisms of species accumulation (immigration and speciation) on islands, the increase of area and of habitat diversity, usually due to increase in altitude (Losos & Schluter 2000, Triantis *et al.*, 2003). A simple mathematical model that unifies these two dominant explanatory variables was used by Triantis *et al.* (2003). However, few authors have developed more direct approaches to address the habitat effect on diversity (Buckley 1982, 1985; Deshaye & Morriset, 1988; Domínguez *et al.*, 2010; Zobel *et al.*, 2011). In this context, habitats from individual islands have rarely been compared between islands, within or between biogeographic regions.

In this paper, we present a comparison of richness patterns and floristic similarity for bryophytes in the five most important altitudinal habitat types in the Macaronesian islands. We evaluate the importance of different factors discussed in the literature in predicting species diversity applying the traditional island approach and within the framework of the new habitat approach, including area, isolation, climatic factors, geological age and human influence. From the analysis of patterns of bryophyte species distribution for selected habitats across islands and archipelagos, we specifically test the hypothesis that (i) floristic similarity is primarily determined by climatic factors, but not by geographical distance due to high dispersal ability in this species group and (ii) bryophyte richness is best predicted by area, but not by geological age of the habitat due to very low endemicity or speciation rate and high colonization rate.

Methods

Data collection

Information on species distribution and frequency on each archipelago was recorded from a complete literature survey for the Macaronesian islands (Macaronesia s.s.) as well as herbarium data from AZU (Azores), LISU, S, MADS, MADJ and MADM and TFC-Bry.

Habitat data

The three Macaronesian archipelagos considered are very different with respect to climatic conditions with bioclimatic belts varying from a temperate hyperoceanic (Itc 470-120) and mediterranean oceanic (Itc 480-420) in the Azores; from temperate hyperoceanic (410-170) to mediterranean xeric oceanic (510-430) and mediterranean pluvi-seasonal oceanic (520-350) in Madeira and from mediterranean pluvi-seasonal oceanic (550-120 to mediterranean xeric oceanic (560-400) and Mediterranean desertic oceanic (570-490) in the Canaries (Rivas-Martínez, 2009). We assigned bryophyte species to major and minor habitat types (caves, human disturbed, water habitats, recent lava flows) that latter were grouped in different bioclimatic belts following altitudinal gradients: lowlands, mesic areas, humid and hyper-humid mountain belts, and high mountain belt. Major habitat types were distinguished following Dias (1996) for the Azores, Mesquita *et al.* (in preparation) and Capelo *et al.* (2004, 2007) for Madeira and Del Arco *et al.* (2010) for the Canary Islands. Disturbed areas at each habitat were generally not separated. However, open areas in the humid or hyperhumid mountain belt (where laurel forests were destroyed or natural open areas) were specifically distinguished because they represent a very different type of habitat for bryophytes with a very distinct floristic composition, at least in the Canary Islands (González-Mancebo *et al.*, 2008b). Additionally, in the Canary Islands, pine forest was distinguished from those areas situated above the timberline, in the Supramediterranean and Oromediterranean belts.

Variables

Geological, geographical and habitat variables (see table 1) were compiled from Forjaz (2004) DROTRH (2008) and Del Arco et al (2006b), using digital maps. Climatic data, such as precipitation and temperature, were largely obtained from Azevedo (2003) and Forjaz (2004) for the Azores, PRAM (2002) for Madeira and Del Arco et al (1999, 2002; 2006a, 2009; Reyes-Betancort (2001) for the Canaries. In Madeira, climatic data were originally gathered from the National Institute of Meteorology for the period between 1961 – 1990. For Desertas, the data was obtained from "WORLDCLIM" (www.worldclim.com). The population density data was calculated from Forjaz (2004) for the Azores, from Instituto Canario de Estadística (ISTAC), censos 2001 for the Canaries and "Instituto Nacional de Estatística. Direcção Regional de Estatística da Madeira - Censos 2001 for Madeira.

Habitats	**LOWLAND**			**MESIC**			**LAUREL FOREST**			**PINE FOREST**	**HIGH MOUNTAIN**		
Archipelagos	Azores	Madeira	Canaries	Azores	Madeira	Canaries	Azores	Madeira	Canaries	Canaries	Azores	Madeira	Canaries
Potential area (km2)	*16,35*	*72,35*	*592,87*	*131,71*	*85,30*	*138,13*	*79,90*	*192,35*	*144,45*	*288,79*	*35,00*	*96,90*	*91,64*
Current veg. area (km2)	*9,32*	*12,72*	*127,16*	*0,00*	*26,63*	*23,08*	*6,38*	*77,50*	*52,46*	*159,43*	*35,00*	*48,35*	*91,31*
%Veg Conserv	*67,28*	*26,54*	*29,73*	*0,00*	*26,98*	*18,97*	*6,79*	*20,20*	*34,41*	*56,19*	*100,00*	*49,94*	*90,00*

Habitats	LOWLAND			MESIC			LAUREL FOREST			PINE FOREST	HIGH MOUNTAIN		
Archipelagos	Azores	Madeira	Canaries	Azores	Madeira	Canaries	Azores	Madeira	Canaries	Canaries	Azores	Madeira	Canaries
Min Altitude (m)	*0,00*	*0,00*	*0,00*	*8,89*	*233,33*	*228,57*	*371,89*	*350,00*	*491,67*	*825,00*	*1200,00*	*1450,00*	*1916,67*
Max Altitude (m)	*350,00*	*333,33*	*421,43*	*717,22*	*560,00*	*788,71*	*851,67*	*1010,00*	*1371,50*	*1862,50*	*2351,00*	*1861,00*	*2683,00*
Mean Altitude (m)	*175,00*	*166,67*	*210,71*	*367,50*	*513,33*	*622,93*	*797,72*	*855,00*	*1177,42*	*1756,25*	*2275,50*	*2380,50*	*3258,17*
Nºhabitants/ km2	*1009,98*	*407,74*	*340,68*	*23,99*	*74,05*	*363,61*	*0,00*	*32,19*	*277,86*	*73,74*	*0,00*	*0,00*	*0,00*
Min, Age (m.y.)	*0,56*	*5,10*	*0,44*	*0,59*	*5,27*	*0,44*	*1,16*	*8,25*	*4,40*	*0,78*	*0,24*	*1,50*	*1,30*
Max, Age (m.y.)	*2,52*	*7,83*	*13,11*	*2,15*	*7,67*	*13,11*	*1,20*	*9,75*	*9,72*	*7,20*	*0,30*	*3,00*	*3,10*
Mean Temp. (°C)	*18,69*	*18,50*	*20,29*	*16,05*	*17,67*	*17,43*	*13,86*	*14,75*	*15,17*	*12,70*	*8,50*	*9,50*	*8,87*
Annual Prec. (mm)	*1376,06*	*516,67*	*350,00*	*1939,56*	*626,67*	*488,57*	*2893,42*	*1195,00*	*850,00*	*825,00*	*5100,00*	*2500,00*	*530,00*
Dist,Mainland (km)	*1853,33*	*662,67*	*268,57*	*1853,33*	*662,67*	*268,57*	*1853,33*	*674,00*	*291,33*	*326,00*	*1860,00*	*668,00*	*305,33*
Dist, nearest habitat (km)	*34,89*	*46,00*	*36,41*	*34,89*	*46,00*	*36,41*	*67,00*	*243,50*	*77,50*	*92,00*	*1241,00*	*430,00*	*122,33*

Table 1. Relation of the mean values among islands of each archipelago for the most important variables considered at each habitat.

Data analysis

Floristic similarity was analysed using multivariate statistical techniques. We applied Detrended Correspondence Analysis (DCA, Hill & Gauch 1980) to evaluate floristic similarities within and across archipelagos, islands and habitat types. To explore correlations between floristic gradients and environmental variables, we applied Canonical Correspondence Analysis (CCA) as implemented by CANOCO (ter Braak & Smilauer, 1998). TWINSPAN (Hill 1979) was selected as a hierarchical classification method.

Total bryophyte richness, hepatic and moss richness was modelled applying generalized linear models (GLM; McCullagh and Nelder 1989; Dobson 1990). AIC (Akaike information criteria) with forward stepwise selection was used to obtain the optimal set of predictor variables, choosing the lowest AIC value for every possible combination of explanatory variables, starting with the explanatory variable with the best fit. The fit of the model was tested by the log-likelihood statistics. Distributions of residuals as well as potential problems of over-dispersion were analysed.

Before running GLMs, we applied a Principal Component Analysis (PCA) to explore relationships among explanatory variables. In order to avoid multicollinearity and eliminate redundant information, we used the first two components of the PCA constructed from the island characteristics matrix directly as explanatory variables in the GLM. In case of the habitat approach, we selected the best explanatory variables to enter in the GLM analysis, by eliminating those variables that were highly correlated with each other and the first two PCA components. The following variables were retained: mean annual precipitation, mean annual temperature, habitat area, mean geological age, distance to nearest habitat and population density. Variables were log transformed if distribution across islands was skewed. GLMs can also detect non-linear relationships, if quadratic terms are included (Zuur *et al.* 2007), therefore we included quadratic terms of mean annual temperature and geological age in all models, since we expected possible non-linear relationships for these variables.

Results

General aspects

In all three northern Macaronesian archipelagos we found a total of 725 species (501 mosses and 224 liverworts (including hornworts)). The bryophyte species diversity of the three archipelagos was very similar with 451 (Azores), 461 (Madeira) and 485 species (Canary Islands). The same holds true for the two groups of hepatics and mosses, although there seems to be a slight tendency of increase of hepatics (161, 158 and 147, respectively) and decrease of mosses (290, 303 and 338, respectively) with latitude, corresponding to a precipitation gradient. Species number per island and habitat is given in Table 2. Comparisons of habitat richness between archipelagos (table 2) showed a similar altitudinal pattern of richness for the Azores and the Canary Islands. Richness is increasing from lowland areas to open areas and laurel forest in the most humid mountain belt on each archipelago. However, lower levels of richness are recorded for the laurel forest compared to open areas in the Canaries. In the Azores an opposite pattern occurs in this bioclimatic belt. The laurel forest of Madeira shows the highest species richness; whereas the open areas have the lowest species number. Also, the mesic habitats of this archipelago revealed a higher richness for both liverworts and mosses.

	Corvo	Flores	Faial	Pico	Graciosa	Sao jorge	Terceira	Sao Miguel	Santa María
T	*168*	*259*	*254*	*266*	*123*	*236*	*348*	*333*	*199*
M	*102*	*154*	*152*	*149*	*76*	*132*	*204*	*215*	*129*
L	*66*	*105*	*102*	*117*	*47*	*104*	*144*	*118*	*70*
	AZORES								

	Lowland	*Mesic*	*Open*	*Laurel forest*	*High mountain*
T	*139*	*219*	*238*	*256*	*143*
M	*93*	*137*	*132*	*141*	*80*
L	*46*	*82*	*106*	*115*	*63*

	Madeira	Porto Santo	Desertas	Hierro	Palma	Gomera	Tenerife	Gran Canaria	Fuertevent.	Lanzarote
T	523	128	95	194	345	292	434	305	131	113
M	343	57	60	135	245	193	300	223	93	88
L	180	71	35	59	100	99	134	82	38	25

	MADEIRA					CANARIES					
	Lowland	*Mesic*	*Open*	*Laurel forest*	*High mount.*	*Lowland*	*Mesic*	*Open*	*Laurel forest*	*Pine forest*	*High mountain*
T	124	231	182	301	56	113	173	249	205	180	140
M	90	163	118	165	54	78	117	182	117	146	126
L	90	163	118	165	54	35	56	67	88	34	14

Table 2. Species number by island and type of habitat distinguished at each archipelago. T (total species number), M (mosses), L (liverworths). Open (areas included in the potential area of the laurel forest, including both, natural areas (rock communities) and disturbed areas where the laurel forest was destroyed).

Most of the species are able to occupy more than one type of habitat in Macaronesia, since only 29% were exclusive for one habitat type, half of them belonging to the laurel forest, the rest of them divided equally into the other four habitat types. Out of these habitat specialists, only seven species were found in all three archipelagos. One hundred and twenty species were observed in two habitat types and 73 species were real generalists appearing in all five habitat types and mostly in all three archipelagos.The proportion of species exclusive to the laurel forest was lowest for the Azores (29%), followed by the Canaries (67%) and Madeira islands (89%).

Floristic similarity (see figures 1, 2 and 3, TWINSPAN and CCA analyses for island-unit and habitat-unit models are not shown in this summary).

The macroecological analyses presented here reveal the particularities of the Macaronesian archipelagos and main relationship among them. When islands data were used with ordination analyses (CA, CCA) and cluster analysis, Azores and Canary islands were clearly differentiated; while drier islands in Madeira archipelago showed a close relationship with drier Canary Islands such as Lanzarote and Fuerteventura. However, island data represent an extremely oversimplification of the relationship between islands, which leads to a strong affect of the geographical position of the island. When islands and habitats were considered the inter-archipelago distances were reduced. In fact, floristic distances between habitats of the same island can be higher than distances between the same habitat types of different archipelagos. The habitat-unit model allows us to see how geographical distances are less important than environmental conditions for wide-dispersal groups like bryophytes.

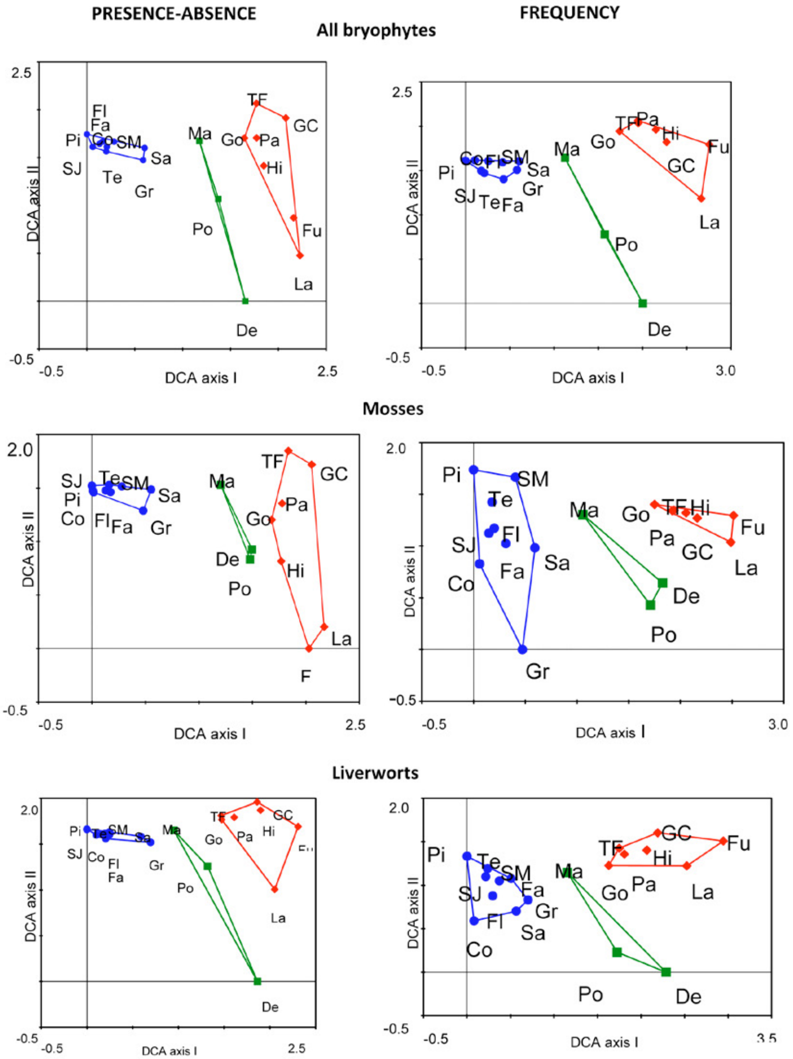

Figure 1. Detrended Correspondence Analysis with presence-absence data (Left) and Frequency (right) of all islands and total bryophyte species (above), mosses (middle) and liverworts (down). For presence-absence data Total species (eigenvalues axis 1,2: 0.363, 0.095, total inertia: 1.563; cumulative percentage variance for the first two axes 29,3), mosses (eigenvalues: 0.334, 0.080, total inertia:1.263; cumulative percentage variance for the first two axes 28,3) and liverworts (eigenvalues axis 1,2: 0.334, 0.080, total inertia: 1.263; cumulative percentage variance for the first two axes 32.8). For frequency data Total species (eigenvalues axis 1,2: 0.435, 0.100, total inertia: 1.763; cumulative percentage variance for the first two axes 30,3), mosses (eigenvalues: 0.396, 0.109, total inertia:1.537; cumulative percentage variance for the first two axes 32,9) and liverworts (eigenvalues axis 1,2: 0.440, 0.103, total inertia: 1.816; cumulative percentage variance for the first two axes 29,9).

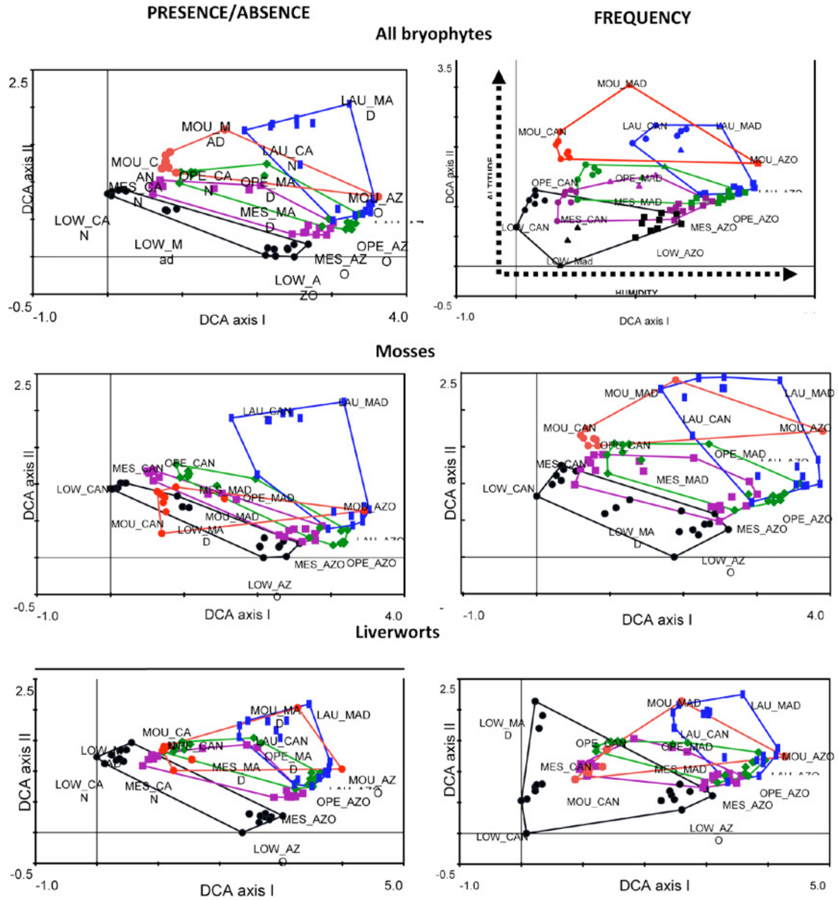

Figure 2. Detrended Correspondence Analysis with presence-absence data (Left) and Frequency (right)

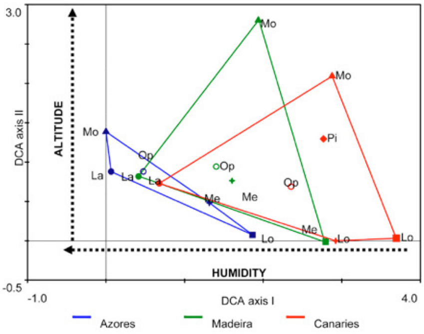

Figure 3. Detrended Correspondence Analysis with frequency data of open habitats and laurel forest habitats of humid and hyper-humid bioclimatic belts. For all bryophytes (eigenvalues axis 1,2: 0.566, 0.280, total inertia: 2.674; cumulative percentage variance for the first two axes 31.6).

Two types of inter-island similarity relations may be observed between all these northern Macaronesian islands. The first one is related to precipitation, which in these islands seems to mostly reflect inter-island similarity of the laurel forest ecosystem. The second one is related with the temperature gradient and the capacity of bryophytes to persist in microhabitats where a suitable microenvironment persists, demonstrating a much higher altitudinal and latitudinal distribution gradient for most of the species.

Richness (see table 3)

The overwhelming majority of studies on species diversity pattern in island biogeography applied the classical island approach, i.e. used the whole island as the sample unit and identified factors like area, isolation, geological age and habitat diversity (or its surrogate, maximum altitude) as best predictors for richness in different species groups (Rosenzweig 1995, Losos & Schluter 2000, Whittaker & Fernández Palacios, 2007). This has the advantage that many islands and even archipelagos can simultaneously be compared since environmental data for whole islands are easily available. But this approach has, at least when applied to many oceanic archipelagos, the disadvantage that some ecological factors are strongly correlated at the island level. When analyzing bryophyte richness in Macaronesia with the classical island approach we are confronted with the same problem. In contrast, when applying the habitat-unit-model to the bryophyte diversity of the northern Atlantic islands, we separate altitudinal habitat types, i.e. we split the altitude gradient and disentangle, at least partially, habitat diversity from island area. Including all fragments of all habitat types of each island and archipelago in the statistical model, we were able to identify habitat area and climatic variables (mean annual precipitation and temperature) as very good predictors of total bryophyte richness.

Model	Variable	Estimate	Wald-stat.	P	AIC	P	Dev. expl. (%)
ALL HAB	**Precipitation (log)**	**0,744**	**166,9**	**< 0.0001**	*798,4*	*< 0.0001*	*70,2*
	Temperature	*0,647*	*144,1*	*< 0.0001*			
	*Temp * Temp*	*-0,022*	*143,5*	*< 0.0001*			
	Habitat Area (log)	*0,195*	*73,3*	*< 0.0001*			
LOW	**Precipitation (log)**	**0,896**	**15,5**	**< 0.0001**	*42,6*	*< 0.0001*	*64,5*
	Habitat Area (log)	*0,239*	*7,0*	*0,008*			
MESIC	**Precipitation (log)**	**0,565**	**33,1**	**< 0.0001**	*149,5*	*< 0.0001*	*64,6*
	Habitat Area (log)	*0,242*	*14,5*	*0,000*			
OPEN	**Precipitation (log)**	**0,805**	**55,4**	**< 0.0001**	*127,5*	*< 0.0001*	*83,6*
	Habitat Area (log)	*0,089*	*4,2*	*0,040*			
LAUREL	**Precipitation (log)**	**0,815**	**62,1**	**< 0.0001**	*152,0*	*< 0.0001*	*70,1*
	Habitat Area (log)	*0,220*	*28,4*	*< 0.0001*			
MOUNT	**Habitat Distance (log)**	**0,992**	**40,5**	**< 0.0001**	*38,6*	*< 0.0001*	*66,0*
	Habitat Area (log)	*0,424*	*12,9*	*< 0.0001*			

Model	Variable	Estimate	Wald-stat.	P	AIC	P	Dev. expl. (%)
ALL HAB	**Habitat Area (log)**	**0,304**	**313,6061**	**< 0.0001**	*887,1*	*< 0.0001*	*62,50*
	Temperature	*0,207*	*50,7411*	*< 0.0001*			
	*Temp * Temp*	*-0,009*	*80,9688*	*< 0.0001*			
LOW	**Habitat Area (log)**	**0,315**	**24,04500**	**< 0.0001**	*149,0*	*< 0.0001*	*45,3*
	Precipitation (log)	*0,703*	*20,57964*	*< 0.0001*			
MESIC	**Habitat Area (log)**	**0,465**	**60,27610**	**< 0.0001**	*14,4*	*< 0.0001*	*70,9*
	Temperature	*-2,005*	*8,55560*	*0,003*			
	*Temp * Temp*	*0,059*	*8,55140*	*0,003*			
OPEN	**Habitat Area (log)**	**0,405**	**145,2425**	**< 0.0001**	*-4,3*	*< 0.0001*	*81,6*
	Precipitation (log)	*-0,345*	*16,4894*	*< 0.0001*			
LAUREL	**Habitat Area (log)**	**0,489**	**167,930**	**< 0.0001**	*149,5*	*< 0.0001*	*82,7*
	Population density (log)	*-0,164*	*39,784*	*< 0.0001*			
MOUNT	**Habitat Area (log)**	**0,241**	**16,133**	**< 0.0001**	*66,9*	*< 0.0001*	*70,3*

Table 3. Results of generalized linear models (GLMs), showing the best set of explanatory variables explaining richness of hepatics (Upper) and mosses (down) as response variable, using AIC best set selection and Poisson distribution with log-link function. Temperature and geological age were introduced both as a linear and as quadratic terms. (Dev. expl. = deviance explained by the model).

If we consider a step further and analyze habitat types separately and, in consequence, reduce the variation of climatic variables in the whole data set, then habitat area is by far the best predictor for overall bryophyte richness, followed by precipitation or temperature. When separating liverworts from mosses, we obtained further new insights in the richness pattern, since liverworts, as we mentioned before, are much more sensible to variation in mean annual precipitation than mosses, and this could be detected in all habitat types. That means that the latitudinal precipitation gradient affects especially liverworts and that the differences between the more arid Canary Islands and Madeira are more important for liverworts richness than the differences between Madeira and the Azores, in spite of the longer geographical distance.

Human disturbance, despite to high degree of landscape transformation on these archipelagos, shows only a weak negative effect on bryophyte richness within the laurel forest ecosystem (see table 3 mosses). Therefore, this habitat seems to be the most sensitive to destruction and fragmentation.

Habitat age, ie. historical factors, were not important (table not shown) in explaining richness pattern, which can be again attributed to the high dispersal abilities and high colonization rates of this species group.

Conclusions

Inter-islands similarity and richness pattern are better described with the habitat-unit-model than by the islands-unit model. The habitat approach allows us to see how the floristic distances vary between islands depending on the type of habitat, and the factors that influence both species distribution and richness.

Using frequency instead presence/absence data represents a great improvement for data interpretation in the habitat-unit-model; while the island-unit-model only offers an adequate approach when the compared islands are more homogeneous, as occurs in the Azores. Azores show the highest similarities of bryophyte compositions among different habitats. This is notable when disturbed (open) and preserved laurel forest areas were compared, which means that, especially for liverworts in the laurel forests, human disturbance has a stronger impact on species composition for drier regions, such as on the Canary Islands where precipitation conditions within this type of forest are limiting for bryophyte richness. The highest diversity and richness was found in the preserved laurel forest area of Madeira, where the distinct tree species strongly shape the liverworts and mosses composition and distribution (Sim-Sim *et al.* 2011).

The bryophyte flora of Macaronesia is heterogeneous when whole islands are considered. The habitats approach shows that this heterogeneity might be primarily interpreted as the differences between oceanic and Mediterranean habitats. Oceanic habitats, mostly represented by laurel forests, are the most similar and probably also the most differentiated from the surrounding mainland areas. In consequence, they are the best to define the Macaronesian concept for the phylogenetic groups of mosses and liverworts. Drier habitats, here represented by the Mediterranean bioclimatic belts, show higher ecological and floristic dissimilarities between islands and archipelagos, which presumably might be also related to a more effective species interchange with the surrounding mainland areas. Some functional groups of mosses are more tolerant to aridity and consequently show closer relationship with mainland areas at the same latitudinal level. The island-unit-model does not allow us to distinguish these differences in similarity and gives us an overestimation of the differences between mosses and liverworts distribution within Macaronesia.

In short, applying the habitat-unit-model we reduce environmental, mostly climatic variation in the data set by splitting the altitudinal gradient and analyzing habitat types separately, which enables us to evaluate and interpret better bryophyte richness and similarity pattern. As already was confirmed in other studies, the species-area relationship holds also true at the habitat level but depending on species groups. We can confirm this habitat area effect here for the first time for an entire biogeographical region, despite the considerable variation in environmental factors across these three Atlantic archipelagos. Nevertheless, we argue that we cannot detect here the area *per se* effect since there is still a lot of environmental variation within habitat types at the island or archipelago level. There is even a certain environmental variation within a specific habitat type on each single island and this variation will increase with habitat area.

The use of bryophytes, a group with high dispersal capacity and a high representation of both restricted and widely distributed species, at both altitudinal and latitudinal levels, is confirmed in this work as a very adequate approach to analyze and infer phytogeographic affinities.

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Macaronesia: a refugium and obligate custom office for Atlantic bryophyte species in transit from and towards Europe

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Island biogeography has historically been and remains an area of research of prime importance for the advance of biology. It is in an island setting that Wallace (1870) and Darwin (1859) have proposed their theory of evolution by natural selection. It is also in this context that McArthur & Wilson (1967) described a suite of mechanisms ruling the assembling of biological communities. In particular, they attempted to describe biodiversity patterns in terms of isolation from source populations and area size, which determine rates of immigrations and extinctions. Oceanic islands hence appear as a natural laboratory and Wallace (1902) already suggested that the understanding of evolutionary processes within the island context is the key to the understanding of the same mechanisms in the more complex continental setting.

Owing to geographic isolation and important volcanic activity, which promotes local speciation through adaptive radiations, insular organisms traditionally display high endemism rates coupled with a suite of specific ecological, biological and phenotypic specificities termed as island syndromes (Whittaker & Fernandez Palacios 2007). In particular, the reduced immigration rate associated to the oceanic barrier involves that the founding population is small and comprised of a single or a few individuals. Those individuals bear a small fraction of the global genetic variation of the species across its distribution range. The small size of the founding population is likely to rapidly lead to the fixation of alleles due to genetic drift. As a consequence, phenotypic variation within island species or populations is traditionally lower than that displayed on continents. One of the most obvious effects of this process is the strikingly different size exhibited by island populations and/or species as compared to continental ones, either in the direction of nanism or gigantism.

Island species and populations further typically lose their dispersal power. Since the size of the founding population is small, the loss of individuals through migration represents a high cost that is likely to impede the chance of survival of the population. Hence, dispersal ability is counter-selected in the island setting. In the annual daisy *Lactuca muralis* for example, a significant and substantial loss of seed dispersability owing to a reduction of the volume of the pappus and an increase in the seed weight was demonstrated after only 10 generations (Cody & Overton 1996).

As a result, islands have traditionally been considered as the 'end of the colonization road'. The idea, that islands are a dead-end for colonizers has been, however, recently questioned (Bellemain & Ricklefs 2008). In the Canary Islands, a scenario of continental back-colonisation giving raise to a new endemic continental species from a Canarian endemic species has been demonstrated in the genus *Convolvulus* (Carine et al. 2004). The hypothesis that islands may actually serve as sources, perhaps even refugia, for continental back-colonisation, has been increasingly accepted. In the heather *E. scoparia* s.l. for example, chloroplast DNA diversity peaks in the Azorean archipelago and decreases on the continent, in sharp contrast to McArthur & Wilson's equilibrium model, derived theoretical population genetic expectations (Barrett 1996), and previous studies on island/continent patterns of genetic diversity (Franks 2010, but see Algar & Losos 2011). In *E. scoparia*, the effects of the bottleneck associated to the founding event might have been erased by several colonization events from different continental sources, active in-situ diversification through cross-fertilization, and allopatric diversification. Evidence for continental back-colonisation, giving rise to new allelic combinations on the continent, and for a recent colonization of Madeira from the Azores, reinforces the idea that islands might play a key role in dynamic interchanges with continents and other archipelagos (Désamoré et al., unpublished results).

This is especially true for organisms that are assumed to display a high dispersal power, and in particular, spore-producing plants. Bryophytes, a paraphyletic assemblage of about 25,000 species including the most diverse phylum of land plants after the angiosperms, are precisely the land plants that display the widest range of cell abscission mechanisms that are at the basis of vegetative propagation (Duckett & Ligrone 1992). Evidence from modelling of species distributions in relation with wind connectivity, molecular dating and reconstruction of migration events, and experiments on spore viability in high altitude air currents, all suggest that the disjunct distributions typically exhibited by bryophyte species result from recent long-distance dispersal (see Vanderpoorten et al. 2010 for review). At the landscape scale, observations on the colonization of artificial substrates unambiguously show that colonization rates can be extremely high in bryophytes (Hutsemékers et al. 2008). Those considerations suggest that the high dispersability of bryophytes could erode any island syndrome in the group.

Studies in the moss *Platyhypnidium riparioides* (Hutsemékers et al., unpublished results) show that island populations exhibit a significantly lower genetic diversity than continental ones, confirming predictions from population genetics theory (Barrett 1996). As opposed to these predictions, however, coalescence analyses failed to demonstrate any signature of bottleneck from patterns of nuclear microsatellite variation in Macaronesian populations. The reduced levels of genetic diversity observed in the island setting might, hence, have two origins. First, variation in water physico-chemistry, which was identified as one of the main drivers of genetic diversification in *P. riparioides* (Hutsemékers et al. 2010), is comparatively lower in the island than in the continental setting. Second, the low genotypic diversity found for island populations and the significantly higher linkage disequilibrium between loci in the island than in the continental setting point to a shift in mating system towards increased clonality. Several studies reported similar shifts in mating system from continental dioecious or allogamous ancestors towards insular self-compatible populations to ensure a rapid population growth following the founding event (e.g., Comes et al. 2008).

Three lines of evidence indicate that any signature of bottleneck in extant patterns of genetic variation in island populations of *P. riparioides* has been erased by several immigration events and/or subsequent gene flow. First, the mixing of continental and island populations and the scattered position of populations from the different archipelagos in the ordination diagram of a principal component analysis of genotypic variation point to weak geographic barriers and recurrent migration events. This hypothesis is reinforced by phylogenetic analyses of cpDNA and nrDNA sequence data, wherein constraining all Macaronesian, Azorean, and Madeiran accessions to monophyly results in a significant decrease in log marginal likelihood. Third, patterns of isolation by distance are not significantly different among islands and on the continent, as revealed by the complete overlap in the regression slope estimates of the Mantel tests. Altogether, these observations reinforce the notion that the sea is not a major impediment for migration in mosses, for which oceanic islands may function as 'mainland' (Grundmann et al. 2007). In sharp contrast to the traditional view of islands as the 'end of the colonization road' (Bellemain & Ricklefs 2008) and the widely accepted notion that island populations quickly lose their dispersal power (see Whittaker & Fernandez Palacios 2007 for review), coalescence analyses in *P. riparioides* further unambiguously show that the high dispersal ability of the species has not decreased in the island setting. In fact, constraining the immigration and emigration rates on islands in the model did not result in a significant decrease in log-likelihood. Although the difference is not significant, the posterior probability distribution of the island to continent rate even peaks at a higher value than the continent to island one.

These analyses tend to indicate that the loss of dispersal power syndrome does not apply to mosses and, perhaps more globally, spore-producing plants. As a direct consequence, patterns of endemism in the Macaronesian bryophyte flora differ from those exhibited in angiosperms in that endemic species tend to be widespread, both within and among archipelagos. Indeed, whilst single-island endemics compose the bulk of the endemic element within angiosperms (e.g. >90% in the Canary Islands), they represent only 37.5% of the endemic liverworts across Macaronesia (Vanderpoorten et al. 2011).

The maintenance of a strong dispersal ability in island bryophytes has several important implications. First, such a strong genetic connectivity with the continent hampers genetic isolation and in fact, Barton & Mallet (1996) demonstrated that even marginal gene flow between populations can strongly delay the accumulation of genetic differences by drift, and hence, allopatric diversification. This might explain why much less private alleles were found on islands than on the continent in *P. riparioides* and, more globally, the very low rate of endemism displayed by bryophytes (see Vanderpoorten et al. 2010 for review). For example, the highest rates of species-level endemism within Macaronesia is found in the Madeiran liverwort flora, with 6% of endemism, which pales as compared to the >40% of endemism rates in the angiosperm flora of the Canary Islands and the Azores. High rates of continental back-colonisation also explain why most endemics are neoendemics of recent evolutionary origin, e.g., in *Rhynchostegiella* (Aigoin et al. 2009), *Homalothecium* (Huttunen et al. 2008), and *Leptoscyphus* (Vanderpoorten & Long 2006).

A second implication of the maintenance of a high dispersal power on islands and, in particular, of high rates of continental back-colonisation, is that islands, which experienced a buffered climate during the glaciations, may actually serve as refugia. The idea, that islands are not only sinks of biodiversity, but also sources for continental back-colonisations, has already been evoked in a few instances (Heaney 2007, Nicholson et al. 2005, Caujapé Castell 2011, Laenen et al. 2011) and was further investigated by demographical analyses in *P. riparioides*. Those analyses failed to demonstrate any significant bottleneck in island populations, whereas a severe bottleneck was detected for continental populations from the western Mediterranean, about 43,000 years ago, corresponding to the late glacial maximum in Europe, even in those areas of the Iberian Peninsula that have traditionally been identified as refugia.

Finally, ongoing research also suggests that Macaronesia is a stepping stone for tropical species in transit towards Europe and might play a key role in explaining the origin of the unique cryptogamic flora of the Atlantic fringe of Europe. Phylogeographic inference in the liverwort *Leptoscyphus cuneifolius* indeed indicates that the species originated in the Neotropics, colonized the Azores and Madeira and, from there, the westernmost seabord of the UK. Similarly, the moss *Ptychomitrium nigrescens*, a species of presumably sub-Saharan African origin, diversified in the Cape Verde and across Macaronesia before eventually reaching southern Portugal and southern France.

These findings are of tremendous relevance for conservation. Whilst conservation efforts have traditionally focused on the endemic element indeed, the results presented here show that the non-endemic element is of prime importance for the evolution of continental biodiversity in the cryptogamic flora, and hence, should be taken into account in conservation planning

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2

Genetic diversity and ecology in the Canaries

Sexual systems in canarian flora. Evolutionary pathways to gender dimorphism and dioecy

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Introduction

The spectacular diversification of Angiosperms from Cretaceous middle stills remains one of the enigmas that DARWIN named "abominable mystery" to symbolize just about all aspects of the origin and early evolution, big radiation and expansion of flowering plants (Friedman, 2009). The spectacular floral diversity is associated with an impressive variety of pollen vectors, mating strategies and sexual systems (see Figure 1) that directly influence to reproductive success with production of offspring of high genetic quality. The floral diversity largely interpreted as the historical environments interactions and natural selection that result in floral adaptations or transitions (Darwin, 1877; Richards 1997; Barrett, 2010). Flowers transitions affect different types of reproduction having profound ecological and evolutionary consequences influencing genetic diversity within natural populations, phenotypic evolution and patterns of diversification (microevolutives processes). Flowers transitions take on added significance when they are maintained through multiple speciation events and become characteristic features of lineages in supra-specific levels (macroevolutives processes).

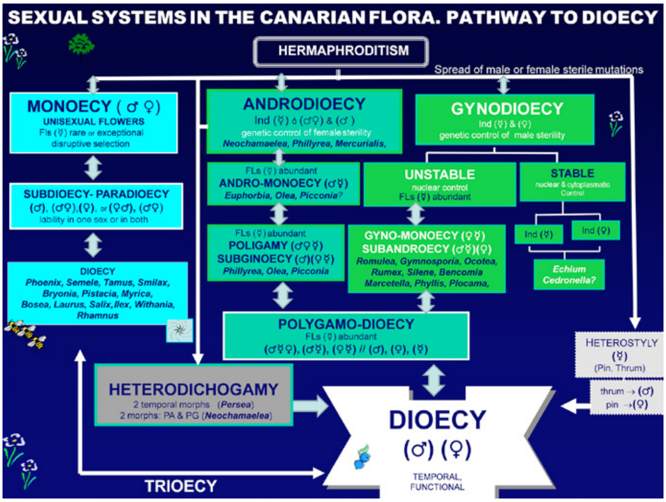

Figure 1.

There are two big evolutionary TRANSITIONS of angiosperm flowers (Barrett, 2010): i) The origin of separate sexes (females and males) from hermaphroditism and ii) The shift from animal to wind pollination. Evolutionary transitions are functional changes to adaptive traits that spread to replace ancestral conditions because they increase fitness. From the ancestral angiosperm's flower (hermaphrodite enthomophilous and selft-incompatible), transitions to DIOECY occurring: sexual segregation (spacial and temporal) of gyneceous and androceous from hermaphrodite to unisexual flower where environmental factors are involving. Two morphological types of unisexual flowers have been recognized: I) one type by abortion, exhibits rudiments of the opposite sex, while the other type (II) from inception, exhibits no rudiments of the opposite sex. This evolution of flower development however, occurs within the context of species phylogeny and the different PATHWAYS for the evolution of dioecy invoke the origin of unisexual flowers (Webb, 1999; Mitchell & Diggle, 2005; Pannell & Verdú, 2006). The presence of type I flowers, suggest that the evolution to sexual dimorphism occurred via a gynodioecious or others intermediate systems. In contrast, the presence of type II suggests that the evolution of dioecy occurred via monoecy, which involves the origin of unisexual flowers prior to the separation of sexes.

By other hand, DIOECY is commonly associated with a suite of life history and reproductive traits. In broad comparative studies, correlations have also been documented between dioecy and insect pollination, bird dispersal, growth form, self-incompatibility, heterostyly, living on islands and tropical ecosystems (Bawa 1980, 1982; Renner & Ricklefs 1995; Sakai & Weller, 1999). Using a molecular phylogeny of the angiosperms, many authors found that dioecy was associated with the woody growth form, small inconspicuous flowers, fleshy fruits and abiotic pollination. The particular correlations that were revealed and the strength of the association differed among the three main monophyletic groups of angiosperms, Rosids, Asterids, and Eumagnoliids, but it has not been possible to determine with any confidence the order of acquisition of these traits in relation to the origins of dioecy (Vamosi et al., 2003; Barrett, 2010).

Why DIOECY? Beginning with Darwin, evolutionary biologists have tried to explain the origin and significance of dioecy in flowering plants. Hypotheses for the ORIGIN of gender dimorphism and dioecy from hermaphroditism consider genetic and ecological factors that include (1°) avoidance of inbreeding (IA) *vs* (2°) sexual selection (SS) and optimal resource allocation. Today, dioecy may evolve from hermaphroditism as a result of selection for IA to promote outcrossing (Charlesworth & Charlesworth, 1978). An alternative, though not mutually exclusive idea is that dioecy may evolve through a gradual process of gender specialization (SS) and frequency-dependent selection (Darwin, 1877; Charlesworth & Charlesworth, 1978; Bawa, 1980; Freeman *et al.,* 1997; Webb, 1999; Gleiser & Verdú, 2005).

Early observations about the ORIGINS of GENDER DIMORPHISM in plants and the pathways to dioecy, were based on a qualitative examination of the floral morphology the dimorphic taxa and on analysis of near relatives. Today it is also necessary: a) to distinguish the *phenotypic gender* from *functional gender* based on the relative maternal and paternal investments, gamete or seed estimates, and b) theoretical models to provide testable predictions for the empirical data which provide validity of theoretical models, so ensuring that these models do not become mathematical fantasies. Theoretical model and empirical studies have identified possible evolutionary PATHWAYS to dioecy under these selective forces (IA *vs* SS). Transitions to dioecy from monoecy, gynodioecy, androdioecy, duo-hetero-dichogamy, and distyly have been explored (Charlesworth, 1999; Webb, 1999; Pannell& Verdú, 2006; Gleiser *et al.,* 2008; Barrett, 2010).

Functional unknown of the FLOWER is many times a fact, in spite being one of the basic props of the reproductive systems, constituting the essence of the reproductive success, genetic diversity, maintenance of populations, and to the diversification processes (micro and macro) specially relevant in the ISLANDS, so often invoked natural laboratories for the studies of evolution and especiación (Crawford *et al.*, 1987; Fco *et al.,* 2000; Anderson *et al.,* 2001).

Canary Islands, despite being near Africa, are known as the oceanic island by its volcanic origin, climate, orography and vegetation. Like others oceanics archipelagos have many and diverse habitats with many endemic species. Crawford *et al.* (2011 in press) highlight that the increase of studies of island organisms during the last two decades, is largely made up of molecular phylogenies and genetic diversity investigations, whereas reproductive biology knowledge remains barely the same as 30 years ago.

The present study is a first approximation to analyses of SEXUAL SYSTEM evolution and diversification in Canary Islands within a phylogenetic context for identification closest relatives. Actually 15 genera (8 endemic) with canarian natural populations are studying in the JBCVC, previous analyses of plants cultivated in the botanic garden. The other taxa information of sexual system, are punctuals observations and bibliographics records. Because molecular phylogeny facilitates understanding the evolution of sexual systems, sexual features of some canarian lineages, are plotted on the cladograms. But no always this analysis provides appropriate information because the phylogenies of canarian and continental relatives have some problems of leaky species in Canary Islands and disjunt phytogeographical areas (unfinished sampling, extincted species), the poor phylogenetic support for clades with Macaronesian taxa and polytomies. Other problem is the non-existent reproductive studies in closest relatives.

Preliminary results

It can be reckoned that only a 42% (87) of the total (≈208) genera in CANARY ISLANDS presents any information about reproductive biology. The assessment of unisexual flowers in endemic taxa is still underestimated in the bibliography with only 3% canarian species with dioecy (Helfgott *et al.,* 2000). A new count for the canarian sexual dimorphism (12% dioecious or subdioecious genera or with functional dioecy) is contributed. Four possible pathways have been recognized for the dioecy as Webb's Model (1999) and terminology of Sakai & Weller (1999) for the general terms of sexual systems.

1. Dioecy associations

In Canary Islands dioecy may be commonly associated with the traditional reproductive traits and life history, generalist insect pollination but no wind pollination, woody growth form, small inconspicuous flowers and fleshy fruits and high chromosome number. *Fleshy fruits*: 21 genera analyzed (67%) have fleshy fruits. *Inconspicuous flowers* with entomophilous (E) generalist pollination or anemophilous (A): 26 genera have inconspicuous flowers, 14 genera have floral syndrome E, and 15 genera remaining both E&A. These data must be taken into account with caution because the anemophily may be obscured by retention of zoophilous features, and studies of pollination biology may reveal anemophilous species where morphology would not predict it (Crawford *et al.*, 2011). *Growth form*: all of analyzed genera have woody habit, except 5 herbaceous, herbaceous-climber or geophytes (*Tamus, Smilax, Bryonia, Romulea* and *Mercurialis*). *Vegetative propagation* has been detected at the moment in only 8 genera, but more detailed studies would be necessary. There are not data for agamospermy in the canarian flora. *Chromosome number and ploidy level:* in 29 genera analyzed, 12 (41%) are polyploids, and the diploid genera have a high chromosome number with basic number 11-23. Concerning to the associations with the *self-incompatibility*, some genera are being evaluated at the moment (mainly the ginodioecy and androdioecy pathway) and there aren`t definite data yet. In general, it is one of the reproductive characters less studied in Canary Islands which would require investigation with priority character.

2. Canarian autochthonous dioecy vs no autochthonous

At the moment, 12% dioecious or subdioecious genera have been detected to present, which would locate Canary Islands as an example of oceanic islands where the dioecy is relatively abundant and similar to the one detected in Hawaii with 11% of the genera with strict dioecy (Sakay *et al.*, 1995). A total of 31 genera, 48 species (50 tx) and 23 families with unisexual flowers were analized. 19 of the 31 genera have dioecy (*Phoenix, Tamus, Semele, Smilax, Bosea, Pistacia, Ilex, Bryonia, Laurus, Myrica*, *Salix, Withania, Rhamnus, Rumex Bencomia, Marcetella, Phyllis, Euphorbia, Mercurialis)* and others 4 genera may have functional dioecy (*Plocama*, *Neochamaelea, Phillyrea, Persea*). The molecular phylogenies show that the putative colonizing ancestors of the some canarian genera may be dioecious, but others (10 genera) may be derived of the colonists sexually dimorphic or monomorphic, that have developed autochthonous dioecy in Canary Islands (*Semele, Bosea, Neochamaelea, Bryonia, Bencomia, Marcetella, Phyllis, Plocama*, *Rumex?, Withania?*).

Autochthonous evolution of sexual dimorphism occurred in at least 14 lineages and it is worthy to remark that three genera (of 31) are subdioecious or trioecious with possible dioecy (*Gymnosporia, Romulea, Ocotea*), others two gynodioecious (*Echium*, *Silene*) and two may be androdioecious (*Neochamaelea, Phyllirea*?), or polygamodioecious (*Phyllis, Picconia, Olea*?), with other one only monoecious (*Dendropoterium*) or polygamomonoecious (*Olea?*).

3. Pathway to dioecy in Canary Islands. Origin

The occasional presence of bisexual flowers in some taxa dioecious may occur, though they usually have lower reproductive fertility of such rare bisexuals. This reinforce Darwin's hypothesis: the allocation of resources between the sexes as force involved in the evolution and maintenance of dioecy.

A) Monoecy pathway. ia) Monoecy-subdioecy pathway is caracterised by unisexual and occasional hermaphrodite flowers. The ancestral population is most likely to comprise monoecious individuals (Renner & Ricklefs, 1995; Webb, 1999; Pannell & Verdú, 2006) and inconstancies are in one sex and unisexual flower type II. This way is recognized in the genera (12) and species (16, 17 tx): Phoenix (Arecaceae), no-autochthonous dioecy. Tamus or Dioscorea (Dioscoreaceae): no-autochthonous dioecy. Semele (Ruscaceae) 2 species with no-autochthonous dioecy. Smilax (Smilacaceae): no-autochthonous dioecy. Bosea (Amaranthaceae): autochthonous dioecy. Pistacia (Anacardiaceae): 2 species with no-autochthonous dioecy. Ilex (Aquifoliaceae): 2 species (3 tx) with no-autochthonous dioecy. Bryonia (Cucurbitaceae): no autochthonous dioecy. Laurus (Lauraceae): no-autochthonous dioecy. Myrica (Myricaceae): 2 species with no-autochthonous dioecy. Salix (Salicaceae): no-autochthonous dioecy. Withania (Solanaceae): autochthonous dioecy?. All genera (except Semele, Bosea, Ilex, Withania), show unisexual flowers without organs rudiments of the other sex. In Semele, the male plants with ocassionally hermaphrodite flowers are considered an intermediate step of subdioecious pathway with inconstancies in only one sex. ib) Monoecy-paradioecy pathway with occasional hermaphrodite flowers and inconstancies in both sexes is recognized in Rhamnus (Rhamnaceae) with no-autochthonous dioecy. This genus show unisexual flowers with organs rudiments of the other sex.

B) Gynodioecy pathway: hermaphrodite and female flowers with transitional stages of maleness and unstable gynodioecy (Webb, 1999). This way are recognized in 11 genera and 22 species with unisexual flowers T-I: Romulea (Iridaceae): autochthonous subdioecy?. Echium (Boraginaceae): many endemic species with stable gynodioecy. Silene (Caryophyllaceae): 2 endemic species gynodioecious. Gymnosporia (Celastraceae): 2 species with autochthonous trioecy?. Ocotea (Lauraceae): autochthonous gynodioecy-dioecy?. Rumex (Polygonaceae): autochthonous dioecy?. Rosaceae-Sanguisorbeae: autochthonous dioecy; with 8 species and 3 genera, Dendropoterium (M), Bencomia (M, PD, D) and Marcetella (D). Phyllis (Rubiaceae-Anthospermae): 2 species with autochthonous dioecy. Plocama pendula (Rubiaceae-Paederiea): autochthonous functional dioecy. In Gymnosporia cassinoides the relative presence of hermaphrodites flowers indicates an intermediate stage of the gynodioecious pathway. The ginodioecy (GD) and trioecy in Celastraceae like Eumonymus reinforces this pathway (Darwin, 1877; Webb, 1979). Autochthonous evolution of sexual dimorphism occurred in Rosaceae-Sanguisorbeae where from a colonist sexually monomorphic Sarcopoterium (M) or Sanguisorba minor complex (GM), the dioecy was gradually developed (Helfgott et al. 2000, Pérez de Paz, 2004) with monoecious Dendropoterium, Bencomia with monoecious, trioecious and dioecious species, and Marcetella (only dioecious). The non-existence of sexual dimorphism in canarian endemism Echium confirms stable gynodioecy (Pérez de Paz, 2002).

C) Androdioecy pathway: with cosexual and male plants and possible subginoecious intermediate stage and unisexual flowers T-I. Euphorbia balsamifera (Euphobiaceae): no autochthonous dioecy?. Mercurialis canariensis (Euphobiaceae): no autochthonous dioecy. Neochamaelea (Cneoraceae): autochthonous androdioecy with functional dioecy. Phillyrea **(Oleaceae):** no autochthonous androdioecy and functional dioecy?. Picconia (Oleaceae): autochthonous polygamo-dioecy?. Olea (Oleaceae): autochthonous polygamous?. A more complex system of androdioecy associated to heterodichogamy, has been recognized in Neochamaelea (Sapindales) like genus Acer of Sapindaceae (Gleiser & Verdú, 2005). The androdioecy system was considered a rare sexual system. Recent studies in Acer have demonstrated that the access to dioecy from androdioecy is possible when there are heterodichogamy with two reciprocal morphs protandrous and protogynous (Pannell & Verdú, 2006; Gleiser et al., 2008). Neochamaelea (see Figure 2) constitute an important empirical contribution which possible evidence the access to dioecy from androdioecy in some populations and others with male, monoecious and heterodichogamous individuals (Pérez de Paz et al., in prep).

Figure 2

D) Heterodichogamy pathway: hermaphrodite protogynous plants with a dimorphic synchronized dichogamy. This way is recognized in Persea indica (Lauraceae) with no autochthonous functional dioecy. This pathway presents a kind of temporal dioecy system of tropical families with inconspicuous flowers, generalist pollination and fleshy fruits dispersed by birds (Kubitzki&Kurz, 1984).

4. Sexual dimorphism, phylogenetic component & genetic diversity

Sexual dimorphisms in Canaries are consistent with results of prior continental comprehensive studies of dioecy (Renner & Ricklefs, 1995) and reinforce the hypothesis of the high phylogenetic component of sexual systems, which conditions the answer of the colonizing taxa to the local selective forces, in this case islanders. All canarian sexual systems are probably tied to their common phylogenetic history. Nevertheless, the dependence on environmental factors of the different sexual manifestations becomes obvious in Canaries after the pursuit of polygamous situations or populations and intermediate stages to dioecy. By other hand, meta-analysis results of allozymes diversity in Canary Islands (Pérez de Paz & Caujapé-Castells, 2010), show the low detected genetic diversity for dioecious taxa supports sexual selection hypothesis (Darwin, 1877; Bawa, 1980; Charlesworth, 1999) that some times, construes dioecy as a trend related to resource allocation and SS oriented towards progeny's vigour. Genetic diversity patterns in Canaries depend on the floral attributes (phylogeny) of colonizing lineages, that determine breeding systems and reproductive success: breeding system of congeners taxa (SI) have similarly diversity levels, that can shift depending on their population size (in keeping with Gitzendanner & Soltis 2000), chromosome number, apomixis … and other important factors like island age or closeness to the mainland.

5. Canarian dioecy and species richness (speciation vs extinction)

The majority of the genera dioecious analyzed, have not specied in Canary Islands. 19 of them have only one canarian taxon, 9 genera have only 2-4 endemic taxa and only one genus (*Euphorbia*) has more than 4 canarian endemic taxa. This would mean that once they established they didn`t diversify, or, in case of having done, they finally extinguished. The genera analyzed, all of them with unisexual flowers, do not characterize for having few dioecious species out of the Canary Islands: 8 genera have more than 50 species, 13 genera ranges between 5-50 species and 10 genera less than 5 species. These data remind the ones found by Heilbuth (2000) for the tropical ecosystems where more abundance of dioecious species has been traditionally considered like the island ecosystems (Bawa, 1980; Renner & Ricklefs, 1995). This agrees with studies done on Hawaiian flora (Sakai *et al.* 1995*a, b*), which suggest that Baker's law does not reduce the amount of dioecious colonizers (»10%) and no trend for higher extinction (or lower speciation rates).

6. Conservation

In the 29 genera analyzed 11 genera present threatened species (Semele, Ilex, Myrica, Rhamnus, Marcetella, Dendropoterium, Bencomia, Ocotea, Phyllis and Gymnosporia?) which characterize for being dioecious basically strict and with woody habit. This could be in accordance with Heilbuth (2000) and Vamosi & Vamosi (2005): dioecious plants may go extinct more often if they are segregated into small populations in which there are no members of the opposite sex. There are many consequences to having a dioecious breeding system that may contribute to its lack of success, such as a possible decrease in seed set or different ecological tolerances between sexes. Dioecious clades have been observed to have lower species richness than their nondioecious sister groups indicating that dioecious species experience higher extinction rates or lower speciation rates. The results of Vamosi & Vamosi (2005) indicate that dioecious plants may warrant special attention in conservation practices. By other hand, the available knowledge on the allozyme diversity and reproductive biology of canarian endemic plants reveals the existence of small populations with very high genetic variation levels (Fernández Palacios et al., 2007, 2010; Suarez et al., 2009; Pérez de Paz & Caujapé-Castells, 2010) whose reproductive success, some times, is whittled down by the loss of dimorphic population diversity associated with SSI-He (cob-pap in Limonium dendroides) to produce viable crosses. These first evidences reveal that the canarian species needs a reproductive and genetic integrate knowledge for a suitable and better evaluation to their conservation strategies. Future population studies should address questions regarding reproductive biology with the genetic diversity and its conservation implications. Conservation issues may be particularly complex when Important factors include breeding and mating systems (Crawford et al., 2011).

Current & future studies

A) More study in canarian sexual systems uninvestiged to present and associations with life history traits, SI and ploidy level in Canarian flora: more study in phenotypical and functional gender when considering sexual dimorphism (flowers appearing to be hermaphroditic and functionally unisexual-dioecious) and evolution of dioecy are need.

B) The role of biotic pollinations *vs* wind-pollination in canarian flora. Observation of visitors *vs* pollinators, floral morphology (dimorphisms) and breeding system studies would provide insight into the factors yielding the observed mating system. Very few species have been critically examined, and such studies have shown that the situation is more complex than it appears from casual observation (Anderson *et al.* 2001; Bernardello *et al.* 2001, 2006; Crawford *et al.,* 2011).

C) Studies relationed to Homomorphic Sporophitic Self Incompatibility System, micro-morphology and allozyme diversity are in prep for published: i) In canarian endemic genus *Parolinia* of Brassicaceae (Fernandez Palacios *et al.,* 2007 and Fernandez Palacios, 2010: doctoral thesis) and ii) in macaronesian endemic *Argyranthemum* of Asteraceae (Olangua *et al.,* 2004; Olangua, in prep: doctoral thesis). In addition, studies relationed to Heteromorphic Sporophitic Self Incompatibility System, micro-morphology and allozyme diversity are in prep for published in the canarian endemism of genus *Limonium* (cob-pap) of Plumbaginaceae and genus *Jasminum* (pin-thrum) of Oleaceae. More basic, meticulous studies of the reproductive biology of Canary Islands plants such as mating systems and self-incompatibility detections in colonization and the subsequent diversification of island lineages are needed and the relations with other oceanic islands (Anderson *et al.* 2001; Bernardello *et al.* 2001, 2006; Crawford *et al.,* 2011).

D) It would also be desirable to have more highly integrated population biology studies in which more reproductive-micromarkers investigations could be combined with molecular markers (allozymes, microsatellites, etc.) to estimate outcrossing rates, paternity analysis, S alleles detection and the significance of phylogeographic history and diversification of canarian lineages. Future population studies should address questions regarding reproductive biology and genetic diversity to compare Canaries with other archipelagos for which there is comparable data (Sakai *et al.* 1995a,b; Fco-Ortega *et al.,* 2000; Crawford *et al.,* 2001; Anderson *et al.* 2001; Bernardello *et al.* 2001, 2006; Pérez de Paz & Caujapé-Castells, 2010).

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She has authored some 30 scientific publications, two book chapters, about 50 participations in congresses (with several invited lectures), and coorganized two meetings. She participated in six R+D projects (in two of them as the PI), and in 2 Interreg projects. A supervisor of ca. 10 projects in pallinology, reproductive biology and genetic diversity of Canarian endemics (e.g., Limonium, Echium, Neochamaelea, Plocama, etc), she has co-directed two PhD’s on Parolinia (January 2010) and Argyranthemum (2011), and participated in doctoral committees at the Universities of León, Barcelona and Las Palmas. She co-directed and taught four courses (Introduction to Scanning Electron Microscopy, pallinology, systematics and evolution, Introduction to numeric taxonomy and Reproductive biology) in the doctoral programme of the ULPGC Biodiversity and plant conservation during 1994-96 and 1997-99. She was a professor of the First seminar about conservation biology of threatened plants.

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Islands within islands: historical and ecological constraints explain disjunct distribution between Navaea phoenicea populations

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The Canary Islands represent one of the best studied models on oceanic island biogeography. However, the phylogeography of their endemic species is still in a earlier stage. Navaea phoenicea (Vent.) Webb & Berthelot (Malvaceae), an arborescent mallow endemic to the island of Tenerife, is a monotypic isolated lineage, sister to the Lavatera-Malva clade, as revealed by ITS and cpDNA studies, suggesting the Tertiary relict condition for this Macaronesian species. Its present distribution, restricted to Teno and Anaga massifs, shows a recurrent biogeographic pattern common in other endemic lineages. An integrative approach based on molecular population genetics (AFLP), reproductive biology and ecological modeling techniques is helping to understand the origin of such pattern. The ecological study demonstrates that, despite a potential niche larger than the presently occupied by the species, the gap between Teno and Anaga of Navaea is also present in their potential niche distribution, as shown in niche modelling based onm bioclimatic variables. The results from populational and demographic studies show an East –West negative gradient in genetic diversity allelic richness values in populations. Floral morphology and physiology of Navaea exhibit a marked assemblage of traits adapted to ornithophily. The pollination study indicates that no restriction is observed in the fruit and seed set induced by the pollination efficiency of different vectors (mostly birds). Germination rates, seed dispersal and seedling recruitment, suggests however an ongoing active process of population depauperation due to a high rate of seedling mortality by herbivory. The results of our study lead us to conclude that while past historical events (volcanism, landslides) in Canary Islands have greatly influenced the present distribution patterns in plants, the effects of current events mediated by human activities (land reclamation, invasive species) not only obscure such patterns, but can most likely conduct Navaea towards rapid extinction.

Javier Fuertes Aguilar

Javier Fuertes Aguilar is a Senior Scientist at the Real Jardín Botánico (RJB-CSIC). First trained as a Tropical Botanist, after receiving his Ph.D. in Biology in 1993 at the University of Salamanca, he was a Postdoctoral Fellow at the University of Texas at Austin (1994-1996). In 1997, he joined the RJB, where he set up the Molecular Systematics Lab. Since then, his research has primarily been focused on the use of molecular markers in the study of Plant Evolution and Diversity. His recent interest in Navaea was born from his previous works on the Systematics and Evolution of Malvaceae and his fascination after the contact with the Canarian Flora. He has been principal investigator in several projects related to the Macaronesian Flora and is presently responsible for a research project centered in the origin of morphological novelties associated to ecological niche shifts in several lineages of Canarian plants funded by the Spanish National Plan of R&D&I.

Genetic diversity of Canarian endemisms revealed by microsatellites: knowledge after one decade of analysis

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An increasing number of studies have demonstrated the value of genetic data in addressing issues of plant conservation biology, especially in identifying populations where genetic issues are likely to affect their prospects of long-term survival in reintroduction biology or in resolving taxonomic uncertainties (DeSalle and Amato 2004; Sosa et al. 2010a,b; Figure 1). Understanding the level and apportionment of genetic diversity within and among populations is especially important for the conservation of island endemics because being island plants; this may make them even more susceptible to extinction (Sosa et al. 2010a, Franks 2010).

Neutral hypervariable markers are useful in estimating the relative evolutionary importance of genetic factors such as mutation rates, gene flow, and genetic drift (González-Pérez et al. 2009a,b; García-Verdugo et al. 2010, Segarra-Moragues & Catalán 2010, Sosa et al. 2010b). In this sense, there are growing conservation genetic studies on Canary endemic flora (Sosa et al. 2010a,c; Table 1).

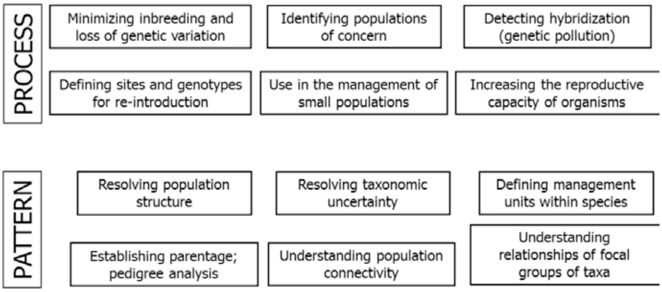

Figure 1. The roles of Conservation Genetics in Plants. Adapted from De Salle & Amato 2004.

TAXON	NL	A	H_O	H_E	REFERENCE
Bencomia caudata	*4*	*5.13*	*0.49*	*0.62*	*González-Pérez et al. 2009b*
Bencomia exstipulata	*4*	*3.50*	*0.39*	*0.44*	*González-Pérez et al. 2009b*
Ilex azorica	*8*	*5.63*	*0.26*	*0.34*	*Sosa et al. 2010b*
Ilex canariensis	*8*	*5.88*	*0.60*	*0.55*	*Sosa et al. 2010b*
Ilex perado spp. platyphylla	*8*	*3.87*	*0.42*	*0.56*	*Sosa et al. 2010b*
Ilex perado ssp. lopezlilloi	*8*	*2.13*	*0.50*	*0.43*	*Sosa et al. 2010b*
Myrica faya	*6*	*9.30*	*0.57*	*0.67*	*González-Pérez et al. 2009a, Sosa et al.2010c*
Myrica rivas-martinezii	*6*	*6.50*	*0.49*	*0.56*	*González-Pérez et al. 2009a, Sosa et al. 2010c*
Neochamaelea pulverulenta	*13*	*7.85*	*0.19-0.74*	*0.42-0.81*	*Rigueiro et al. 2009*
Olea europea ssp. guanchica	*6*	*4.0-4.8*	*0.64*	*0.71*	*García-Verdugo et al. 2010*
Phoenix canariensis	*8*	*11.00*	*0.51*	*0.60*	*González-Pérez et al. (in prep.)*
Pinus canariensis	*8*	*17.7-22.80*	*-*	*0.75-0.77*	*Navasques & Emerson, 2007; López de Heredia et a.. 2010*
Sambucus palmensis	*5*	*6.80*	*0.55*	*0.50*	*Sosa et al. 2010a*
Silene nocteolens	*6*	*15.83*	*0.64*	*0.78*	*Sosa et al. 2010b*
Sorbus aria	*9*	*5-14*	*-*	*0.653-0.847**	*Sosa et al 2010b*

Table 1. Genetic variability indexes from microsatellite data of Macaronesian plant taxa. NL: Number of loci; A: Average number of alleles; Ho: Observed heterozygosity; He: Expected heterozygosity.

The main goals of these studies are: (i) to assess the existing levels of genetic variability in the species using highly polymorphic markers (microsatellites); (ii) to analyse the distribution of this genetic diversity among the different populations; (iii) to infer potential evolutionary processes that could have led to present genetic differentiation among the islands; and (iv) to use this molecular information as a tool for assessing the current conservation management plan for endangered species and for designing conservation strategies.

We synthesized the results of several studies of population genetics for Canarian (and Macaronesian) plants from microsatellite data, comparing the results (genetic variability, mean expected heterozygosity, and genetic differentiation, F_{ST} parameter) with other congener and endemic species (Table 2).

TAXON	NL	H_E	REFERENCE
lex perado ssp. lopezlilloi	***8***	***0.435***	***Sosa et al. 2010b, González-González (in prep)***
I. perado ssp. platyphylla	***8***	***0.560***	***Sosa et al. 2010b, González-González (in prep)***
I. azorica	***8***	***0.339***	***Sosa et al. 2010b, González-González (in prep)***
I. canariensis	***8***	***0.551***	***Sosa et al. 2010b, González-González (in prep)***
I. leucoclada	*13*	*0.804*	*Torimaru et al. 2007*
Phoenix canariensis	***12***	***0.600***	***González Pérez et al. (In prep)***
P. dactylifera	*16*	*0.700*	*Zehdi et al 2004*
P. dactylifera	*17*	*0.666*	*Akkak et al. 2009*
Myrica faya	***6***	***0.670***	***González Pérez et al. 2009a, Sosa et al. 2010c***
M. rivas-martinezii	***6***	***0.560***	***González Pérez et al. 2009a, Sosa et al. 2010c***
M. cerifera	*3*	*0.825*	*Erickson et al. 2004*
M. rubra	*14*	*0.656*	*Xie et al. 2010*
M. rubra	*11*	*0.612*	*Zhang et al. 2009*
Silene nocteolens	***6***	***0.780***	***Sosa et al (2010b), González-González (in prep).***
S. vulgaris	*7*	*0.816*	*Juillet et al. 2003*
S. tatarica	*5*	*0.455*	*Tero & Schlötterer 2005*
S. latifolia	*6*	*0.956*	*Teixeira & Bernasconi 2007*
Sambucus palmensis	***5***	***0.500***	***Sosa et al. 2010a***
S. nigra	***8***	***0.710***	***Clarke and Tobutt 2006***
Pinus canariensis	***8***	***0.777***	***Navasques & Emerson 2007***
P. monticola	*8*	*0.808*	*Mehes et al. 2009*
P. strobus	*8*	*0.802*	*Mehes et al. 2009*
P. pinceana	-	*0.418*	*Mehes et al 2009*
P. radiata	-	*0.700*	*Mehes et al. 2009*

Table 2. Mean Expected heterozygosity from microsatellite data of Macaronesian taxa (in bold) compared with other congeners. NL: Number of loci.

The studies were drawn from data obtained in our research group and from a literature search in the database of SCOPUS and the Institute for Scientific Information using the search terms "plant", "Microsatellites", "Canary Islands", "endangered", "endemic" and "genetic diversity". Only nuclear microsatellites loci were included.

The results obtained in several cases, *Bencomia exstipulata* natural populations, *Ilex* genus, *Sambucus palmensis*, *Myrica rivas-martinezii*, *M. faya* and *Sorbus aria* are shown as examples of the application of microsatellite analysis to conservation genetics.

Some aspects related with the future of conservation genetics of rare and threatened plant species are also described and discussed (Figure 2).

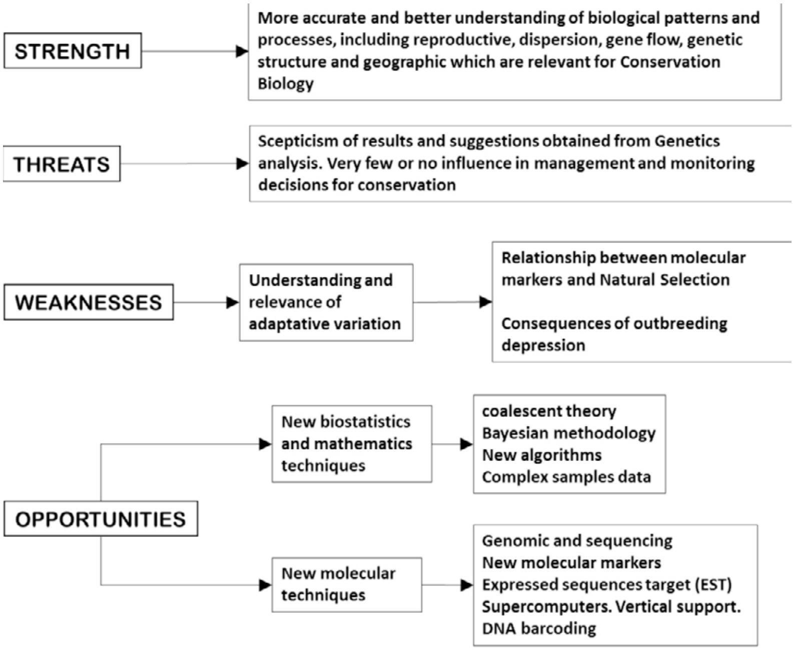

Figure 2.- Some strengths, threats, weaknesses and opportunities of genetic studies applied to Plant Conservation Biology.

BANMAC: Bank of Molecular Markers of the Macaronesian

The Bank of Molecular Markers of the Macaronesian Flora (BANMAC) is a free data matrix that compiles all the available genetic information generated with molecular markers in the Macaronesian flora. BANMAC is a webpage integrated in DEMIURGO, a European project funded by Transnational Cooperation Programme – Madeira-Canarias-Azores (FEDER).

BANMAC is designed to facilitate the generation of new molecular data on Macaronesian flora. In this sense, BANMAC will provide laboratory protocols, primer sequences, PCR conditions, etc., in order to speed up and facilitate laboratory processes needed to generate new molecular data. In addition, BANMAC will contain basic information about genetic diversity detected in the species with different molecular markers.

BANMAC will collect information from the three molecular markers (microsatellite, allozyme and RAPD) that have been used in the research group of Biogeografia, Conservación y Territorio (www.bcyt.es) from the University of Las Palmas de Gran Canaria. However, new molecular markers could be incorporated in the near future.

Users will be able to freely access the database and carry out a search entering the species name (scientific or common name) or through a specific molecular marker (microsatellite, allozyme and RAPD).

Entering the name of the species, users will be able to access information from all molecular markers that have been worked on this species, as well as, a brief description of the specie. On the other hand, entering in a specific molecular marker, users could enter into a list of all Macaronesian species that have been studied using this particular molecular marker.

The database could be searched using either the search page (searches based on family, genus or species names) or in the future, using an advanced search page, which will allow for more flexible and detailed queries as the database grows.

For each species a brief descriptive file that includes the scientific and common name, family, location, a general description, distribution, conservation status, researches carried out on this species, as well as, publications on conservation and population genetics will be available.

Once the species and the molecular marker have been selected, a variety of data could be consulted. In general, each molecular marker file gives specific data about the number of individuals sampled, number of loci analyzed, values of different genetic diversity indices and a brief conclusion about genetic studies carried out in the target specie. Also, the references of the papers from these genetic studies will be provided.

Genetic diversity indices will provide codominant molecular markers (microsatellite and allozyme) which are: mean number of alleles, expected and observed heterozygosity and percentage of polymorphic loci. Whereas, for dominant markers (RAPD), the number of fragments, genetic diversity, Shannon index and percentage of polymorphic loci are shown.

Too, a brief laboratory protocol guide will be given on this main page. Laboratory protocols include the protein extraction protocol, electrode/gel buffer system and starch gel electrophoresis in the case of allozyme data. In regard to microsatellite and RAPD, a laboratory protocol file will contain information about DNA extraction and purification, thermal cycling conditions and PCR reactions.

Within each molecular marker file, general information about each specific locus will be contained in the detailed locus file:

In the case of the microsatellite locus, each detailed locus file will give information about repeated motifs, gene bank accession numbers, publication references, forward and reverse primer sequences, thermal cycling conditions, PCR reaction reactives, the number of alleles recorded, size range and other species amplified with this locus.

In the RAPD detailed locus files, information about commercial primer sequences, thermal cycling conditions, and PCR reactions will be shown. Also, data about the number of fragments recorded, fragment size range and the percentage of polymorphism, other Macaronesian species analyzed with this locus, etc. are illustrated.

In regard to allozyme locus, each detailed locus file will include isozyme systems, allozyme loci recorded, chemical reactions, electrode/gel buffer systems, reactives, stain layers, stain conditions, the number of alleles detected, publication references and other species analyzed.

In all detailed locus files, microsatellite, allozyme and RAPD, a link to the DEMIURGE data base of the selected species will be provided.

Besides laboratory protocols, basic genetic information will be facilitated: a list of publications on conservation and population genetics on Canaries flora and a manual for the different molecular markers. These manuals are simple and easy guides for each molecular marker that includes basic concepts, technical help, data interpretation and genotyping, etc.

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Pedro A. Sosa

Pedro A. Sosa is a graduate in Molecular Biology at the University of La Laguna (Canary Islands, Spain), and has a PhD in Marine Sciences. He is a Professor of Botany at the University of Las Palmas de Gran Canaria (ULPGC) and Director of the Research group Biogeography, Conservation and Territory from the ULPGC. He has centered his scientific career in the area of Botany, on the genetic characterization of natural populations of endangered endemic plants from the Canary Islands, and its application to conservation biology, especially in Conservation Genetics. He has participated in different national and international research projects, being principal investigator in at least 15 research projects. Highlighting, the project Biodiversity and Genetics of Algae Populations, supported by the MAST-III European program, where he was the lead/head scientist for the Spanish group. He has directed three PhD theses, and has set up and implemented the laboratory of Molecular Biology and Conservation Genetics at the University of Las Palmas de Gran Canaria.

Miguel Ángel González-Pérez

Miguel Ángel González-Pérez graduated in Biology at the University of La Laguna (Canary Islands, Spain) and is a doctorate from the University of Las Palmas de Gran Canaria (Canary Islands, Spain). His PhD thesis was awarded with different research prizes. He did short-term postdoctoral research in the Jodrell Laboratory, (Royal Botanic Gardens, Kew, UK) with Dr.Michael Fay, in order to develop microsatellites for an endangered endemic species from the Canary Islands (Bencomia exstipulata). Currently, he is working in the research group of Biogeography, Conservation and Territory at the University of Las Palmas de Gran Canaria, where he participates in different research projects oriented to evolutionary and conservation biology of plants endemic to the Canary Islands. Likewise, he is head of the laboratory of Molecular Biology and Conservation, and co-director of several PhD theses. At the moment, he is working with several endangered endemic species from the Canary Islands using a wide range of molecular techniques (allozymes, RAPDs, microsatellites) for the study of population genetics, systematics and evolution. The research group shows a strong personal interest in endangered and endemic Canarian flora and is committed to its conservation. At the moment, he has multiple scientific publications in international journals, a book, several book chapters, and more than 30 congress communications. He is also a referee of several scientific international journals.

A "Tree of Life" for the Canarian endemic trees: a first step toward the Phylogenetic Diversity of the archipelago's flora.

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Introduction

A DNA barcode is a short DNA sequence taken from standardized portions of the genome, normally used to identify species (Kress et al, 2005; Savolainen et al, 2005). Lately, DNA barcodes have been employed for a number of different purposes in addition to the identification such as the construction of phylogenies (Kress et al, 2009), the assistance to classical taxonomy in the elaboration of censuses of plant biodiversity (Janzen 2009), and the application of these data to conservation strategies. Many studies have shown that DNA barcoding is very efficient in the discrimination of species and in helping to flag possible new species by using one or a combination of several DNA regions (Hebert et al. 2003, 2009). The application of the mitochondrial marker *cytochrome c oxidase I* (COI) has been succesfull and it usually offers a high discrimination rate (>90%) for species identification in a wide range of animal taxa (see references Ran et al, 2010); however, this marker was found inadequate for barcoding plants. A multi-locus approach based on the chloroplast genome was increasingly accepted as an effective strategy for species identification and recognition in land plants (Newmaster et al. 2008; Chase et al. 2007; Kress and Erickson 2007; Fazekas et al. 2008; Lahaye et al. 2008a). In 2009, the Consortium for the Barcoding of Life (CBOL) Plant Working Group (PWG from now on) recommended the two-locus combination *rbcL+matK* as the universal DNA barcode for land plants (CBOL Plant Working Group 2009). The discrimination among highly speciose genera, or cases of recently evolved species are the most complicated challenges when barcoding plants. Compared with the successful identification rates of over 90% in animal, fungi and algae species, the average rate of successful species discrimination in land plants was found to rarely exceed 70%. (e.g. Newmaster et al. 2008; CBOL Plant Working Group 2009). Another issue to overcome in plants is that the discrimination rate varies greatly among different lineages, and it gets still lower the more congeneric species are sampled, or when processes such as hybridization and/or introgression are taking place.

To use the two accepted Angiosperm "barcode" cpDNA sequences (*rbcL* and *matK*) to assess their usefulness to identify any Canarian endemic tree species should be greatly challenging for various reasons: i) in oceanic islands the incidence of evolutionary processes, namely radiation and hybridization, can be much higher than in other insular or mainland regions and ii) it has been shown that woody plant lineages show consistently lower rates of molecular evolution as compared with herbaceous plant lineages, suggesting the application of DNA barcoding concepts should be more difficult for tree floras than for non-woody floras. Significantly, trees in general are perhaps the organisms with a lower capacity of adaptation to the current global changes, due to their long generation times. Hence, testing the applicability of DNA-barcodes for trees of an oceanic archipelago such as the Canary Islands seems to be of utmost importance in elaborating more accurate censuses that support world-wide conservation efforts.

The project "arBOLcan" has as the main goal to barcode all 46 Canarian endemic tree taxa. The objectives, methodologies, and results of this study ("arBOLcan") will be shared with other international projects under way in Europe and other continents (such as the AR-BOL project in Mesoamerica) because Dr. Juli Caujapé Castells (Jardín Botánico Canario "Viera y Clavijo"-Unidad Asociada CSIC) and Dr. Félix Forest (Jardín Botánico de Kew) are co-coordinators of Tree-BOL in Europe.

We examined more extensively samples of a Macaronesian endemic tree *Heberdenia excelsa* (Sol. in Aiton) Banks ex A. DC. *(sensu lato)* that is distributed in Canary Islands and Madeira. Notable morphological and ecological differences have been found between the forms that grow associated to the laurel trees ("saquitero") and the ones that grow in the termophilous woodlands ("aderno") although, they have not been reflected in the taxonomy of this specie (Jaén Molina et al, 2007). Currently, this taxon is not included in any threatened status but if the molecular markers confirm the morphological divergency found between its populations and betwen the islands, than *Heberdenia excelsa* could be divided into two taxa, both of which should be under a threatened category.

The use of the DNA barcodes sequences to estimate a region's Phylogenetic Diversity (PD, an essential parameter to define *in situ* conservation strategies and priorities) is relatively new. Basically, this methodology uses molecular information to suggest conservationist priorities based on phylogenetic singularities of the territory (Faith et al. 2005, Forest et al. 2007, Schaefer et al. in press), thereby complementing estimates based on taxonomic diversity per unit area (e.g., Reyes-Betancort et al. 2008). To estimate a "tree of life" for the Macaronesian Flora and the PD of the Canarian Flora have been two of the aims of the DNA Bank of the Canarian Flora (JBCVC-CSIC) since its inception (Caujapé-Castells et al. 2006, and see also http://www.bioclimac.com/mbdna/). The DNA bank at the JBCVC-CSIC contains at present more than 7,000 samples that represent ca. 70% of the Canarian endemic flora (i.e., at least one population per taxon, though not every island of occurrence is sampled in all cases). Ongoing strategic sampling aims at presenting the first robust PD estimates for at least 85% of the endemic flora, in two years.

In this communication, based on preliminary results from project arBOLcan and others, we use a multi-locus approach (*rbcL*, *trnH-psbA* and *trnK-rps16*) to detect possibly cases of cryptic species in a focal case of the Myrsinaceae (*Myrsine-Pleiomeris-Heberdenia)*. We also use the *rbcL* and *matK* sequences to (i) to assess the genetic diversity of endemic trees relative to non-tree endemics in the Canarian Flora, (ii) build a preliminary tree of life for the Canarian endemic trees (including the available outgroups from other geographical regions) and provide a baseline data to estimate Phylogenetic Diversity for the Canarian endemic trees by the end of this year. We cross-compared the topologies obtained by Parsimony, ML and Bayesian inferences with the results of the application of the "automatic barcoding" (*Declic*, Franc et al, in prep.) that to our knowledge is a pipeline for data analysis that has not been performed yet with the Canarian Flora.

Material and methods

Sampling

For the focal case (Myrsinaceae) we sampled in total 77 individuals, 49 of them correspond to Heberdenia excelsa (13 from populations in La Palma; 11 in La Gomera; 7 in Tenerife; 5 in el Hierro; 4 in Gran Canaria and 9 from populations in Madeira) plus 11 samples of Pleiomeris canariensis (5 from populations in Gran Canaria and 6 in Tenerife) and 17 accesions of Myrsine africana (8 from different populations of Sao Miguel; 7 from different populations of Faial). Further details of the localities where the samples were collected are included in Table 1. For the Canarian endemic trees our sampling includes 138 accessions, 35 of which are sequences downloaded from GenBank (the majority of the continental representatives used as outgroup samples) that represent 16 Angiosperm families and 2 Gymnosperm families (Cupressaceae and Pinaceae), encompassing 26 genera and 33 taxa of the 46 Canarian endemic trees. With the exception of 11 taxa that are from Madeira, the rest of the ingroup´s taxa are from the Canary Islands. As for outgroups we have samples from Morocco, Cape Verde, Azores and other continental areas, as well. All the Macaronesian endemic taxa are represented by a minimum of one individual (*Arbutus canariensis, Limonium dendroides, Pinus canariensis* and *Sideroxylon mirmulano*) and a maximum of 9-10 individuals (*Dracaena draco, Rubus bollei* and *Sambucus nigra*) (see Table 2 for more details). For Myrsinaceae vouchers were collected for each population and, in most cases of the Canarian trees, herbarium sheets corresponding to the sampled accessions are deposited in the LPA herbarium.

Table 1. Myrsinaceae Samples analyzed as part of the focal case (vouchers in Gran Canaria: Marrero Á & Caujapé-Castells J; in El Hierro: Fdez-Palacios JM & Dámaso Perera López, P; in Tenerife: Fdez-Palacios JM, Rüdiger O & Fernández Lugo S; in La Palma: Fdez-Palacios JM & Romero P; in La Gomera: Fdez-Palacios JM, Romero P, Marrero Á, Naranjo J, Caujapé-Castells J & Jaén-Molina R; in Madeira: Barone Tosco R; in Azores: Fdez-Palacios JM, Caujapé-Castells J & Rodrigues N.)

DNABANK VIALCODE	BOTANICAL TAXONOMY	LOCALITY/ISLANDS	Nº seq. rbcL	Nº seq. psb-trnH	Nº seq trnK-rps16	Nº seq All 3-reg.
6764-6769	*Myrsine africana*	Pico da Vara / Sao Miguel	3	2	3	2
6770-6779	***Myrsine africana***	Lagoa /Sao Miguel	3	2	3	2
6780-6788	***Myrsine africana***	Caldeira / Faial	3	3	2	2
6789-6793	***Myrsine africana***	Capelo/ Faial	2	3	3	2
6794-6799	***Myrsine africana***	Freguesia/Faial	2	3	3	2
3724-3733	***Myrsine africana***	Sierra de Tronqueira /Sao Miguel	2	2	2	2
		TOTAL Individuals/Locality/ Islands =	17/6/2	15/6/2	16/6/2	**12/6/2**
3709, 3711,3712	*Pleiomeris canariensis*	Teno/ Tenerife	3	2	1	1
3715,3716	***Pleiomeris canariensis***	Pista Las Hiedras/ Tenerife	2	2	0	0
3714	***Pleiomeris canariensis***	Mirador Pico del Inglés/ Tenerife	1	1	1	1
7038	***Pleiomeris canariensis***	Albercón de la Virgen/ Gran Canaria	1	1	0	0
7042-7044	***Pleiomeris canariensis***	Bco. de Azuaje (Andén)/ Gran Canaria	2	2	0	0
7048-7050	*Pleiomeris canariensis*	Bco. de Azuaje (Telesf.)/ Gran Canaria	1	3	0	0
3803	*Pleiomeris canariensis*	Bco. de Azuaje (Cornisa)/ Gran Canaria	1	1	1	1
		TOTAL Individuals/Locality/ Islands	**11/7/2**	**12/7/2**	**3/3/2**	**3/3/2**
3754-3758	*Heberdenia excelsa*	Barranco seco / Palma	1	3	2	1
3759-3763	***Heberdenia excelsa***	Ladera Tagoja/ Palma	4	3	4	3
3764-3768	***Heberdenia excelsa***	Los Tilos/ Palma	4	4	3	3
3769-3773	***Heberdenia excelsa***	Tajadre/ Palma	4	2	2	1
3774-3778	***Heberdenia excelsa***	Bajada a Jinama/ Hierro	5	1	2	1
3734-3738	*Heberdenia excelsa*	El Cedro/ Gomera	0	3	2	0
3739-3743	***Heberdenia excelsa***	Chorros de Epina /Gomera	3	4	3	3
3744-3748	***Heberdenia excelsa***	Bco. de Majona/ Gomera	5	2	1	1
3749-3753	***Heberdenia excelsa***	Altos de Hermigua/ Gomera	5	2	4	2

3942-3944	**Heberdenia excelsa**	Valle Gran Rey/ Gomera	2	1	2	1
3642-3646	**Heberdenia excelsa**	El Palmar/ Tenerife	2	2	2	0
3705-3708	**Heberdenia excelsa**	Teno/ Tenerife	2	2	1	1
7045-7046	**Heberdenia excelsa**	Bco.de Azuaje (Andén)/ Gran Canaria	1	1	1	1
7047	**Heberdenia excelsa**	Bco. de los propios/ Gran Canaria	1	1	1	1
3795	**Heberdenia excelsa**	Barranco Oscuro/Gran Canaria	1	0	0	0
3796	**Heberdenia excelsa**	Bco. de Palo Blanco/ Gran Canaria	1	1	0	0
3779-3783	**Heberdenia excelsa**	Laguna de Dona Beija/ Madeira	1	1	1	1
3784-3788	**Heberdenia excelsa**	Lagoa do Vento/ Madeira	4	0	0	0
3789-3793	**Heberdenia excelsa**	Levada de Dos Fontes/ Madeira	4	3	2	2
		TOTAL Individuals/Locality/ Islands	**49/18/6**	**36/17/6**	**35/15/6**	**21/14/6**

Table 2 (below). Macaronesian endemic trees (distribution following Acebes-Ginovés et al. 2004). In black are highlighted the islands were samples have been collected and, with an asterisk the taxa that are not represented, yet.

	ESPECIE	FAMILIA	DISTRIBUCIÓN INSULAR						
			H	P	G	T	C	F	L
M	*Ilex canariensis Poir*	Aquifoliaceae	H	P	**G**	T	C		
	Ilex perado Ait. ssp. lopezlilloi (G. Kunkel) A. Hansen & Sunding	Aquifoliaceae			G				
	Ilex perado Ait. ssp. platyphylla (Webb et Berth.) Tutin	Aquifoliaceae		P	**G**	**T**			
	Phoenix canariensis Chab	Arecaceae	H	P	G	T	**C**	F	L
	Viburnum rigidum Vent.	Caprifoliaceae	H	P	G	T	**C**		
	Sambucus palmensis Link.	Caprifoliaceae		P	G	T	C		
	Maytenus canariensis (Loes.) Kunk. et Sund	Celastraceae	H	P	G	**T**	**C**	F	
	Juniperus turbinata Guss. ssp canariensis *	Cupressaceae	H	P	G	T	C		
M	*Juniperus cedrus Webb & Berthel.*	Cupressaceae		P	G	T	C		
M	*Dracaena draco L. ssp. draco*	Dracaenaceae	H	P	G	T	**C**		
	Dracaena tamaranae Marrero Rodr., Almeida-Pérez & González-Martín	Dracaenaceae					C		
	Arbutus canariensis Veill.	Ericaceae	H	P	G	T	**C**		
	Erica platycodon (Webb et Berth.) Rivas-Mart. & al.	Ericaceae	H		G	T			
M	*Euphorbia mellifera Aiton var. canariensis Boiss.*	Euphorbiaceae		P	**G**	**T**			

M	Apollonias barbujana (Cav.) Bornm. ssp. barbujana	Lauraceae	H	P	**G**	T	C	F	
	Apollonias barbujana (Cav.) Bornm. ssp. ceballosi (Svent.) G. Kunkel	Lauraceae			G				
M	Laurus azorica (Seub.) Franco	Lauraceae	H	P	**G**	T	C	F	L
	Laurus novocanariensis (Seub.) Franco	Lauraceae			G				
M	Ocotea foetens (Ait.) Berth. et Hook. f.	Lauraceae	H	P	**G**	T	C		
M	Persea indica (L.) Spreng. Rkse.	Lauraceae	H	P	**G**	T	C		
M	Morella faya (Aiton) Wilbur y Morella rivas-martinezii A. Santos & J. Herber	Myricaceae	H	P	**G**	T	C	F	L
	Morella faya (Aiton) Wilbur y Morella rivas-martinezii A. Santos & J. Herber	Myricaceae	H	P	**G**				
M	Heberdenia excelsa (Ait.) Banks et DC.	Myrsinaceae	**H**	**P**	**G**	**T**	**C**	F	
	Pleiomeris canariensis (Willd.) A. DC.	Myrsinaceae		P	G	T	C		
	Olea europaea L. ssp. cerasiformis (Webb et Berth.) Kunk. et Sund.	Oleaceae	H	P	G	T	C	F	L
M	Jasminum odoratissimum L.	Oleaceae	H	P	G	T	**C**	F	
M	Picconia excelsa (Ait.) DC.	Oleaceae	H	P	G	T	C	F	
	Pinus canariensis Chr. Sm. ex DC.	Pinaceae	H	P	G	T	**C**		
	Limonium dendroides Svent.*	Plumbaginaceae			G				
M	Rhamnus glandulosa Aiton	Rhamnaceae		P	G	T	**C**		
	Rhamnus crenulata Aiton	Rhamnaceae	H	P	G	T	C	**F**	L
	Rhamnus integrifolia DC.	Rhamnaceae				T			
M	Prunus lusitanica L. ssp. hixa [Willd.] Franco	Rosaceae	H	P	**G**	**T**	C		
	Marcetella moquiniana (Webb & Berthel.) Svent.	Rosaceae			G	T	**C**		
	Dendriopoterium menendezii Svent.	Rosaceae					C		
	Dendriopoterium pulidoi Svent. ex Bramwell	Rosaceae					C		
	Bencomia brachystachia Svent. ex Nordborg	Rosaceae					C		
	Bencomia exstipulata Svent.	Rosaceae		P		T			
	Bencomia sphaerocarpa Svent.	Rosaceae	H						
	Bencomia caudata (Aiton) Webb & Berthel.*	Rosaceae	H	P		T	C		
	Rubus palmensis A. Hansen	Rosaceae		P		T	C		
	Rubus bollei Focke	Rosaceae	H	P	**G**	T	**C**		
M	Salix canariensis Chr. Sm. ex Link	Salicaceae	H	**P**	G	T	C		
M	Sideroxylon mirmulano Banks ex Lowe	Sapotaceae	H	P	G	T	**C**	**F**	
	Tamarix canariensis Willd.	Tamaricaceae		P	G	T	**C**	F	**L**
M	Visnea mocanera L. f.	Theaceae	H	P	**G**	T	C	**F**	

DNA extraction, amplification and sequencing

DNA extractions were performed from both silica-gel dried or fresh material using the CTAB 2X method. The quality of total extracted DNA was checked on 1% agarose gels, and concentrations were measured in an Eppendorf biophotometer. Subsequently, aliquots of the DNA extracts were deposited in the DNA Bank of the Canarian Flora at the Jardín Botánico Canario “Viera y Clavijo” (JBCVC-Unidad CSIC) (see “DNABank” codes in Table 1 and 2). PCR amplifications were performed for *rbcL*, *psbA-trnH* and *trnK-rps16* (for the focal case) and *rbcL* and *matK* (general case), with primers and conditions recommended by CBOL and in Shaw et al. (2007), for *trnK-rps16*. The PCR products were sent to Macrogen in Korea or to the INRA in France, for sequencing. Sequence alignment was performed using the accessory application CLUSTAL W implemented in BIOEDIT with manual adjustments as needed. The concatenated alignment matrices (3-loci for the focal case and 2-loci for the general case) were obtained using GENEIOUS. Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were conducted for each marker separately and combined. Parsimony analyses were carried out in PAUP* v4.010b, ML analyses with PhyML and Bayesian analyses were conducted with MrBayes v3.0b4. The obtained topologies were crossed-compared with the output file of pipeline *declic* (Franc et al, in prep.).

Results and discussion

1. Focal case:

A) Diagnostic characters

We explored the matrix containing the three-locus aligned sequences to detect diagnostic characters (one or a combination of nucleotides in a position of aligned sequences that is unique to all individuals available for a specific taxa) that will discriminate the species of Myrsinaceae and also to determine whether or not the morphological divergence found, so far, in *Heberdenia excelsa* (“saquitero” vs. “aderno”) was supported by molecular data. From the three locus analyzed, the one that found more diagnostic characters for each taxa was *trnK-rps16*, the second was *psbA-trnH* and, interestingly the third was *rbcL* that only found one unique nucleotide for *Myrsine africana*. When we estimated, the total number of variable characters (diagnostic and polymorphic) detected by each locus, we found out that *rps16* detected twice of diagnostic characters than *psbA* and 20 diagnostic more than *rbcL*. We only examined the *three-locus* alignment for DNA barcode positions between populations, because was the only that presented enough variability to show some intraspecific polymorphism. No diagnostic characters were found to distinguish between all populations examined. One character distinguished one sequence of Caldeira do Faial, from all the sequences of the same and different populations of *Myrsine africana*.

One character was shared by all samples from the three different populations of Sao Miguel. Four characters are exclusive of one sample of *Pleiomeris* from Teno and two characters were found that distinguished one sequence (from Tenerife-Pico del Inglés) from the remaining *Pleiomeris* samples (Gran Canaria). Within *Heberdenia*, a group of samples from La Gomera (Chorros de Epina) shared a nucleotide with samples from La Palma (Los Tilos and Tajadre). One sample from Gran Canaria has an exclusive character. The remaining samples of the different populations even some of Madeira were 100% identical.

B) Phylogenetic reconstructions

MP (Paup), ML (PhyML) and BI (MrBayes) analyses were carried out to evaluate whether or not *rbcL* (“official barcode”) recovered the three Myrsinaceae species as monophyletic and whether or not the “non-official barcodes regions” recovered the same clades as *rbcL.*

The consensus bayesian tree obtained for all the 77 *rbcL* sequences (427chs.) grouped each genus in three main clades, one for each taxa. For *psbA-trnH* (63 taxa/ 418 chs.) again, all sequences for each taxa were clustered together in three main clades but now with a higher resolution within *Myrsine* (Faial´s samples are in a clade separated from samples of Sao Miguel) and within *Heberdenia* (although populations of the different Canary Islands and Madeira are mixed). There is no discrimination between samples of *Pleiomeris* from Gran Canaria and Tenerife. The Bayesian consensus tree for *rps16-trnk* (58 taxa/ 589 chs.) showed a less resolved topology between *Myrsine. Pleiomeris* from Pico del Inglés (T) was slightly different than the others from Tenerife and Gran Canaria.

The resolution within *Heberdenia* is better, although populations of different islands are not completely segregated from each other. The three-locus combination (37 taxa and 1,427 characters) provided the most fully resolved phylogeny with the higher support values recovered (Fig. 1). The Bayesian consensus tree topology resulted, in practically the same clades as for *rps*16 alone, for *Heberdenia and Myrsine* with a mix of populations in them. There are identical sequences for *Heberdenia excelsa* samples from different islands and populations, so a clear discrimination between populations and islands was not possible and neither between the two morphological forms (“saquitero” and “aderno”). It was the position of Pleiomeris what, notably distinguished these results with respect to those from other analyses. This discrepancy is not completely unexpected because in the past, sometimes species of *Heberdenia* have been named after *Ardisia (Heberdenia excelsa=Ardisia teneriffae*) or species of *Pleiomeris* after *Myrsine* (*Pleiomeris canariensis=Myrsine canariensis*). The morphological and molecular variability of the Macaronesian Myrsinaceae need further analyses, in order to bring more light to the taxonomy of the group. One important aspect to consider is the difficulties to collect fruits and flowers of *“Heberdenia excelsa”* that make it very difficult a precise study of the presence of the different forms (saquitero and aderno) in each island.

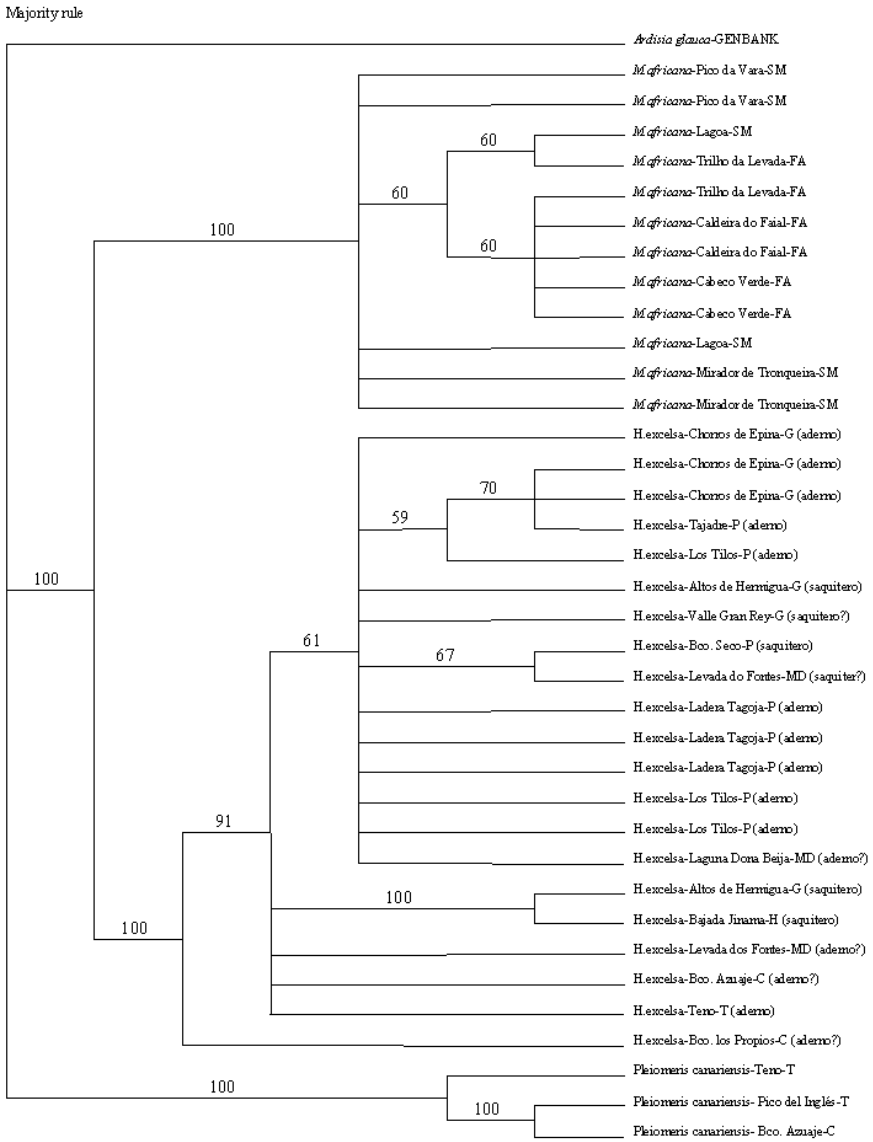

Figure.1. Bayesian consensus tree for 36 taxa of Macaronesian Myrsinaceae based on three locus combined analyses (rbcL+ psbA-trnH + rps16-trnK) with each genus coloured differently (Heberdenia excelsa-blue, Pleiomeris canariensis-green, Myrsine africana-orange)

C) Declic with Myrsinaceae data

Most of barcoding or phylogeny approaches rely on multiple alignments. *Declic* is an alignment free method. Its first step is to build a pairwise edit distance matrix (the minimum number of edits needed to transform one string into the other, with the allowable edit operations being, insertion, deletion, or substitution of a single character), the one implemented here is Levenshtein distance. Second step in pipeline *declic* is to transform genetic distances between observed sequences into estimates of branch length using an evolution model. Third step in the pipeline is to translate the updated edit distance matrix into a graph. A graph is a list of nodes, some of them being linked by an edge. A connex component (cc) in a graph is a maximum set of nodes such that any two of them can be linked by a path. A clique in a graph is a set of nodes such that any pair of nodes is linked by an edge. The graph is a set of cliques. If taxonomy would be perfect, a cc would be a clique: any pair in the clique is linked by an edge. Each clique is a taxon. Pipeline *declic* allows a link between phylogenies, barcodes, and resolutive and variable markers. No alignments, no block selection, no phylogenies, no BLAST, are needed, just graph on edit distances!! We run declic on a *.fasta file of 63 *psbA-trnH* sequences representing the three genus of Myrsinaceae. In Table 3 are included the composition of connex components with a gap = 10. The results showed that *declic* was able to find cliques for *Heberdenia excelsa* and *Myrsine africana* (with very few exceptions) with *Pleiomeris canariensis* in a clear position, too.

CONNEX COMPONENTS	
gap = 0 **field = Genus**	
1 ->	*Heberdenia : 36 / 46* *Pleiomeris : 2 / 10*
2->	*Myrsine : 13 / 14*
3 ->	*Pleiomeris : 8 / 10*
4 ->	*Heberdenia : 3 / 46*
5 ->	*Heberdenia : 1 / 46*
6 ->	*Heberdenia : 1 / 46*
7 ->	*Heberdenia : 1 / 46*
8 ->	*Heberdenia : 1 / 46*
10 ->	*Heberdenia : 1 / 46*
11 ->	*Heberdenia : 1 / 46*
12 ->	*Myrsine : 1 / 14*
9 ->	*Heberdenia : 1 / 46*

Table 3. *Declic* connex components for *trnH-psbA*

2. General case

Our sampling includes more sequences for *rbcL* (138) than for *matK* (85), because we encountered technical problems not only in he amplification of this region but also in the obtention of high quality sequences. We analyzed three matrices: one per locus and a two-loci combination. We did look for diagnostic characters only in conflictive cases as *Ilex* (data not shown).

A) Phylogenetic reconstructions

For each dataset MP, ML and BI analyses were performed. The MP consensus tree for rbcL distinguished well between families (color shaped) and also between genera, with the exceptions of Lauraceae (although the genera that conform this family are easily recognizable at the field). For genus represented by a larger number of individuals, the assignment of the individuals to the correct specie was 100% with the exception of Ilex (spread across different parts of the tree). The only two Gymnosperm families represented (Cupressaceae and Pinaceae) are placed together. The limited taxon sampling and the lack of species per genus in some cases, may be the reason of not much congruence in the recovered of some clades, as the one that grouped Dracaena and Phoenix. The clades recovered by parsimony analyses for *matK* were very similar as the ones obtained for *rbcL*, with the exceptions of Ilex (these time all sequences are grouped together) and within Lauraceae, species of Apollonias and Laurus are distinguished.

Within some genera as *Rubus* and *Morella* the resolution was higher than for *rbcL*.The two-loci combination (85 taxa/ 599 chs.) analyses resulted in a better resolved tree (Fig.2), where all families are clearly differenciated and more clades were supported with higher values.

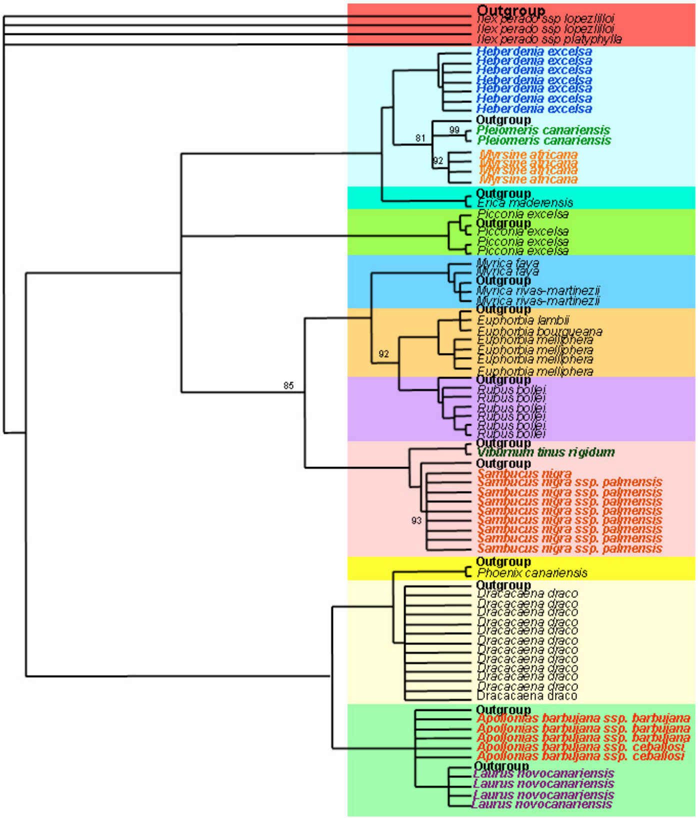

Figure.2. Bayesian consensus tree for 85 Macaronesian endemic taxa and based on two locus combined analyses (*rbcL+ matK*) with families color shaded and genera within the no- monotypic families colored differently.

B) Declic with Canarian tree data

We run declic (gap=10) on a *.fasta file for the 138 *rbcL* sequences representing the Canarian endemic trees. The results showed (Fig.3) that *declic* was able to find cliques for almost all the genera, with very few exceptions, as for the genera within Lauraceae and Myrsinaceae that were not well distinguished.

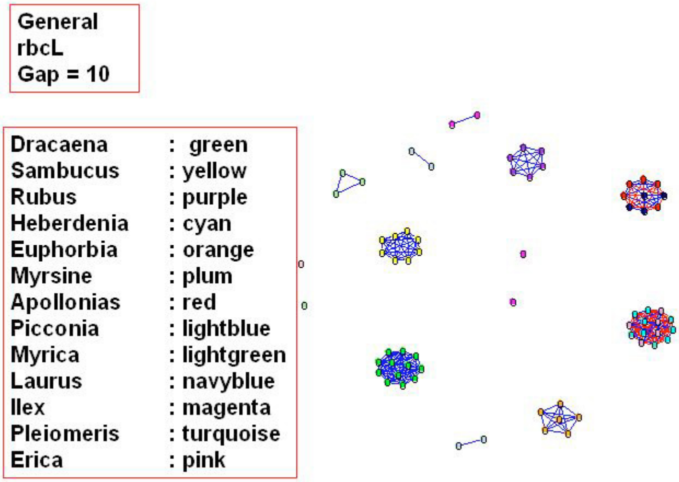

Figure.3. Decli´s graph output showing cliques for the majority of the genera (see legend box for family colors, one genera- one color)

3. Phylogenetic Diversity and "Tree of Life" for the Canarian flora.

With the data generated for the arBOLcan and Garajonay´s projects (Jaén Molina et al, 2010), we began to have some extensive data sets (especially for *rbcL*) of the Angiosperm endemic Canarian flora, that should allow us to construct a preliminary "Tree of Life" for the Canarian Flora (Fig.4) and to estimate the PD of the islands that are more represented (Gomera and Gran Canaria), once some strategic additional sampling is completed.

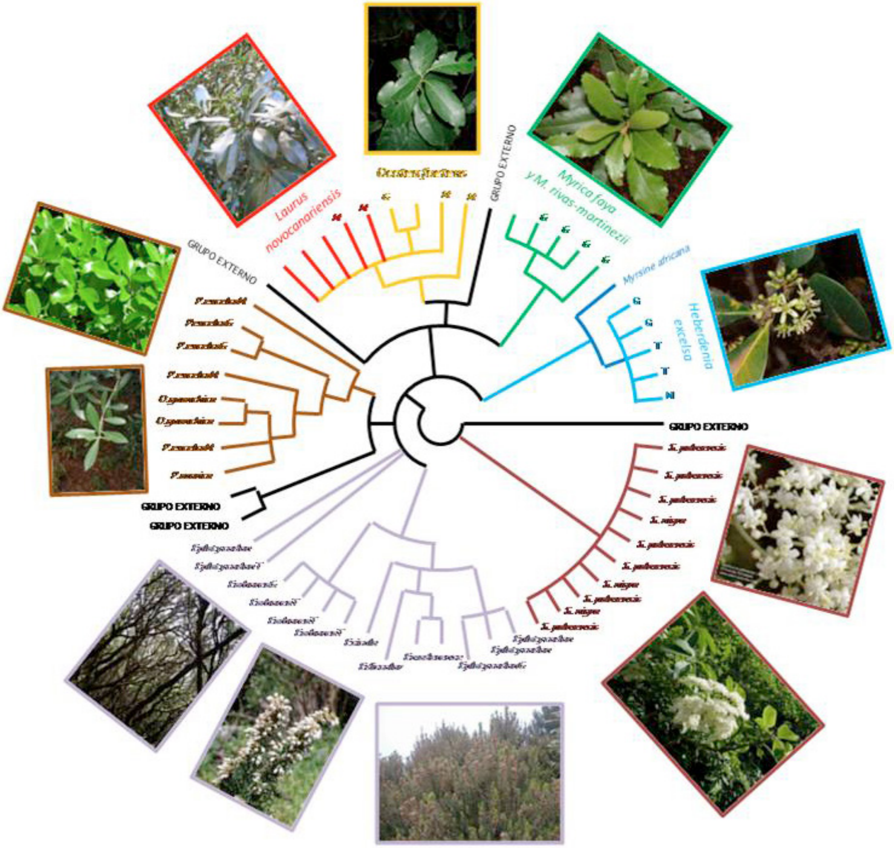

Figure 4. "Tree of Life" for some Macaronesian endemic trees based on *rbcL* sequences.

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Ruth Jaén-Molina has been combining different activities as a researcher of the Department of Molecular Biodiversity in addition to managing the DNA Bank of the Canarian Flora at the Jardín Botánico Canario “Viera y Clavijo”-Unidad Asociada CSIC (since May 2005). Her research interests are in molecular systematics, molecular phylogeny, molecular taxonomy, biogeography, and diversification of island plants, particularly from Macaronesia. The majority of the projects in which she participates have a multidisciplinary approach and the conservation of the species as a priority aim. Some examples of the emblematic groups that she has been studying are *Matthiola R.BR, Parolinia Webb, Dracaena Vand. ex L., Minuartia L., Heberdenia Banks ex A.DC., Myrsine L., Echium L.*, etc. The main goals of her research are to use molecular tools (DNA sequencing but also ISSR and microsatellites) to better understand the origin, evolution and biogeographical patterns of mainly, but not only, the species of the Canary Islands. For the past few years, she has been involved in projects to explore and develop the potential of DNA barcoding (*matK* & *rbcL*) as a practical tool for species identification as complementary to the classical taxonomy (Projects “Garajonay”, “Tree Bol” and “arBOLcan”).

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3

Evolutionary processes

Contribution of natural hybridization to plant evolution in oceanic islands (and elsewhere). Is a conceptual synthesis possible?

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1. Natural hybridization and evolution

Hybridization is a multifaceted topic particularly in plants. One of its faces is the possibility to contribute to generate diversity as a sort of shortcut that skips differentiation. In this context, we may speak of hybridization as the genetic exchange that takes place between populations or species distinguishable on the basis of one or more heritable characters. Hybridization is also a wide-ranging phenomenon, and as such it may have different possible evolutionary outcomes. Some of the most common include the production of sterile or unfit hybrids (F_1s) with no evolutionary consequences. Reinforcement of reproductive barriers as a result of contact between hybridizing species is another outcome, which does not alter their genetic integrities. The formation of the F_1s can be followed by repeated backcrossing towards the parents, which may result in the acquisition of useful genes from the other progenitor through introgression, but also could possibly give rise to new introgressed lineages. When hybridization between two populations occurs repeatedly in a limited space across time, we can speak of a hybrid zone. The most negative outcome in terms of diversity is the genetic assimilation or demographic swamping of one of the hybridizing species. The most positive one is the formation of a new hybrid lineage once F_1s acquire isolation from progenitors and restore fertility, either with or without the involvement of (alo)polyploidy.

To attempt to understand each case study involving hybridization or introgression, three aspects should be considered. The first is the search for patterns allowing the recognition of hybridization. Several patterns have been looked for; classically morphological intermediacy, malformed pollen, or irregular meiosis. With molecular markers, one can either seek for specific markers in the putative hybrids revealing the involvement of a specific progenitor or for incongruence between phylogenies from differently inherited markers. The second aspect is the genetic architecture of hybrids, or how the hybrids react to the so called 'genomic shock' of Barbara McClintock that results from merging two genomes of different background in the same cell. In model allopolyploid organisms, where this has been studied, there is evidence that the shock is due more to hybridity that to polyploidy (Hegarty et al., 2006). There are also a number of changes (loss of DNA, chromosomal rearrangements, repatterning of epigenetic marks, transposon activation) which however are largely unpredictable. The third aspect is the less appropriately considered in investigations. It relates to the ecological component of hybridization and introgression and the question of whether the formation and permanence of the hybrid has an adaptive significance.

Scanning the literature for case studies that involve hybridization or introgression, a wide range of situations is found, which is not unexpected given the variety of possible outcomes. It is useful, I think, to bear in mind how diverse and contrasted those outcomes can be so as to frame the space of possible patterns. Our own work in *Armeria* certainly provides an interesting example of extreme or bizarre patterns in the form of a taxonomic independent geographical structure of nuclear ribosomal ITS variation (Fuertes et al., 1999; Nieto Feliner et al. 2001). But there are much more.

2. Hybridization in the Canary Islands

Hybridization in the Canary Islands has been reported to contribute to shape the biota of the islands in different ways (e.g., Francisco-Ortega et al., 2000). The background for this proposal may be found in reports from the 1970s that plants in oceanic islands, specifically in Hawaii, frequently exhibit weak reproductive barriers, which facilitate hybridization (e.g., Gillet 1972) although this is not the case in Juan Fernandez, where natural hybridization is essentially rare (Stuessy and Crawford, 1998).

In the Canaries, to my knowledge, detailed studies assessing reproductive barriers are lacking although there are some reporting breeding and pollination systems. However, there have been a number of reports of natural hybridization based on eco-morpho-geographical patterns as well as an attempt to categorize the situations in this respect depending on the strength of pre- and postzygotic reproductive barriers in combination with the sympatry of congeneric species or lack thereof. For instance, in cases in which low pre- and postzygotic barriers are coupled with sympatry hybrid swarms may be formed, as in *Argyranthemum, Carlina, Echium* and *Micromeria* (Marrero, 1992).

Hybridization has been also approached from a conservation perspective, focusing at actual or potential hybridization between continental congeners and island endemics. Examples include *Phoenix canariensis*, *Arbutus canariensis* and *Senecio teneriffae* that hybridize with *Arbutus unedo*, *Phoenix dactylifera* and *Senecio vulgaris*, respectively. Such perspective has been also focused on native but aggressive congeners, one case being the swamping of *Argyranthemum coronopifolium* by *A. frutescens* (Vila et al., 2000; Francisco-Ortega et al., 2000).

The risk of demographic displacement or genetic assimilation of a rare endemic by a widespread congener does exist and has been reported in a number of studies starting in the 1990s (e.g. Levin et al. 1996). However, such potential risk should not override the need for a careful and detailed consideration of factors in every single study. Recent spatially-explicit simulation studies of the patterns arising from range expansions following colonizations have lead to unexpected results about genetic patterns emerging (Currat et al. 2008). One of those patterns is that, if interbreeding is not severely prevented, massive introgression may take place and that it does from the local into the invading species, particularly if the source of the invading population is far away.

In addition, thanks to the number of phylogenetically-oriented studies in the last two decades on groups from the Canary Islands, there are also reports of hybridization events inferred from incongruence between phylogenies from differently inherited molecular markers (Mort et al. 2002, Barber et al., 2007). However, they represent clues that need to be looked at with additional independent data.

Hybridization has been also invoked to add to the debate on the cause for the abundance of monophyletic Canarian groups in molecular phylogenies despite the proximity of the continent, which should have led to several independent colonization events. The dominant competing hypothesis is the niche pre-emption hypothesis. According to it, niche pre-emption by earlier already established colonizers would prevent success of later arrivals by competition. However, Emerson (2002) and Herben et al. (2005) have proposed a role for hybridization and introgression between independent immigrants in masking independent colonization events by close congeners.

There are studies that specifically aimed at detecting introgression, e.g. between *Ilex canariensis* and *Ilex perado*, that have rendered inconclusive results (Manen, 2004). There are also particular case studies that have been particularly well surveyed using different approaches such as morphometrics, experimental crossing programs, cytogenetics (classic and molecular) and fingerprinting data, at least since the 1970s. One example is *Argyranthemum sundingii*. This hybrid has been reported to have been originated several times and to be a hybrid species (Brochmann et al., 2000) but it has also inspired statements: "illustrates some of the many problems connected with the species concept in plants" (Borgen et al. 2003).

As a whole, one cannot avoid the impression that evidence is insufficient for identifying the specific role of hybridization in each case in which it has been reported in the Canary Islands. One possible reason is that the weakness of genetic barriers to hybridization in many island groups is a by-product of a small genetic differentiation in recently radiated species (Crawford et al. 1987; Kim et al. 1996). But whatever the causes, to answer the question of what has been the role of hybridization in the colonization and evolution of the Canary Islands we need more detailed studies, or as Jorgensen and Olesen (2001) put it "with our present knowledge, it is impossible to predict the importance of this factor in the colonization history of *Aeonium* and other island genera".

3. Considerations on the role of hybridization in plant evolution

Global importance, quantification

The following points revolve around the idea that hybridization ought to be more important in plant evolution than we usually admit even if its role has been increasingly acknowledged during the last decades with the advent of the molecular markers.

Quantification of the impact of hybridization across plant groups is an intriguing issue. Ellstrand et al. (1996) reported that it is not distributed at random across the angiosperms but concentrated in a relatively small number of families. I think it should be realized that these data are exclusively based on morphologically recognizable hybrids reported in five floras, albeit considered biological floras.

Chloroplast capture is an explanation for a pattern that has been reported not so infrequently along the past two decades in plants, namely, the occurrence of populations having plastid DNA haplotypes from one species and nuclear DNA from another. This is theoretically possible if there is an immigrant from a species A into a congeneric population of species B and the F_1 hybrid is followed by repeated backcrossing towards species B, the latter always acting as pollen donor. The final outcome would be individuals with cytoplasm haplotypes from species A and nuclear genes mostly from species B. This pattern likely requires adaptive explanations as well as some kind of cytoplasmic male sterility in the different hybrid generations that prevent them from selfing and makes them dependent on pollen from species B to set seeds. It also needs that the hybrids produce more seeds than the local species B, i.e. have a higher fitness.

Yet, despite these requirements, cases of cytoplasmic introgression and its extreme case (chloroplast capture) are not that rare in the literature even in the Canaries (Mort et al. 2002; Barber et al., 2007). And this raises the following question. Should not be nuclear introgression, i.e. just the transfer of a number of nuclear genes between two hybridizing species via repeated backcrossing, a much more frequent phenomenon than cytoplasm introgression? Nuclear introgression does not have restrictions such as backcrossing operating in the same direction but it is much more likely not to leave distinct traces. So, it is likely that many nuclear introgression histories have remained undetected because they did not leave a clear trace as chloroplast capture.

Another quantification of the impact of hybridization refers the cases of homoploid hybrid species. Rieseberg (1997) surveyed the botanical literature for possible cases and identified more than 50 putative examples from 20 families of seed plants. However, he concluded that only 17 examples had been rigorously tested with molecular markers and eight convincingly documented. The reduced figures raise the question of what happen to all the reports of hybridization in the literature that are not confirmed as hybrid species. Even assuming that hybrid speciation is infrequent and that many unviable or sterile hybrids don't leave traces beyond a F_1 generation, still a high number of cases, particularly those that were not detected without molecular markers, must have an outcome. It seems likely that the contribution of introgression remains largely undetected. But, there might be another important sac where case studies involving hybridization but not completing hybrid speciation may end up going. Hybrids occupying new niches but depending on external barriers for persistence do represent evolutionary meaningful units. Grant listed such scenario (isolation by external barriers) as one of the mechanisms to accomplish hybrid speciation in his book *Plant Speciation,* in the 1970s. But even if hybrid speciation is not completed, these cases are relevant ecologically. And it can be inferred that significant number of niches may be occupied by plants of hybrid origin that have not developed internal reproductive barriers.

Species concept

Although it may sound strange to outsiders that we still have to discuss about species concepts, interspecific gene flow remains one of the major problems to agree on a universal species concept. The possibility of looking more deeply into the genome in the last two decades has confirmed that species exchanging genes can maintain their integrity. In such cases, it is clear that the biological species concept is of no use. A species concept should be compatible with interspecific gene flow at least in those groups where it occurs frequently and Mallet (2007) has proposed to recognize species as multilocus genotypic clusters. Accordingly, species could be defined as distinguishable groups of genotypes that remain distinct in the face of potential or actual hybridization and gene flow. This focus on gene clusters that resist gene flow, in turn, stresses the idea about the utility of hybridization to study speciation genes (those contributing to cessation of gene flow; Rieseberg and Blackman 2010) or rather (because there's some controversy with this concept) for those responsible for maintaining the integrity of species despite gene flow.

Related issues

Because hybridization initially affects the whole genome (although introgression of genes following backcrossing may minimize this initial effect), it has many implications in the biology and evolution of plants. Some relevant issues clearly require further studies. I will only mention two of them.

Genetic basis for hybrid vigor (heterosis or hybrid superiority) is one. It has been explained through the suppression of recessive deleterious mutations accumulated in one parent by dominant alleles in the other, or by overdominance, by which some alleles in heterozygosis are particularly advantageous. Both hypotheses have problems and a recent study by the group of Enrico Coen claims that hybrid vigour may be related to variations in gene expression (Rosas et al., 2010). Specifically, they examine regulatory genes related to flower asymmetry in *Antirrhinum*, and found that there is what they call cryptic variation (no detectable phenotypic effects) between close species for regulatory genes –they speak of a plateau in gene expression-morphology space. However, F_1 hybrids may provoke shifting of genotypes off the plateau, and this may result in some increased performance for certain traits when multiple loci across the genome are considered.

Another relevant issue is the effects of hybridization on secondary metabolites. Secondary metabollites (SMs) are important primarily for antagonistic (herbivory) but also mutualistic relationships, and thus for plant fitness. Hybridization is suggested to contribute to the evolution of diversity of SMs because outcomes on the corresponding synthetic routes vary considerably. Hybrids may show transgressive segregation in SMs and herbivore resistance and it may increase the variation of SMs and affect herbivore resistance (Cheng et al., 2010).

In conclusion, certainly a conceptual synthesis for the Canary Islands (or elsewhere) is not possible because hybridization is too wide a phenomenon and too open for categorizing and predicting clear-cut patterns. However, due to its utter importance in plants (if only judged by its occurrence not by its consequences), multidisciplinary efforts are needed to understand in depth more case studies.

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Long-distance dispersal by wind

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Abstract

Global wind patterns influence dispersal and migration processes of aerial organisms, propagules and particles, which ultimately affect colonizations, invasions or spread of pathogens. However, studying how wind-mediated movements actually happen has been hampered so far by the lack of high resolution global wind data as well as the impossibility to track aerial movements. Although small organisms -or their propagules- are the best suited to use wind as dispersal vehicle, to demonstrate it by direct observation or experimentation is actually very hard. We have used shared species by remote areas in the Southern Hemisphere, and subsequently several big- relative to cryptogams or small invertebrates-birds, to test if wind is a more general dispersal vehicle than traditionally accepted. Our studies demonstrate that wind is crucial to explain why remote islands share so many species, and also that model organisms can be used to study why continents, areas with a much more complicated biogeographical history than isolated islands, have also a wealth of humble organisms in common.

Jesús Muñoz

Jesús Muñoz's current research interests are mainly three: moss systematics, ecological modeling, and long-distance dispersal. In his studies of moss systematic, he uses classical and new tools to study moss relationships. Quite different, although related, are his interests on ecological modeling and climate change consequences on biodiversity. He integrates museum data (presence-only) and survey data (presence/absence) with remote-sensing data to examine individual species' distributions that, combined, are used to explore richness patterns. Finally, his interest in long-distance dispersal by wind derives from the striking fact that localities separated by immense spans of ocean share many species of cryptogams as well as small animals. His group has demonstrated that landmasses in the Southern Hemisphere share more species of mosses, liverworts, lichens, pteridophytes, and tardigrades if they are connected by "wind highways", and that Gondwana breakup sequence shows no correlation with the number of shared species. Now his research group is replicating the experiment on the northern Atlantic, an area where they can also use, besides the number of species in common between landmasses, genetic similarity of a set of target species.

The surfing syngameon hypothesis: implications for the genetic and taxonomic diversities of the present Canarian flora

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At present, the molecular markers that form by far the largest genetic diversity database for the Canarian endemic Angiosperm flora are allozymes, and they encompass heterogeneously usable data for 316 populations and 115 taxa (Pérez de Paz and Caujapé Castells 2010 and in prep., Fig. 1a). Although data for other population genetic markers are fast becoming available, this means that any sound hypothesis on the genetic variation levels of the Canarian endemic flora must be based at present on allozymes. These data have experienced a substantial upward surge since the last review by Francisco-Ortega et al. (2000) (Fig. 1), and they are the subject of a meta-analysis (Pérez de Paz and Caujapé-Castells 2010 and in prep.) with 13 reproductive and biotic variables and with 15 a-biotic ones.

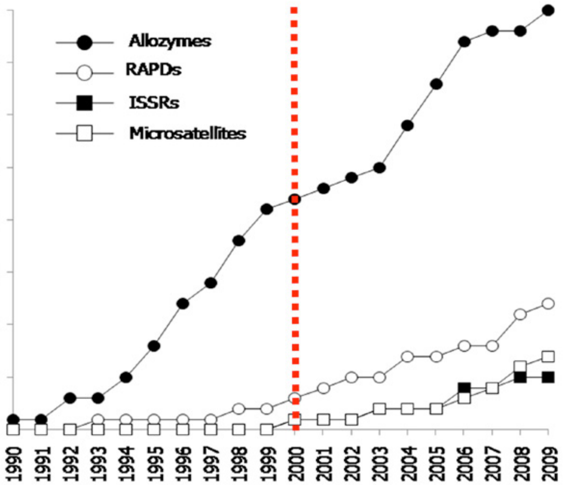

Figure 1. Cumulative plots of the evolution of population genetic knowledge for the Canarian flora (up to April 2009), as estimated from the number of papers published each year for each considered technique (taken from the author's database, only major molecular techniques considered). The red line in 2000 corresponds to the review of allozyme diversity by Francisco-Ortega et al. (2000). Modified from Fig. 3 in Caujapé-Castells (2011).

In this contribution, I will focus on just one of our a-biotic results. Namely, island age, area, and maximum height correlate with the levels of genetic diversity, that I chose to represent (Fig. 2) by linear regression analyses on observed heterozygosity, a parameter that measures the proportion of heterozygotes detected in each population i.e., the *real* degree of combination among alleles. Specifically, older, lower and larger Canarian islands contain higher levels of genetic admixture and variation. Critically, the genetic variation levels in the three easternmost islands are significantly higher than those in the four westernmost islands for all population genetic polymorphism parameters examined by us, as evidenced by Kruskal-Wallis tests.

I aim at presenting a hypothesis to explain how and why the Easternmost island group could have attained and maintained much higher levels of genetic variation than the Westernmost islands. Paraphrasing Carine and Schaeffer (2009), this question could be called the "Canarian genetic discontinuity enigma", which encompasses the traditionally asked (though still unsatisfactorily answered) question of "Why is the Canarian flora so variable genetically?" Albeit with remarkable exceptions, maybe after all the population genetic variation levels in the four westernmost Canaries are comparable to other archipelagos of the world, and the key to understand the high overall genetic diversity reported for the archipelago's flora lies on the apparently "simple" Easternmost islands.

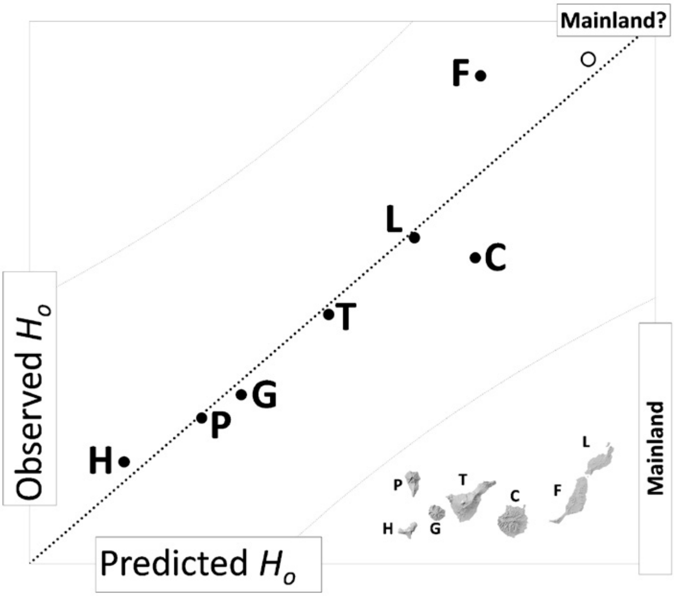

Figure 2. Relationship between the observed values of average observed heterozygosity per island (H_o) when it is predicted through linear regression by the logs of Island age, area, and maximum height (predictor variables). The observed values of H_o correspond to the averages across the populations distributed in the corresponding island, irrespective of their lineage ascription. Islands in their original geographic situation are at the bottom right corner. The unknown mainland's average H_o is tentatively represented by a white circle situated at the top right, although it could be lower in the graph if admixture in the Easternmost Canaries was in fact higher than on the mainland and encompassed genes from populations that are far in the mainland. This is one of the major weaknesses of the present population genetic database for the Canarian flora, and has thus far hindered the testing of one crucial question: are *all* the Canarian populations *really* less variable than those of their closest mainland congeners?.

Although, for brevity, I will focus especially on a-biotic factors to elaborate on the observed allozyme genetic diversity patterns, any conclusion needs to be fine-tuned by lineage-driven biotic factors, especially reproductive and ecological ones, as Pérez de Paz and collaborators posited in another of the contributions, and I will underscore at the end of this lecture.

Eastern islands

On a first examination, there are relevant differences in the present characteristics of the two groups of islands. The Easternmost Fuerteventura and Lanzarote are the oldest in the archipelago, with estimated ages of ac. 21 and 19 million years, and feature (at present) a practically shallow relief, except in the massifs of Famara and Jandía. Not unnaturally, highest endemic species richness and range-size rarities concentrate in these remarkable geographical features (Reyes-Betancort et al. 2008). Also, both Fuerteventura and Lanzarote are geographically very close to the African mainland, and data in Ortiz et al. (2002) show that Saharan dust deposition rates are much higher on these two islands than in the westernmost ones. Thus, the probability of colonization must also be higher than in other islands.

The Westernmost islands have radically different geo-morphological characteristics. They are much farther from the mainland and younger (especially El Hierro), they have a much more rugged relief and greater height, so they host many more ecological areas (esp. Tenerife and la Palma), and feature more complex ravine networks (especially La Gomera). Many of these characteristics create proper conditions for speciation. Ortiz et al.'s (2002) paper also shows that dust deposition rates decrease abruptly from La Gomera Westwards.

Gran Canaria encompasses features from both Eastern and Western island types. It is the third oldest island in the archipelago, quite close to Africa and to the two Easternmost islands, and its Easternmost part is quite flat. However, it features a considerable altitude, and its central and Western parts are very complex geographically, with a wealth of ravines and chasms that make botanical exploration difficult and possibly impose limits to gene flow.

We also have to keep in mind that these islands have not always been as we know them today. According to geological data discussed in Stillman (1999), at least Fuerteventura may have attained a height of at least 3,500 m in the past, meaning that its physical appearance, and its biodiversity contents may have been radically different to what we know today. Although its present orography seems to have been changed only a little in the last million years, a high palaeo-Fuerteventura may have furnished biodiversity adapted to elevations to other high islands in the archipelago.

A surfing syngameon?

Considering the historical-geographical setting of the Canaries and the chronological sequence of emergence of the islands, a feasible explanation of how the Eastern islands attained and maintained their much higher average levels of population genetic variation in comparison to the westernmost ones is that they could be acting as genetic variation "melting pots", where frequent colonizations from the African mainland, a relative ecological uniformity, and a generally shallow relief gave rise to rife genetic admixture and high levels of genetic diversity. Given enough phylogenetic closeness between the colonizing genomes, these colonization processes likely generated syngameons (*sensu* Grant 1957) derived from gene flow among genotypes previously isolated in the mainland, and consisting of morpho-species, or of incipient species that in some cases still retain crossing compatibility.

The surfing syngameon hypothesis contends that predominance of processes entailing high levels of gene flow in Fuerteventura and Lanzarote (largely facilitated by the generalized absence of relevant geographic barriers in these islands in the last million years), could be a factor to consider, along with island age and extinction, to explain the high levels of genetic variation and the low number of endemics that they host. This is not to say that extinction has not played a role in the present botanical composition of these islands; however, a profuse gene flow can be an additional and so far overlooked factor to consider in explaining these patterns.

Although there are other geographic regions from where propagules can colonize the Western islands, genetic variation furnished by founder stocks from syngameons in the Eastern islands (or the mainland) may have been critically important to overcome selective pressures and the deleterious consequences of further genetic drift in the Western islands; subsequently, the populations could develop adaptations to a particular environment, and colonize similar habitats within these islands, or in other islands. Despite La Gomera is the oldest in the western group, the emergence of Tenerife could have been instrumental to increase colonization frequency in that island. Also, if palaeo-Fuerteventura was indeed a much higher island than at present (more than 3,500 m, Stilman 1999), taxa adapted to its mountain tops could have colonized similar habitats in other islands with similar altitudinal zones at the time, thereby contributing to their taxonomic diversity.

Since the predominant evolutionary processes within the Western islands entail speciation and cessation of gene flow, we would expect a much higher number of endemics in these islands than in Fuerteventura and Lanzarote, as is the case. As outlined above, Gran Canaria would have both a "promiscuous", syngameon side (especially in its Eastern face), and a "virtuous", non-admixture side where speciation processes are prevalent, especially in its summits and ravines from the westernmost areas.

As predicted by population genetic theory, such syngameons would "surf", because the East-West colonization wave is associated with a decrease in genetic diversity levels from the wave origin in the eastern islands (at the syngameons themselves) to the wave end (somewhere in the western islands). Of course, this picture would be complemented (in the same or different epochs, and largely conditioned by variations in the climatic and tectonic conditions), by extinction (especially in the Easternmost islands), back-colonizations of the mainland and gene flow between mainland and eastern islands (Caujapé-Castells 2004, 2011; García-Verdugo et al. with *Olea*), within-island colonization and speciation, and colonization from other geographical enclaves.

Context

This "surfing syngameon" hypothesis does not conflict with eventual competition preventing multiple colonizations in most radiating monophyletic lineages (i. e., the 'niche preemption' hypothesis, Silvertown 2004), because most colonizers would be sufficiently close genetically, and admixture processes in the Easternmost islands would have happened *earlier* than radiations in the Easternmost islands. The theory is thus compatible with the prevalent pattern of monophyly in the Canarian flora. Indeed, recent reviews of introgression (Currat et al., 2008; Pétit and Excoffier, 2009) find a significant negative correlation between intra- and inter-specific hybridization, emphasizing a major role of gene flow in the maintenance of species cohesion and in promoting rapid monophyly.

The general possibility of multiple colonisations and hybridization before radiations took place was first suggested by Francisco-Ortega et al. (2000) to explain the apparent contrast between the high population genetic diversity in Canarian plant endemics and their predominantly monophyletic origin. Subsequently, other theories (Herbén et al. 2005, Saunders and Gibson 2005) have favored hybridization over inter-specific competition (Silvertown 2004) to account for the monophyly landslide.

The concept of syngameon itself is not new either, as it was first defined by Grant (1957) as "the sum total of species or semi-species linked by frequent or occasional hybridization in nature; [hence] a hybridizing group of species . . . ". Furthermore, many recent molecular investigations have invoked a syngameon either directly (e. g. Van Oppen et al. 2001 with the coral genus *Acropora*, Seehausen 2004 with African cychlid fishes), or indirectly (Herbén et al. 2004; Saunders and Gibson 2005 in the Canarian Flora, or Curto and Meimberg 2010 with Canarian *Micromeria*) to refer to a population or meta-population that contains great morphological and genetic variation, and hosts high levels of gene flow.

Apart from a funny name, the surfing syngameon hypothesis for the Canarian flora introduces two new elements of discussion:

(i). It contends that the variations in the geo-morphological features of islands through geological time (see Whittaker et al. 2008) also influence the genetic diversity levels that they may host;

(ii). It predicts that the high genetic variation levels of the lineages that formed syngameons in the Easternmost islands after they attained their present geomorphological conformation could have been determinant to colonization success in the Westernmost islands.

It is important to outline that the hypothesis is not proposed as a general one to explain the colonization of the Canaries.

Elements of a future research plan

To test this hypothesis, several steps are necessary, among which the following three seem especially important:

1. Selecting candidate groups for assessing genetic diversity patterns & other.

According to the present molecular phylogenies, the ca. 35% of the groups examined with molecular phylogenies have a Mediterranean origin, and only a 25% a West African origin (Carine et al. 2009, Caujapé-Castells 2011), so we will first have to restrict our selection within the latter. However, it may be questioned how many of the Canarian endemic lineages presently considered of Mediterranean origin are so because of insufficient sampling, and whether a West African origin would be revealed if this region was properly explored and represented (e.g. *Micromeria*, as intimated by Curto and Meimberg, 2010 and pers. comm.). Discrimination between real and spurious monophyly resulting from poor sampling could still be necessary in many cases.

2. Seeking evidence of hybridization

The genome of the radiating taxa derived from hybridization between immediate ancestors may carry conflicting information in chloroplastic and nuclear markers, as in the (allopolyploid) Hawaiian silversword alliance, which appears to be derived from a hybrid population between two or more species of North American tarweed (Baldwin et al. 1991, Baldwin and Sanderson 1998, Barrier et al. 1999). Although there are clear cases of discordance in the Canarian flora (e.g., Sideritis, Barber et al. 2007), many Canarian endemics are diploid, a fact that may hinder detection of hybridization events. Application of molecular cytogenetic techniques such as GISH and FISH is unprecedented in the Canaries, but may give rise soon to promising evidence to reject, support, or fine-tune the syngameon hypothesis, thanks to the support of Pep Toni's and Marcela's group (Rosato et al. 2008).

3. Assessing if genetic diversity arose in a syngameon phase, and furnishing evidence of generation of functional diversity by hybridization

We should show that the groups for which the Easternmost island endemics are consistently basal in the phylogenies are also genetically more variable. This is difficult in the Canarian Flora because extinction may have wiped out ancestral populations in Fuerteventura, Lanzarote or mainland West Africa. If such cases were detected, generation and inheritance of functional diversity should be tested by reproductive biology experiments.

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Preliminary investigations of correlated diversification between plants and their associated arbuscular mycorrhizal fungi in Macaronesia

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Introduction

Arbuscular mycorrhizas (AM) are symbiotic associations between land plants and fungi of the phylum Glomeromycota. The fungi are obligate symbionts that receive plant carbon in the form of photosynthates and typically provide some benefit in return, such as improved nutrient (especially phosphorus) uptake (Smith and Read, 2008). Although most plants can live without AM fungi under laboratory conditions, their roots are consistently colonized by AM fungi in natural ecosystems (Trappe, 1987). Recent estimates find up to 90% of land plants forming AM associations (Smith and Read 2008). This symbiosis is an ancient one; fossil evidence for mycorrhizas dates back approximately 455 million years, to the same era when plants invaded land (Redecker et al., 2000). In addition, the genes necessary for colonization of roots by AM fungi are present throughout the land plant phylogeny and appear to have been vertically inherited since the origin of land plants (Wang et al., 2010). Thus, understanding the diversification patterns of AM fungi is likely to be an important aspect of understanding patterns of plant speciation.

Patterns of AM fungal diversification are likely to be complex given their apparent lack of strict host specificity. The level of fitness improvement plants experience in association with a given AM fungal taxon depends on ecological conditions (Bever, 2002). Soil nutrients, precipitation, plant community diversity, and the presence of exotic plants, for example, influence the availability and relative benefits of AM fungi (Hawkes et al., 2006; Landis et al., 2004; Yang et al., 2010, Kivlin and Hawkes 2011). Ecological specificity can lead to increased mutualism when both plants and AM fungi become adapted to local site conditions (Johnson et al., 2010). Specific plant-fungal combinations may also be stabilized if there is a self-reinforcing mutualism (Kiers and van der Heijden, 2006), although this remains speculative (Helgason and Fitter, 2009). Alternatively, partner choice with trade-based mechanisms may operate for both the plants and the fungi; the plants are simultaneously colonized by multiple AM fungi that can be selected or sanctioned based on their provision of different benefits, while the fungi are similarly able to form a network across multiple host plants and can be selective based on carbon provisioning by the plant (Sachs et al., 2004; Johnson, 2010).

Given the nature of the AM symbiosis, oceanic islands likely provide the best model system in which to study speciation patterns of AM fungi. Compared to continental conditions, competition from other species is generally reduced and allows the evaluation of individual speciation drivers (Silvertown, 2004). Research on AM fungi benefits particularly from the reduced number of compatible plant hosts. On the continent, fungal speciation may be the result of numerous species interactions, whereas on islands this is likely to be confounded by many fewer interactions. The reduced complexity of island evolution is perhaps the only means to evaluate drivers of AM fungal speciation in non-laboratory settings.

Among oceanic islands, the Macaronesian archipelagos are well suited to study AM fungal evolution. The islands' wide range of distances to continental habitats, from close proximity to hundreds of kilometers separation, and their diversity of geological ages, from 0.8 to 21 million years (Carracedo et al., 2002), present a unique opportunity to explore AM fungal diversifications. Macaronesia's stratified ecosystems also provide an opportunity to study the impact of both ecological habitats and host preference on plant-AM fungus evolution. Several major ecological habitat zones are present, each defined by elevation and precipitation levels (Fernandez Palacios and Whittaker, 2008). The same plant genera are present across most ecological habitat zones, with largely unique species in each habitat and a few that are found across multiple habitats.

Determining the patterns of host association in AM fungi and their underlying drivers is the primary aim of our investigation. We hypothesize the existence of three drivers of non-random associations between the plants and fungi: geographic constraints, ecological habitat, and phylogenetic partner specificity. None of these potential drivers is mutually exclusive in its influence on AM formation, and we hope to distinguish to what degree each may explain the patterns of fungal symbiont diversification. Here we summarize some preliminary results of investigations of AM fungi associated with host plants in two genera, *Plantago* and *Tolpis*, both of which are represented by multiple endemic species in Macaronesia.

Hypotheses

We are examining the role of three potential factors in the diversification of arbuscular mycorrhizal fungi in Macaronesia: geography, ecological specificity, and phylogenetic partner specificity. We hypothesize that each of these drivers will be correlated with non-random patterns of AM fungi. Specifically, we are testing the following hypotheses:

H_1: AM fungal and host plant diversifications are correlated via ecological habitat.

H_2: AM fungal and host plant diversifications are correlated via geographic constraints.

H_3: AM fungal and host plant diversifications are correlated via phylogenetic partner specificity.

Methods

We used four criteria to select focal Macaronesian host plant groups: (1) species occur on multiple islands and ecological zones; (2) the groups or their relatives are known to have mycorrhizal associations; (3) continental relatives should be known or at least there should be reliable information about the putative relatives; and (4) plant material, including roots, should be readily accessible in the field. Two genera, *Tolpis* (Asteraceae) and *Plantago* (Plantaginaceae), fit these criteria.

Clarifying full AM fungal diversity in root samples is currently hindered by a lack of DNA markers that are both applicable within the entire phylum and at the same time variable enough to differentiate all fungal taxa in a root sample (Stockinger et al., 2010). A preliminary study on highly variable nuclear and mitochondrial DNA markers was therefore performed. We found several genomic markers sufficiently variable to differentiate AM fungal genotypes in Macaronesia, while at the same time being amply represented in sequence databases for taxon identification. Two markers – nuclear ITS and mitochondrial LSU – were selected to generate molecular phylogenies of AM fungal symbionts. Comprehensive amplification of AM fungal genotypes in *Plantago* and *Tolpis* roots was performed by nested sets of multiplex PCR reactions, with general fungal amplifications followed by amplifications specific to the Glomeromycota. Subsequent cloning of amplicons and Sanger-sequencing was performed for several samples. After computationally filtering out contamination sequences of bacteria and fungal pathogens, statistical analyses to test the hypotheses were performed.

Correlated symbiont diversification was concluded from significant clustering of terminal taxa in observed AM fungal phylogenies compared to phylogenies with randomized terminal taxa. The randomized phylogenies were generated via bootstrapping at observed taxon frequencies. Statistical significance was determined by directional one-sample t-tests at significance levels of $\alpha = 0.01$. The tests were performed on AM fungal phylogenies, coded according to the three hypothesized drivers of AM fungal diversification. Tests of plant-fungus relationships were done in three ways: (1) in selected ecological zones to test for correlated symbiont diversification by ecological specificity, (2) on selected islands to test for correlated symbiont diversification by geographical constraints, and (3) of closely related host plants to test for correlated symbiont diversification by phylogenetic partner specificity.

Preliminary Results And Discussion

Non-random associations were found for AM fungi in both host plant genera, although the underlying drivers differed (Figure 1). AM fungi associated with *Plantago* grouped by geographic region and ecological habitat at statistically significant levels, but not by phylogenetic partner specificity. *Tolpis* associations with AM fungi were constrained by both geography and phylogenetic partner specificity at significant levels, but not by ecological habitat. The correlation between geography and AM fungal phylogeny in *Plantago* is shown in Figure 2, where AM fungal sequences grouped together by island. When considering habitats, Figure 3 shows a clear separation of AM fungi colonizing *Tolpis* species native to laurel and pine forests, while those colonizing *Tolpis* species of the lowland scrub zone are intermixed with AM fungi of the other two ecological zones. This pattern could be explained by the ecological and floristic homogeneity within the laurel and pine habitats. In contrast, the lowland scrub zone is inhabited by various native and non-native plant species and is the main zone of human settlement; many of its sites are disturbed and its flora influenced by anthropogenic nitrogen input. Therefore, the lowland scrub zone is likely to contain higher AM fungal heterogeneity. In contrast, the laurel and pine forests are relatively undisturbed habitats with largely intact native floras, disturbed by only a few introduced species.

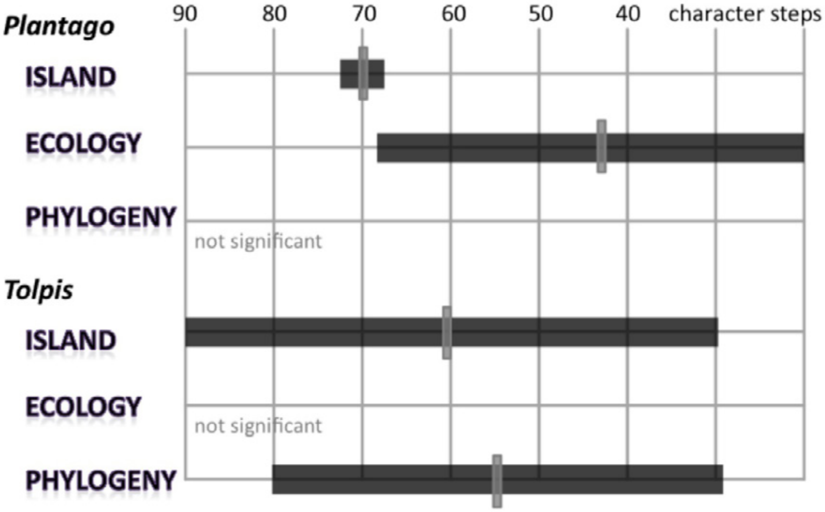


Figure 1. Summary of results of directional one-sample t-tests to evaluate significant correlations between island distribution, ecological zone or phylogeny in AM fungi and their plant hosts. The tests were performed on character reconstruction step numbers of AM fungal phylogenies; black bars represent the null distribution of randomized grey vertical bars indicate the values of the test statistic.

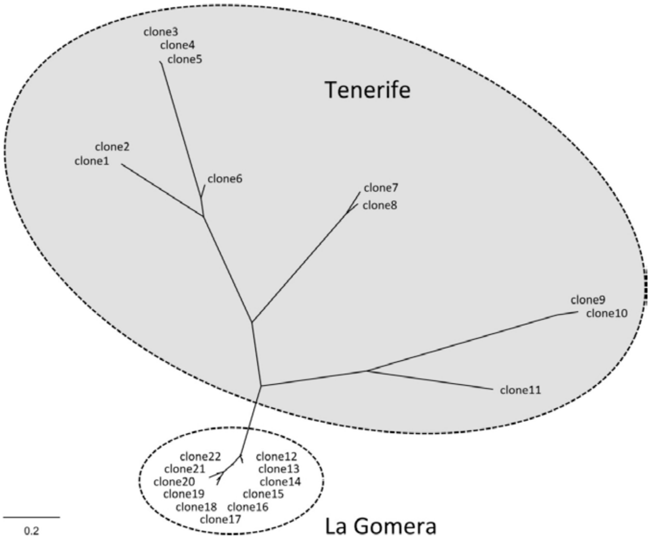


Figure 2. Maximum likelihood tree of AM fungi associated with *Plantago arborescens* from the laurel forest ecological zone on the islands of La Gomera and Tenerife.

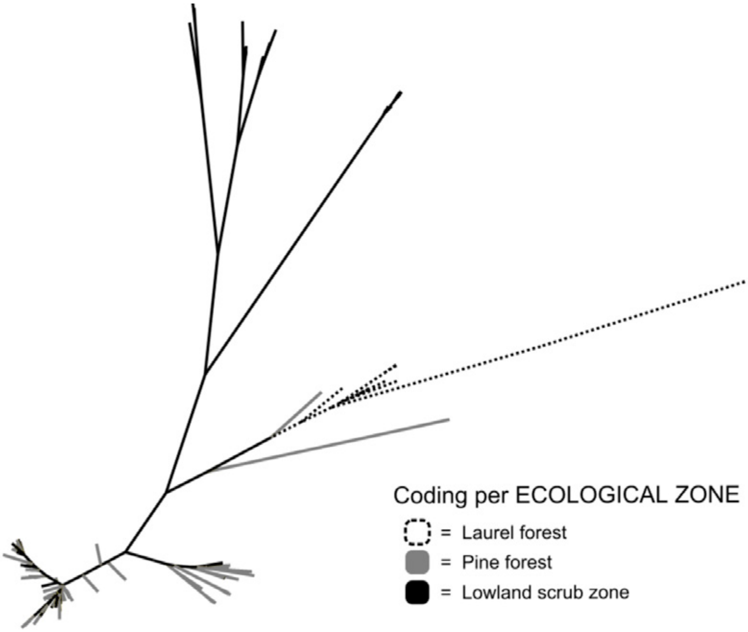


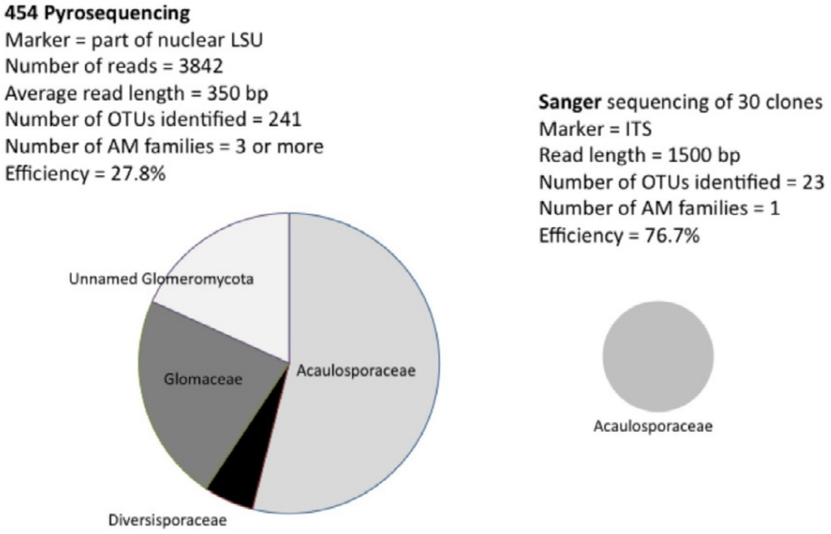
Figure 3. ML tree of AM fungi colonizing roots of four Tolpis species. The four plant hosts are *T. coronopifolia* from the lowland scrub of Tenerife, *T. laciniata* from the laurel forest on La Palma, *T. lagopoda* from the pine forest on La Palma, and *Tolpis sp. nov.* from the lowland scrub on La Gomera. The tree branches are shaded by ecological zone (black = lowland scrub zone, dotted = Laurel forest, grey = Pine forest).

Preliminary Conclusions

Based on our preliminary data, we conclude that there are non-random associations in arbuscular mycorrhizal fungi of Macaronesia. All three hypothesized drivers of non-random AM formation, ecology, geography, and phylogeny, appear to have contributed to AM fungal diversification in these islands, although the relative importance varied between the host plant genera.

Future Directions

Our preliminary studies are very encouraging, but two areas of concern with these early studies are that (1) we have not been able to fully resolve phylogenetic relationships among plant hosts, and (2) we may not have adequately sampled the AM fungal diversity. To overcome these issues we are moving into a next-generation sequencing approach to uncover more variation. For the plant hosts, we are sequencing complete plastid genomes and low copy nuclear genes. In the case of AM fungi we are using 454 pyrosequencing to sequence amplicons from nuclear ribosomal and mitochondrial regions. Our preliminary pyrosequencing results from a single root sample reveal that we can uncover a much greater diversity of AM fungal taxa by this approach, including multiple families in the Glomeromycota that were not evident with traditional cloning and sequencing (Figure 4).



454 Pyrosequencing
Marker = part of nuclear LSU
Number of reads = 3842
Average read length = 350 bp
Number of OTUs identified = 241
Number of AM families = 3 or more
Efficiency = 27.8%

Sanger sequencing of 30 clones
Marker = ITS
Read length = 1500 bp
Number of OTUs identified = 23
Number of AM families = 1
Efficiency = 76.7%

Figure 4. Comparison of AM fungal diversity detected in a root sample of *Tolpis coronopifolia* based on 454 pyrosequencing of amplicons versus Sanger sequencing of 30 clones.

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Does the Linnean shortfall explain the Azores diversity enigma?

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Isolated oceanic archipelagos have long been considered natural laboratories for the study of evolution, ideally suited for in-situ studies of speciation processes. The recent availability of checklist data for many archipelagos worldwide has stimulated research on the macroecology and evolution of island biota and this has led to the development of new models to explain the distribution of endemic diversity on islands. Intriguingly, the angiosperm flora of the Azores is not readily accommodated by such models and this is because it is distinctive both in terms of the low number and extent of evolutionary radiations and in the widespread distribution of a high proportion of its endemic species. The unusual characteristics of the Azores flora has been known for a considerable time. Indeed, following the publication of an enumeration of the Azores flora by Watson, Darwin wrote to Hooker 'Watson's Paper on [the] Azores has surprised me much; do you not think it odd, the fewness of peculiar species…?' [December 25th, 1844]. Carine and Schaefer (2010) referred to the unusual features of the Azorean endemic flora as 'The Azores Diversity Enigma'.

Based on a review of distribution data and available phylogenetic data, Carine and Schaefer (2010) concluded that neither island age, age of endemic lineages nor ecological diversity, at least considered individually, could explain the patterns observed in the Azores flora. Rather they offered two possible explanations. The first was that palaeoclimatic conditions across Macaronesia may have played an important role in generating diversity patterns, with a more unstable climate regime in the Canaries (e.g. Ortiz et al., 2006) promoting speciation through dispersal mediated allopatry, a process less evident in the Azores where the climate may have been more stable. This hypothesis was subsequently challenged by Cardoso et al. (2010) who argued, in the context of similar distribution patterns for endemic spiders, that human-mediated extinctions coupled with the high dispersal ability of Azorean endemic lineages offered a more plausible explanation for the patterns observed.

The second explanation offered by Carine and Schaefer, and one not addressed by Cardoso et al (2010), concerned the extent of taxonomic knowledge of the Azorean flora and specifically the possibility that our knowledge of the Azores flora may be inadequate – the so called Linnean shortfall of Brown & Lomolino (1998). Whilst recent checklists for each of the Macaronesian archipelagos and a regional checklist are available, there is no Flora for the entire region despite the significance and vulnerability of its plant diversity. Only the Madeiran archipelago benefits from a complete, recent, critical flora and whilst the Azores was included in Flora Europaea, it is notable that that work tended to adopt a broad circumscription of species (Väre et al., 2003). The possibility that the lack of a consistent and critical taxonomic framework for the floras of the Macaronesian archipelagos may contribute to the differences observed between archipelagos and to the apparent distinctiveness of the Azorean endemic flora cannot be ruled out.

To test the possibility that undetected diversity in the Azores flora may contribute to the distinctive patterns observed, Schaefer et al (2011) assessed molecular variation within a range of Azorean endemic plant lineages to determine whether patterns of molecular diversity are congruent with current species circumscriptions. Molecular variation in five groups was assessed which collectively contain eight accepted endemic taxa (approximately 11% of the endemic flora). The study focussed on variation in the ITS region to provide a point of comparison with studies of other archipelagos. In addition, for some lineages, variation in chloroplast markers was also determined.

The focal taxa included those for which current taxonomic circumscriptions suggest a single widespread Azorean endemic species (*Angelica lignescens* J. P. Reduron & Danton, Apiaceae and *Azorina vidalii* (H. C. Watson) Feer, Campanulaceae), those that exhibit a pattern of dispersal mediated allopatry (*Euphorbia stygiana* H. C. Watson, Euphorbiaceae, with two subspecies) and lineages that exhibit a pattern consistent with ecological speciation (*Ammi*, Apiaceae, with two endemic species and *Pericallis malvifolia* (L'Hér) B. Nord., Asteraceae with two endemic subspecies). Samples were collected on all Azorean islands except Graciosa where only one of the studied taxa (*A. vidalii*) is recorded. Wherever feasible, multiple populations per island and multiple individuals per population were sampled.

ITS variation was observed in all five lineages surveyed with at least some of the ITS diversity within each lineage not reflected in current taxon circumscriptions. A strong geographical pattern was apparent in most of the lineages examined. In *A. lignescens,* for example, endemic ribotypes were observed on three of the five islands from which this species is known (Flores, Terceira, São Miguel; Fig. 1). Faial and Pico populations shared the other ribotype identified, a pattern that is readily explained by the fact that these two islands are today in close proximity (c. 6 km) and would have formed a single island during the last glacial maximum given that the channel separating them is only 80m deep.

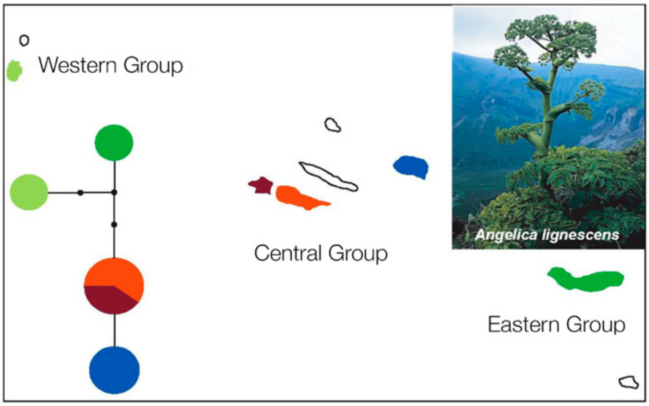

Figure 1. Distribution and ribotype network for the Azorean endemic *Angelica lignescens* based on the ITS region produced with TCS vs. 1.2.1. Colours of islands correspond to those used in the network diagram. The size of circles in the network diagram is proportional to the number of individuals possessing each ribotype. Black circles are unsampled haplotypes (from Schaefer et al., 2011).

Azorina vidalii exhibited the most complex pattern of ITS variation. No island was characterised by an endemic ribotype and there was extensive ribotype sharing among islands (Fig. 2). However, some geographical structure was evident with single island endemics on São Jorge and Pico and one ribotype confined to São Jorge, Terceira and Faial in the central archipelago. The distributions of the other shared ribotypes are less readily explained by geography with one distributed on Flores, Corvo (western archipelago) and Santa Maria (easternmost island) and the other on Pico (central archipelago) and São Miguel (eastern archipelago). The patterns observed are consistent with the idea that this attractive plant was moved around the archipelago by humans. However, the fact that some geographical structure was evident suggested that whilst the true extent of the native range of *A. vidalii* is likely to be difficult to ascertain, it is unlikely that it was restricted to Flores prior to human impact as some authors have suggested.

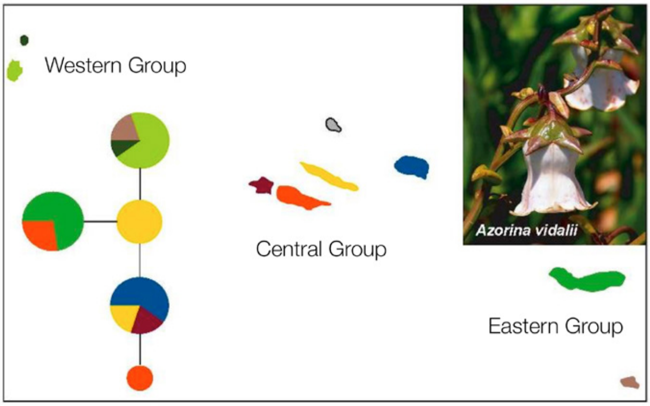

Figure 2. Distribution and ribotype network for the Azorean endemic *Azorina vidalii* based on the ITS region produced with TCS vs. 1.2.1. Colours of islands correspond to those used in the network diagram and the size of circles is proportional to the number of individuals possessing each ribotype. Graciosa (shaded grey) was not sampled in the analysis (from Schaefer et al., 2011).

It was notable that of the four lineages sampled that occur on Santa Maria, three exhibited ribotypes that were unique for the island. The distinctiveness of populations from Terceira in relation to the other central group islands was also highlighted by the data, factors that should be considered in conservation priorization.

The results of the Schaefer et al (2011) study indicated that dispersal mediated allopatry has been much more extensive in endemic Azorean lineages than has previously been suggested and that the considerable distances between some of the islands or island groups have been effective barriers to gene flow. This challenges the hypothesis that Azorean endemic lineages are likely to be good dispersers, capable of maintaining gene flow between islands (Cardoso et al., 2010). The data also suggest a much higher incidence of single island endemism and a lower proportion of widespread taxa in the endemic flora than current species circumscriptions would suggest. Thus, current circumscriptions suggest only one single island endemic among the eight taxa surveyed (12%) whereas for ITS ribotypes, 17 of the 24 ribotypes identified (71%) appear to be endemic to a single island. The high incidence of single island endemics among ribotypes is similar to the patterns observed for other oceanic island archipelago floras based on current species circumscriptions (Carine & Schaefer, 2010, Fig. 3).

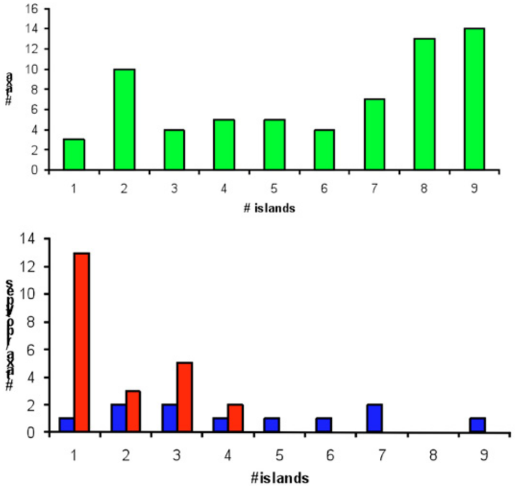

Figure 3. Per-K-island (PKI) distributions for the Azores flora. Current concepts (green bars) for the endemic flora indicate that a large proportion of endemic taxa are widespread (distributed on 8 or 9 islands) and that there are few single island endemics (SIEs). This differs from the distribution observed in other oceanic island floras where the proportion of SIEs exceeds other classes (Carine & Schaefer, 2010). The blue bars indicate the PKI distribution for taxa sampled by Schaefer et al (2011) and the red bars indicate the ribotype PKI distribution. The latter is similar to the typical distribution observed for oceanic archipelago floras.

Plotting percentage Single Island Endemics against island Age reveals a linear relationship, similar to that observed for other Azorean groups (Fig. 4). Molecular data therefore suggest that diversity patterns in the Azores endemic flora may not be as distinctive as was previously thought and that they may be more readily accommodated by recent theories to explain the diversity and diversification of island biota (Whittaker et al., 2008; Borges & Hortal, 2009).

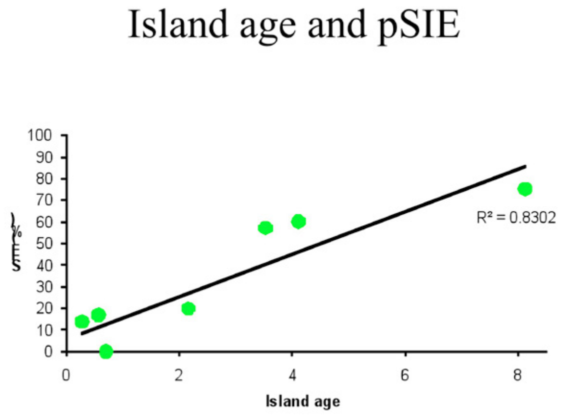

Figure 4. Relationship between island age and the percentage SIE for the Schaefer et al (2011) ribotype data. The linear relationship observed is similar to the pattern Borges and Hortal (2009) observed in Azorean beetles and molluscs and is consistent with the predictions of the General Dynamic Theory of Oceanic Island Biogeography (Whittaker et al., 2008)

From a morphological perspective, the molecular data are congruent with the subspecies classification of *E. stygiana* proposed by Schaefer (2003). Within *A. lignescens,* variation in seed morphology first alluded to by Press and Dias (1998) is consistent with the patterns of ribotype variation observed. These observations suggest that the molecular data may be revealing taxa that are also morphologically characterisable. Further morphological work on the other lineages examined in now necessary and work on *Pericallis* is underway.

Herbarium collections are the foundation for our knowledge of plant diversity. They provide a globally distributed infrastructure for discovering, documenting and describing taxonomic diversity and their importance was emphasised in a recent study by Bebber et al (2010). In the case of the Azores, many apparently widespread endemic taxa are actually known from relatively few, small populations on each of their islands of occupancy. A survey of herbarium collections of Azorean endemic plants (K. Jones, unpublished data) indicates that the herbarium material available when Flora Europaea was produced would have provided a biased and inadequate sample of Azorean diversity. Since then, thanks to the efforts of botanists at AZU and AZB and at other institutions, the collections resource for studying the Azores flora has improved significantly.

The results of the Schaefer et al (2011) study suggest that the Linnean shortfall – inadequacies in our understanding in patterns of endemic diversity in the Azores flora, may explain, at least in part, the Azores Diversity Enigma. The time is now ripe for a new flora for the region, critically reassessing patterns of diversity both to inform conservation of this fragile flora and enhance our understanding of the evolution of the region's endemic diversity.

Acknowledgments

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4

Plant life out of Macaronesia

The Reproductive Biology of Island Plants: the Juan Fernández and Canary Islands

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I. Introduction

Endemic insular floras constitute about 25% of the species of vascular plants occurring on about 5% of the Earth's land surface: 5-10% of the endemic species are highly threatened. We recognize that many factors impact the origin and evolution of island plants; in the present review we focus on reproductive biology (RB). Island natives are excellent subjects for studying the evolution of reproductive systems. Especially interesting and informative is how colonizing ancestors adapt to the insular setting, and meet such challenges as the loss of biotic pollinators and compatible mates. RB is critical in the establishment and diversification of sexually reproducing insular lineages, making knowledge of RB critical in developing effective conservation strategies.

This paper provides a general overview of the RB of island plants, and focuses on studies needed to address questions about their evolution and reproductive biology. We will emphasize the Canary Islands (CAN) flora, but will make some comparisons with the RB of native plants of the Juan Fernández (JF) Islands. We hope these comparisons will produce hypotheses to consider for islands in general. Our use of RB will include pollination biology, breeding systems (features promoting selfing or outcrossing), and mating system (level of selfing and outcrossing) (Neal & Anderson 2005).

Figure1. El Teide, at over 3,700m asl, is the highest point in Spain, and home to many Canary Island endemics.

II. Comparisons of Juan Fernández and Canary Islands

The CAN are an auspicious archipelago for comparisons. Most important are the excellent studies by a wide range of researchers that have yielded a strong foundation. Also, the flora and fauna are diverse and, together with the geology of the islands, are relatively well documented. The JF were selected for primary comparisons because we know that flora well from decades of work there.

1. Location, age and size

The JF is a simple system of two major islands at 33^0 S latitude 670 km W of Santiago, Chile: Masatierra (4 million yrs old [myr]) and Masafuera 1-2 myr, and 150 km further W), each about 50 km^2 in area. There are no known submerged stepping stones to the JF. The present land area of Masatierra is about 10% its original size. The highest point is now 950m but originally it was about 3,000m. There has been no volcanic activity or massive land slides since the origin of the archipelago. The situation is different for the CAN: the 7 islands are 75 times larger in area, are 10 times closer to a continent, are 4-5 times older, and have been subjected to volcanic activity (most recently in 1971) and massive landslides. Although the JF are younger, they may be declining, without any active volcanic eruptions and are actually shrinking.

2. Floras

The JF has a flora of about 155 native species, about 65% endemic. The major source areas are South America (Followed by Pantropics, Australia, New Zealand; Bernardello et al. 2006). The largest lineage has only 11 species. About 55 colonizations probably account for the endemic flora. The CAN have a much larger native flora with ca. 600 endemics, which is 45% of the native flora. Major source areas are the Mediterranean and Africa. The endemics may have originated from 156 colonizations (Santos 1998, unpubl.). It is interesting that only 17 introductions have generated 270 endemic species. In contrast to JF, 14 lineages in the CAN have 10 or more species.

Figure 2. Solanum vespertilio is an endemic, and a highly unusual solanum (mostly located on Tenerife, Canary Is.), in being andromonoecious, and having zygomorphic, four parted flowers, with unequal anthers, and sometimes showing enantiostyly.

3. Pollination

The pollination of the JF is dramatically simple, with wind (nearly 50% of the flora) and hummingbirds playing major roles; there are no native bees (one newly introduced-Anderson et al. 2001b), wasps, flys, butterflys or moths that are regular flower visitors (Bernardello et al. 2001; Anderson, et al. 2001a). The only native biotic pollinators are two hummingbirds, one endemic. The hummingbirds are obviously flower specialists, and visit 14 spp. (~8% of the flora; 14% of the endemics). There are very few reports of wind pollination in the CAN, but studies of endemics from several wind-pollinated families are lacking. The pattern in the CAN is more typical of other island systems (Hawaii, Galápagos, Bonin Is.) where insect-pollinated colonists have adapted to insects, though sometimes to generalists. There have been some shifts to wind; this is an area meriting more detailed study.

Hummingbirds on the JF have not had to compete with other biotic pollinators for plants. This contrasts with the CAN (and most other archipelagos), where, although a similar number of plants (11) are visited by 6 bird species, a proportionately small part of the flora (> 2% of endemics, > 1% of flora). Importantly, the birds are all passerines (Ollerton et al. 2009), i.e., not nectar-feeding specialists (notably, not from the African Sun Bird flower-specialists -Nectariniidae), and, reciprocally, most of the bird-pollinated plants are not exclusively ornithophilous. Valido et al. (2004) conclude that most bird-flower syndromes on the CAN came to the islands already developed – the 'relict condition'– and that Macaronesian ornithophilous species sometimes represent survival in their island 'refugia' when ancestral forms went extinct on continental areas. The majority (60-70%) of JF ornithophilous plants also seem to have been derived from bird pollinated colonists (Bernardello et al. 2001). The higher proportion of ornithophily on the JF probably represents exploitation of an open niche for hummingbirds: they did not have to compete with the full panoply of insect pollinators (see below).

Pollination by the other biotic groups typical of continental floras is much more common on the CAN. For instance, it is estimated that the 125 CAN bee species visit many plants, exemplified by the at least 88 CAN endemics pollinated by the ubiquitous endemic *Bombus* (Olesen et al. 2002). These authors also show that plants in 3 different floristic zones range from moderate specialists to generalists. Even so, the CAN pollinator fauna is much less rich than the continent.

Immigrants to islands are few, highly selected/ filtered by distance, dispersal capabilities, and adaptations for establishment. Furthermore, their survival depends on dealing with what is there. This is manifest in two interesting forms. One is that there are often many niches open, with little or no competition – at least for the first immigrants. As a result, there are impressive morphological/ functional radiations in some groups. There is the caveat, however, that one of the ways in which near-source archipelagos like the CAN may be somewhat different is the much more likely constant source of immigrants from the nearby continent. As a consequence, what the new immigrants have to deal with is very different for the early (open niches) vs. later arrivals ("full" niches, more like diaspores within a continent). Secondly, as noted, the immigrants have to deal with what is there; there is no analog of the 'universe of possibilities' (and restrictions) that continental species encounter. So, when we study the evolution of various lineages, we have to be careful of over-interpretation.

Figure 3. Cheirolophus burchardii, a member of the Asteraceae, is one of the species visited by introduced honey bees, but also native bees.

III. Baker's Law, Colonizing Ancestors and Island Radiations

1. Colonizing ancestors

Baker's "Law" (he preferred "generalization") posits that self compatible (SC) colonizers, especially autogamous ones, would be most effective in establishing sexually reproducing populations following long distance dispersal because, unlike self incompatible (SI) plants, no pollen vector may be needed and only a single disseminule is required. Baker's Law has been widely accepted despite concerns about whether the low diversity carried by a single propagule from a selfing source population would preclude the development of a diverse island lineage, e.g., such doubts as expressed at the time Baker was promoting these ideas in the 1950s and 60s. There appear to be conflicting optimal characters for establishment on the one hand, and on diversification and radiation on the other. This is one of the basic questions in the RB of island plants, and has several important implications for understanding the origin and evolution of insular lineages. One of the continuing problems with applying it and testing the ideas is the lack of comprehensive data on the compatibility of island plants. There is speculation from a few carefully studied species – it is time consuming to determine SC. We have good data for the JF where, like the Galápagos (McMullen 2009) a number of species have been surveyed, and 70 to 80%

of the species are SC (Anderson & Bernardello 2011). There are few comprehensive data for most other archipelagoes; such data are needed for comparisons that generate testable hypotheses on plant evolution. It has been our intention to summarize work on the CAN to facilitate these comparisons.

There are several mechanisms by which plants may escape from this seeming conundrum. In the JF, the 80% of SC species are not highly autogamous and many require a vector to move pollen from anther to stigma. Thus, the ancestral source populations likely were not highly homozygous, as would be true for highly selfing populations. The same is true for the remote Hawaiian Islands, where there is little evidence for autogamous ancestors. Thus, knowing the mating systems of the colonizing ancestors of SC island lineages is important.

Examples are slowly accumulating of the feasibility of establishing sexually reproducing populations from a single SI diaspore. The source population would be outcrossing and highly heterozygous. Preliminary data indicate that over 40% of the 27 lineages examined from the CAN originated from SI ancestors. Detailed studies from the genus *Tolpis* indicate that "obligately" SI species in fact exhibit various levels of selfed seed (Crawford et al. 2008). There is some evidence that the large CAN radiation, *Argyranthemum*, is pseudo-self-compatible (PSC, i.e, has "leaky SI). PSC occurs in the largest lineage (*Scalesia*) in the Galápagos and in a large radiation (silversword alliance) in Hawaii; both likely evolved from PSC ancestors. Five of the 6 largest radiations in the CAN have SI members, and likely originated from SI or PSC ancestors (Anderson et al. unpubl.). The breeding systems of these and other lineages, and their continental ancestors should be examined in detail to ascertain the potential role of PSC in the establishment and radiation of island plants. How general is PSC as a way of establishing genetically diverse populations from a single disseminule?

The cryptic dioecy we found on the JF (*Pernettya*), and CAN (*Withania*), and leaky dioecy in *Solanum conocarpum* from the Virgin Islands (more below) of course might have arisen similarly.

2. Mating systems in island populations: how little we know

Regardless of the diversity brought to an island by colonizers, establishment in new niches and outcrossing are probably important in the initial diversification and radiation of a lineage. The two most effective mechanisms for promoting outcrossing are complete separation of the sexes on different plants (dioecy) and obligate SI in hermaphroditic plants. Dioecy is rare in flowering plants (6%) and is likewise infrequent on CAN (perhaps as high as 6% when cryptic and temporally functional dioecy are considered; Pérez de Paz et al. 2011), though more frequent in other archipelagos such as Hawaii (15%) and Bonin Islands (13%). On the JF it is about 9%. Other mechanisms promoting outcrossing in SC species of JF include monoecy (9%) and herkogamy, with about 30% of species protandrous and 7% protogynous; meager published data suggest similar mechanisms in the CAN (Francisco-Ortega et al. 2000). Thus, although SI *per se* has not been important in promoting outcrossing in the JF and its frequency in the CAN is largely unknown, there are other ways of promoting outcrossing in SC species. Data for continental ancestors are needed to infer whether factors promoting outcrossing were brought to an island or evolved *in situ*; data from JF and Hawaii indicate that both have occurred.

Despite observations on factors promoting selfing and outcrossing, there is very little information on how these factors actually shape mating systems in natural populations of insular endemics. Recent studies of highly SI *Tolpis* in the CAN revealed a mixed mating system in several populations (Crawford et al. 2010). These unexpected results illustrate the possible pitfalls of inferring the mating system from the breeding system data. Similarly, studies of the mating system in Hawaiian *Bidens*, a large radiation of SC plants, revealed that protandry and gynodioecy function to produce mixed mating. You can not necessarily judge the pollination of a flower from its morphology (Bernardello et al. 2001), nor mating system from the breeding system.

While outcrossing may be an important factor in the diversification of island lineages, the conditions under which higher selfing, including autogamy, would be favored must be considered. Two commonly cited advantages of autogamy are lack of pollinators and compatible mates. There are few extensive studies of mating system variation in island lineages; a notable exception is *Schiedea* in Hawaii (Sakai et al. 2006). From SC hermaphroditic ancestors autogamy evolved three times in small populations in wet areas; dioecy originated twice in dry, open areas with no biotic pollinators but wind pollination occurs. In *Tolpis* in the CAN, increased selfing could occur by higher levels of PSC; preliminary results indicate higher selfed seed and lower allozyme diversity in populations from the El Golfo region of El Hierro (Crawford et al. unpubl.); more studies are needed to see whether there are correlations between selfed seed and areas of disturbance. True SC has evolved within *Tolpis* in the CAN; *T. coronopifolia* exhibits floral features typical of selfers and is highly autogamous (Crawford et al. 2008).

The genetics or adaptive significance of its mating system have not been elucidated. Analyses of synthetic F_2 hybrids between *T. coronopifolia* and a SI plant show that SI is controlled by a single major dominant locus with modifiers and morphological characters co-segregate with the S locus (Crawford et al. unpubl.). Thus, evolution of true SC and the associated morphological characters could be rapid. The breakdown of SI occurs in *Dendroseris* in JF but the selfing syndrome has not evolved, with retention of outcrossing features. Studies of comparative breeding-mating systems within lineages in the CAN are needed. Examination of one to several species within lineages may not detect evolutionarily important variation. The CAN are an ideal system because of diversity of habitats, opportunities for colonization of new islands and new open areas on individual islands. Some larger survey studies are underway (Anderson et al. unpubl.)

Figure 4. Euphorbia atropurpurea are monoecious, bear a good deal of nectar and are emblematic of the dryer zones on Tenerife.

IV. Reproductive Biology and Conservation of Island Plant

Successful sexual reproduction is critical to the survival and evolution of island lineages, and thus any conservation effort without knowledge of reproductive biology is in danger of failure. Any study of RB has conservation implications, and this is especially true for species present as a few small island populations. Some important issues are outlined below.

1) Conservation of S alleles in SI species from very small populations important for *in situ* and *ex situ* conservation efforts to assure compatible matings.

2) For dioecious species, the conservation plan has to take account of the mix of sexes and behavior of pollinators toward them. Dioecy leads to skewed sex ratios in smaller populations, reducing effective population size, possibly to only one gender in a species. Leaky dioecy could be a mechanism for generating seeds. Recent example is newly discovered cryptic dioecy (and 'leaky dioecy' too) in the critically endangered (> 200 pls. in wild) *Solanum conocarpum* on Virgin Is. (Anderson et al. 2010).

3) Highly selfing species may have high interpopulation differentiation, knowledge that aids conservation planning.

4) Loss of pollinators outright, or competitively to invasives, or loss of plants to sustain pollinators have significant implications.

5) Invasive pollinators and plants. Networks may be destroyed.

6) Hybridization between natives, and between natives and invasives by virtue of habitat alteration.

V. Future Studies

Some topics regarding the RB of island plants were described above and elsewhere (Crawford et al. in press). Here we outline some areas for future research.

1) RB research *sensu lato* involves both in-depth and multifaceted studies within lineages to elucidate subtle variation in effective pollination (not just floral visitation), and breeding and mating systems.

2) Generation of accurate general principles requires comparative studies of similarities and differences among lineages within the same and among different archipelagos.

3) Comprehensive comparative studies require research teams within an archipelago and coordination of efforts among archipelagos. To quote H. G. Baker (1967) "Clearly, the greatest need in this area of biology is for further field and experimental studies (by as many workers as can spare the time) on the reproductive biology of plants which make up floras of all kinds----".

4) Molecular data are needed to place evolution of RB within a phylogenetic-biogeographical context. Molecular markers allow estimation of mating systems in populations. The potential rewards are great in yielding an understanding of the complicated biological world we are privileged to study.

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Dan Crawford has held faculty positions at the University of Wyoming, The Ohio State University and the University of Kansas. He has served in a variety of professional organizations, including the presidencies of the American Society of Plant Taxonomists and the Botanical Society of America. His interests are the systematics and evolution of the tribe Coreosideae, especially the genus Coreopsis (Asteraceae), and the origin and evolution of plants endemic to oceanic islands. He has been involved in broadly-based collaborative studies of the flora of the Juan Fernández Islands, Chile for 30 years. A current research interest, in collaboration with workers from the US and the Canaries, is the genus Tolpis in the Canary Islands. These studies are focused on producing a phylogenetic hypothesis for Tolpis, inferring its biogeography in the Canaries, and elucidating the evolution of breeding systems and isolating factors during the radiation of the lineage in the archipelago.

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Genetic diversity and phylogeography in Western Mediterranean islands: what we do know and what we should know

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The numerous islands present in the Mediterranean constituted major refugial areas, and are nowadays a significant component of its plant diversity, with a large number of narrowly endemic taxa. In addition, the highly fragmented insular landscapes have promoted geographical and genetic isolation among plant populations, favouring allopatric speciation via selection and/or genetic drift. In spite of the central role that islands play to understand the evolution of plant biodiversity in the Mediterranean, and despite a long tradition of studies based on the endemic species, it is only recently that the Mediterranean islands have regained interest from evolutionists, as shown by several studies on the evolution of the Aegean, the Balearic, and the Corso-Sardinian floras. There have been very few studies on levels of intraspecific cpDNA differentiation in narrowly distributed plants from the Mediterranean islands. The scarcity of these records is due to the persisting perceptions that (i) rare species show, on average, low levels of overall genetic variation, (ii) insular species tend to show low values of genetic diversity compared with close continental relatives, and (iii) the cpDNA genome is an unsuitable target for assessing genetic markers for intraspecific analysis due to its relative slower mutation rate compared with nuclear DNA. However, these general expectations seem to be lineage- specific and the detected levels of cpDNA variation depend to a large extent on the recent evolutionary history of the species. The currently available studies suggest that (i) plant endemics from Mediterranean islands are not genetically depauperated and have conspicuous genetic diversity levels, which are also highly structured, indicating that (ii) gene flow is scarce, even within species with a high dispersal capacity, thereby highlighting a predominant role of drift on the observed diversity patterns. This work has been funded by projects CGL2010- 22347-C02-01 (Ministerio de Ciencia e Innovación) and 2009 SGR 608 (Generalitat de Catalunya, Agaur).

Josep A. Rosselló

Josep A. Rosselló's early research was devoted to the taxonomic and evolutionary knowledge of the Balearic vascular flora (especially as regards the diversification of the endemics) and, later on, he specialized in the study of different genera of particular evolutionary interest (e.g., Limonium, Armeria). The ease with which these taxa hybridise and beget agamospermic species makes it difficult to address their evolutionary study through solely morphological characters. Consequently, he complements his studies with molecular markers (multiple- and single-copy nuclear markers, diverse intergenic chloroplastic spacers), with classical and molecular cytogenetics techniques, and with the determination of genomic DNA contents. He also applies molecular methodologies to the taxonomic study of plants with an agronomic interest, and to the characterization of processed plants in food, being the author of a patent. In the last decade, he has been a PI in four projects in the national Spanish calls of R+D+R, all of them related to micro-evolutionary aspects of Balearic plants or their relationships with Macaronesian congeners. Likewise, he participated in another project related to the Thyrrenian islands. He became staff of the Universidad de Valencia eighteen years ago, and he is also the first scientific director of the Jardí Botànic Marimurtra (since 2008).

What can tell us genome organization about plant evolution in Mediterranean continental islands?

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The general patterns that might explain microevolutionary processes underlying plant speciation in continental islands have been rarely explored. Such absence of data is most surprising in an enclave like the Western Mediterranean basin, where the endemicity rate on major islands far outnumbers a minimum of 10%. Only recently, a critical review about karyological evolution on the Balearic endemic component has been tackled (Rosselló & Castro 2008), revealing that (i) the origin of the islands (continental or oceanic) is not an accurate predictor of the proportion of polyploid component in the flora, (i) there are no apparent unifying processes that explain the evolution of ploidy levels on insular endemics, and (i) the levels of autochthonous polyploidy (i.e., developed in situ) on continental islands are higher than on oceanic islands, where the percentage of polyploidy evolution on insularity conditions is comparatively low. Nevertheless, other important genomic and karyotypic aspects that might be linked to the patterns and processes of insular speciation have not been yet assessed. In this talk we discuss the patterns of insular speciation and differentiation linked to changes at deep genomic levels, i.e., in the distribution and structure of repeated DNA, and the nuclear DNA content (1C values) in the endemic element of the Balearic Islands. Specifically, we assess whether genome reorganization, involving amplification or loss of multigene families and satellite DNA, is one of the undetected cryptic events that have acted on the endemic component of continental islands. This would allow discuss to what extent similar selective pressures at the genome level are involved in the speciation processes occurring in oceanic and continental islands. This work has been funded by the CGL2010-22347-C02-01 (Ministerio de Ciencia e Innovación) and 2009 SGR 608 (Generalitat de Catalunya, Agaur) projects.

Marcela Rosato

Marcela Rosato has a wide interest in plant evolution and speciation in narrowly distributed Mediterranean plants, especially those from the Western Mediterranean islands. She uses DNA sequencing from non coding regions of the cpDNA genome and molecular cytogenetics to assess the phylogeographic structure of endemic plants from the Balearic Islands, Corsica, Sardinia, and surrounding territories.

Contrasting patterns of plant evolution in the Canarian and Galápagos islands: the origin of dispersal and colonization

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Islands are ideal models to study long-distance plant dispersal (hereafter LDD) (Gillespie and Clague 2009). Seed dispersal is often the first step for plants to colonize new territories and thus is crucial to understanding species composition, range expansion and genetic structure (Cain et al. 2000, Nathan 2006). Traditionally, taxonomic and floristic data have provided the basic tool to infer the geographic origin of flowering plants on oceanic islands (Hooker 1847, Carlquist 1967, MacArthur and Wilson 1967). Given their relatively recent origin from the sea-floor in comparison to continental areas, researchers have focused on the biogeography of LDD events. The number of taxa (mainly genera) has been widely used to estimate the number of colonisation events. In addition, description of the vegetative and reproductive structures of plants has aided in categorising them into four main groups of diaspores related to dispersal by: wind (anemochory), sea (hydrochory), internally by animals (endozoochory), and externally by animals (epizoochory) (van der Pijl 1982). The lure of reconstructing the colonisation history of each archipelago has led scholars to infer, and often speculate, about actual vectors responsible for the presence of plant groups on remote oceanic islands.

In the case of the Canary Islands, Bramwell (1985) estimated that the extant flora was generated by 186 founders (c. 1500 species), of which 63 (34%) were considered endozoochorous, 35 (19%) epizoochorous, 48 (26%) anemochorous, 8 (4%) hydrochorous and 32 (17%) uncertain. For the Galápagos flora (c. 500 native species), Porter (1983) also suggested that a large proportion (60%) of all flowering plants had been introduced by birds, a lower fraction by wind (31%), and the rest by ocean drift (9%).

Which dispersal agent was responsible for the presence of a plant group in an archipelago is a matter of speculation, because fruits and seeds may have arrived on an island by different unpredictable means (Higgins et al. 2003). Rather than speculating on the colonisation vector, a more testable exercise would be to evaluate the relative presence of different plant dispersal syndromes occurring on islands, regardless of how the first propagules actually arrived (Vargas 2007).

Previous authors, however, claimed for a high number of seeds accidentally introduced by mud adherence (41 colonists) for the Galápagos Islands (Porter 1983), even with no solid evidence for such events. Direct observations of the arrival of new diaspores are unlikely and inferences of means of dispersal used by early founders cannot be reliably tested. In contrast, the contribution of various dispersal syndromes of plants that have colonized remote archipelagos (like the Galápagos and the Canary Islands) can be estimated.

In order to correctly assess whether the four LDD syndromes have been favourable in the natural colonisation of oceanic islands, it is necessary to control for some factors obscuring correct inferences. The most confounding factor is colonisation by plants brought by humans and therefore not attributable to natural dispersal. The Galápagos (subaerial volcanic rocks of < 6 Ma, Geist 1996) and Canary (subaerial volcanic rocks of < 21 Ma Carracedo et al. 2002) islands have been continuously colonized since their formation. This period is considered long enough for the islands to receive natural introductions that may have differed into morphological varieties (endemic taxa). Thus, the native (and endemic) status of these taxa is simply identified using morphological characters. For non-endemic taxa, natural *vs.* human-mediated introductions are often difficult to tell apart (Tye 2006).

The appropriate unit to test the success of LDD syndromes is the number of colonisations itself, which is unknown. Initial working units for the number of introductions are genera, assuming an independent origin for each. However, a single genus can be the result of more than one introduction, particularly large genera including infrageneric taxa. Therefore, multiple dispersal events from the same plant group (genus, subgenus, species, lineage) may greatly increase the number of successful dispersal events undetected by morphology. In addition to morphological inferences, phylogenetic and phylogeographic methods are essential tools at the species and population levels, inasmuch as they provide precise reconstructions of lineage relationships for oceanic islands and continental plants (see Andrus et al. 2009). In particular, phylogenetic methods aid in evaluating the number of colonisation events (testing monophyletic groups), geographical sources of origin (inferring sister-group relationships) and shifts of ancestral syndromes related to LDD (reconstructing ancestral characters) (Vargas 2007). On the other hand, phylogeographic methods allow us to determine the unique genotypes of particular areas (endemic genotypes), which equally support the native origin of populations followed by genetic differentiation (Avise 2009).

In this study we contrasted the proportion of syndromes that have been favourable in the colonization of the Canarian and Galápagos archipelagos. We used floristic data and a new approach that proposed explicit assumptions and corrections to find out the contribution of four diaspore syndromes to the colonization history of oceanic archipelagos. A fifth category was considered in the analysis for diaspores displaying no specific traits for LDD.

Material and methods

The colonization of a new territory entails four essential phases: dispersal from the source flora, arrival on the new land, establishment in particular habitats, and in situ differentiation and further distribution. The origin of floras from ancient and dynamic continental plates has historically been difficult to be reconstructed. In contrast, oceanic islands are relatively new territories were spatial and temporal frameworks are simplified. In any case, the complete reconstruction of this process for a whole flora is unfeasible using testable data because nobody has witnessed the arrival of early plant colonists. However, it can be tested whether particular plant features have significantly contributed to the success of plants in the colonization. A new approach herein presented focuses on three essential aspects needed to infer whether particular diaspore traits have been favourable: plant diversity, colonist and syndrome estimates.

Floristic diversity estimates

Floristic data were systematically evaluated along the following complementary steps:

1) Analysis of only native taxa as considered from floristic studies.
2) Use of lists of the native species that were amended using palaeobotanical evidence (palaeobotanical correction).
3) Additional analysis of only endemics (flora endemic approach) to prevent from including human-mediated introductions.
4) Comparable analyses from multiple floristic accounts differing in number of all the species and in number of native species.

Early colonist estimates

The number of early colonists is inferred from the native and endemic flora. There are, however, some assumptions to consider in order to reliably estimate the number of colonizations that brought about the current flora:

1) One colonization result in a lineage that either differentiated or not.
2) Genera are used as operational starting units.
3) More than one ancestor was adopted for genera containing multiple taxonomic groups (subgenera, sections, subsections).
4) The inferred number of original colonists was corrected by phylogenetic evidence (phylogenetic correction), in which each independent, monophyletic group accounts for a single origin. This correction implies the analysis of species and population levels using phylogenetic and phylogeographic methods, respectively.

Dispersal syndrome estimates

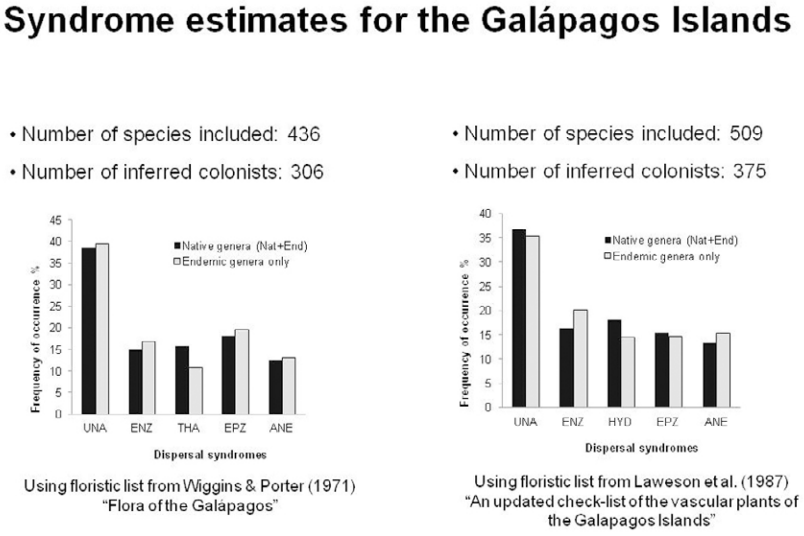
A testable, evolutionary analysis relies on plant features of the extant flora. Only traits related to LDD in the colonization are used to categorise the inferred colonists. The following considerations are used for the analysis:

1) Syndromes unrelated to LDD were not considered, i. e. myrmecochory, autochory and ballistic.
2) Four LDD syndromes related to LDD dispersal by: water (hydrochory), wind (anemochory), internal animals (endozoochory) and external animal (epizoochory).
3) One more category is analysed for plants with no specific mechanism for LDD: unassisted.
4) Probability of one colonization event is calculated depending on species displaying one or more syndromes.
5) Syndrome coding of the early colonists as revealed by morphologies of the extant species from the oceanic island and closely-related taxa from the continent. Result searches were performed with three scientific web search engines (Scopus, ISI, Google).

Results

We did not find noticeable differences between the two floristic accounts used to analyze the number of colonists in the Galápagos Islands (Fig. 1). They basically differed in number of species (68 more species in Laweson et al. 1987) and consideration of native vs. non-native species. Once corrected by means of the fossil pollen and phylogenetic information available to date, we obtained the following different proportions of dispersal categories in the native Galápagos flora: endozoochory 16.4%, epizoochory 15.7%, hydrochory 18.6%, anemochory 13.3% and unassisted 36.1%. When we considered exclusively genera with at least one endemic species, the proportions changed only slightly: endozoochory 20.1%, epizoochory 14.9%, hydrochory 14.5%, anemochory 15.3% and unassisted 35.1% (Fig. 1). In both datasets, the frequency of the four LDD syndromes was not statistically different (genera containing native species $G = 0.9$, $df = 3$, $p = 0.830$; genera containing endemic infraspecific taxa $G = 1.2$, $df = 3$, $p = 0.751$). When including the frequency of unassisted diaspores in the analysis, this was significantly higher than that of the four syndromes associated with LDD (genera containing native species $G = 14.8$, $df = 4$, $p = 0.005$; genera containing endemic infraspecific taxa $G = 13.6$, $df = 4$, $p = 0.009$).

The use of the same approach on a subset (38) of plant groups from the Canary islands reveals the following results: endozoochory (34%), epizoochory (10.5%), anechory (8%), hydrochory (16%), unassisted (21%) and uncertain (10.5%) (Vargas 2007). Interestingly, these results are similar to previous estimates using a different approach. Bramwell (1985) estimated that 186 founders generated the extant flora of the Canary Islands, of which 63 (34%) were endozoochorous, 35 (19%) epizoochorous, 48 (26%) anemochorous and 8 (4.3%) hydrochorous . It is difficult to interpret the category of 32 (17%) uncertain plant groups (Bramwell 1985), but this figure may include both the unassisted category considered in our study plus plants with dispores of uncertain classification into the four LDD syndrome categories.


Syndrome estimates for the Galápagos Islands

- Number of species included: 436
- Number of inferred colonists: 306

- Number of species included: 509
- Number of inferred colonists: 375

Using floristic list from Wiggins & Porter (1971) "Flora of the Galápagos"

Using floristic list from Laweson et al. (1987) "An updated check-list of the vascular plants of the Galapagos Islands"

Figure 1

Discussion

As the actual the arrival of early plant colonists to isolated islands cannot be tested, the actual means of long-distance dispersal (LDD) have historically been a matter of speculation. Given the practical impossibility of determining the actual number and vectors of dispersal events, estimates of the number of colonisations have to rely on taxonomic data, LDD syndrome categories and phylogenetic reconstructions.

We showed that a large number of plant groups with no special morphological adaptations for LDD (36.1% of the native flora and 35.4% of the endemic flora) may have reached the Galápagos Islands prior to human colonisation. Unassisted diaspores appear to have been successful in the colonisation of the Galápagos Islands and represent a proportion even higher than diaspores with syndromes historically considered to be successful in long-distance colonisation, such as endozoochory (16.4% of native genera and 20.1% of endemic genera). The same is true for the estimates of the origin of the Canarian flora. No special morphological adaptations for LDD were needed to reach the Canarian archipelago in nearly 20% of the cases, and possibly in some of the 10% more categorized as uncertain (Vargas 2007). These figures are even more striking given that the Galápagos and Canary archipelagoes share few characteristics (similar land area). In fact, many dissimilar features considered essential in the theory of island biogeography do not meet in the Galápagos and Canary islands, respectively: location (Pacific and Atlantic oceans), proximity to the continent (c. 100 vs. 1000 Km), age of subaerial volcanic rocks, number of habitats (highest elevation of 3742 m vs. 1710 m). A preliminary analysis of a subset of the Hawaiian flora, of which there are reliable phylogenies published, revealed that also about 30% of colonists lacked any special mechanism to LDD (Vargas 2007; z et al., unpublished). Therefore, the question remains as to why most of the world´s oceanic archipelagos were colonised by a successful group of plants (between 20 and 40%) with unassisted diaspore.

In summary, we suggest that the speculative exercise of inferring actual dispersal to remote archipelagos should be discouraged. Instead, analyses of sets of traits favouring LDD generate explicit hypotheses to be tested within the theory of island biogeography.

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Pablo Vargas

Pablo Vargas (Real Jardín Botánico, CSIC) leads projects on the evolution and molecular systematics of primarily Mediterranean and oceanic island plant groups. Although a majority of his publications supports a carrier devoted to Iberian and Mediterranean angiosperms, his first phylogenetic studies (1998-2000) were undertaken in angiosperms from Hawaii and Macaronesia (Sanicula, Saxifraga, Hedera, Olea). Further projects and collaborations resulted in an ongoing investigation on plant evolution of Macaronesia and Galápagos, including genetic diversity and evolution of particular genera (Cistus, Olea, Carex, Reseda, Echium, Juniperus, Galvezia, Linum). New points within the theory of relictualism and theory of island biogeography have also been proposed to the scientific community.

Manuel Nogales

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Floristic relationships between Macaronesia, mainly Canary Islands, and East Africa

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Introduction

In the last forty years, several authors have conducted interesting research about the origins of Macaronesian floras, especially the Canarian flora (due to its higher endemic richness). The first papers related to this question were published in the nineteenth century (Hooker, 1898) and, more recently, in the middle of the twentieth century by Sunding (1979), Bramwell (1972, 1985), Humphries (1979) or Santos (1999). These contributions considered other works related to extinct floras in Europe (Aubreville, 1976) and Africa (Quezél, 1978), as well as more general publications related to with the distribution of the famous “rand-flora” (Axelrod & Raven, 1978) along mainland North and East Africa. Most of the hypotheses and ideas were based on macro-morphological variables, sometimes combined with pallinological, phytochemical or cariological evidence.

The geology and, in particular, the evolution of the vegetation in the territories concerned (especially the Saharan-Mediterranean areas and Atlantic archipelagos), still need more information and studies according the evolution of floras correlated with them. We have not yet enough data about the potential vegetation and floras extending through northern Africa and the current Saharan area during, and after, Mio-Pliocene times. In the same way, the information about fossil floras existing in the Canaries needs urgent revision, both for macrofossils and for pallinological studies of paleosoils. The information published about this topic is very interesting, though scarce.

Only after the beginning of molecular studies applied to insular floras in the 1960´s, new windows could be open to further understand the wonderful scenario which we are beginning to know. Several previous molecular papers had a good general ”starting point” but, according to the new techniques, it is necessary to use different molecular markers together with morphological and molecular analyses to do some new research about the first genera studied (*Argyranthemum, Lotus, Sonchus,…*).

A few of these “reviews” are in progress in relation to Canary Islands or Macaronesian area. Several especial and interesting works aimed to clarify the East African –

Macaronesian relationships have been undertaken, for example those by professors M. E. Mort, D. J. Crawford and their collaborators (2003) about the resolution and understanding of *Tolpis* (Asteraceae), in which they reject the south African and the Ethiopian species formerly included in this genus (*Tolpis capensis* and *Tolpis staticifolia*, respectively). Recent research, not yet published or in press, has been undertaken in *Lotus* sect. Pedrosia and sect. Rhyncholotus by Isidro Ojeda at Vancouver, including both continental and insular taxa. Similarly, the team at the Jardín Canario – Unidad Asociada CSIC (and collaborators) is making substantial progress with, for instance, *Androcymbium*, *Heberdenia*, *Pleiomeris*, *Matthiola, Neochamaelea, Parolinia,* or *Ruta*.

Research that improves previous sampling in other groups is also just starting, for example the complex clade of macaronesian *Sonchus* (in progress by Dr. S.-C. Kim's group); this work encompasses newly described species and corrects the misidentifications of old conflictive ones. Other similar cases could be mentioned but, perhaps not least important, the progress in taxonomy must be taken into account. This topic has not been, according our point of view, so much considered as necessary, probably due to economical-political reasons and, especially, to the "absence of good motivation" for scholars. Admittedly, taxonomy is not an easy study because most of the ancient herbaria, where most of the types of the Canarian flora are located, are out of Canaries but also because this kind of university speciality is not the best way for a professional to get a permanent "work" or "research position".

The complexities of the Canarian flora, as well as its distribution through the archipelago, also stand in the way of a thorough knowledge. However, if we don't have good taxonomists, we cannot get good molecular results using only modern techniques and machines. The knowledge of the wild flora is an absolutely necessity if we want to understand and explain the evolutionary patterns according to molecular results, especially in insular territories.

Exhaustive sampling of not only all well-established taxa, but also of different "cryptic ones" (i. e., those where the assignation of populations to an existing taxonomic unit is not clear), have led us to the recent discovery of several new taxa in the genera Aeonium, Argyranthemum, Lotus, Sideritis, Sonchus, Tolpis, ..; most of them are not yet published, but they may already provide new data and very good information to "elucidate" the complicate and rich network of insular endemism. Furthermore, sometimes due to the lack of taxonomical information (or to the fact that authors do not take the already existing taxonomical knowledge into account), wrong results are appearing in recent papers concerning species of Macaronesian flora, including genera such as Aeonium or Patellifolia. The greater the collaboration between molecular researchers and taxonomists is, the more reliable the results get.

The papers trying to inform about the floristic similarities among Macaronesian archipelagos and, especially between the Canaries and Africa, were scarce in the past and based on morphology but, in the last two decades, several ones haven been published taking in account molecular results. Most of these papers, devoted to the relationships between

Macaronesia and East Africa, were summarized by Andrus et al. (2004), Carine et al. (2010) and Thiv et al. (2010). Some years earlier, B. Mies (1995, 2000) published some information in particular related to Soqotra island comparing not only its types of vegetation with the Canarian ones, but also including the distribution of some genera with related species in both areas (e.g., *Caralluma*, *Euphorbia*, *Kleinia*,...). Some of the "future works" proposed by Andrus et al. (2004) are still waiting for research, although part of them are under way in a project coordinated by I. Sanmartín (at the Royal Botanic Garden, Madrid) together with other colleagues from the universities of Barcelona and Santiago de Compostela (Spain). This project includes the study of several selected genera with that distribution pattern (Mediterranean and continental-insular North Africa), mostly with endemic species in both areas (*Campanula*, *Euphorbia*, *Geranium*, *Hypericum*, *Kleinia*, etc.).

Several genera have not been well studied yet, and a lot of work needs to be done to get a better general idea about the putative origins of the Macaronesian (especially Canarian) representatives from their relatives in NE continental-insular Africa and Arabian peninsula. Peculiarities such as those about *Aeonium*, *Campylanthus*, *Dracaena* or *Parolinia* have been always mentioned in the literature, but really a lot of molecular work and good sampling are still necessary to get a better molecular information in particular related to other genera shared for both areas, not only the previously mentioned ones, but also for example *Asparagus*, *Camptoloma*, *Caralluma*, *Ceropegia*, *Habenaria, Micromeria* or *Sideroxylon*. The relationship seems to be weaker with areas in central or south east Africa, but still important (*Apollonias*, *Plocama*, *Kunkeliella*,...), and only some of them are partially studied.

There are few published papers including apparently unrelated, co-generic species that are endemic to insular environments at both sides of North Africa. Some of these samples correspond to *Lotus*, *Polycarpaea* or *Pulicaria*.

Besides, no research whatsoever has been done to know the relations of different supposed native species distributed only in the Mediterranean-Macaronesian and east Africa-adjacent Asian areas such as *Aizoon canariense*, *Gymnocarpos decandrus* or some *Plantago* species. Probably the results of such investigation could be interesting to understand better the migration pathways of these floras.

After two trips (1999, 2009) to Yemen-Soqotra and two to Ethiopia (2001, 2010), with some weeks of field work, to try to interpret the relationships between the insular territories of Soqotra and the Canaries is really a fascinating exercise. Both archipelagos share at least 38 species, such as the probable native *Aizoon canariense*, some grasses (*Enneapogon desveauxii, Tricholaena teneriffae,..*), and a few clearly introduced taxa (*Argemone mexicana*, *Nicotiana tabacum*, *Ruta chalepensis*,…). Another kind of example includes *Pennisetum setaceum*, which seems to be native to Soqotra, but is a clear introduced and very dangerous weed on the Canaries. Still more interesting is to take into account the 37genera common to both territories, 23 of which have endemic species. We must not forget that these territories have different origin: Soqotra is a continental ancient island,

and the Canaries and the close Macaronesian archipelagos are recent oceanic ones. This biogeographical setting gives us the opportunity to compare floras with diverse origins and evolutionary paths, but showing similar evolutionary groups such as *Helichrysum* or *Hypericum*. Similarities are also, according to climatological characteristics, recognized in the vegetation structures (sand dunes vegetation, cliffs communities, succulent coastal shrub, and hygrophilous forest) where some shared genera possess dominant endemic species in the landscape, such as *Tamarix*, *Euphorbia* or *Dracaena* (Mies 2000).

Macaronesian-East continental Africa connections (out of Mediterranean areas)

Out of the east African insular territories, a few classical samples of existing relationships must be mentioned. Some of these belong to the same genera, like the beautiful, old member of the Campanulaceae, *Canarina* and its three species: *C. canariensis* at the Canaries, and the two east African tropical *C. abyssinica* and *C. eminii*. We don´t know yet which was the origin of this genus or its way of propagation, but probably it was due to bird dispersal from east to west in the Canary Islands. In this group, we should also include the relations of the Canarian endemic *Rumex lunaria* with eastern African species.

Another example is *Solanum*, where the relationships between the two Canarian endemics (*S. lidii* and *S. vespertilio*) have been investigated by Anderson *et al.* (2006), showing an interesting affinity to the East African *S. capense*, *S. tomentosum* and *S. aethiopicum*. Similar results emerge in *Helichrysum*. A remarkable case is *Dracaena*, with species in both insular and continental environments in the West of Africa (*D. draco* in Macaronesia and the Moroccan Antiatlas, and *D. tamaranae* from the island of Gran Canaria), and in easternmost locations of the African-Arabian continent (*D. ombet*, *D. serrulata*, *D. schyzantha*) including the insular soqotran *D. cinnabari*. Preliminary studies have been conducted (Carine *et. al.*, not published) but some work still remains to clarify the evolution and dispersal of this fascinating group.

Similar distributions and relationships are probably shown by some *Euphorbia* taxa (*E. balsamifera* and *E. canariensis*), with some data presented by Steeman at the Missouri Botanical Congress (1999), as well as *Kleinia* and *Ceropegia*. It is interesting to note that most of the west-east connections belong to floristic elements established in the lowland vegetation at the Canaries (*Aeonio-Euphorbion* communities), which is dominated by succulents and shrubs of African origin, and where trees are not present.

More genera that share species at both territories have been also investigated, including the molecular works by Thulin et al. (2010) with *Campylanthus* (Canary Islands, Cape Verde and East Africa).

A stronger relationship with the East African area could be the case of *Sideroxylon*. The three relictic Macaronesian species that seem to be closely related to the Canarian *S. canariense* were included in the work of Smedmark et al. (2006). Although *S. fimbriatum* from Soqotra was not investigated, those authors include accessions of *S. marmulano* (showing the connection to the clade including *S. marginatum* from Cape Verde), and *S. inerme* (the only south African species). Studies about this genus are in progress currently. This case could be similar to other investigated like the one with *Ocotea* spp. where the Macaronesian *O. foetens* looks to be related to south African one (Chanderbali et al., 2001), genus not present at Soqotra.

In this context, it is also important to mention the paper by N. Jürgens (1997) about the floristic biodiversity and history of African arid regions. According to this author, we can see that a very important component of the African (mostly the Sahelian or south Saharan) and the Macaronesian floras evolved independently. Only very rare examples as those of *Euphorbia balsamifera* or the genus *Campylanthus* still reveal a relationship between Macaronesia (dry areas), sahelian areas and east Africa, showing probably that this was not a common migratory path for most of the Macaronesian floristic elements related to continental Africa, and suggesting a Mediterranean-North African one before the evolution of Sahara desert.

Macaronesian-East continental African connections (including Mediterranean taxa)

Other relationships have been studied with genera with Mediterranean elements linking the Canaries-Mediterranean with East continental Africa. In *Adenocarpus* (Percy et al. 2000), only indirect connections have been found between the Canarian endemics and eastern African species, via a relationship throughout the Mediterranean area. Several genera have a similar distribution, or are present at both territories, but most of them have not been completely resolved, i.e., *Carex*, *Euphorbia*, *Heliotropium*, *Hypericum*, *Lavandula*, *Limonium*, *Micromeria*, *Lotus*, *Reseda* (with some data from Martín-Bravo et al., 2007), *Teucrium* or *Whitania*. In these cases, it will be necessary include Mediterranean elements to investigate if the Eastern and Western taxa belong to the same or different clades.

In the curious case of *Helichrysum* (Galbany-Casals et al., 2009), some of the Macaronesian species are related to Mediterraean elements (the Canarian *H. gossypinum* and the Madeiran endemic taxa), and the others to a clade with samples from Oman and South Iranian taxa (the Canarian *H. alucense* and *H. monogynum*).

According to Javadi and Yamaguchi (2004), *Cicer canariense*, an endemic to the Canarian islands of Tenerife and La Palma, could be another species related to east Africa (*C. cuneatum*) with a distribution similar to this of *Adenocarpus*, but more species need to be sampled for a clearer result.

Not all of the Macaronesian endemic *Euphorbia* species have been compared with North and East African congeners, though we can hypothesize that this group includes multiple introductions. At least four of these introductions coming from the Mediterranean and east Africa are necessary to explain the present distribution of the genus in Macaronesia. Partial results about Canarian *Euphorbia* taxa were presented by Steenman at the St. Louis Botanical Meeting (1999). According to Molero et al. (2002), the Macaronesian members of sect. Pachycladae are related to the Mediterranean *E. dendroides*. However, neither other African taxa feasibly related to this group based on morphological data, nor the interesting *E. balsamifera*, *E. canariensis* or *E. handiensis* have been investigated.

A dubious relationship was proposed by Thulin et al. (2010) concerning the distribution of some Chenopodiaceae belonging to the genera *Beta* and *Patellifolia*. According to these authors, some material from Somalia and Soqotra island reported as *Tetragonia pentrandra* seems to belong to *Patellifolia patellaris* (native?, introduced?) a species whose known distribution at present restricts to West Africa and the Mediterranean. They proposed to merge the three presently accepted *Patellifolia* species (*P. patellaris*, *P. procumbens* and *P. webbiana*) into only one (*P. procumbens*), which doesn't look to be sufficiently supported in view of the questionable sampling and limited morphological data. New studies (already in progress), are necessary to better streamline this conclusion.

In the opposite situation, other papers (e.g., Andrus et al. 2004), have clarified the non existing relations between East and West previously supposed connection according only to similar morphology. That study included some *Pulicaria* taxa endemic to both Soqotra (e.g., *P. vieraeoides*), and the Canaries (*P. canariensis*), together with the monotypic Canarian genus *Vieria*. The same finding applies in the cases of the *Bencomia* alliance, *Micromeria* or *Polycarpaea*, as reviewed by Andrus et al. (op. cit.). Further investigations are under way in the case of *Micromeria,* but we don't know yet if other east African species will have to be included in future molecular analyses for better resolution.

Relationships with South Africa

Most of the ancient, "morphology-based" proposed relationships between the floras of Macaronesia (mainly the Canaries) and South Africa have been forgotten. A seminal biogeographical paper (Sunding 1979) hypothesized a connection between these areas in the genera Argyranthemum, Echium, Gonospermum, Kunkeliella, Phyllis or Plocama, and several authors have discussed about this proposal. However, none of these relationships has been confirmed with molecular works except for Kunkeliella (which shows affinities with the South African endemic genus Thesidium (Der & Nickrent 2008), and Phyllis (apparently related to the South African Gallopina (Bremer & Eriksson 2009).

After several rounds of molecular research, we know now that *Argyranthemum* is related to north western African elements (*Chrysanthemum*, *Ismelia* and *Heteranthemis*), *Gonospermum* with the Mediterranean *Tanacetum*, and *Plocama* with *Putoria*. Only *Kunkeliella and Ocotea* seem to have a stronger relationship with some elements of the Cape flora. Recent studies revisiting the origin of the Macaronesian endemic genus *Pericallis* show a relationship with the East-South African *Cineraria* (Cron et al., 2008), which is apparently much closer to that group than the North American *Packera* (as proposed by Panero et al. 1999), but the question is not completely solved.

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5

Macaronesian Floras

Taxonomy of island plants: a proposal for a Flora of Macaronesia

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In the early 1970`s a small group of botanists with an interest in the Macaronesian flora attempted to set up a programme for the preparation of a "Flora of Macaronesia". Unfortunately the Project failed to obtain financial support and only preliminary checklist was produced by the Scandinavian participants. Perhaps, in view of the progress that has been made in the study of the Macaronesian flora over the past 35 years or so, the failure of the project was a blessing in disguise as the scientific basis for preparing a Flora is a much more solid one. With regard to the none-endemic flora there have been major advances including the completion of Flora Europaea, Flora de Andalucia Occidental and, of course, the monumental Flora Iberica Project which is now in an advanced stage of completion. With regard to the endemic Macaronesian flora, we are now scientifically in a much better position to justify the preparation of a comprehensive account. We have morphological, micromorphological, reproductive biology and molecular data that can be used to help define species in a better way, to understand their genetic diversity and their biogeographical and taxonomic relationships though we still have a long way to go on this latter subject. However, one major point for debate is what kind of species concept to apply? The advantages and disadvantages of broad and narrow species concepts will be discussed from both practical taxonomic and conservation points of view. This will be mainly for the endemic flora as the non-endemic species wil generally have been covered and defined in the Floras mentioned above. Preparing a Macaronesian Flora will still not be an easy task but the author considers that the moment is right especially as European funding from programmes such as INTERREG might well be available for such a Project.

David Bramwell

David Bramwell was the former director of Director of the Jardín Botánico Canario 'Viera y Clavijo', Cabildo de Gran Canaria". His current research interests include the monitoring and mapping of wild populations of threatened species, the preparation of a Flora of Macaronesia, mapping species decline world-wide, molecular studies of genetic diversity in island populations and the effects of climate change on island plants.

SW Morocco: An obvious step for understanding the Canary Islands Flora.

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Introduction

Phytogeographic relationships between Canary Islands and Africa (especially with SW of Morocco and the coastal fringe of Sahara Occidental) have been highlighted from the first botanical trips in both areas. Bory de Saint Vicent (1803), probably below the influence of the French citizen Pierre Marie Auguste Broussonet, naturalist who knew, recollected and studied the flora of W Morocco (from Tanger to Mogador), is one of the first botanist to sketch the similarities between the easternmost islands (Lanzarote and Fuerteventura) with the neighbouring African coast. These relationships have fascinated many botanists who have described them in different ways (Humboldt & Bonpland, 1814; Berthelot, 1839; Hooker, 1878; Bolle, 1893), such as common floristic elements (Pitard & Proust 1908; Peltier, 1973; Santos-Guerra, 2001; Reyes-Betancort et al., 2003). This has led to establish different phytogeographical approaches to put in order these relationships (Sunding, 1979; Lobin, 1982; Lüpnitz, 1995; Medail & Quézel, 1999). Recent phylogenetic studies have come to strength those relations providing relevant extensive data to the interpretation of plant colonization of the Canary Islands (Santos-Guerra, 1999; Carine et al. 2010). Leaving aside the problems raised by the discrepancies between those biogeographic approaches, these similarities reach also to the central and westernmost islands of the archipelago, especially in dry and warm areas belonging to the infra- and thermomediterranean bioclimatic belts (Buch, 1825; Hooker, 1878; Barbero et al., 1982). In this talk similarities regarding flora and vegetation landscapes between SW Morocco and the Canary Islands are highlighted and not the obvious differences.

Results

Geographically the boundaries of the here called SW Morocco are those defined by Medail & Quézel (1999) as “Argan Sector” enlarged to the south by a narrow strip of littoral steppes to La Güera in Western Sahara. The zone in question stretches from Safi to Cabo Blanco and includes, in addition to the coastal areas, the valley of the Oued Souss, the southern slopes of the western High Atlas and the northern and southern slopes of the western Anti Atlas.

Precipitation ranges from 40 mm in the south coast and 500 mm on the flanks of the High Atlas and Anti Atlas. The oceanic influence is noticeable along this area and fogs and mists are common. From the bioclimatic point of view the boundaries are related with the distribution of the infra- and thermomediterranean belts.

Vegetation

We could say that there are three vegetation landscapes that show similarities between these two territories: (i) along the coastal strip we find analogous plant communities adapted to the intense salt-spray in rocky or sand systems (ii) in low lands we can observe succulent scrubs dominated by cactiform and pachycaulescent *Euphorbia* species practically exclusive to SW Morocco and Western Sahara (iii) finally with the increase in rainfall we can see open sclerophyllous woodlands dominated by the argan tree (*Argania spinosa*), which we can compare with canarian wild-olive and juniper groves (*Olea cerasiformis-Juniperus turbinata* subsp. *canariensis* communities).

From the landscape point of view we might complain that this impression can be observed in any desert or semi-desert environment, which it is not wrong at all, but if we split up a bit more these views, we realize that a significant number of elements of this landscape grow on both sides, and therefore relates us. Moreover some of those elements are endemic to the Canary Islands and the south-western Morocco and Western Sahara, which supports the floristic affinities of the two territories and a common origin of their flora.

Littoral vegetation

Along the sea shore of SW Morocco different communities adapted to salt tolerance, wind exposure and low precipitation are established. In this area the structure and floristic composition of the communities are very similar to those of the Canary Islands.

Sandy coastal strip

Littoral dunes communities are dominated by shrubs adapted to retention of big amount of sand (*Traganum moquinii* and *Atriplex halimus* in both areas for example), while on unstable sands, flattish embryonic mobile dunes and interdune spaces of both areas are characterized mainly by hemicryptophytes like *Euphorbia paralias*, *Lotus spp.*, and *Polygonum maritimum* and the geophyte *Cyperus capitatus* (Sunding, 1972; Reyes-Betancort et al., 2001; Gehú et al., 1996; Gehú & Biondi 1998) or chamaephytes like those of *Ononis hesperia* and *Polycarpaea nivea* (Gehú et al., 1996).

Rocky coastal strip

The same occurs with rocky slopes where we found the equivalent communities like those dominated by *Astydamia latifolia* and *Zygophyllum fontanesii* where *Limonium* and *Frankenia* species are commons (*L. papillatum, L. imbricatum,* and *L. pectinatum* in the Canaries and *L. mucronatum, L. chrysopotamicum*, and *L. asperrimum* in SW Morocco, *Frankenia ericifolia* and *Frankenia capitata* in the Canaries -the later also in SW Morocco-, and *Frankenia corymbosa* in continental areas). These similarities were well described by Gehú & Biondi (1998).

Salt-marshes

In SW Morocco they are better represented than in the eastern Canary Islands, nevertheless the same sequence of communities can be observe: from the lower level of the salt marshes we can see a *Sarcocornia perennis* belt due a permanent circulation of seawater; in a higher level is substituted by a *Arthrocnemum macrostachyum* belt on highly saline soils influenced also by circulation of seawater. In the case of the salt-marsh of Khnifiss Lagoon, near Tarfaya, other communities not present in the Canary Islands can be draw, for example *Sarcocornia fruticosa* community in less saline habitats or *Halocnemum strobilaceum* community establish in hyper-saline environments. A Sesuvium portulacastrum belt is also present here like we can see in Maspalomas pond.

Succulent Scrubs

Low lands of the Canaries are characterised by succulents scrubs dominated by cactiform or pachycaulescent *Euphorbia* species (*Euphorbia canariensis* and *E. handiensis* belong to the cactiform group, and *Euphorbia balsamifera, E. regis-jubae*, *E. lamarckii*, and *E. berthelotii* belong to the other group). In the hyperarid, arid and lower semiarid ombrotypes they represent the mature stage of the vegetation but in upper semiarid to dry ombrotypes probably represent degradation stages of the sclerophyllous woodlands. This interpretation is also used for SW Morocco where *Euphorbia officinarum*, *E. echinus* and *E. regis-jubae* stands are considered degradation stages of *Argania* forests in semiarid ombrotypes (Barbero *et al.* 1982; Peltier, 1986, 1995). To the South, in driest areas, Euphorbia balsamifera subsp. balsamifera and E. echinus communities seems to be representative of mature stages.

Between Tamri to the North and the Oued Massa is easy to see close to the sea the *Euphorbia officinarum* scrubs. To the south this species is substituted by *Euphorbia echinus* which extending inland to an altitude of 1500 m in the Anti Atlas, reaching to 1800 m in rocky sunny places. From Guelmin to the south this scrub is accompanied by numerous Chenopodiaceae: *Traganopsis glomerata*, *Haloxylon scoparium*, *Salsola vermiculata*, and *Suaeda ifniensis* (Barbero et al., 1982; Peltier, 1995).

Euphorbia regis-jubae scrubs are conspicuous between Tiznit and Sidi Ifni while *Euphorbia balsamifera* scrubs, also accompanied by numerous Chenopodiaceae, are present south of the former locality. *Euphorbia balsamifera* subsp. *balsamifera* grows in slopes of ravines and cliffs along the littoral steppe. In our opinion inland populations of this species belong to subsp. sepium.

Sclerophyllous woodlands

These woodlands are characterized in Canary Islands by sclerophyllous woody bushes or small trees with optimal development in semiarid and dry infra- and thermomediterranean belts. In some cases, as an open forest, between *Olea cerasiformis, Juniperus turbinata* subsp. *canariensis*, *Pistacia atlantica* and *P. lentiscus*, and *Phyllirea angustifolia* are common *Euphorbia canariensis* (cardoon), species of the complex *Euphorbia lamarckii-regis-jubae* (bitter spurges) and *Kleinia neriifolia* (berode) among others.

In the other side (SW Morocco) the sclerophyllous woodlands are dominated by *Argania spinosa* (Sapotaceae), the gum tree (*Acacia gummifera*) and various *Euphorbia* shrubs (*Euphorbia regis-jubae*) or cactiform (*Euphorbia officinarum* and *E. echinus*) which represents a specific entity from the physiognomic point of view called "the Argan sector". As *Argania spinosa* itself is present in a diverse ecological area of 820000 ha (Medail & Quezel 1999) a lot of different communities have been described and nominated by this tree. But if we refer to those which occur below the influence of the Atlantic Ocean and in the infra-thermomediterranean belts we found that some Moroccan-Canarian endemic or vicariant plants persist along theirs floristic composition. For instance *Olea maroccana*, *Cytisus albidus*, *Kleinia anteuphorbium*, *Asparagus pastorianus*, *Helianthemum canariense*, *Kickxia sagittata*, *Scilla iridifolia*, *Bupleurum canescens*, *Coronilla viminalis*, and *Sonchus pinnatifidus* are representatives of these relations. *Argania spinosa-Olea maroccana* woodland is well represented in the low and middle slopes of the Ida-ou-Tanane Mountains in the inframediterranean xeric semiarid-dry bioclimatic belt. Above these *Tetraclinis articulata* appears to differentiate a more humid thermomediterranean stage. Others two argan tree forests are worthy of mention such as those characterized each one by the two cactiform *Euphorbia* species: *E. officinarum* to the north and *E. echinus* to the south.

In 1996 was discovered *Dracaena draco* (subsp. *ajgal*) on the western part of Anti Atlas (Benabid & Cuzin 1997), in remotes gorges in the Assif Oumarhouz mountains, between 400-1400 m above see level. Medail & Quézel (1999) defined this dragon-tree formation as "rupicolous community". In our opinion we prefer to define this situation as refuge from anthropogenic activities and not as rupicolous itself. Similar formation of *Dracaena draco*, but in miniature, can be observed in NE of Tenerife, where they grow in very steep fonolitic rocks in the coastal of Anaga massif.

CANARY ISLANDS	SW MOROCCO
SANDY COAST	
Traganetum moquinii	*Traganetum moquinii*
Cypero capitati-Euphorbietum paraliae	*Cypero capitati-Euphorbietum paraliae* *Zygophyllo* waterlotii*-Euphorbietum paraliae*
Cypero capitati-Ononidetum tournefortii	*Cypero capitati-Ononidetum tournefortii* *Euphorbio paraliae-Ononidetum tournefortii*
Polycarpaeo niveae-Lotetum lancerottensis *(vicariant Lotus* spp.)	*Polycarpaeo niveae-Lotetum pseudocretici* *Cypero capitati-Polycarpaeetum niveae* *Polycarpaeo niveae-Bubonietum imbricate* *Polycarpaea nivea-L. assakensis* and *L. chazaliei* communities
Launaeo arborescentis-Ononidetum hesperiae	*Frankenio corymbosae-Ononidetum ramosissimae*
Frankenio capitatae-Zygophylletum gaetuli	*Zygophyllum gaetulum community*
ROCKY COAST	
Frankenio ericifoliae-Zygophylletum fontanesii	*Frankenio laevis-Zygophylletum fontanesii*
Frankenio ericifoliae-Astydamietum latifoliae	*Limonio mucronati-Astydamietum latifoliae*
Chenoleoides tomentosa-Astydamia latifoliae community	*Astydamio latifoliae-Chenoleetum tomentosae*
SALT-MARSHES	
Zygophyllo fontanesii-Arthrocnemetum macrostachyi	*Arthrocnemum macrostachyi* community
Sarcocornietum perennis	*Sarcocornia perennis* community
Frankenio capitatae-Suaedetum verae	*Astydamio latifoliae-Suaedetum verae*
RUPICULOUS COMMUNITIES	
Soncho congesti-Aeonietum holochrysi *Sonchus pinnatifidus* community	*Aeonio arborei-Sonchetum pinnatifidi*
LOW LANDS	
Euphorbietum balsamiferae s.l.	*Euphorbia balsamifera community*

Euphorbietum canariensis s.l.	*Euphorbio officinari-Arganietum spinosae* p.p.
Kickxio sagittatae-Euphorbietum handiensis	*Traganopsido glomeratae-Euphorbietum echini*
Euphorbia regis-jubae communities	*Euphorbio officinari-Arganietum spinosae euphorbietosum regis-jubae* *Euphorbio echinii-Arganietum spinosae euphorbietosum regis-jubae*
MID-LANDS	
Oleetum cerasiformis s.l.	*Oleo maroccanae-Arganietum spinosae* *Euphorbio officinari-Arganietum spinosae* p.p. *Euphorbio echinii-Arganietum spinosae* p.p.

Table 1. Some Canarian vegetation landscapes and theirs analogous in SW Morocco.

Flora

The flora of SW Morocco has been estimated in 1400 species and subspecies. Of these around 120 are endemics (Medail & Quezel, 1999) so this area is one of the most remarkable regions of North Africa. The gradual botanical exploration of this sector has particularly highlighted similarities between this region and the Canary Islands (Hooker, 1878; Peltier, 1973). However, some remote areas remain unexplored while others need a more systematic exploration and a comparative study from the taxonomic point of view is necessary between these two areas.

At present day the Canary Islands share with SW Morocco 28 endemics taxa (Table 2), almost the same number of endemic species that Mahan (Lanzarote and Fuerteventura) shares with the others islands of the Archipelago (around 30 taxa).

In the last two decades relations between Canarian endemics and Moroccoan taxa have been confirmed also from molecular point of view. For example as proposed in previous morphological studies (Bolle, 1892; Cauwet-Marc & Sunding, 1981) the Eastern Canarian endemic *Bupleurum handiense* is related on the base of ITS analyses with the SW Moroccoan endemic *B. canescens* (Neves & Watson, 2004). The macaronesian endemic genus Argyranthemum show affinities with the SW Moroccoan endemic genus *Ismelia* (Francisco-Ortega et al., 1997) or *Pulicaria canariensis* another Eastern Canarian Endemic is sister to *P. mauritanica*, a species endemic to Morocco and Algeria (Francisco-Ortega et al., 2001). A recent paper in *Androcymbium* suggests a close relationship between the two Canary Island species (*A. hierrense* and *A. psammophilum*) with the *A. gramineum* lineage from Essaouira (Del Hoyo et al., 2009).

Another question to avoid is to know patterns of genetic diversity of a widespread shared species along the littoral of SW Morocco to investigate different sources or ways of colonization of the Canaries as origin of early speciation mechanism (Jaén-Molina et al., 2009). Samples of this could be *Matthiola bolleana s.l.* or *Rhodalsine geniculata-webbii s.l.*

Table 2. Common and related flora elements between Canary Islands and SW Morocco.

1	2
Aaronsohnia pubescens subsp. maroccana	*Aeonium balsamiferum / A. kornelius-lemsii*
Ammodaucus nanocarpus	*Andryala pinnatifida subsp. buchiana / subsp. jahandiezii*
Anacyclus radiatus subsp. *coronatus*	*Argyranthemum* spp. / *Ismelia carinata*
Artemisia reptans	*Bupleurum handiense / B. canescens*
Asparagus pastorianus	*Apteranthes burchardii* subsp. *burchardii* / subsp. *maura*
Asplenium filare subsp. *canariense*	*Cytisus proliferus / C. albidus*
Asteriscus graveolens subsp. *odorus*	*Dracaena draco* subsp. *draco* / subsp. *ajgal*
Asteriscus schultzii	*Erucastrum cardaminoides and E. canariense / E. ifniensis*
Astydamia latifolia (Salv.)	*Euphorbia handiensis / E. echinus*
Chenoleoides tomentosa (Mad., Salv.)	*Kleinia neriifolia / K. anteuphorbium*
Euphorbia balsamifera subsp. *balsamifera*	*Limonium papillatum / L. chrysopotamicum*
Euphorbia regis-jubae	*Lotus lancerottensis-L. kunkelii/L. chazaliei-L. assakensis*
Frankenia capitata	*Olea cerasiformis/O. maroccana*
Helianthemum canariense	*Ononis catalinae / O. zygantha*
Limonium tuberculatum	*Pulicaria canariensis / Pulicaria mauritanica*
Lobularia canariensis subsp. *marginata*	*Androcymbium psammophilum/A. gramineum (Essaouira)*
Matthiola longipetala subsp. *viridis*	*Matthiola bolleana / M. longipetala* subsp. *viridis*
Ononis hesperia	
Polycarpaea nivea (CV.)	
Pulicaria burchardii subsp. *burchardii*	
Rhus albida	
Scilla iridifolia	
Senecio massaicus	
Sonchus bourgeaui	
Sonchus pinnatifidus	
Suaeda ifniensis	
Traganum moquini (CV.)	
Zygophyllum fontanesii (Salv., CV.)	

1. Common endemic (shared also with Madera –Mad.–, Salvagems –Salv.– or Cape Verde –CV.–)
2. Islands endemic / closest continental one

Conclusion

SW Morocco constitutes an obvious very specific region from a floristic point of view. However from a physiognomic approach the presence of analogous coastal communities, succulent *Euphorbia* species, and open sclerophyllous woodlands implies a close relationship with certain areas of the Canaries that are climatically and ecologically very similar. This is also supported by a unique list of shared floristic elements.

In the light of these evidences we want to promote the need for multidisciplinary joint studies of these two nearby areas as a way to understand the evolution of diversity in the Canary Island and SW Morocco.

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Conservation strategies for the Azorean priority taxa: what are the molecules telling us?

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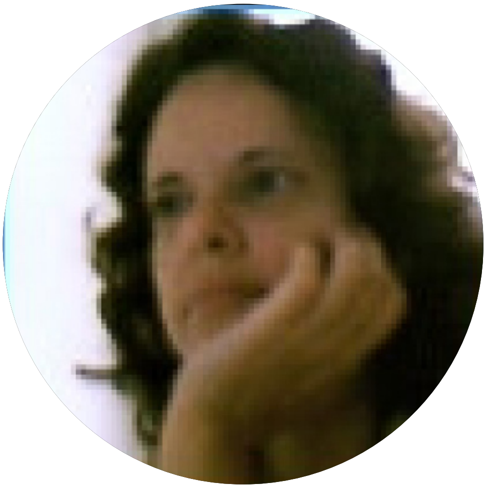
The vascular flora of the Azores comprises about 1,000 taxa, of which no more than 200 are considered native and about 72 endemic. The number of endemic taxa has increased in the last decade, since several taxa, previously considered as native, were found to be endemic, while new subspecies have also been described. A recent evaluation categorized the Azorean vascular plants based on rarity and taxonomic uniqueness, on the knowledge about the threats pending, their ecological role, social interest, and the feasibility of their recovery. As a result of this review, the number of endangered taxa defined as priority for conservation is currently of about 90, which consist of a 50% increase comparing to a decade ago. In order to define a successful conservation strategy for the Azorean priority taxa it is of the utmost importance to understand the degree and distribution of genetic diversity of the existing populations. Since several cases of taxa with low number of effectives were identified, the knowledge of the population genetic structure is particularly important whenever reintroduction actions are required. With the use of molecular genetics techniques we have started to complete information already available on propagation methodologies of some of these priority taxa, developing a populations structure knowledgebase, and pinpointing critical cases of low genetic variability, which will contribute to a more complete understanding of the populations dynamics and to the definition of global conservation plans. Information obtained about genetic diversity patterns is also uncovering clues on these species evolutionary history, providing means to access future risk of diversity reduction.



Mónica Moura

Mónica Moura has been lecturing in the University of the Azores since 1991 where she has obtained her PhD in Plant Physiology. Since 1993 she has been working in the field of propagation of endemic plants by in vitro culture and seed. More recently she has started taking interest in molecular genetics, and is currently developing research in the areas of systematics and population genetics of Azorean endemic taxa.

A phylogeny for the Macaronesian flora and how we can use it to predict potential invaders and identify hotspots of genetic diversity

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In the past 20 years, molecular phylogenies have become the most important tool in plant systematics and their use has greatly improved our understanding of relationships in vascular plants and other organisms. More recently, this 'molecular revolution' has also led to new approaches at the community level ('community phylogenetics') where we now use phylogenies to analyze e.g., the composition of vegetation zones, plant communities or the plant groups that invade these communities.

In the middle-Atlantic islands, the lack of a comprehensive phylogeny has so far hampered considerably the study of flora and vegetation. Even though there is a steadily increasing DNA database for the islands and one archipelago (the Azores) has now its entire angiosperm flora sequenced for two plant barcodes, it will still take a long time to gather DNA data for all 3111 species listed as natives or naturalized species in the most recent checklists for the region. We therefore used a faster supermatrix-supertree approach that allowed us to build a phylogenetic tree for the Macaronesian flora that uses all systematic information available today and can be easily adjusted whenever additional sequence data or taxonomic revisions become available.

1. Building a phylogenetic tree for the complete Macaronesian vascular plant flora

Most phylogenetic studies rely on a combination of more or less variable genetic regions depending on the scope of the study (population level, species level, genus level, etc.). For plant communities, only conserved markers like the plant barcoding markers rbcL and matK can be used because alignments across a range of vascular plant families that usually make up a community are otherwise not possible. These gene regions, however, are not informative enough to resolve phylogenetic relationships in most of the fast-evolving clades of the middle-Atlantic islands like *Echium*, *Micromeria, or Aeonium*. Even genetic markers that were shown to be highly variable in regions outside Macaronesia like the nuclear ribosomal ITS region seem to fail to distinguish morphologically well characterized species in the Canary islands (Schaefer et al., in press). So it is evident that we cannot build a highly resolved phylogenetic tree for all Macaronesian species using only the plant barcoding markers plus e.g., the ITS region. We will rather need group-specific fast evolving markers and for some clades only microsatellites seem promising at the moment. To build our Macaronesia tree we therefore chose a supermatrix-supertree approach, which allows to combine all available evidence: DNA sequence data of fast and slow evolving regions as well as results of AFLP, microsatellite or isoenzyme studies.

First, we produced a DNA phylogeny for the 800 species of the Azorean flora based on the maturase K and rbcL genes (for details see Schaefer et al. 2011). This tree is the backbone structure for our Macaronesia tree. We then downloaded from Genbank all vascular plant sequences with the geographic origins “Canary islands”, “Cape Verde”, and “Madeira”. These sequence datasets were checked manually to eliminate duplicates and non-Macaronesian species. Then we aligned the remaining sequences and added them as 15 extra blocks to the Azores matK + rbcL alignment. This supermatrix with a huge proportion of missing data was then used to build a maximum likelihood (ML) tree with the program RAxML (Stamatakis et al. 2008) using the GTR+CAT model. The resulting well-resolved ML tree included 1920 of the 3111 species listed in the latest checklists for the Azores, Madeira, Canaries, and Cabo Verde and all major native and introduced lineages present in the region. We then added the missing species to the corresponding genera or families in the tree following a supertree approach: if topology information was available from published phylogenetic studies, we resolved the clades accordingly. If not, the additional lineages were added in polytomies with the closest relatives based on morphologic evidence or taxonomy.

2. Calibrating the phylogeny

Time-calibration of molecular phylogenies is a complex topic and for supermatrices/supertrees, standard approaches like Bayesian relaxed clock dating (BEAST) are likely to fail (the often conflicting signal from the different markers and the high proportion of missing data will prevent the runs from reaching a stable likelihood value even after hundreds of millions of generations). We used instead the *bladj* algorithm implemented in *phylocom* (Webb et al. 2008) and fixed the ages of all family crown nodes based on the most recent angiosperm-wide relaxed clock study that had used 22 fossil calibration points (Bell et al. 2011). For ferns and gymnosperms, we used age estimates published by Pryer et al. (2004) and Schneider et al. (2004). At genus level, we used the crown group estimates from our time-calibrated tree for the Azorean flora (Schaefer et al. 2011) and a couple of recent genus level studies from other islands in the region to fix the corresponding clades in the Macaronesia tree. The *bladj* algorithm then allows partitioning of the branch lengths between the fixed calibration points equally among the nodes. The resulting time-calibrated phylogeny for the entire Macaronesian vascular plant flora (Fig. 1) is already reasonably well resolved and it will be very easy to incorporate the results of future studies to resolve the remaining polytomies and adjust the fixed ages of a clade.

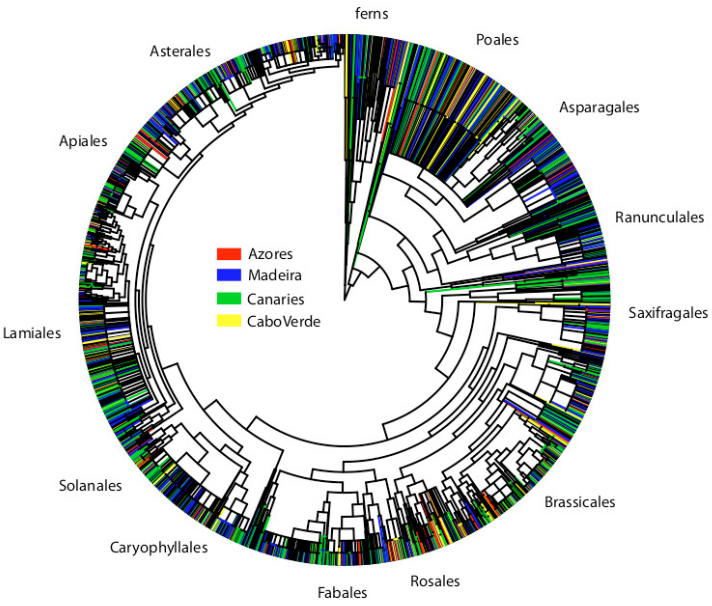

Figure1. Time-calibrated phylogenetic tree for all 3111 vascular plant species of the Macaronesian flora based on a supermatrix-supertree approach (native species of each archipelago color-coded: Azores-red, Madeira-blue, Canary Islands-green, CaboVerde-yellow; naturalized species in black).

3. Phylogenetic Diversity analyses

Vane-Wright et al. (1991) and May (1990) were the first to suggest phylogenetic diversity (PD) as a measure of genetic heritage in conservation planning. PD in the original sense is simply the sum of the phylogenetic branch lengths of a clade or community. PD is often correlated with species richness and to control for this effect, 'PD per taxon' can be calculated.

Ideally, a fully bifurcating ultrametric tree should be used for PD calculations. Our Macaronesia tree is clearly far from fully bifurcating. The polytomies, however, are mostly at the genus level and the more basal regions of the tree are fully resolved. Swenson (2009) showed that PD values based on this kind of tree correspond well to those from fully bifurcating trees. So, while we do agree that all efforts should be made to resolve as many of the remaining polytomies as possible, we are still convinced that the results based on the actual Macaronesia tree will hold.

Specifically, we find a strong increase in total PD going south from the Azores towards the Canary Islands and north from Cabo Verde towards the Canary Islands (Fig. 2), which is the same as the species richness pattern. However, when we control for species richness (PD values divided by number of native species per archipelago), we find an increase in PD per taxon with increasing distance to the mainland. The lowest values are found in the Canaries, followed by Madeira and Cabo Verde. For the Azorean flora, PD per taxon values are almost double the values for Canarian species. This means that the loss of unique genetic heritage is much higher when an Azorean or Cabo Verdian plant species goes extinct than when we loose a Canarian species.

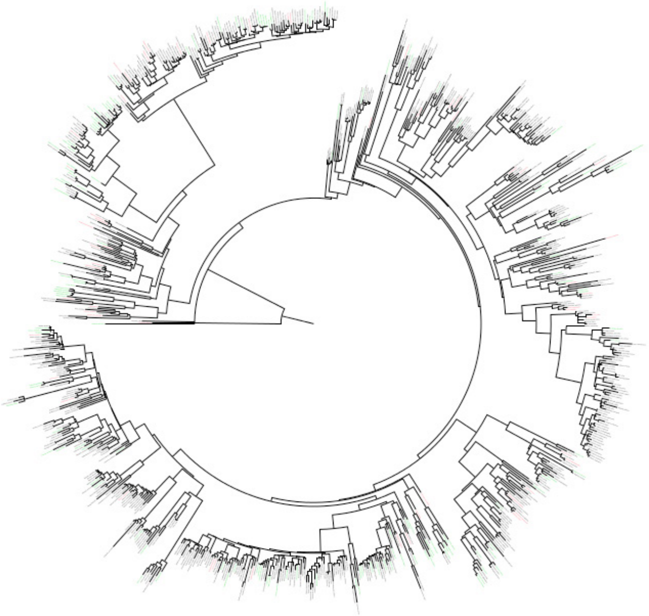

Figure2. Distribution of phylogenetic diversity in the middle-Atlantic islands based on the calibrated branch lengths from the phylogenetic tree for the Macaronesian flora.

4. Invasive species in Macaronesia: testing Darwin's naturalization hypothesis

Invasive species are a serious threat to native plant communities and species throughout Macaronesia but so far it has been very difficult to predict, which species might become problematic (spread into natural ecosystems) and which species can be used as ornamentals without risk. Charles Darwin suggested in 1859 that introduced plant species should be more likely to become invasive in the absence of close relatives in the native flora ('Darwin's naturalisation hypothesis', Darwin 1859). Darwin's idea was that closely related species are more likely to have similar ecological niches due to common ancestry, and therefore would be competing for the same resources. By contrast, 'unrelated' species would be relatively free from competition, and could more easily invade the new environment. To test this hypothesis, adequate phylogenetic information is crucial. So far, no study has used a species-level phylogeny for an entire flora, which is required to evaluate the general importance of this hypothesis.

We used our DNA based phylogeny for the Azorean flora and species lists at archipelago, island, square km, and 100 square meter scale to test Darwin's hypothesis in the Azores and found that introduced plant species are more likely to become invasive in the absence of closely related species in the native flora, verifying Darwin's 'naturalisation hypothesis' (Fig. 3 & 4; for details see Schaefer et al. 2011). This means that ornamental species without close relatives in Macaronesia are likely to escape from cultivation and to cause trouble, whereas those with relatives already present should be less problematic.

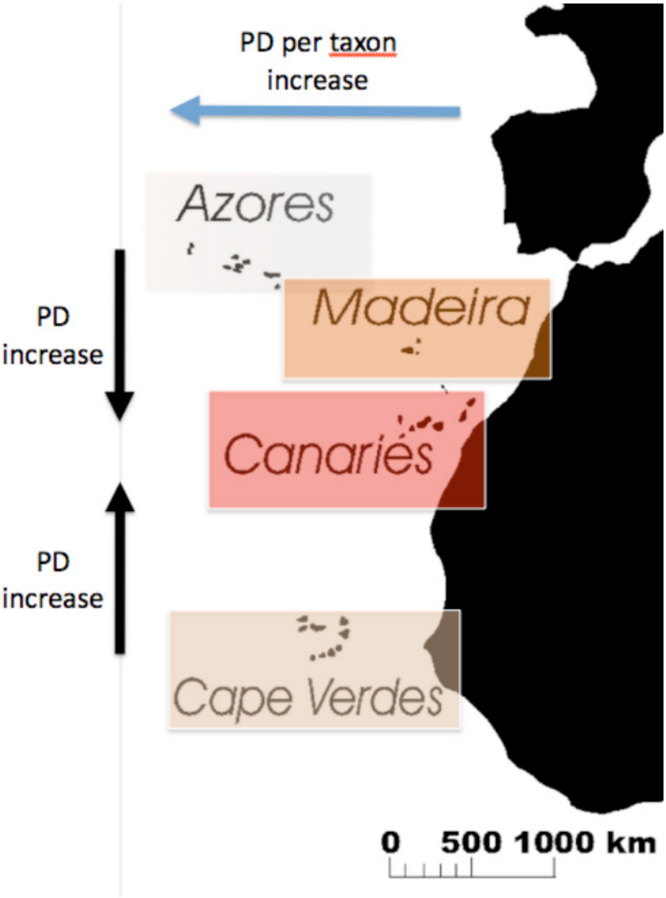

Figure 3. Phylogenetic tree (maximum likelihood) for the angiosperm flora of the Azores (794 species) based on matK + rbcL gene sequences with tips coloured by status group (native - green, non-invasive introduced - black, invasive - red).

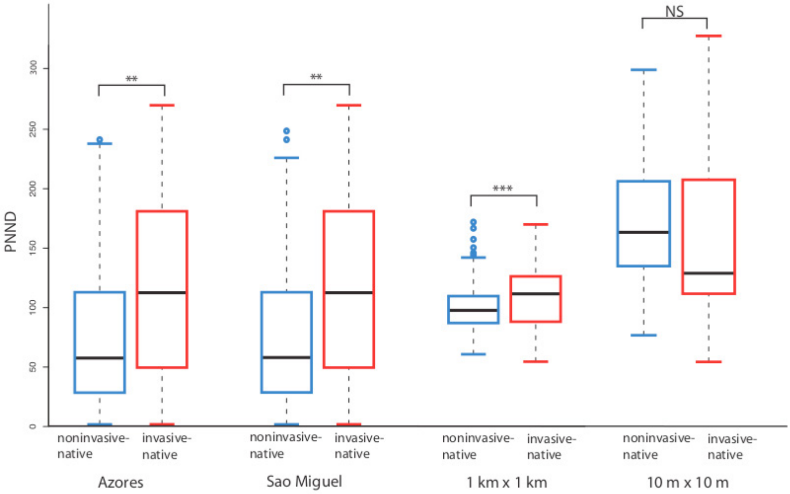

Figure 4. Comparison of phyolgenetic nearest neighbor distance values, PNND, (in million years, based on the time-calibrated tree) in the Azores archipelago for each noninvasive-native pair (n = 589) compared to each invasive-native pair (n = 56) (P = 0.0008); on a single island, Sao Miguel, for each noninvasive-native pair (n = 411) compared to each invasive-native pair (n = 50; P = 0.0008); in square km plots on a single island, Flores, (n=174; comparison of the means for each plot; P = 0.0001); and in 100 m2 plots (n=59) on three islands, comparison of the means for each plot (P = 0.1413). The boxes show the first and third quartiles, the median is indicated by the horizontal line, the range of the data by the vertical dashed line, and outliers (more than 1.5 times the interquartile range above/below the first/third quartile) by circles.

While we have confirmed Darwin's hypothesis so far only for the more isolated Azores, our Macaronesia phylogeny will allow similar tests for the remaining archipelagos as soon as comprehensive classifications for the introduced species become available. So far, for islands other than the Azores, it is still unclear, which species are 'invaders' (spreading into natural habitats) or 'non-invasive introduced' species (restricted to urban and agricultural areas).

5. Outlook

We have shown that our Macaronesia phylogeny already allows a wide variety of tests but still needs some refinement. Recent work in the Azores has shown that there is also a need to revise currently used species concepts (and checklists) in the archipelagos (Schaefer et al., in press). Furthermore, for most community-level and biogeographic analyses, it will be crucial to be able to distinguish native from introduced species and invasive from non-invasive exotics. This will need a lot more work and preferably collaborations between colleagues based in all four archipelagos to make sure that classifications are comparable.

We hope that our work will contribute to clarify the mechanisms that produced the fascinating Macaronesian flora and help to protect these highly threatened species and communities.

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Hanno Schaefer

Hanno Schaefer studied Biology with a focus on Biogeography and Systematics at the Universities of Wuerzburg and Regensburg, Germany. In 1998 he started working in the Azores archipelago.

He published the first fieldguide for the Azores in 2002. In 2003, he defended his PhD on the ‘Chorology and Diversity of the Azorean flora’ (supervisor: Prof. Peter Schoenfelder) and then moved to Susanne Renner’s group at University of Munich, Germany. In Munich, he started working on molecular phylogenies and became interested in the gourd family, Cucurbitaceae, for which he produced several genus-level phylogenies and revisions and a new classification based on morphology and DNA data. In 2008, he won a Marie Curie fellowship that enabled him to move to Imperial College London, where he spent two years in Vincent Savolainen’s group. In London, he was working mainly on a phylogeny for the complete Azorean flora and on different approaches to identify potentially invasive plant species. Since May 2010, he is at Harvard University, Cambridge (USA), where he works on new phylo-geographic approaches using nextgeneration sequencing but also continues to study Cucurbitaceae and the Macaronesian flora.

Comments on the Vascular Flora of Madeira: history, recent advances and relations with other Macaronesian archipelagos

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New information new approaches

The scientific study of Madeira's Flora dates back to the XVII century. Further in the late years of the XVIII century and the XIX century, the interest on the Macaronesian flora gave rise to a continuous flow of botanical visitors or residents such as R.T. Lowe. This intense scrutiny resulted on the description of most of the currently recognized endemic *taxa* (Figure 1). In the XX century a first complete Flora was published by Menezes (1914) and only eighty years later it was replaced by Press & Short: Flora of Madeira (1994). The checklists produced by Hansen (1969) and Hansen & Sunding (1985, 1993) were recently continued by the Checklists for the Canary Islands (Acebes Ginovés et al. 2004; Martín et al. 2005), Cape Verd (Sánchez-Pinto et al. 2005), Azores (Silva et al. 2005) and the Checklist on the Madeira and Selvagens (Jardim & Menezes de Sequeira, 2008b). The use of similar nomenclature and taxonomy along with a similar format on what concerns naturality, allowed for a comparison on the floristic biodiversity patterns by Jardim & Menezes de Sequeira (2008a). Although the Portuguese Phytossociological Association (ALFA) coordinated the "Checklist da Flora de Portugal, Continental, Madeira e Açores" now available online (http://www3.uma.pt/alfa/checklist_flora_pt.html) and due to the use of different criteria (consideration of hybrids and below subspecies ranks) these data can not be compared with other Macaronesian checklists. Although several recent papers (e.g. Silva et al. 2009) on the Madeira's flora refer the naturalization of new species and the description of new endemics, the 2008 Checklist's patterns analysis did not change significantly. A total of 1204 *taxa* of vascular plants is currently recognized, including 154 exclusively endemics, 74 Macaronesian endemics, 546 native *taxa* and 430 introduced *taxa*. Madeira Island comprising 94,4% of the total *taxa* and 89,6% of the endemics from the archipelagos of Madeira and Selvagens, while the Selvagens have only 8,7% of total *taxa* and 8,4% of endemics (Figure 2). The exclusive endemic and Macaronesian *taxa* follow the same pattern. The number of introduced *taxa* is higher in Madeira and Porto Santo.

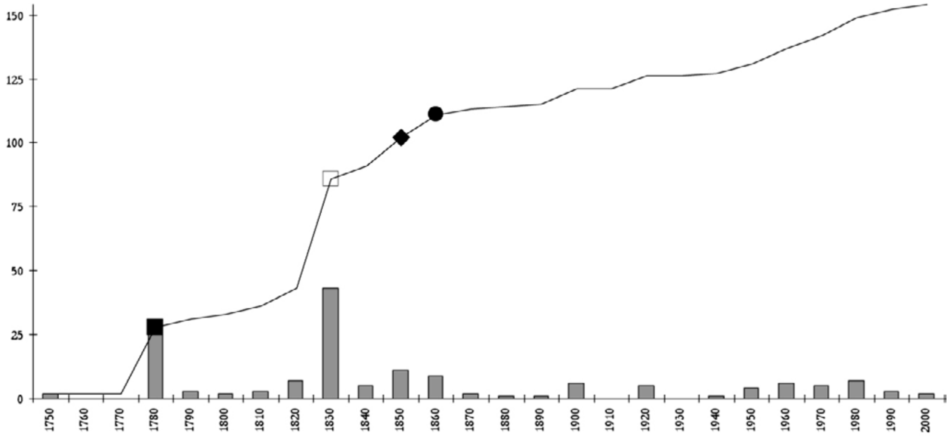

Figure 1. Trends in the description of endemic *taxa* from the archipelagos of Madeira and Selvagens since 1753, with indication of the number of described *taxa* per decade and the cumulative number (▪ Aiton (1731-1793) in Hortus Kewensis (1789) and Carl Linnaeus filius (1741-1783) on Supplementum Plantarum Systematis Vegetabilium Editionis Decimae Tertiae (1782); □, Richard Thomas Lowe Transactions of the Cambridge Philosophical Society vol. 4, 1831; ◆, Richard Thomas Lowe, Manual Flora of Madeira and the Adjacent Islands of Porto Santo and the Desertas published between 1857 and •1872, based on Jardim & Menezes de Sequeira, 2008a).

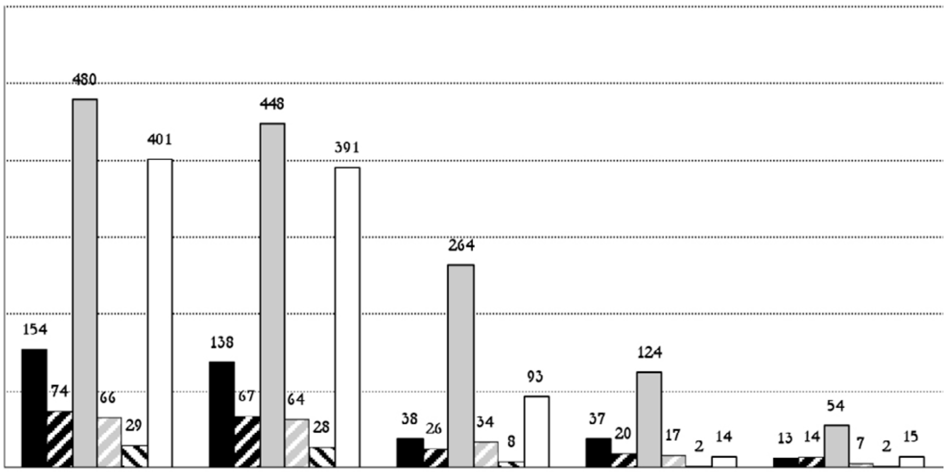

Figure 2. Number of vascular plant *taxa* (species and subspecies), distributed according to colonisation status, in the archipelago of Madeira and Selvagens and individually by island/group of islands (END - Madeira endemics, MAC – Macaronesian endemics, n - native, np - possible native, ip - possible introduced, i – introduced) based on Jardim & Menezes de Sequeira, 2008a

139 families of vascular plants are reported to the archipelagos of Madeira and Selvagens (the larger families being Poaceae, Asteraceae and Fabaceae altogether 32,5% of total *taxa*). Endemic *taxa* belong to 43 different families and 99 genera. Endemic genera are *Sinapidendron* Lowe, *Musschia* Dumort., *Monizia* Lowe, *Melanoselinum* Hoffm. and *Chamaemeles* Lindl. and the genera with the larger number of endemics are *Argyranthemum* Webb and *Sinapidendron* with 6 *taxa* each, *Sonchus* L., *Helichrysum* Mill., *Sedum* L. and *Teucrium* L. having 4 endemic *taxa* each, while *Vicia* L., *Lotus* L., *Geranium* L., *Crepis* L., *Scrophularia* L., *Saxifraga* L. and *Musschia* have only 3 endemic *taxa*.

The geographic distribution of the exclusive endemic *taxa* of the archipelagos of Madeira and Selvagens follows the same pattern obtained for the whole group of vascular plants, *i.e.*, larger islands have a higher number of exclusive endemics. The number of endemics that occur in Porto Santo is apparently inferior to what could be expected, considering that this island is 3.2 times larger than the Desertas.

Macaronesian endemics present in the Madeira flora comprehend 74 *taxa*, although the recent publication of new editions of the Canary Islands (Acebes Ginovés et al. 2010) and Azores (Silva et al. 2010) changed this number (e.g. *Misopates salvagense* from Mad to Mac, *Festuca jubata* from Mac to Az, *Lotus glaucus* from Mac to Mad, *Andryala glandulosa* from Mac to Mad). The analysis of the spatial distribution of these endemics shows that the archipelagos of Madeira and Selvagens share 65 *taxa* with the Canaries, 16 *taxa* with the Azores and only 9 *taxa* with Cape Verde. As known, the Canary Islands have the higher number of native and exclusive endemic *taxa* (67,8%) and Madeira presents the second largest number of native and endemic plants (16,2%). The Azores and Cape Verde have a similar number of endemic vascular plant *taxa* (respectively 7,5 and 7,2%), but the southernmost archipelago has a larger number of native plants. The number of introduced plants is larger in the Azores and in the Canaries, while Cape Verde and Madeira have an identical low number of this category of *taxa*. The islands with a larger number of exclusive endemics are Tenerife (131), Gran Canaria (97) and Madeira (94). On the other hand, five islands of the Azores and Cape Verde present no exclusive endemics. The islands with higher density of exclusive endemics are: La Gomera, Madeira, Desertas and Porto Santo (more than 10 *taxa* per 100 Km^2), followed by El Hierro, Tenerife, La Palma and Gran Canaria (with 2 to 7 *taxa* per 100 Km^2), Lanzarote and S. Nicolau (with values of endemic *taxa* per 100 Km^2 superior to 1). Other Macaronesian islands have lower densities (Figure 3).

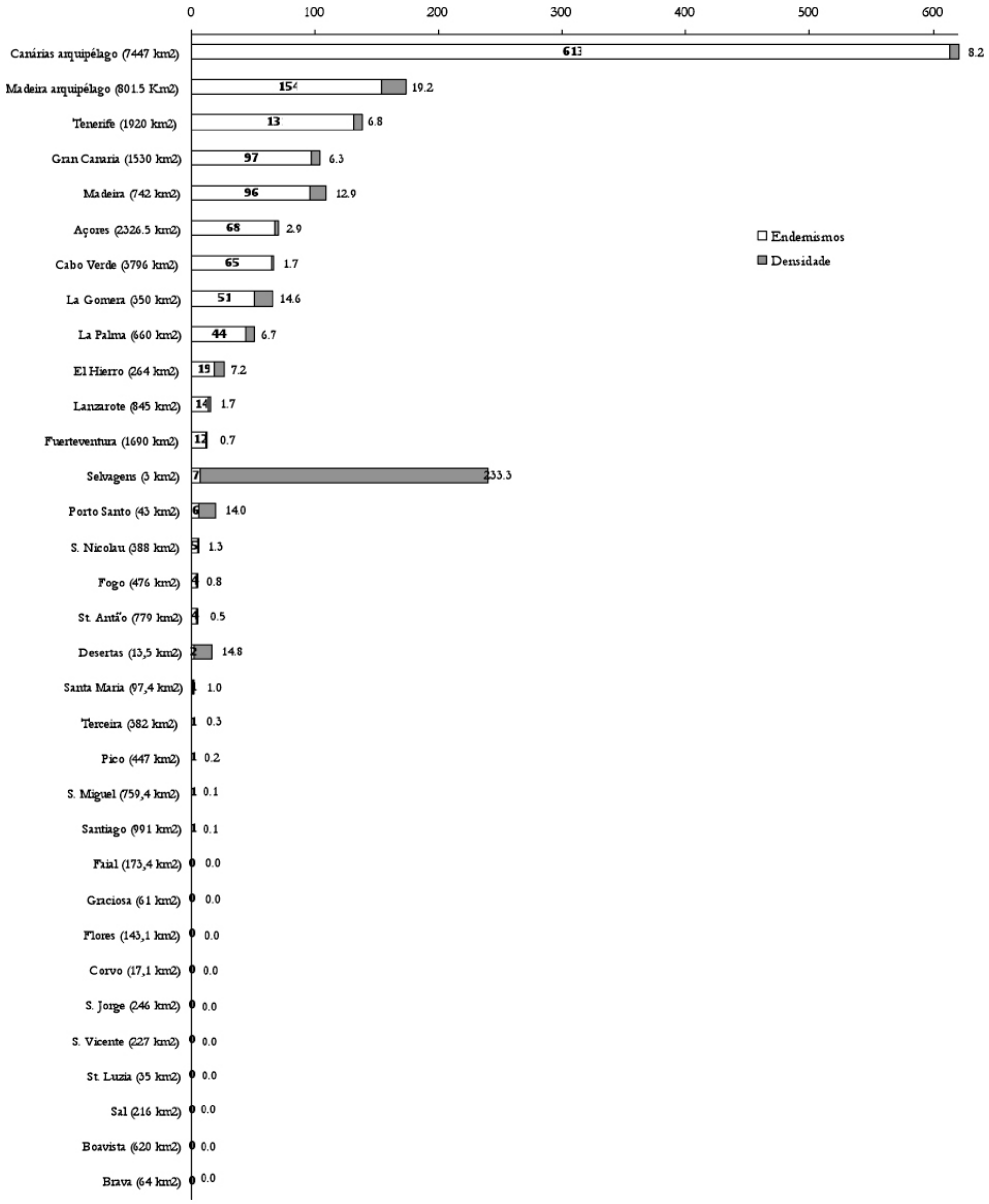

Figure 3. Number of vascular plant *taxa* in the Macaronesian archipelagos according to colonisation status (endemic, END; native, n; introduced, i). B. Number and density (per 100 Km2) of exclusive endemics of the islands and archipelagos of Macaronesia. Based on Jardim & Menezes de Sequeira, 2008a

What can we learn from landscape historical use and pre-Linnaean botanical references?

Early accurate landscape descriptions show that the dense and luxurious forest cover of Madeira Island deeply impressed the first visitors (Frutuoso, 1590). Early documents show that by the end of the XVII century most of the Funchal area was already seriously affected by human activities (Menezes de Sequeira et al. 2007) and it is likely that little of the original vegetation of this area was present during Sloane's visit to southern Madeira (Menezes de Sequeira et al. 2010). Pristine vegetation destruction, caused by overexploitation, grazing and fire was a well-documented fact at the end of the XIX and the beginning of the XX century. Although this is not indirect evidence, it is suggested by early photographs dating back to 1867. This photos show that the legal measures to prevent deforestation along five centuries of human occupancy were unsuccessful. Latter afforestation led to a massive cover of exotic trees. Recently, Pupo-Correia (2007) and Pupo-Correia et al. (2011) using RLPT and analyzing almost one hundred old/new photograph pairs, shown that actual native vegetation occurred where it already existed or in areas without intense human pressure and conversely presenting a very low regeneration in humanised or invaded areas. Well preserved areas usually taken as examples of pristine landscape proved to consist mainly of recently recovered vegetation (Capelo et al. 2004; Capelo et al. 2007) and based on pre-existent secondary communities. Thus, we suggest that species description in the XIX century must have been accomplished on an already severely degraded landscape, possibly on the peak of highest landscape disturbance. Lowe's own descriptions of plants' habitats and comments on the rarity of some endemics nowadays easily found seem to support this assumption. Another important factor was the recent finding of new endemics, some hard to have been overlooked by experienced botanists (possibly just the result of expanding distributions caused by the removal of grazing, *e.g.* the expansion of *Armeria maderensis*, and the finding of new species of *Holcus*). However, data seem to relate species' expansion after pressure removal with the presence of refugees (due to orography). The extinction of *taxa* was facilitated in orographically favourable areas for human settlement. The reference to unknown species by Gaspar Frutuoso or pre-Linnaean references to the Porto Santo flora serve as a good example of probable species extinction. These references are even more interesting since most of the species are nemoral (e.g. *Apollonias barbujana*, *Laurus novocanariensis* and *Dracaena draco* subsp. *draco*), and their former habitats unequivocally related with a nowadays almost completely humanized landscape. By contrary, mountain habitats that are inaccessible for human usage, such as agriculture (*e.g.* a large fraction of Madeira Island and Desertas) seem to have acted as refuges and favoured species' survival. Another curious fact is that documented (post-Linnaean) extinctions are larger in Madeira than in the Azores archipelago and at the same time that most extinct species are mesic or even nemoral. and corresponding to the extinction of dominant species of known and described vegetation types. In any case the number of reported extinctions is very low.

Historical disturbance needs to be taken into account when comparing islands in terms of their plant diversity. In fact, almost five centuries of landscape disturbance could explain at least part of the pattern found both on the Madeira archipelago and other Macaronesian archipelagos. A self-evident and simple fact is that the 'extinction' concept applies only to already described species/*taxa*. This means that species becoming extinct before the XIX century "never existed" and species distribution could just result from local/island extinctions. Although differences in the number of exclusive endemics between islands and archipelagos are certainly related with well-known biogeographic factors such island size, orography, altitude, latitude and distance to other territories, at least partially the same factors could either facilitate or make human-led extinction by landscape change more difficult.

The comparison of the number of *taxa* and the colonisation status between the floras of the Macaronesian archipelagos may provide an estimative of the number of *taxa* that became extinct before even being described. In fact, the lack of exclusive endemics in the largest islands (e.g. some islands from the Azores and mainly Cape Verde), is probably the result of overexploitation. So, the density of exclusive endemics is a valuable tool to evaluate the conservation status of islands and data from better-preserved islands, like Gomera, Madeira and Tenerife, can be used to estimate putative losses in the other Macaronesian islands. Several Macaronesian islands show extremely reduced areas bearing natural vegetation, some clearly being the result of recent successional processes, as disturbance can be derived from vegetation mapping. Azores and Madeira stand for clear-cut distinct situations with most of the Azorean Islands and Porto Santo (Figure 4) showing very low cover of natural vegetation. Although NPV (Natural Potential Vegetation) maps cannot be used to reconstruct relict/vanished habitats/landscapes, they could be used to compare to actual vegetation maps and therefore to estimate disturbance. Diversity patterns on the different islands of the Madeira archipelago reflect not only a different geological origin and a variety of evolutionary processes related to island size, orography, distances etc. but the patterns of human occupancy and the subsequent losses of diversity.

Figure 4. Porto Santo Island landscape without any remains of climax vegetation.

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Top 4 recent publications:

1) Menezes de Sequeira, Santos-Guerra, Jarvis, Oberli, Carine, Maunder, & Francisco-Ortega 2010 Sir Hans Sloane's Madeiran visit: seventeenth century specimens from Macaronesia. Taxon 59 598–612.

2) Menezes de Sequeira, Capelo, Costa & Jardim 2008 Teucrium francoi M. Seq., Capelo, J.C. Costa & R. Jardim, a new species of Teucrium gr. Scorodonia (Lamiaceae) from Madeira. Botanical Journal of the Linnean Society 156 639–647.

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Present and emerging knowledge on the evolution, diversity and conservation of the canarian flora.

A review of recent advances in the study of the endemic flora from Cape Verde Islands

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Cape Verde archipelago

The Cape Verde archipelago comprises the southernmost islands of Macaronesia, about 1500km south of the Canary Islands and is located in the subtropical region in close proximity to the western African coast, i.e., only 570km separate Boavista Island from the African mainland (the coast of Senegal). Cape Verde consists of nine inhabited islands and several islets (Fig. 1) comprising a land area of 4033km^2 with about 1050km of coastline. The islands are grouped in (1) Eastern Islands: Sal, Boavista and Maio; (2) Northern Islands: São Nicolau, São Vicente and Santo Antão; and (3) Southern Islands: Santiago, Fogo and Brava (Table 1). These islands differ in geomorphologic characteristics, Eastern Islands are flat and desert-like, with a relatively wide extend of sand dunes, while the Northern and the Southern Islands are characterized by high mountains [e.g. Tope de Coroa (1979m) in Santo Antão and Pico do Fogo (2829m) in Fogo], offering a wide range of habitats in relatively short areas. This archipelago has a volcanic origin, and Fogo Island has the only volcano still active, the latest eruptions occurred on 1951 and 1995. All the islands have a long history of drought, due to the arid climate, only with one to three humid months. During all the year Cape Verde is influenced by the NE trade winds that specially affect the north-eastern slopes above the 400m of altitude of mountainous islands.

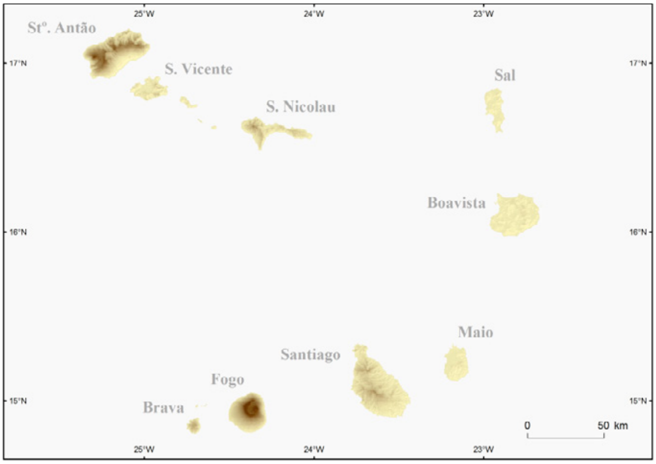

Figure 1. Macaronesian Region (previous page) and Cape Verde archipelago.

Islands Group	Islands Names	Area (km^2)	Max altitude (m)
Northern	*Santo Antão*	*779*	*1,979*
	São Vicente	*227*	*725*
	Santa Luzia	*35*	*395*
	São Nicolau	*343*	*1,304*
Eastern	*Sal*	*216*	*406*
	Boavista	*620*	*387*
	Maio	*269*	*436*
Southern	*Santiago*	*991*	*1,392*
	Fogo	*476*	*2,829*
	Brava	*64*	*976*

Table 1. Some geophysical features of Cape Verde Islands

Floristic data

The Cape Verde archipelago was discovered by Portuguese navigators who, throughout the fifteenth century, explored the African coast. Documents related to its discovery do not record the existence of any traces of human occupation, but mention the existence of several plant species that attracted the attention of their first occupants. Before the XVIIIth century no scientific expeditions had taken place, the first one occurred in 1783 with the Portuguese naturalist João Silva Feijó. Only in the XIXth century Cape Verde Islands began to raise more interest and some field expeditions were made by Forbes in 1822, Darwin in 1832, Hooker in 1839, Bolle in 1851, and Welwitsch in 1853 and 1861. The most important pioneer botanical studies were done by Schmidt "*Beiträge zur Flora der Cap Verdischen Inseln*" (1852) and by Webb "*Spicilegia Gorgonea*" (1849), although Webb alerted for the fact that his catalogue only contained a small portion of costal vegetation and some from neighbouring mountain areas.

In the XXth century, Cape Verde flora was subject of several field expeditions, of which we find of striking importance, Chevalier's work that led in 1935 to the publication of "*Les îles du Cap Vert. Géographie, biogéographie, agriculture, Flore de l'archipel*" and, in 1956 and 1961, Grandvaux Barbosa, that has one of the most important collections of botanical material from the Cape Verde Islands. Most recently the Cape Verde flora was mainly studied by Hansen and Sunding (1993); Lobin and Zizka (1987); Leyens and Lobin (1996); Brochmann et al. (1997) and also by other authors, who have contributed to the publication of several fascicles of the *Flora de Cabo Verde* (Martins et al. 2002-ongoing).

The Cape Verde vascular flora comprises about 750 taxa, including more than one hundred families, being the Asteraceae, Euphorbiaceae, Fabaceae, Malvaceae, Solanaceae, Cyperaceae, and Poaceae the most well represented. Most of the archipelago flora is presently constituted by exotic naturalized species. Although a low number of native species (less than 400 species) is present, Cape Verde is rich in endemic taxa, namely one fern (*Dryopteris gorgonea*) and ~90 flowering plants, including one endemic genus (*Tornabenea*: Fam. *Apiaceae*) and several endemic species from 42 genera. Cape Verde has only few trees most of them being threatened: the endemics marmulan (*Sideroxylon marginata*) and date palm (*Phoenix atlantica*); the dragon's blood tree (*Dracaena draco*); the fig trees (*Ficus gnaphalocarpa,* and *F. sur*); and the tamarisk (*Tamarix senegalensis*), which fossilized stalks and roots are found in sand dunes of Boavista, S. Vicente, Sal, and Maio.

Plant communities

Climatic factors related with altitude and aspect, as well as soil characteristics are mainly responsible for the different plant communities found on Cape Verde (Duarte and Romeiras, 2009). Owing to their high altitudes Santo Antão, São Nicolau, Fogo, Santiago, São Vicente and Brava present higher diversities in plant communities than the islands of the Eastern group (Sal, Maio, Boavista).

Even though the natural landscape has been altered especially by human activity, there are still natural communities where most of the native flora occurs, namely:

1) in the coastal sand dunes, native species such as *Sporobolus spicatus*, *Cyperus crassipes*, *Cistanche brunneri*, *Lotus brunneri*, *Limonium brunneri*, and

Zygophyllum waterlotii are common;

2) in semi-arid cliffs and rocky escarpments up to 400m, herbaceous and subshrub vegetation becomes denser and includes some endemic species like *Asparagus squarrosus* and *Sarcostemma daltonii* (Fig. 2a);
3) also up to 300-400m some formations like "savannah" with *Acacia albida* and *Zyzyphus mauritiana* can be found;
4) the rocky scarps at high altitudes, are rich in endemic species such as *Campylanthus glaber* ssp. *glaber*, *Conyza fea*, *Euphorbia tuckeyana, Echium hypertropicum*, *Globularia amygdalifolia*, *Lavandula rotundifolia*, *Lobularia canariensis* ssp. *fruticosa*, *Nauplius daltonii ssp. vogelli, Periploca chevalieri*, *Satureja forbesii*, *Sideroxylon marginata,* and *Sonchus daltonii* (Fig. 2b);
5) *Artemisia gorgonum*, *Echium vulcanorum*, *Euphorbia tuckeyana*, *Erysimum caboverdeanum, Globularia amygdalifolia*, and *Verbascum cystolithicum*, are some of typical endemic species found on volcanic soils of Fogo highlands, above 1600m (Fig. 2c).

Figures 2 (a, b and c). a) Semi-arid cliffs and rocky escarpments up to 400m with Sarcostemma daltonii; b) Sonchus daltonii in rocky scarps at high altitudes of Santo Antão Island; c) Volcanic soils of Fogo Island, above 1600m with Artemisia gorgonum (above) and Euphorbia tuckeyana (below).

Threats to plant diversity

Despite the subtropical dry climate that characterizes the Cape Verde archipelago, and which has not permitted the development of abundant vegetation, a negative impact resulting from anthropogenic factors was especially noted since the second half of the XX^th^

century. According to Leyens and Lobin (1996) about 30% of the native vascular flora and 50% of endemic species are threatened. Thus, wood collection for domestic fuel (e.g. *Echium vulcanorum*, *Periploca chevalieri*, *Sideroxylon marginata*), or fodder (e.g. *Lotus purpureus*, *Sonchus daltonii*, *Tornabenea bischoffii*), or tanning (*Euphorbia tuckeyana*), or as medicinal (e.g. *Artemisia gorgonum*, *Campylanthus glaber s.l.*, *Satureja forbesii*) are responsible for the high threatened rates of the native flora. Introduction of plants of agricultural interest since early colonization times, and with them adventitious species from different geographic origins, constituted another factor in defining the present day flora, leading to changes in the composition of autochthonous plant communities in the different Cape Verde Islands. In fact, most of the endemic species appears as small populations, distributed in habitats with clearly defined ecological characteristics, in particular in northeast-exposed areas and high mountains above 400m of altitude. Most of those habitats previously occupied by native species are being progressively occupied by exotic species, a situation that might lead to a drastic reduction of the autochthonous vegetation of these islands. The uncontrolled spread of some exotic species like *Lantana camara*, *Furcraea foetida* (Fig. 3) and *Prosopis* spp., introduced for forestry or land conservation purposes, has led to the invasion of native vegetation habitats'. The introduction of *Prosopis* spp. in temporary river beds, led to the depletion of the scarce water resources of the islands.

Figure 3. Exotic species: Furcraea foetida invading native plant communities.

Cape Verde flora: distribution patterns and phytogeographic links

In general, the Eastern Islands and the lowlands of the Northern and Southern islands show more floristic affinities with North-West African regions than with the other archipelagos of Macaronesia. However a different picture occurs in highlands of the Northern and in the Southern Islands where more rich vegetation occurs, especially in the northeast-exposed slopes above 400m. In Northern and Southern Islands, plants share greater floristic affinities with the other Macaronesian archipelagos, especially with Canaries and Madeira. In this case, we quote with particular interest certain genera of angiosperms, which have a large number of endemic taxa in Macaronesia such as *Aeonium*, *Echium*, *Euphorbia*, *Campanula*, and *Sonchus*.

Molecular phylogenies published until now were mainly based on Azores, Canaries and Madeira floras, being frequent a limited taxon sampling among the Cape Verde endemic species. For instance, from the six *Tornabenea* endemic species (Fig. 4a) only two were included in Spalik and Downie (2007) study. In the phylogeny of the Macaronesian *Lotus* species (Allan et al., 2004) only *L. jacobaeus* and *L. purpureus* were included in the analysis, from the 5 endemic species in Cape Verde. To our best knowledge, no comprehensive molecular phylogenetic analysis was already published concerning the genus *Diplotaxis* (Fig. 4b) in the Cape Verde, where nine endemic taxa are accepted. So, there is an urgent need for additional taxonomic, ecological and molecular studies, in order to understand the origin and colonization patterns of some genera that show a wide diversification within this archipelago. Furthermore, these studies would help to re-evaluate the taxonomic status of infrageneric taxa in genera like *Campylanthus, Echium, Frankenia, Kickxia,* or *Lobularia* for which several species and subspecies were described based on a presumed morphological variation according to the altitude where they occurred. For instance, based on the patterns of morphological and ecological variation three subspecies of *Kickxia elegans* were recognized in Santo Antão Island, namely subsp. *elegans* for the coastal areas; subsp. *webbiana* 200-550m and subsp. *dichondrifolia* 450-1700m. Likewise, *Campylanthus glaber s.l.* is a very variable species and a taxon for the low altitude areas was described for Santo Antão Island (*C. spathulatus*), and other (*C. glaber*) for the mountainous regions (Brochmann et. al, 1997).

Figures 4 (a and b). Cape Verde endemic species. a) Tornabenea sp. and b) Diplotaxis sp.

The Cape Verde endemics which present wider distributions show a more or less continuous morphological variation from the coastal to the mountainous regions, in response to the gradual changes in the conditions of humidity mainly related with pluviometry and with the effects of trade winds. So it is very difficult to distinguish some of these taxa based on a presumed morphological variation according to the altitude where they occurred. Within this scope, an integrated study of ecological, morphological and molecular characters must be carried out at inter- and intraspecific level, for these endemic taxa. Moreover, these studies should preferably include whole Macaronesian taxonomic groups and with recourse to additional molecular markers, which can enable a better result in the establishment of the origin and phylogeny of the flora of these islands.

Final notes

In general, the recovery of natural vegetation from tropical and subtropical dry regions, like Cape Verde Islands, is difficult particularly when compared to that of ecosystems from tropical humid regions. It is important to notice that especially the Eastern Islands (Sal, Boavista and Maio) faces large periods of serious drought and therefore present poor flora comprising mainly annual herbaceous species. These three islands suffer of desertification problems, and a great amount of pressure is caused by an exponentially emerging tourism in coastal areas that possess white sand beaches and dunes. In the past few decades, these areas have been targeted to the construction of several hotels and resorts, which exert strong environmental pressures on the native vegetation of the surrounding areas.

The knowledge and characterisation of Cape Verde flora is fundamental to the conservation of biodiversity in this archipelago. The preliminary Red Data List (Leyens and Lobin 1996) was published more than 15 years ago, and, as stated above, about 50% of the endemic species were classified as threatened species. However, our field experience allows us to consider that this number is certainly much higher. So, there is an urgent need to update species abundance, and to identify what are the main threatening factors affecting native plants in these islands, in order to suggest precise conservation measures. In the Macaronesian region, Cape Verde has low financial resources and is the only archipelago which is not part of the European Union, in contrast to Madeira, Azores and Canary archipelagos. Thus, we consider that an effective support from developed countries is required to undertake the preservation of Cape Verde's endemic flora.

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