

Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands

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The Canary Islands are historically and biologically of particular interest. Pliny the Elder heard about 'Canaria, so called from the multitude of dogs (canes) of great size', through an expedition (c. 40 BC) to the islands by Juba II, King of Mauritania. The island of El Hierro was chosen by Ptolemy as the prime meridian of longitude because it was the most westerly place known in his time. The aboriginal inhabitants of the Canary Islands were the Guanches, probably of Berber origin^{1,2}, who were conquered by the Spanish in the 15th century. Following this, the Canaries became an important base for sea journeys to the Americas and Christopher Columbus replenished all four of his westbound fleets at the islands.

The Canary archipelago

The Canary Islands are situated in the northeast Atlantic ocean between 27°37' and 29°25'N, and 13°20' and 18°10'W (Box 1). The nearest island (Fuerteventura) to the continent lies approximately 110 km off the northwest African mainland (Cape Juby) and the most distant island (La Palma) lies 460 km off the mainland. The archipelago has seven islets, and seven main islands aligned from east to west: Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro.

All the islands were formed in the past 20 million years (Ma) by volcanic eruptions. The central and western islands exceed 1400 m at the highest volcanoes, with Teide on Tenerife rising to 3718 m. The geological history of the islands is well documented³⁻⁶ (Box 1), providing a known temporal sequence for the origin of the islands and thus for their availability for colonization by diverse biota. The Pacific Hawaiian Islands and the Atlantic Canaries have similarities, but also have distinctive differences, with the Atlantic Canaries having longer life, more complex volcanic evolution and close proximity to the continent. Island formation is controversial; the two main competing theories being a 'hot spot' origin and 'propagating fracture' from the Atlas mountains^{7,8} (Box 1).

Recently, the Canary Islands have become a focus for studies of the colonization and the diversification of different organisms. Some authors have considered Canarian endemisms as relicts of Tertiary origin, but new molecular data suggest a general pattern of continental dispersion followed by *in situ* speciation. Recent phylogeographic studies are revealing variants of the simple stepping-stone colonization model that seems to hold for many Hawaiian groups. Many factors can generate deviations from such a pattern: the stochastic nature of colonization, competitive exclusion, phylogenetic constraints on adaptive evolution and extinction. An understanding of island colonization and diversification can best be developed from an ecosystem level synthesis as more data for the Canarian archipelago come to hand.

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Biogeography and ecology

The Canary Islands have a sub-tropical climate; temperatures are warm and show little seasonal variation. The climate is strongly influenced by the humid trade winds from the northeast, which, in combination with the altitude of the volcanoes and the drier north-west winds blowing at higher levels, produce an inversion zone and marked vegetational zones (Box 2). Some of the most peculiar and interesting habitats in the Canary Islands are those produced by the volcanic terrains, which are inhabited by specialized animals⁹ (Box 3).

The Canarian fauna and flora, which is related to that of Madeira and, to some extent, to that of the Azores and the Cape Verde Islands, has affinities with the Mediterranean region, although some elements are related to more remote regions [*Ceropegia* (Asclepiadaceae) and *Sideroxylon* (Sapotaceae) to East Africa; *Apollonias* (Lauraceae) to India; *Bystropogon* (Lamiaceae) to South America; *Picconia* (Oleaceae) to Australia; *Vanessa indica* (Nymphalidae) and *Cycliurus webbianus* (Lycaenidae) to Asia; *Danaus* spp. (Nymphalidae) to America and Ethiopian Africa, etc.]. Given the prevailing winds and the sea currents, two

plausible sources of colonizers are neighbouring North Africa and the Iberian Peninsula. Endemism is high in the archipelago: about 27% of the approximately 1000 native vascular plant species and 50% of the terrestrial invertebrate fauna (c. 6500 species) are endemic. Amphibians were absent from the original fauna; only one snake has been recorded (already extinct), and apart from bats only five mammals were present – two shrews and three rodents (although rodents are now extinct).

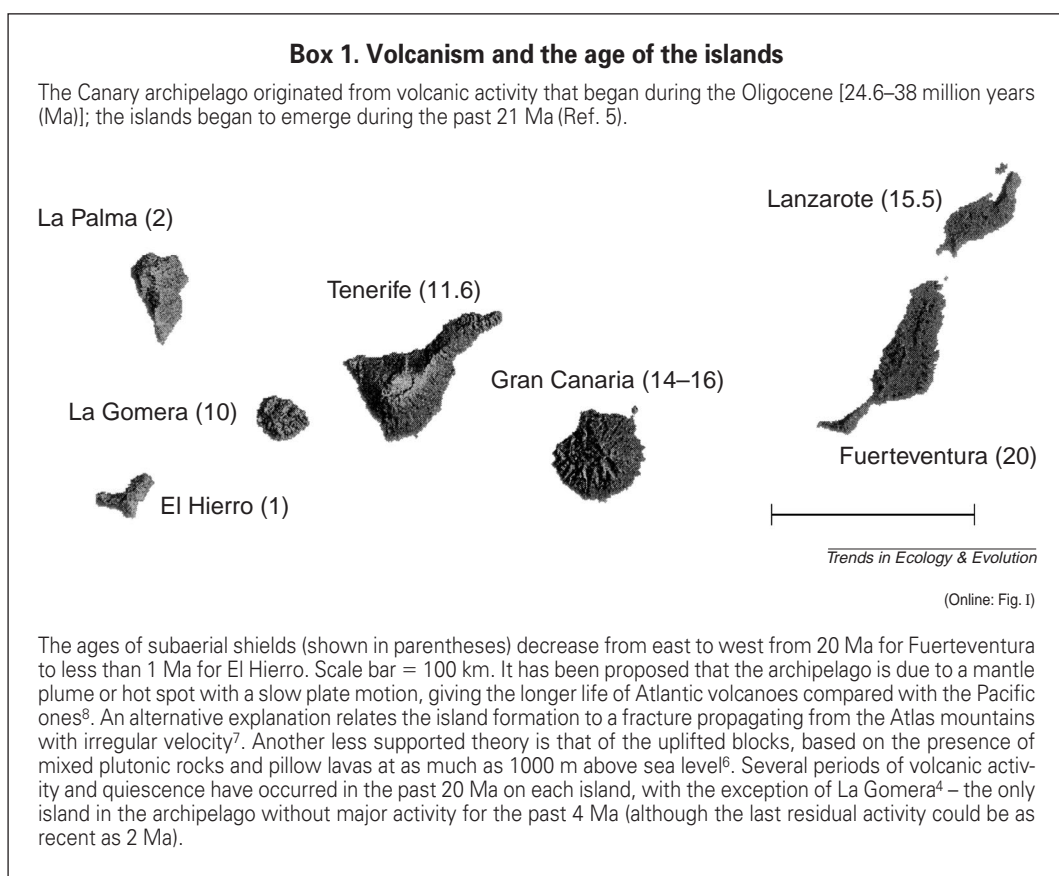
Some notable invertebrate families that occur on the mainland are absent, for example, from the order Coleoptera (Lucanidae, Geotrupidae and Lampyridae) and from the order Hemiptera (Cicadidae, Naucoridae and Nepidae). In some instances complete orders are absent, such as the Plecoptera and Phasmoptera. Other orders were originally absent but have been introduced, such as Scorpiones, Symphyla and Embioptera.

Examples of diversification within the archipelago can also be found among vertebrates, notably geckos (*Tarentola*), lizards (*Gallotia*) and skinks (*Chalcides*). Incipient speciation processes (subspecies divergence) are occurring in some birds, such as finches (*Fringilla coelebs*) and blue tits (*Parus caeruleus*). But, by far the most striking cases of diversification are among invertebrates, because of either geographic isolation or adaptation to diverse niches. Some of the groups (number of endemic species in parenthesis) are: Gastropoda¹⁰ – *Napaeus* (50) and *Hemicycla* (35); Araneae¹¹ (M.A. Arnedo, PhD thesis, Universitat de Barcelona, 1998) – *Spermophorides* (22), *Dysdera* (44) and *Oecobius* (34); Isopoda – *Porcellio* (18); Diplopoda¹² – *Dolichoilulus* (46); Collembola¹³ – *Pholosomes* (16); Hemiptera¹⁴ – *Cyphopterus* (24) and *Erythro-neura* (23); Coleoptera^{15,16} – *Calathus* (24), *Cardiophorus* (31), *Attalus* (51), *Tarphius* (30), *Hegeter* (23), *Nesotes* (20), *Laparocerus* (66) and *Acalles* (18).

Colonization pathways and the stepping-stone model

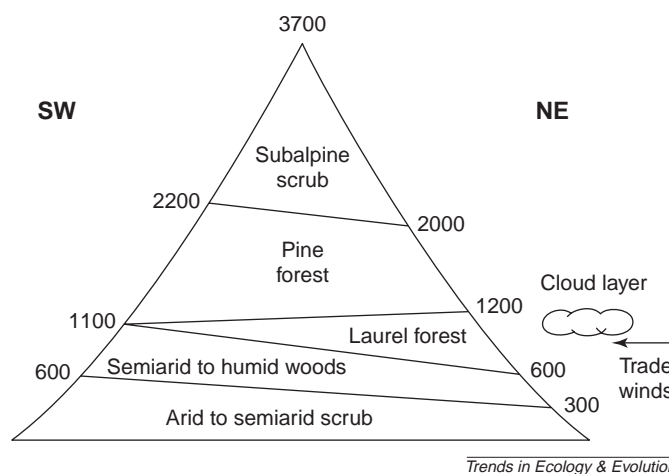
DNA data are better suited for a phylogenetic tree than morphology when interpreted in terms of dispersal (i.e. colonization sequence)¹⁷. A morphological phylogenetic tree is constrained by the ecogenetic adaptation to current and to recent selective pressures, but these are unlikely to confound a DNA-based phylogeny. Recently, molecular phylogenies have been obtained for Canarian lizards^{17–22}, skinks²³, geckos²⁴, beetles^{25–31}, butterflies³², *Drosophila*^{33,34}, cockroaches (I. Izquierdo, PhD thesis, Universidad de La Laguna, Tenerife, 1997), bees^{35–37}, spiders (M.A. Arnedo, PhD thesis)³⁸, mites³⁹ and plants^{40–47}. Most of these studies have made use of mitochondrial or chloroplast DNA sequences as a marker for inferring dispersal events between islands. In some of these taxa, the predominant pattern is similar to the one found for Hawaiian organisms; a stepwise colonization sequence from older to younger islands in the chain. However, this simple pattern can be complicated by several factors. Back colonization, recent colonization, within-island differentiation, adaptation and extinction are some of the historical events that confound a simple ‘island colonized as it emerges’ pattern.

Thorpe *et al.*¹⁷ have proposed two ways of interpreting a tree in terms of colonization sequence, taking into account biogeographical and genetic data. One method uses tree topology and geography, based on the increased probability of an island being colonized by founders from a closer island than from a distant one. The other method of determining the polarity of dispersal uses tree topology plus branch length. This method relies on the proposal that there is an acceleration of divergence caused by a founder effect, thus allowing discrimination between colonist and ancestor.



Box 2. Vegetational zones

The central (Gran Canaria and Tenerife) and western (La Gomera, La Palma and El Hierro) islands have high volcanoes that for most of the year have cloud banks at around 1000 m on the windward slopes. These clouds are caused by the upper hot dry air and by the lower humid trade winds. As a result, five vegetation zones can be distinguished: (1) arid subtropical scrub up to 250 m; (2) humid and semi-arid subtropical scrub, and woods from 250 to 600 m altitude; (3) humid laurel forest in the cloud belt from 600–1000 m; (4) humid to dry temperate pine forest from 1000–2000 m; and (5) dry subalpine scrub over 2000 m.



The eastern islands are influenced less by trade winds because of their lower altitude and because rainfall is also scarcer owing to their geographic situation. An arid scrub with many xerophilous African plants dominates almost everywhere. Only on the top of a few mountains (Jandia in Fuerteventura and Famara in Lanzarote) can local hygrophilous endemisms be found. These are most probably the remains of more humid woods occurring before the glaciations.

Box 3. Lava flow and lava-tube habitat

The recent, barren lava flows are inhabited by 'lavicolous' species, which are adapted to extreme conditions (drought, exposure to sun, temperature changes and no primary production)⁹. They feed on biological fall-out and are quickly replaced by competitors as soon as primary succession provides the lava with soil and plants (a few hundred years). The underground environment is also peculiar in volcanic, oceanic islands, where basaltic flows prevail. The existing caves are lava tubes with the typical environmental conditions of caves (humidity, darkness and scarce food), but are highly different in other features⁴⁹. Unlike karstic caves, lava tubes are shallow, with roots inside, little running water and few ponds. The formation is quick and succession usually takes place upwards instead of downwards, with troglobites moving closer to the surface as the lava ages and shallow passages become more isolated from the surface climate. This contrasts with karstic caves where hypogean communities tend to extend deeper down as time goes by. Succession in lava flows and volcanic caves is closely related. The timespan during which a lava tube provides a suitable environment for particular adapted life forms is longer than in lavas, but much shorter than in karstic caves. Collapsing and silting limit the tube duration from 20 000 to 500 000 years. However, troglobites in the Canary Islands are not really fugitive species because the lava tubes are not their only habitat, they also occur in the cracks and voids of the bedrock and in the mesocavernous shallow stratum, in medium and old volcanic terrains. The variety of underground habitats, geological history and underground barriers has led to the evolution of a rich troglobitic fauna in most of the islands.

The phylogeographic hypothesis for the lizard of the western Canary Islands, *Gallotia galloti*^{17–22}, based upon recent studies using different DNA markers, is compatible with the time of emergence and the sequence of island formation. *Gallotia galloti* appears to have dispersed from Tenerife along two independent pathways: one from north Tenerife to La Palma, and the other from south Tenerife to La Gomera and to El Hierro. The sister group relationship of the western *G. galloti* lineage to either *G. stehlini* (Gran Canaria) or the eastern *G. atlantica* (Fuerteventura and Lanzarote) is less robust^{21,22}. Also compatible with the stepping-stone model are the darkling beetles of the genera *Pimelia* and *Hegeyer*^{26–30}. Both show colonization patterns that are essentially compatible with a stepwise dispersal from older islands in the east to younger islands in the west. A back colonization probably occurred from Tenerife to Gran Canaria for *Pimelia*. The phylogenetic relationships of brimstone butterflies³² (genus *Gonepteryx*) point to a North African ancestry for the Canarian taxa (three species) and subsequent within-archipelago dispersals from Tenerife to La Gomera and Tenerife to La Palma. The colonization of the archipelago by *Drosophila subobscura* is also consistent with a stepping-stone model of directional east–west migration^{33,34}.

The phylogeography of the endemic skinks of the genus *Chalcides*²³ is only partially concordant with a stepping-stone colonization. A relatively recent dispersal from La Gomera to El Hierro is apparently the only colonization following the ages of emergence of the islands. More ancient colonizations between La Gomera and Tenerife, and between Gran Canaria and Tenerife or La Gomera can be deduced, but the relationship of those clades with the Fuerteventuran *Chalcides simonyi* lineage or with the North African outgroups is unclear.

For the genus *Tarentola*²⁴ (Gekkonidae), mitochondrial 12S and cytochrome b sequences indicate several independent colonization events from the continent. The phylogeny shows higher genetic divergences compared with *Gallotia* and *Chalcides*. The greater age of the *Tarentola* group could have involved more extinction followed by replacement, generating a contemporary phylogenetic pattern that is not consistent with a simple stepping-stone

model. Recent studies of *Calathus*³¹, a genus of ground beetles, have also revealed a phylogeographic pattern that conflicts with a stepping-stone colonization model. *Calathus* has colonized the Canary Islands at least twice and probably three times (mtDNA COI and COII sequence data). The most recent colonization from the mainland has occurred on the oldest islands of Fuerteventura and Lanzarote.

Within-island phylogeography and volcanism

Once an island has been colonized, within island differentiation is facilitated by vicariance events produced by lava flows and by local extinctions followed by recolonization. This has been documented in many Hawaiian biota⁴⁸. Across the islands of Fuerteventura and Lanzarote, subaerial volcanic activity has been almost continuous in the past 20–22 Ma (Ref. 5). The current top layers of the islands are the result of two distinct cycles, one ranging from 21 to 12 Ma, and another ranging from 6 Ma to prehistoric and historic eruptions. This volcanic activity has resulted in a SSW (oldest) to NNE (youngest) age gradient for the surface terrains of Fuerteventura and Lanzarote. This geological age gradient is consistent with the mitochondrial genealogy (mtDNA COI sequence data) found for the endemic *Hegeyer politus* (Coleoptera, Tenebrionidae)³⁰. This can be explained by an ancestral population in the south, which colonized in a northerly direction following the progressive northward cessation of volcanic activity.

A repeated phylogeographic pattern is found in diverse organisms on the island of Tenerife. *Gallotia* lizards, *Chalcides* skinks, *Pimelia*, *Calathus* and *Eutrichopus* beetles, *Steganecarus* mites, *Dysdera* spiders and *Loboptera* cockroaches all possess vicariant sister taxa affiliated with the Anaga and Teno massifs. The level of divergence between these sister lineages varies considerably among the different taxa and molecular markers used, but, in all cases, this pattern can be related to the disjunct volcanic evolution of the island³. The relatively recent volcanic activity (less than 2 Ma) producing the Pico Viejo and Teide eruptions, generated massive lava flows that joined the previously separate massifs of Anaga in the northeast and Teno in the northwest. These geologically old massifs (>4.5 Ma) have not been covered by recent lava flows (Box 4). The present-day distributions and phylogeographies of many organisms in Tenerife can be related to the recent connection of the massifs. For example, the beetle *Pimelia* shows two distinct mtDNA and ITS (internal transcribed spacer) lineages associated with the west and the east of Tenerife, which probably originated on Teno and Anaga, respectively, and spread over the central regions after the connective eruptions²⁸.

The Gran Canarian skink *Chalcides sexlineatus* shows considerable within-island morphological variation that is, in part, coincident with genetic differentiation²³. Similar morphological and genetic differentiation is present in *Tarentola* geckos from Gran Canaria²⁴. These phylogeographic patterns are probably related to vicariance caused by the last volcanic cycle on the island approximately 2.8 Ma. There is a distinct possibility of mass extinctions about 3.4–4.5 Ma on Gran Canaria caused by the violent emission of volcanic agglomerates over a great part of the island. This could explain some of the back and relatively recent colonizations of Gran Canaria shown by the phylogeographies of *Dysdera*, *Calathus*, *Tarphius* (B. Emerson, unpublished), and possibly *Chalcides* and *Tarentola* species.

Radiations, habitat shifts and adaptations

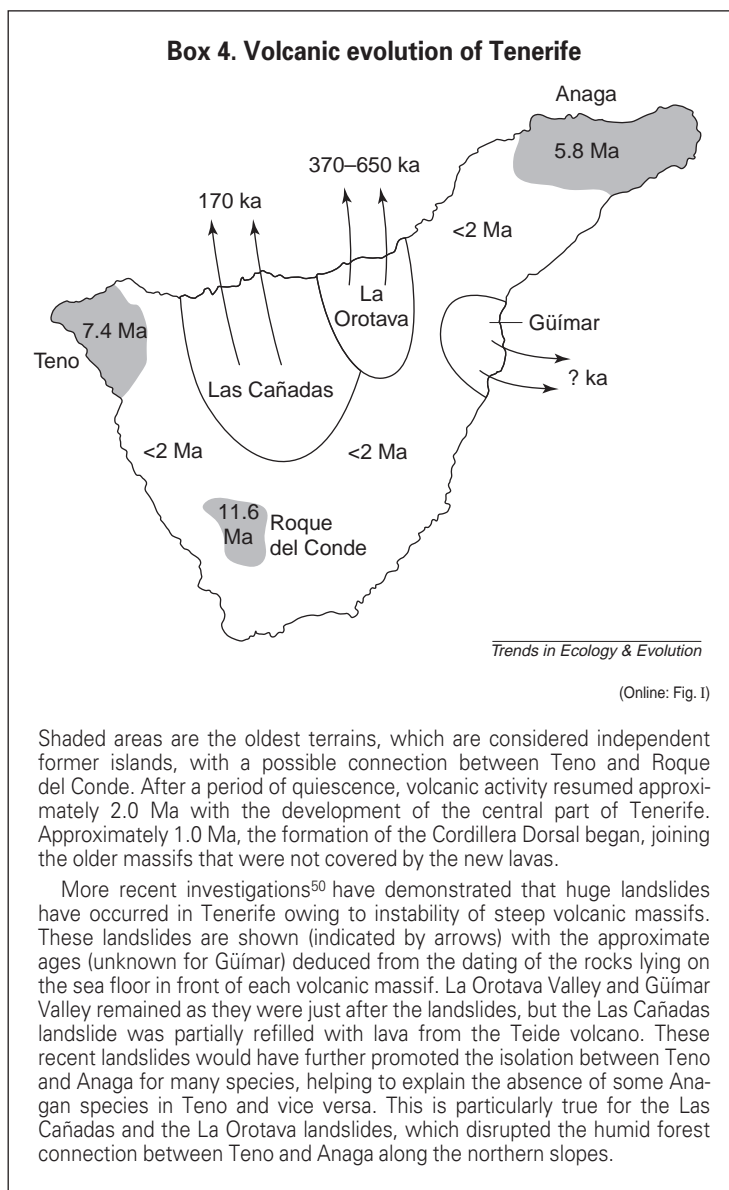
Oceanic islands show considerable habitat diversity, produced by topology and humidity gradients, which, combined with their isolation, produces lower competition and empty ecological niches. This provides a template for the evolution of species radiations. For example, the plant genus *Argyranthemum* has radiated into virtually all habitats of the Atlantic islands^{42–47}. In the Canarian archipelago, two major clades of this genus can be distinguished by DNA markers. One of the groupings is formed by the taxa occurring in arid–subarid zones (high altitude desert, pine forest, arid lowland scrub and coastal desert), but the other clade comprises species in zones influenced by the humid trade winds (laurel forest and humid lowland scrub). Inter-island colonization of taxa pre-adapted to particular microhabitats, rather than to radiation within each island, seems to have been a significant factor in the evolution of *Argyranthemum* and other Canarian plants.

Recently, several plant genera have been studied using molecular markers, such as ITS and chloroplast sequences or RFLPs. The genera *Sonchus*⁴⁰ (Asteraceae) and *Echium*⁴¹ (Boraginaceae) have experienced rapid radiations in the Canaries following single colonization events in Gran Canaria or Tenerife in the late Miocene or early Pliocene. Colonization patterns for *Argyranthemum* (Asteraceae) are less clear, but morphological, phytochemical and molecular data suggest that some elements of the Canarian flora are relictual⁴². Also, there is evidence of extensive inter-island dispersal between similar ecological zones, of hybridization in the Macaronesian island flora and even of back-colonization to the continent by island taxa. ‘Insular woodiness’ has been identified in numerous angiosperm families. The problem of ascertaining the polarity of perennial woodiness of plants in the Macaronesian Islands has been examined in both *Echium* and *Sonchus* (ITS sequences)^{40,41}. Both studies conclude that the most plausible explanation is a founding colonization of the Canary Islands from a continental herbaceous ancestor followed by intense speciation.

Adaptation to hypogean life in the volcanic tubes is exemplified by the spider genus *Dysdera* and the cockroach genus *Loboptera*. Morphological and molecular data suggest two independent *Dysdera* colonizations from the continent to the eastern islands, a third colonization resulting in a central–western islands lineage, and a possible fourth colonization for *D. unguimmanis* (to be confirmed when more data are available) (M.A. Arnedo, PhD thesis). Eight of the 43 species of *Dysdera* are found exclusively in lava tubes, most of them on the island of Tenerife³⁸. These troglomorphic taxa are not relictual species (with the possible exception of *D. unguimmanis*, by far the most adapted species), they share the same ancestor with the epigeal species, and switches to life in the caves have occurred periodically throughout the evolutionary history of the genus. Only two of the troglomorphic species, *D. hernandezii* and *D. esquivelei*, are monophyletic.

Different degrees of adaptation to life in caves are also present in the 11 known taxa of cockroaches of the genus *Loboptera* (I. Izquierdo, PhD thesis). These species occur in Tenerife, La Palma and El Hierro, and show colonization patterns strictly compatible with the sequential emergence of those islands. All but one of the Canarian species of the genus *Loboptera* show some degree of adaptation to the hypogean habitat and most of them are strict troglobites. It appears that the colonization and radiation on the Canaries by epigeal ancestors of the present-day *Loboptera* was followed by local speciation underground⁴⁹.

Box 4. Volcanic evolution of Tenerife



Shaded areas are the oldest terrains, which are considered independent former islands, with a possible connection between Teno and Roque del Conde. After a period of quiescence, volcanic activity resumed approximately 2.0 Ma with the development of the central part of Tenerife. Approximately 1.0 Ma, the formation of the Cordillera Dorsal began, joining the older massifs that were not covered by the new lavas.

More recent investigations⁵⁰ have demonstrated that huge landslides have occurred in Tenerife owing to instability of steep volcanic massifs. These landslides are shown (indicated by arrows) with the approximate ages (unknown for Güímar) deduced from the dating of the rocks lying on the sea floor in front of each volcanic massif. La Orotava Valley and Güímar Valley remained as they were just after the landslides, but the Las Cañadas landslide was partially refilled with lava from the Teide volcano. These recent landslides would have further promoted the isolation between Teno and Anaga for many species, helping to explain the absence of some Anagan species in Teno and vice versa. This is particularly true for the Las Cañadas and the La Orotava landslides, which disrupted the humid forest connection between Teno and Anaga along the northern slopes.

In the Canarian *Calathus* ground beetles, there is no relationship between the age of colonization and speciation³¹. For example, seven species occur in the small island of La Gomera, but only three occur in the topologically similar, but much larger, island of Gran Canaria. Habitat destruction on Gran Canaria could account, in part, for these differences. However, genetic data point to recent diversification rather than the presence of relict species in Gran Canaria. The ecological data available for this genus support the idea that recent speciation on La Gomera has been promoted mainly by marginal isolation or niche specialization into laurel forest microhabitats from a generalist ancestor. At the population level, morphological differentiation of the Tenerife and Gran Canarian skinks (*Chalcides viridanus* and *C. sexlineatus*) is not consistent with vicariance or isolation-by-distance models exclusively²³. The two islands show similar mesic/xeric heterogeneity and the pattern of geographical variation in *Chalcides* is better explained by differential selection along an aridity gradient.

Conclusion

As more and more DNA-based phylogeographic studies are undertaken on the Canary Islands, a picture is emerging of an ecosystem that is perhaps best seen as a mosaic. Although the archipelago can be viewed as a delineable entity with

its own characteristic fauna, general patterns among islands are seldom apparent within individual genera. Stochasticity must be seen as a primary factor in determining species compositions on the islands – you cannot appreciate if you are not there. Even if colonization occurs, subsequent evolution will be contingent upon the floral and faunal composition of the colonized island. All groups on the archipelago have survived the turmoils of volcanism and have responded in different ways to the presence of diverse habitats. This response would typically have depended on the fundamental niche of a colonizing species (i.e. ecological requirements) and the competitive presence of other species within that niche. The potential for both rapid morphological change and convergent evolution further complicates the picture, particularly within some invertebrate groups (e.g. the genus *Nesotes*; D. Rees, unpublished). However, the relative effects of these variables can be truly assessed only at the level of the ecosystem. Phylogenetic data from DNA studies now allow us to assess the process of diversification within genera. Continued efforts to obtain phylogenetic data across a diversity of taxa, from a diversity of habitats, in conjunction with geological and ecological data, would seem the best way to evaluate diversification on the Canary Islands and on other island systems.

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Recreating ancestral proteins

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Molecular evolution leaves behind a trail of amino acid substitutions potentially rich in information about molecular function. Tracing changes in protein structure along the branches of a phylogenetic tree can provide important insights into molecular function, and the role of selection in shaping the relationship between molecular structure and function. Recreation of inferred ancestral proteins using gene synthesis and protein expression methods, whose biochemical functions can then be directly measured *in vitro*, provides a powerful approach to this problem.

Phylogenies, molecular function and natural selection

Phylogenies can be used in several ways to infer the effects of natural selection on molecular function.

Because directional selection is known to elevate the ratio of nonsynonymous to synonymous nucleotide substitutions, this ratio can be used as a tool to detect lineages in a molecular phylogeny along which selection has occurred. Cows and langur monkeys convergently evolved foregut fermentation as a mechanism to digest the large amounts of plant material in their diets; a key component of this involved the recruitment of the lysozyme enzyme to digest foregut bacteria. Selection for this specialized function of

Tracing the history of molecular changes using phylogenetic methods can provide powerful insights into how and why molecules work the way they do. It is now possible to recreate inferred ancestral proteins in the laboratory and study the function of these molecules. This provides a unique opportunity to study the paths and the mechanisms of functional change during molecular evolution. What insights have already emerged from such phylogenetic studies of protein evolution and function, what are the impediments to progress and what are the prospects for the future?

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lysozyme should be evident in a phylogeny of primate lysozyme sequences, on the lineage leading to colobine monkeys. By inferring and comparing ancestral lysozyme sequences, Messier and Stewart¹ were able to demonstrate adaptive change in this lineage. Specifically, they estimated the numbers of nonsynonymous (d_N) and synonymous (d_S) substitutions along each branch using Li's method², and found a d_N/d_S ratio much greater than one in the lineage leading to the colobine monkeys (Fig. 1). This analysis also revealed a previously unsuspected episode of positive selection in the ancestral hominoid lineage.

Subsequently, Yang³ developed a more rigorous statistical approach to this problem, using a codon-based maximum likelihood model of evolution⁴ to detect elevated d_N/d_S ratios along lineages in a phylogeny. Yang's method³ uses likelihood ratio tests to compare the performance of different likelihood models, and to determine if the d_N/d_S ratio for the lineage of interest is elevated compared with other lineages in the phylogeny. This approach avoids using reconstructed ancestral states as if these were actual observations in the calculations of nonsynonymous and synonymous substitution rates. When applied to the primate lysozyme data, this method showed strong evidence for positive selection