Heredity **85** (2000) 20–29 *Received 13 September 1999, accepted 9 December 1999*

Are flightless *Galapaganus* weevils older than the Galápagos Islands they inhabit?

ANDREA S. SEQUEIRA†, ANALÍA A. LANTERI‡, M. AMALIA SCATAGLINI§, VIVIANA A. CONFALONIERI§ & BRIAN D. FARRELL†*

†Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, U.S.A., ‡Departamento Científico de Entomología, Museo de La Plata, Paseo del Bosque s/n, (1900) La Plata, Argentina and §Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428) Buenos Aires, Argentina

The 15 species in the weevil genus *Galapaganus* Lanteri 1992 (Entiminae: Curculionidae: Coleoptera) are distributed on coastal Perú and Ecuador and include 10 flightless species endemic to the Galápagos islands. These beetles thus provide a promising system through which to investigate the patterns and processes of evolution on Darwin's archipelago. Sequences of the mtDNA locus encoding cytochrome oxidase subunit I (COI) were obtained from samples of seven species occurring in different ecological zones of the oldest south-eastern islands: San Cristóbal, Española and Floreana, and the central island Santa Cruz. The single most parsimonious tree obtained shows two well-supported clades that correspond to the species groups previously defined by morphological characters. Based on a mtDNA clock calibrated for arthropods, the initial speciation separating the oldest species, *G. galapagoensis* (Linell) on the oldest island, San Cristóbal, from the remaining species in the Galápagos occurred about 7.2 Ma. This estimate exceeds geological ages of the extant emerged islands, although it agrees well with molecular dating of endemic Galápagos iguanas, geckos and lizards. An apparent explanation for the disagreement between geological and molecular time-frames is that about 7 Ma there were emerged islands which subsequently disappeared under ocean waters. This hypothesis has gained support from the recent findings of 11-Myr-old submarine seamounts (sunken islands), south-east of the present location of the archipelago. Some species within the *darwini* group may have differentiated on the extant islands, 1–5 Ma.

Keywords: cytochrome oxidase I, DNA sequences, island biogeography, progression rule, speciation, taxon cycle.

Introduction

Island archipelagos enable the study as well as the process of speciation (Darwin, 1859; Carlquist, 1974; Grant, 1986). Because island systems comprise sets of often relatively small areas (i.e. patches) separated by uninhabitable gaps, they provide multiple opportunities for isolation of small populations. They also offer the potential for comparative studies of the interactions of habitat patchiness, species vagility, and time in the process of species formation. This potential has begun to be exploited (Grant, 1994; Juan *et al*., 1995; Wagner & Funk, 1995; Roderick & Gillespie, 1998). The three oceanic island archipelagos that have been subject to repeated studies of species' radiations are the Canary Islands (Thorpe *et al*., 1994; Juan *et al*., 1995, 1996) the Galápagos Islands (Darwin, 1859; Grant, 1986; Carson, 1992; Lanteri, 1992; Cook *et al*., 1995; Peck, 1996) and the Hawaiian Islands (DeSalle & Hunt, 1987; Gillespie *et al*., 1994; Carson & Clague, 1995; DeSalle, 1995; Wagner & Funk, 1995; Roderick & Gillespie, 1998).

The origin of the flora and fauna endemic to the Galápagos Islands has been of interest since the publication of *The Voyage of the Beagle* (Darwin, 1845). Evidence of general affinities with coastal South America has accumulated since Darwin's first collections (Snodgrass, 1902; Wright, 1983; Grant, 1986; Lanteri, 1992; Lopez *et al*., 1992; Peck, 1994, 1996; Cook *et al*., 1995; Rassmann, 1997), and there has been recent progress on elucidating the relationships among the forms endemic to the various islands (Lopez *et al*., 1992; Cook *et al*., 1995; Finston & Peck, 1997).

Phylogenetic patterns, for example, might indicate whether the species endemic to various subsets of islands

*Correspondence. E-mail: bfarrell@oeb.harvard.edu

arose most frequently through repeated dispersal from the mainland or from neighbouring islands (Gillespie *et al*., 1994). Phylogenetic patterns common to different groups might even favour a few particular histories of colonization and dispersal among islands (Gillespie *et al*., 1994; DeSalle, 1995; Wagner & Funk, 1995; Roderick & Gillespie, 1998).

Although the sequence of events implied by the branching order of phylogeny estimates can lend support to particular sequences of island colonization (Funk & Wagner, 1995), estimates of molecular divergence are useful in establishing their respective timing. This seems especially important in both the Canaries (Juan *et al*., 1996) and the Galápagos (Christie *et al*., 1992) because the oldest islands (Fuerteventura and San Cristóbal, respectively) in these chains are also the most proximal (110 km and 1000 km, respectively) to dominant oceanic currents from the direction of the mainland (the Hawaiian Islands are much more isolated at 4000 km from the nearest continent).

Moreover, because the respective ages of islands in such volcanic archipelagos are also generally reflected in their spatial distributions, occasional dispersal (e.g. interisland rafting) followed by speciation — all entirely post-island formation — could produce a phylogenetic pattern comparable to that expected if speciation followed in tandem with island formation (Roderick & Gillespie, 1998).

As is also true of the Hawaiian Islands and the Canaries, the presently emerged Galápagos Islands are the most recent products of a long-lived mantle hotspot (Christie *et al*., 1992; White *et al*., 1993). Age estimations of the extant islands are indeed variable: K–Ar radiometry and marine fossils on the extant islands indicate a maximum age of the oldest exposed land on the order of 3–4 Myr (Geist *et al*., 1985; Hickman & Lipps, 1985), whereas different geological plate motion models set a maximum age of emergence in the range of 4.5–6.3 Myr, depending on the velocity of the Nazca plate (55 mm yr^{-1}, 37 mm yr^{-1}, respectively) (White *et al*., 1993; Geist, 1996). Although the emerged islands are evidently young, drowned seamounts east of the existing San Cristóbal island in the Galápagos archipelago are from 5 to 11 Myr old and the history of island production over this hotspot probably extends back 15–20 Myr, and maybe even 80–90 Myr (Christie *et al*., 1992).

Early reports of surprisingly great divergence in proteins have thus recently been reconciled with the dynamic history of this island chain. Investigations of enzyme-electrophoresis and immunological data of the Galápagos iguanid genera *Amblyrhynchus* Bell and *Conolophus* Fitzinger suggest a divergence time of 15–20 Myr relative to other iguanines (Wyles & Sarich, 1983), a range recently confirmed by DNA sequence-based estimates (Rassmann, 1997). Also favouring a range of ages older than the extant islands is the molecular clock calibrations for the gecko and lizard genera *Phyllodactylus* Gray and *Tropidurus* Wied which yield ages of 8.9 Myr and 10.2 Myr, respectively (Wright, 1983; Lopez *et al*., 1992).

Although the ancestors of these lizards are likely to have originally colonized a now-submerged island, other elements of the fauna may be more recent in origin. Enzyme electrophoretic analyses of the 13 Darwin finch species (Emberizinae) suggest divergences within 5 Myr or less (Grant, 1994), within the age-range of the present islands (White *et al*., 1993). This is consistent with some recent studies of insects (Finston & Peck, 1997), which show little allozyme differentiation among marked morphological groups endemic to the various islands.

It is not surprising that the Galápagos fauna reflects a continuous history of colonization, both preceding and postdating the emergence of the present islands. Comparative studies of the effects of vagility or body size on speciation might profit by study of groups of similar age on the islands (Carson & Clague, 1995), whereas focus on the rate of speciation might compare groups of similar vagility or size. In general, larger bodied and/or more vagile animals are probably less likely to be affected by the spatial and temporal history of island archipelagos, *per se*, because they may more readily disperse among islands. However, larger species may also be more likely to suffer higher extinction rates.

The phylogenesis of small, sedentary organisms such as flightless insects or other arthropods (or small vertebrates such as anoles) may more often reflect both their history of colonization and the patchiness of island systems in space and time. These have been the focus of numerous studies, yielding some insights into the assembly of island faunas.

One such group, the curculionid weevil genus *Galapaganus* Lanteri (1992) (subfamily Entiminae, tribe Naupactini), provides a promising system for investigation of patterns and processes of assembly of faunas in the Galápagos Islands, especially for speciation within the archipelago. The 15 species of these weevils include 10 species endemic to the Galápagos Islands that are flightless, fairly heavy-bodied, and probably less vagile than many arthropod groups. The larvae eat roots and the adults eat foliage. Although their habits are known imprecisely, most of them appear to be polyphagous.

Here we provide a phylogenetic analysis of DNA sequences from the mitochondrial locus encoding subunit I of the enzyme cytochrome oxidase (COI). These data are analysed together with morphological characters from an earlier study (Lanteri, 1992) to evaluate whether the phylogenesis of these weevils parallels the

apparent history of island formation; and whether the amount of sequence divergence favours colonization and speciation after, during or before the formation of the emergent islands.

Materials and methods

Sampling

The specimens studied herein were collected by A. Lanteri, S. Peck (Canada) and L. R. Albelo (Ecuador), in the Galápagos islands between March and May 1996. Samples were obtained from Santa Cruz, San Cristóbal, Floreana and Española (Fig. 1). These islands are considered the oldest in the archipelago and show several vegetation and ecological zones from the arid coasts to the moist highlands (Wiggins & Porter, 1971; Peck, 1996) (Table 1). Specimens were captured on shrubs using a beating sheet, with a sweeping net, or by hand from trailing plants (*G. collaris* Lanteri).

We were able to sample the six species occurring on Santa Cruz, San Cristóbal, Floreana and Española islands and a seventh species *G. howdenae* Lanteri, widespread in mainland Ecuador and recently introduced in an agricultural area of Santa Cruz in Galápagos [according to Peck *et al.* (1998), of the over 300 insect species recently introduced to the Galápagos, 60 are plant-feeding beetles]. Of the seven species studied, one belongs to the *femoratus* species group (*G. howdenae* Lanteri), and six species (*G. ashlocki* Lanteri, *G. caroli* (Van Dyke), *G. collaris* Lanteri, *G. conwayensis* (Mutchler), *G. galapagoensis* (Linell) and *G. vandykei* Lanteri were assigned to the *darwini* species group (Lanteri, 1992). Most of the species analysed (five out of seven) are single-island endemics. However, the material available for molecular studies does not include co-specifics from different islands for *G. collaris* and *G. vandykei*; this precludes assessment of intraspecific variation and analysis of inter-island divergence and island colonization below the species level. Outgroups included three species in the naupactine genus *Naupactus* Dejean. Details of all material studied are included in Table 2, and the overall known distributions and ecological zones of species of *Galapaganus* herein studied are given in Table 3.

DNA preparation, PCR amplification and sequencing

Collected specimens were preserved refrigerated in 100% ethanol. A single specimen of each species was selected for the analysis. Ethanol-preserved individuals were left to dry for a few minutes before grinding. Whole weevil specimens were ground to fine powder, in a mortar with liquid nitrogen. DNA was isolated according to the protocol of Normark (1996) for ethanol-preserved individuals.

One microlitre (200 ng) of the DNA solution obtained was amplified to produce a double-stranded product under the following conditions: 2 μM of each primer, 200 μM each dNTP, 2 μM $MgCl_2$, buffer supplied by the manufacturer (Promega) and 1.25 units Taq polymerase (Promega), in a total volume of 50 μL. A typical temperature profile consisted of 40

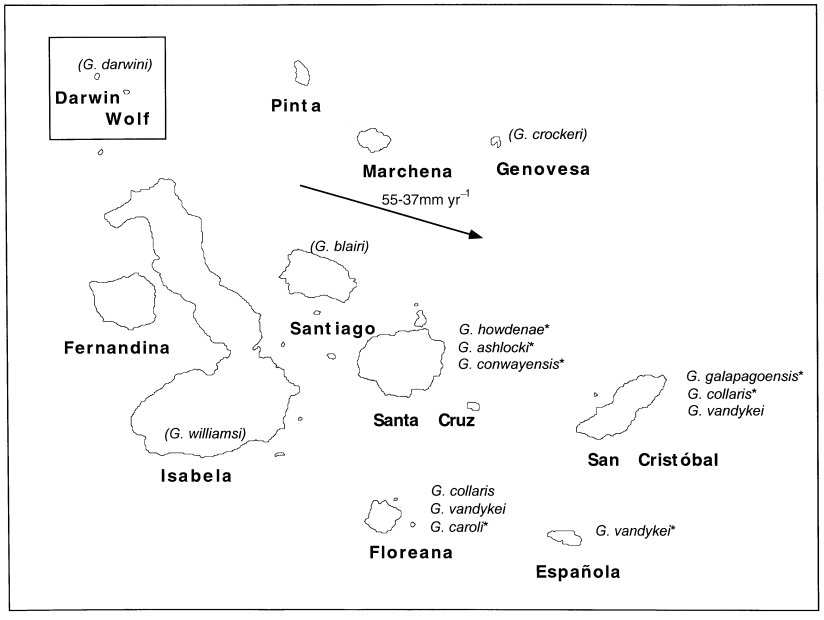

Fig. 1 Distribution of species of *Galapaganus* occurring on the Galápagos archipelago. Species in brackets are not analysed. Asterisks indicate the origin of the specimens analysed in this study. Arrow shows the direction and speed of motion of the Nazca Plate.

Table 1 Galápagos Islands: ecological diversity (sum of an island's vegetational zones) and estimates for geological ages in millions of years since the emergence of the islands

Island	Ecological diversity (no. of ecological zones)[a]	Age (Myr)	
		Minimum (K–Ar)[b]	Maximum (hotspot)[c,d]
San Cristóbal	6	2.3	4.5–6.3
Española	2	2.8	4.1–5.6
Floreana	4	1.5	3.3
Santa Cruz	6	2.2	2.7–3.6

K–Ar, Potassium–argon datings (minimum) and hotspot model datings (maximum). Data taken from Peck (1996)[a], White *et al.* (1993)[b], Cox (1983)[c] and Geist (1996)[d].

Table 2 Details of the material studied

Species	Locality/altitude/date	Notes	Collector
Galapaganus galapagoensis (Linell 1898)	Ecuador, Galápagos, San Cristóbal Is., 5 km SE Wreck Bay, 0 m, 13/3/96	On *Cryptocarpus pyriformis*, *Laguncularia racemosa* and *Gossypium barbadense*, at night	Lanteri
Galapaganus collaris Lanteri 1992	Ecuador, Galápagos, San Cristóbal Is., El Junco rim, 620 m, 14/3/96	On *Verbena litoralis*, in the evening	Lanteri
Galapaganus caroli (Van Dyke 1953)	Ecuador, Galápagos, Floreana, Bahia Las Cuevas, 5–m, arid zone, 16/4/96	Hand collection	Peck
Galapaganus vandykei Lanteri 1992	Ecuador, Galápagos, Española Is., Punta Suarez, arid zone, 23/3/96	At night, hand collection	Peck
Galapaganus ashlocki Lanteri 1992	Ecuador, Galápagos, Santa Cruz Is., trail to Cerro Crocker, 400–800 m, *Miconia* zone, 9/2/96	At noon	Lanteri
Galapaganus conwayensis (Mutchler 1938)	Ecuador, Galápagos, Santa Cruz Is., Tortuga Bay trail, 0–5 m, 10/3/96	On *Cryptocarpus pyriformis*, *Cordia lutea* and *Alternanthera echinocephala*	Lanteri
Galapaganus howdenae Lanteri 1992	Ecuador, Galápagos, Santa Cruz Is., 4 km from Bella Vista, 19/3/96	On *Erythrina* and other plants of the agricultural area, at noon	Lanteri
Naupactus verecundus Hustache 1947	Argentina, La Pampa, Santa Rosa, 6/12/95	On grasses	de Wysiecki
Naupactus xanthographus (Germar 1824)	Argentina, Buenos Aires, Punta Lara, 7/2/97	On Malvaceae	Lanteri & Loiacono
Naupactus dissimulator Boheman 1840	Argentina, Buenos Aires, Punta Lara, 7/2/97	On shrubs	Lanteri & Loiacono

cycles: 94°C for 30 s, 50°C for 30 s and 72°C for 1 min 15 s, followed by a 5-min extension step at 72°C. The product of this reaction was purified after being run on an agarose gel (QIAquick columns, Qiagen Valencia, CA, USA) or directly purified on Centricon 30 columns (Amicon, Beverly, MA, USA). The amount of DNA was estimated using a spectrophotometer and 90 ng was sequenced using ABI dye terminator sequencing kits (PE biosystems, Warrington, UK), following the provided instructions but using half reactions.

The entire cytochrome oxidase I (COI) gene of mtDNA was amplified using the polymerase chain reaction (PCR). COI specific primer pairs S1718, A2411 and S2215, A2940 were usually used, although S1859 was used sometimes. They were obtained from the Harrison Laboratory (Cornell University) and Farrell Laboratory (Harvard University) and used to amplify and to sequence COI of the weevils studied. The sequencing primers were the external primers for each fragment with extra internal primers S2336, S2442, A2191 and A2831 (for primer sequences see Normark, 1996; Normark *et al.*, 1999). Sequencing of this double-stranded product was carried out using 25 PCR cycles of 96°C for 30 s, 50°C for 15 s and 60°C for 4 min with a 2°C increase per s in a 10-L reaction. A 1226-bp region

Table 3 Distribution of the studied species of *Galapaganus*

Species	Distribution	Ecological zones
Galapaganus galapagoensis	San Cristóbal	Littoral to arid zone
Galapaganus collaris	San Cristóbal, Floreana	Fern–sedge zone
Galapaganus caroli	Floreana	Littoral to transition zone
Galapaganus ashlocki	Santa Cruz	Miconia and fern–sedge zones
Galapaganus vandykei	Floreana, Española, San Cristóbal	Littoral zone
Galapaganus conwayensis	Santa Cruz	Littoral to Scalesia zones
Galapaganus howdenae	Mainland Ecuador	Native forest
	Santa Cruz	Agricultural area

Ecological zones according to Wiggins & Porter (1971) are progressively more mesic away from the coast: (1) littoral zone (salt tolerant vegetation); (2) arid zone (microphyllous, xerophytic vegetation); (3) transition zone (dry woodland); (4) *Scalesia* zone (mesophyllous, mainly evergreen forest); (5) *Miconia* zone (mesophyllous evergreen shrub) and (6) pampa or fern–sedge zone (with ferns as the most obvious part of the vegetation). Coastal lowlands are seasonally arid and highlands (*Miconia* and pampa zones, over 500 m) are more stable and humid.

of the mtDNA cytochrome oxidase I (COI) gene was sequenced for eight of the weevil species studied, whereas 691 bases were analysed for *G. caroli* and *N. dissimulator* Boheman. The complete sequence for *N. xanthographus* (Germar) was obtained by compiling two 700-base fragments from two different individuals (one sequence kindly provided by B. Normark). Each sequence was entered and compiled using SEQUENCHER 3.0 (Genecodes Corporation, Ann Arbor, MI). The complete set of sequences has been submitted to Genbank under accession numbers: AF015914 and AF211483–AF211491.

Phylogenetic analysis

A matrix was assembled of 1226 molecular and 33 morphological characters (characters and character states are described in Lanteri, 1992) which were treated as unordered. The molecular dataset was analysed separately and in a combined total evidence matrix together with the morphological characters. Changes at the third codon position were downweighted, giving all other changes a weight of up to 5, and the analysis was also performed using only first and second codon positions. Most parsimonious phylogenetic trees were inferred using the exhaustive search function in PAUP 4.0 (Swofford, 1998). Branch and bound bootstrap searches were performed with random addition sequences of taxa with 10 repetitions for each of 100 replications (Fig. 2). Trees were rooted with the Neotropical naupactine genus *Naupactus* Dejean as outgroup, using sequences of species *N. verecundus* Hustache, *N. xanthographus* and *N. dissimulator*. Based on morphological evidence this genus is one of the closest relatives of *Galapaganus* (Lanteri, 1992). Substitution rates were calculated using the Kimura 2-parameter model (Kimura, 1980) and the pairwise sequence divergence matrix was built using PAUP's distance matrix function (Table 4).

Relative rate tests were performed to test the equality of evolutionary rates between lineages (Li & Bousquet, 1992) using PHYLTEST 2.0 (Kumar, 1996). Likelihood ratio tests (LTR) were performed using the likelihood scores calculated for the most parsimonious tree with and without the constraint of a molecular clock as implemented in PAUP. The statistic ($-2 \log \Delta$, $\Delta = \max$ L null hypothesis/max L alternative hypothesis) can be compared to a χ^2 distribution with $n - 2$ degrees of freedom (n, number of taxa) to determine the significance of the test (Felsenstein, 1981; Huelsenbeck & Rannala, 1997).

The divergence times between lineages were calculated from uncorrected pairwise values and calibrated using 2.3% pairwise divergence per million years based on the arthropod mtDNA survey of Brower (1994). This may bias our estimates towards younger ages, because COI is more conservative (e.g. 72% identity between *Apis* and *Drosophila* — higher than any other gene) than other protein and RNA genes in the insect mitochondrial genome (Crozier & Crozier, 1993).

Results

Phylogeny

A single most parsimonious tree was obtained when analysing the molecular data alone, and in combination with the morphological characters (761 steps long, CI = 0.73, RI = 0.39; Fig. 2). Out of 1226 molecular characters, 570 are constant, 456 are parsimony uninformative and 200 are informative. Most substitutions are at third codon positions, and are silent. However, the same topology was obtained giving changes at the third codon position a weight of 1 and all other changes a weight of up to 5 and also when including only first and second codon positions. The

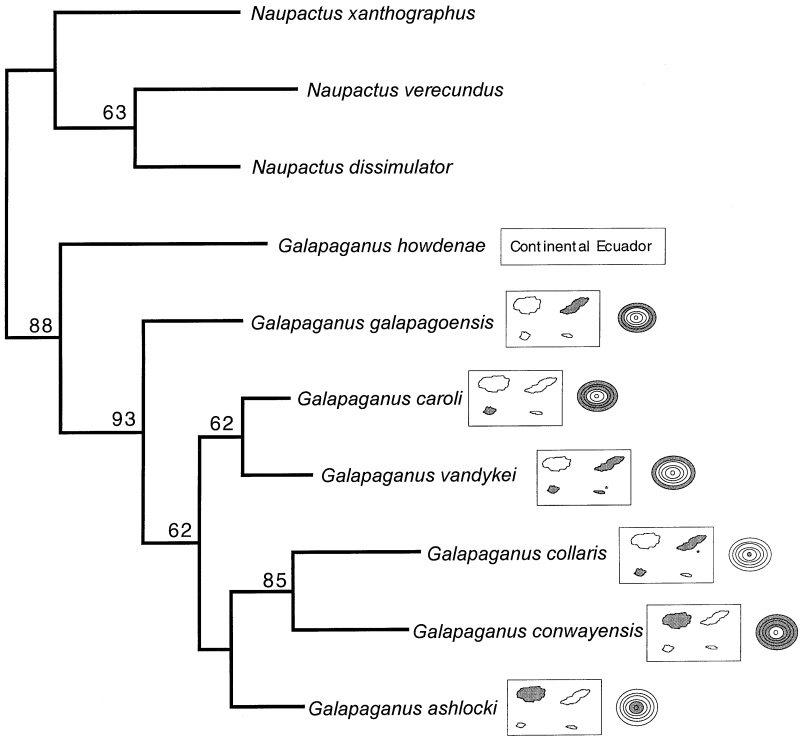

Fig. 2 Most parsimonious tree for the species of *Galapaganus* obtained from the analysis of a combined morphological and molecular matrix. Numbers on the branches indicate bootstrap values for the unweighted combined dataset and branch lengths are proportional to the amount of molecular change without correction for multiple substitutions. Following each species name is a rectangle containing a map of the four islands where the island(s) occupied are shaded. Similarly the schematic circles indicate which of the six roughly concentric ecological zones are occupied on each island by each weevil species.

Table 4 Pairwise sequence divergence values (Kimura 2-parameter model values) within the genus *Galapaganus*

	G. h	*G. g*	*G. car*	*G. v*	*G. a*	*G. coll*
G. howdenae						
G. galapagoensis	0.245					
G. caroli	0.281	0.156				
G. vandykei	0.251	0.149	0.091			
G. ashlocki	0.265	0.147	0.098	0.090		
G. collaris	0.273	0.215	0.137	0.122	0.126	
G. conwayensis	0.261	0.173	0.124	0.121	0.107	0.124

transition/transversion ratio is 1.1:1; the GC content is 32%, and the maximum divergence between *Galapaganus* species groups is 28% (between *G. howdenae* and *G. caroli*) whereas within the *darwini* group it ranges from 17% (between *G. galapagoensis* and *G. collaris*) to 9% (between *G. caroli* and *G. vandykei*) (Table 4).

High bootstrap values (>80) (Fig. 2) support the monophyly of *Galapaganus* and the *darwini* group and agree in this sense with the cladogram based on morphological characters (Lanteri, 1992). The topology shows *G. howdenae* (i.e. the *femoratus* group) at a basal position, as sister group to the *darwini* group. Within the *darwini* group *G. galapagoensis* is basal and there are two subgroups, one including *G. caroli–G. vandykei*, and other comprising *G. ashlocki–G. conwayensis–G. collaris*. The position of *G. ashlocki* is not well supported by bootstrap values.

This combined analysis tree differs in several respects from that based solely on morphology. The relationships among these *Galapaganus* species based on 33 morphological characters as in Lanteri (1992) are (*G. howdenae* (*G. conwayensis* (*G. ashlocki*, *G. caroli* (*G. vandykei* (*G. galapagoensis*, *G. collaris*))))). The combined analyses place *G. galapagoensis* as the basal species of the *darwini* group and sister group to *G. ashlocki* (*G. collaris–G. conwayensis*). On the other hand, the morphological MPT places *G. conwayensis* close to the root, with *G. galapagoensis–G. collaris* forming a monophyletic group together with *G. vandykei*.

The nucleotide substitution rates among the members of the *Galapaganus* genus were compared with respect to the outgroup *Naupactus* ($z = 1.89$). The relative rates were also analysed within the *darwini* group clade using *G. howdenae* as outgroup because the choice of a closely related outgroup is critical for analysing relative rates in a given clade ($z = 1.54$). According to the relative rate test, *Galapaganus* lineages are not evolving at significantly different speeds because no rate heterogeneity was found among the substitution rates at the 0.05 probability level. Furthermore, according to the LRT, the molecular clock hypothesis (where the rates among lineages are equal) cannot be rejected for this dataset ($P > 0.05$).

Age of divergence of Galapaganus *lineages*

Based on the mtDNA clock proposed by Brower (1994) for arthropods, the age for the diversification of the two species groups within *Galapaganus* is 11 Myr (10.7–12.1) (late Miocene). The minimum time for speciation of the *darwini* group within the archipelago is about 7.2 Myr (6.8–7.4) (early Pliocene). Even considering that stochastic errors can be associated with the calculations of age of divergences, this value of 7.2 Myr exceeds the oldest estimates for the islands, especially as Geist (1996) states that the last reported age for San Cristobal (6.3 Myr) is based on very few concrete data and should be viewed only as an estimate. All previous estimates agree that the maximum age for the extant archipelago is 4.5 Myr (Cox, 1983; Geist *et al*., 1985; Hickman & Lipps, 1985; White *et al*., 1993). Within the *darwini* group the divergence between the clade including the sister species *G. vandykei*–*G. caroli* (Española–Floreana–San Cristóbal), and the clade including *G. collaris*–*G. conwayensis*–*G.ashlocki* (Santa Cruz–San Cristóbal) is estimated at 5 Myr (mid-Pliocene). *Galapaganus vandykei* and *G. caroli* would have started their divergence about 3.5 Ma (late Pliocene).The position of *G. ashlocki* in the consensus tree is not resolved and, accordingly, its divergence from related species is not distinguishable from the age estimated for the basal node of the clade (5 Myr). The estimates within the *darwini* group do not exceed the maximum ages of the islands provided by geological analyses, although they are above the minimum estimates (Table 1).

Discussion

Our estimate of *Galapaganus* phylogeny places the oldest weevil species on the oldest island, *G. galapagoensis* on San Cristóbal, as basal to the rest of the *darwini* group. This is also the pattern in the flightless scarab genus *Neoryctes* Arrow (Cook *et al*., 1995) and in the lizard genera *Tropidurus* and *Phyllodactylus* (Wright, 1983). *Neoryctes* has not yet been subject to molecular study. However, *Tropidurus*, *Phyllodactylus* (Lopez *et al*., 1992) and *Galapaganus* all show molecular divergence in apparent excess of the age of even this oldest island. This suggests that the initial colonizations by these groups occurred on a now submerged seamount east of San Cristóbal before the remaining, younger islands had appeared.

The iguanids and these weevils are thus among the older members of the Galápagos' fauna. Because the common ancestor of the *darwini* group is most parsimoniously ascribed flightlessness, this was either the condition of the original colonizing species or evolved between colonization and the first subsequent speciation event. The external morphology of the *darwini* group is typical of weevils from deserts or highlands and includes a very sclerotized integument, scales modified into dense setae as well as absent hind-wings (Lanteri, 1992). These apparent adaptations to aridity were probably essential for colonization and establishment on littoral zones on the islands where the only available vegetation is salt-tolerant shrubs (Finston & Peck, 1997). In contrast, the morphology of the fully winged and flight-capable *femoratus* group suggests adaptation to more mesic environments than that of the *darwini* group. Both *G. howdenae* and *G. femoratus* (as well as two additional species, as yet undescribed) have an integument that is only moderately sclerotized and is covered with iridescent scales.

The source of the founder(s) of the *Galapaganus darwini* group is apparently coastal Ecuador/Perú, probably rafted via the Humboldt current (Wright, 1983; Peck & Kukalova-Peck, 1990; Lopez *et al*., 1992). This current arises off Antarctica, flows northward along the coast of Chile and Perú and joins the South Equatorial current that passes through the Galápagos archipelago carrying along great quantities of flood debris and pleuston, facilitating the passive transport of terrestrial animals (Peck, 1994).

Distribution and speciation of the darwini *group on the Galápagos islands*

The six *darwini* group species sampled here are representative of the ecological diversification that has apparently accompanied *Galapaganus* phylogenesis in these islands. The phylogeny estimate is consistent with the taxon cycle model of island faunal development (Wilson, 1961) in that the basal species occur in arid, coastal areas whereas the more derived species occur in the upland and more mesic habitats (Fig. 2). This is also consistent with the colonization by rafting of a flightless ancestor, as a winged arrival of a form more closely resembling the mesic-adapted *femoratus* group might be at least as likely to have been in the uplands.

The shifts from coast to upland are coincident with speciation of the *darwini* group, which probably occurred in the following manner: initial colonization of a now submerged island east of San Cristóbal from coastal Perú, with subsequent dispersal and speciation in Española, Floreana and Santa Cruz, and a recolonization of San Cristóbal and Floreana from the latter island. The molecular divergence (Table 4) within the *darwini* group, at the upper end of the range expected given the ages of the islands (Table 1), suggests that some speciation events may have occurred in tandem with island emergence. Therefore, the speciation within this clade could have occurred on the presently emerged

islands. Thus if we accept the implication based on the cladogram topology and molecular divergence that the occurrence of the basal species on the oldest island reflects history, then a logical inference would be that the species' distributions on the other, younger islands also partly reflect the history of emergence.

Although Hickman & Lipps (1985) determined the 3–4 Myr age of these islands accurately from fossil evidence, their conclusion that this age sets the upper limit for island evolution is thus not entirely correct. Some speciation of Galápagos endemic *Galapaganus* weevils, *Tropidurus* and *Conolophus* lizards apparently took place before the emergence of the oldest extant islands, and these are probably not unusually old elements of the Galápagos fauna.

Recent molecular phylogenetic studies of flightless beetles in the Canary Islands show similar patterns. Like *Galapaganus*, the flightless tenebrionid genera *Hegeter* and *Pimelia* each reflect the historic sequence of island formation, with the cladistically basal lineages restricted to the oldest island Fuerteventura (Juan *et al*., 1995, 1996). Although flightless beetles may be faithful markers of island biogeographical history, other groups of organisms also lend support to the historical model of island faunal development.

Recent overviews of Hawaiian island biogeography indicate a strong influence of volcanic history on both the flora and fauna (Wagner & Funk, 1995; Roderick & Gillespie, 1998). Like the Galápagos, this hotspot chain is tens of millions of years old, but the presently emergent islands are only 1–5 Myr (Carson & Clague, 1995). Molecular phylogenetic studies of *Drosophila* (DeSalle & Hunt, 1987), spiny-leg *Tetragnatha* spiders (Gillespie *et al*., 1994), and the Malvaceae plant genera *Remya* Hillebr., *Hesperomannia* Gray and *Kokia* Lewton show clear correspondence to the sequence of appearance of these islands, whereas *Geranium* L. and silverswords (*Argyroxiphium* D. C. and *Wilkesia* Gray) exhibit more complex biogeographical patterns (Funk & Wagner, 1995).

Analyses of mtDNA sequences for the Hawaiian Drosophilidae (DeSalle, 1995) corroborate the early inference of colonization in the Miocene or earlier, an age exceeding that of the presently emerged islands (Carson & Kaneshiro, 1976). However, the more vagile Hawaiian honeycreepers, like the Galápagos finches, represent a very recent radiation with little correspondence to island history (Funk & Wagner, 1995).

It is becoming clear that at least some elements of the biota of the Galápagos reflect the long-term history of this hotspot archipelago, as is certainly the case for the Hawaiian Islands and perhaps for the Canaries as well. As with the evolution of floras, where the oldest plant groups are often host to the oldest herbivores (Farrell, 1998), the long-term persistence of historical patterns implies some constraints on dispersal or host shift. Island systems also parallel floras in permitting replicated study of the macroevolutionary consequences of colonization (Strong *et al*., 1984; Farrell & Mitter, 1993).

Galapaganus weevils offer an initial molecular phylogenetic history of colonization of the Galápagos by arthropods. Further study of additional geographical representatives of the analysed species, as well as those of species of *Galapaganus* endemic to other islands (i.e. Isabela) and comparable lineages, will reveal whether the apparent history reported here is general. Comparative molecular systematic studies of other small, sedentary organisms, such as flightless arthropods, and of larger vertebrates, such as birds and lizards, may collectively resolve the history of this and other island archipelagos and help realize the unique advantage they provide for insights into the process of adaptive radiation.

Acknowledgements

Scientific research permits (Department of Forestry, Ministry of Agriculture, Republic of Ecuador) were issued through Dr Stewart Peck, head of the expedition to the Galápagos Islands. Field logistical support was obtained from the Charles Darwin Research Station (Santa Cruz Island), through directors D. Evans and C. Blanton. Fieldwork collaboration was provided for entomologists Stewart Peck (Canada) and Lazaro Roque Albelo (Ecuador). Fieldwork was partially supported by a research grant to S. Peck from the Natural Sciences and Engineering Research Council of Canada and National Geographic Society. Field and laboratory work were sponsored by research grants to A. Lanteri from Fundación Antorchas, Universidad Nacional de La Plata and CONICET (Argentina) and to V. Confalonieri from CONICET (Argentina). This research was also supported by a postdoctoral grant from Fundacion Antorchas to A. Sequeira, at Harvard University, and by a grant from the USDA to B. D. Farrell.

References

BROWER, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 6491–6495.

CARLQUIST, S. 1974. *Island Biology*. Columbia University Press, New York.

CARSON, H. L. 1992. The Galápagos that were. *Nature*, **355**, 202–203.

CARSON, H. L. AND CLAGUE, D. A. 1995. Geology and biogeography of the Hawaiian Islands. In: Wagner, W. L. and

Funk, V. A. (eds) *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*, pp. 14–29. Smithsonian Institution Press, Washington, DC.

CARSON, H. L. AND KANESHIRO, K. Y. 1976. *Drosophila* of Hawaii: systematics and ecological genetics. *Ann. Rev. Ecol. Syst.*, **7**, 311–345.

CHRISTIE, D. M., DUNCAN, R. A., McBIRNEY, A. R., RICHARDS, M. A., WHITE, W. M., HARPP, K. S. *ET AL.* 1992. Drowned islands downstream from the Galápagos hot spot imply extended speciation times. *Nature*, **355**, 246–248.

COOK, J., HOWDEN, H. F. AND PECK, S. B. 1995. The Galápagos islands' genus *Neoryctes* Arrow (Coleoptera: Scarabaeidae: Dinastinae). *Can. Entomol.*, **127**, 177–193.

COX, A. 1983. Ages of the Galápagos islands. In: Bowman, R. I., Berson, M. and Leviton, A. E. (eds) *Patterns of Evolution in Galápagos Organisms*, pp. 11–23. Pacific Division of the American Association for the Advancement of Science, San Francisco, CA.

CROZIER, R. H. AND CROZIER, Y. C. 1993. The mitochondrial genome of *Apis mellifera*: complete sequence and genome organization. *Genetics*, **133**, 97–117.

DARWIN, C. 1845. *The Voyage of the Beagle*. John Murray, London.

DARWIN, C. 1859. *On the Origin of Species*. John Murray, London.

DESALLE, R. 1995. Molecular approaches to biogeographic analysis of Hawaiian Drosophilidae. In: Wagner, W. L. and Funk, V. A. (eds) *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*, pp. 72–89. Smithsonian Institution Press, Washington, DC.

DESALLE, R. AND HUNT, J. A. 1987. Molecular evolution in Hawaiian drosophiloids. *Trends Ecol. Evol.*, **2**, 212–216.

FARRELL, B. D. 1998. Inordinate fondness explained: Why are there so many beetles?. *Science*, **281**, 555–559.

FARRELL, B. D. AND MITTER, C. 1993. Phylogenetic determinants of insect/plant community diversity. In: Ricklefs, R. E. and Schluter, D. (eds) *Species Diversity: Historical and Geographical Perspectives*, pp. 253–266. University of Chicago Press, Chicago, IL.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.*, **17**, 368–376.

FINSTON, T. L. AND PECK, S. B. 1997. Genetic differentiation and speciation in *Stomion* (Coleoptera: Tenebrionidae): flightless beetles of the Galápagos Islands, Ecuador. *Biol. J. Linn. Soc.*, **61**, 183–200.

FUNK, V. A. AND WAGNER, W. L. 1995. Biogeographic patterns in the Hawaiian islands. In: Wagner, W. L. and Funk, V. A. (eds) *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*, pp. 379–419. Smithsonian Institution Press, Washington, DC.

GEIST, D. 1996. On the emergence and submergence of the Galápagos islands. *Not. Galápagos*, **56**, 5–9.

GEIST, D., McBIRNEY, A. R. AND DUNCAN, R. A. 1985. Geology of Santa Fe Island: The oldest Galápagos volcano. *J. Volcanol. Geotherm. Res.*, **26**, 203–221.

GILLESPIE, R. G., CROOM, H. B. AND PALUMBI, S. R. 1994. Multiple origins of a spider radiation in Hawaii. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 2290–2294.

GRANT, P. R. 1986. *Ecology and Evolution of Darwin's Finches*. Princeton University Press, Princeton, NJ.

GRANT, P. R. 1994. Population variation and hybridization: comparison of finches from two archipelagos. *Evol. Ecol.*, **8**, 598–617.

HICKMAN, C. S. AND LIPPS, J. H. 1985. Geologic youth of Galápagos Islands confirmed by marine stratigraphy and paleontology. *Science*, **227**, 1578–1580.

HUELSENBECK, J. P. AND RANNALA, B. 1997. Phylogenetic methods come of age: Testing hypotheses in an evolutionary context. *Science*, **276**, 227–232.

JUAN, C., OROMI, P. AND HEWITT, G. M. 1995. Mitochondrial DNA phylogeny and sequential colonization of Canary Islands by darkling beetles of the genus *Pimelia* (Tenebrionidae). *Proc. R. Soc. B*, **261**, 173–180.

JUAN, C., OROMI, P. AND HEWITT, G. M. 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from Cytrochrome Oxidase I mitochondrial DNA sequences. *Heredity*, **76**, 392–403.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**, 111–120.

KUMAR, S. 1996. *PHYLTEST: A program for testing phylogenetic hypotheses, Version 2.0*. Institute of Molecular Evolutionary Genetics and Department of Biology, The Pennsylvania State University, University Park, PA.

LANTERI, A. A. 1992. Systematics, cladistics and biogeography of a new weevil genus, *Galapaganus* (Coleoptera: Curculionidae) from the Galápagos islands, and coasts of Ecuador and Perú. *Trans. Am. Entomol. Soc.*, **118**, 227–267.

LI, P. AND BOUSQUET, J. 1992. Relative-Rate test for nucleotide substitutions between two lineages. *Mol. Biol. Evol.*, **9**, 1185–1189.

LOPEZ, T. J., HAUSELMAN, D. E., MAXSON, L. R. AND WRIGHT, J. W. 1992. Preliminary analysis of phylogenetic relationships among Galápagos Island lizards of the genus *Tropidurus*. *Amphib-Reptilia*, **13**, 327–339.

NORMARK, B. B. 1996. Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. *Evolution*, **50**, 734–745.

NORMARK, B. B., JORDAL, B. H. AND FARRELL, B. D. 1999. Origin of a haplodiploid beetle lineage. *Proc. R. Soc. B*, **266**, 1–7.

PECK, S. B. 1994. Sea surface (pleuston) transport of insects between islands in the Galápagos archipelago, Ecuador. *Ann. Entomol. Soc. Am.*, **87**, 576–582.

PECK, S. B. 1996. Diversity and distribution of the orthopteroid insects of the Galápagos Islands, Ecuador. *Can. J. Zool.*, **74**, 1497–1510.

PECK, S. B. AND KUKALOVA-PECK, J. 1990. Origin and biogeography of the beetles (Coleoptera) of the Galápagos Archipelago, Ecuador. *Can. J. Zool.*, **68**, 1616–1638.

PECK, S. B., HERATY, J., LANDRY, B. AND SINCLAIR, B. 1998. Introduced insect fauna on an oceanic archipelago: The Galápagos Islands, Ecuador. *Am. Entomol.*, **44**, 218–237.

RASSMANN, C. 1997. Evolutionary age of the Galápagos iguanas predates the age of the present Galápagos islands. *Mol. Phylogenet. Evol.*, **7**, 158–172.

RODERICK, G. K. AND GILLESPIE, R. C. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. *Mol. Ecol.*, **7**, 519–531.

SNODGRASS, R. E. 1902. Papers from the Hopkins Stanford Galápagos Expedition 1890–1899 VIII. Entomological results (7); *Schistocerca*, *Sphingonotus*, and *Halmenus*. *Proc. Washington. Acad. Sci.*, **4**, 411–454.

STRONG, D. R., LAWTON, J. H. AND SOUTHWOOD, T. R. E. 1984. *Insects on Plants: Community Patterns and Mechanisms*. Harvard University Press, Cambridge, MA.

SWOFFORD, D. 1998. *PAUP*: *Phylogenetic Analysis Using Parsimony, 4.0d64*. Beta Version for Macintosh. Smithsonian Institution, Washington, DC.

THORPE, R. S., McGREGOR, D. P., CUMMING, A. M. AND JORDAN, W. C. 1994. DNA evolution and colonization sequence of island lizards in relation with geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12s rRNA and nuclear RAPD analysis. *Evolution*, **48**, 230–240.

WAGNER, W. L. AND FUNK, V. A. 1995. *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago*. Smithsonian Institution Press, Washington, DC.

WHITE, W. M., McBIRNEY, A. R. AND DUNCAN, R. A. 1993. Petrology and geochemistry of the Galápagos islands: portrait of a pathological mantle plume. *J. Geophys. Res.*, **98**, 19533–19563.

WIGGINS, I. L. AND PORTER, D. M. 1971. *Flora of the Galápagos Islands*. Stanford University Press, Stanford, CA.

WILSON, E. O. 1961. The nature of the taxon cycle in the Melanesian ant fauna. *Am. Nat.*, **95**, 169–193.

WRIGHT, J. W. 1983. The evolution and biogeography of the Lizards of the Galápagos Archipelago: evolutionary genetics of *Phyllodactylus* and *Tropidurus* populations. In: Bowman, R. I., Berson, M. and Leviton, A. E. (eds) *Patterns of Evolution in Galápagos Organisms*, pp. 123–155. Pacific Division of the American Association for the Advancement of Science, San Francisco, CA.

WYLES, J. S. AND SARICH, V. M. 1983. Are the Galápagos Iguanas older than the Galápagos? Molecular evolution and colonization models for the archipelago. In: Bowman, R. I., Berson, M. and Leviton, A. E. (eds) *Patterns of Evolution in Galápagos Organisms*, pp. 177–186. Pacific Division of the American Association for the Advancement of Science, San Francisco, CA.