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Colonization history, ecological shifts and diversification in the evolution of endemic Galápagos weevils

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Abstract

Mitochondrial DNA sequence data were obtained for eight species of flightless *Galapaganus* endemic weevils and one winged close relative in order to study their colonization history and modes of diversification in the Galápagos Archipelago. Contrary to most other insular radiations, the phylogeny estimates we recovered for *Galapaganus* do not follow the progression rule of island biogeography. The penalized likelihood age estimates of colonization of the archipelago exceed the age of the emerged islands and underscore the potential role of now sunken seamounts for the early evolution of *Galapaganus*. The phylogeny proposes one intra-island origin for *Galapaganus* endemics, but monophyly tests suggest a larger contribution of *in-situ* speciation on older islands. Generalist habitat preferences were reconstructed as ancestral while shifts to highland habitats were reconstructed as having evolved independently on different islands. Magnitudes and patterns of diversification rate were found to differ between older and younger islands. Our analyses reveal that the colonization sequence of islands and timing of colonization of *Galapaganus* could be linked with the geological and volcanic history of the islands in a rather complex scenario. Even though most islands appear to have been colonized soon after their emergence, there are notable deviations from the pattern of sequential colonization expected under the progression rule when considering only the extant emerged islands. Patterns of diversification rate variation on older and younger islands correspond to the volcanic activity or remnants of such activity, while the pattern of independent evolution of restricted habitat preferences in different islands suggests that habitat shifts could also have contributed to species diversity in *Galapaganus*.

Keywords: diversification, habitat shifts, mitochondrial DNA, progression rule, speciation, volcanic history

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Introduction

Oceanic archipelagoes are useful natural model systems for studying the causes and patterns of evolutionary radiations (Emerson & Kolm 2005; Emerson & Oromi 2005; Ricklefs & Bermingham 2007). In particular, the Galápagos Islands have played a fundamental role in the history of evolutionary biology. The presently emerged Galápagos Islands were formed by successive volcanic eruptions as a result of the movement of a tectonic plate over a hotspot (Morgan 1971; Christie *et al*. 1992; White *et al*. 1993), as is also true of the Hawaiian and the Canary Islands. The age of the islands increases moving eastward along the plate, with the oldest islands located towards the southeast of the archipelago. K-Ar radiometry and marine fossils indicate a maximum age of the oldest exposed land on the order of 3–4 million years (Myr) (Geist *et al*. 1985; Hickman & Lipps 1985), whereas geological plate motion models set a maximum age of emergence in the range of 4.5–6.3 Myr (Fig. 1), depending on the velocity of the Nazca plate (Hickman & Lipps 1985; White *et al*. 1993; Geist 1996). Drowned seamounts east of the existing San Cristóbal Island are 5–11 Myr old,

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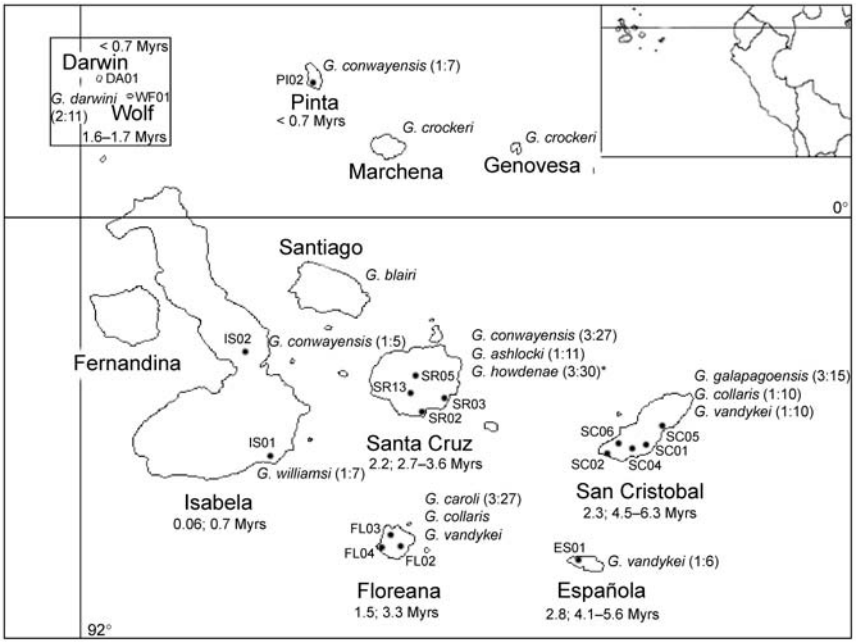

Fig. 1 Map of the Galápagos Archipelago with island age estimates and distribution of *Galapaganus* species. Island ages placed below island names. Minimum ages for the southeastern islands are from potassium-argon (K-Ar) dating (Geist *et al*. 1985; Hickman & Lipps 1985) and maximum age ranges are derived from hotspot models (Cox 1983; White *et al*. 1993; Geist 1996). Age estimates for the northern islands are from Wilson & Hey (1995) and Sinton & coauthors (1993). Numbers in parenthesis after species names indicate species that were sampled for this study with numbers of localities followed by the total number of specimens for each. * indicates an introduced species. Locality codes are detailed in Table 2.

the history of island production over this hotspot probably extends back 15–20 Myr, or even 80–90 Myr (Christie *et al*. 1992). Volcanological data and radiometric dating of the Cocos Ridge and the seamounts in the area show that an archipelago probably existed continuously above the Galápagos hotspot for the past 14.5 Myr (Werner *et al*. 1999), considerably extending the potential time frame for colonization, speciation and extinction.

The well-documented geological history of the Galápagos provides a known temporal sequence for the availability of the islands for colonization, allowing examination of species formation within a temporal framework as in the Canary Islands and Hawaii (Roderick & Gillespie 1998; Juan *et al*. 2000; Hormiga *et al*. 2003). The pattern of colonization derived from DNA data prevalent in Hawaii and documented from the Canary Islands, follows the progression rule: a stepwise colonization down the island chain from the oldest to the youngest island (Funk & Wagner 1995; Juan *et al*. 2000). However, back colonization, within-island differentiation, adaptation and extinction, among other events, can confound a simple 'island colonized as it emerges' pattern (Juan *et al*. 2000).

An intriguing pattern has emerged for the Galápagos, where several endemic taxa display divergences older than the geological age estimates of the islands above water. In particular, calibrations in Galápagos iguanid (Rassmann 1997), gecko and lizard genera (Wright 1983; Lopez *et al*. 1992) reveal divergence times in excess of the estimated island ages. Explanations of this discordance could invoke geological evidence of older, drowned islands now found as sunken sea-mounts southeast of the present location of the archipelago as potential colonization platforms for ancestors, effectively extending the time available for species diversification beyond the ages of the extant emerged islands (Christie *et al*. 1992; Werner *et al*. 1999). In contrast, such explanations are not required to reconcile divergence times and ages of the extant islands in other dynamic volcanic island systems. In Hawaii, most species lineages have diverged within the lifetime of the extant emerged islands (Price & Clague 2002; Hormiga *et al*. 2003). Similarly, in the Canary Islands, molecular estimates of divergence times for several beetle groups have indicated that most of the divergences have occurred within the timelines of the extant islands (Emerson & Oromi 2005; Emerson *et al*. 2000a, c). Furthermore, geological ages of the extant islands have been used as calibration points to derive rates of sequence divergence in both archipelagos (Fleischer *et al*. 1998; Gubitz *et al*. 2000).

Even though the emerged islands in the Galápagos Archipelago are relatively geologically recent, their isolation from the continent (1000 km west of the coasts of Ecuador) and their diversity in climate and topography has promoted speciation in both plant and animal groups (Grant & Grant 1996; Peck 1996a; Sato *et al*. 2001; Nielsen 2004). Six main vegetation/ecological zones are recognized in the largest islands of the archipelago, changing with increasing elevation. Ranging from a littoral zone at sea level containing salt-tolerant vegetation, through arid, transition and pampa zones, to include high-altitude cloud forest (Peck 1991; Wiggins & Porter 1971; Peck 1996b; Peck 2005). Plant composition differs significantly between zones (Wiggins & Porter 1971). Although islands are characterized by a diversity of habitats, the extent of these on each island differs (Peck *et al*. 1998; Causton *et al*. 2005). The rich

Species group and species	Distribution	Ecological zone preference	Character state
femoratus			
*G. h. howdenae**	Ecuador and Santa Cruz	N/A	N/A
G. femoratus	Mainland Ecuador	N/A	N/A
darwini			
G. propinquus	Puna Island and coastal Ecuador	N/A	N/A
G. squamosus	Northern coast of Perú	N/A	N/A
G. lacertosus	Central coast of Perú	N/A	N/A
*G. vandykei**	Floreana, San Cristóbal, Española	Littoral[L], arid[P]	0
*G. galapagoensis**	San Cristóbal	Littoral[L,C], arid[L,P,C], pampa[P]	3
*G. caroli**	Floreana	Littoral[L], arid[L,C], transition[L], humid forest[P]	1
*G. williamsi**	Isabela	Littoral[L], arid[L,P,C], *Scalesia*[P], pampa[L,C]	3
*G. ashlocki**	Santa Cruz	*Scalesia*[P,C], pampa[L]	2
*G. conwayensis**	Santa Cruz, Isabela† and Pinta†	Littoral[L,C], arid[L,P,C], transition[P], *Scalesia*[L,C]	1, 3
G. crokeri	Genovesa	Arid zone	
G. blairi	Santiago	Arid zone	
*G. collaris**	San Cristóbal and Floreana	Pampa[L,P,C]	2
*G. darwini**	Darwin and Wolf	Littoral[C], arid[L,P,C]	0/?

Table 1 Distribution and ecological preferences of *Galapaganus* species in the archipelago. Asterisks after species names indicate material included in this study and †signs after island names indicate a new island record for that species. Multiple populations were included for *G. galapagoensis*, *G. caroli*, *G. conwayensis*, *G. darwini*, *G. vandykei* and *G. h. howdenae.* Ecological zone/habitat preferences are derived from host records obtained through our own collections[C] as detailed in the text, and from Lanteri (1992)[L] & Peck (2005)[P] (0) indicates a preference for lowland habitats including littoral and/or arid zone; (1) for lowland and mid-elevation habitats including littoral, arid and transition zone (and in smaller islands as Floreana also humid forest); (2) for high elevation habitats including *Scalesia*, *Zanthoxylum*, *Miconia* and Fern-Sedge (Pampa) zone and (3) for all ecological zones/habitats. N/A: not analysed for continental species.

biodiversity of the archipelago has been linked in part to the habitat diversity present in the islands. In particular, there is a positive relationship between beetle diversity and island area, elevation, and ecological complexity, but not island age (Peck 1994b; Peck 2005). Hence, it could be suggested that environmental diversity within each island in the Galápagos provides an ecological spectrum for species differentiation. Indeed, several indirect measures of niche space; such as habitat diversity, island elevation and island area are strong predictors of overall species richness of Galápagos endemic bulimulid land snails (Parent & Crespi 2006).

A previous phylogenetic analysis of the weevil genus *Galapaganus* Lanteri (1992) (Coleoptera; Curculionidae) based on mitochondrial DNA and morphological characters revealed a similar pattern as that seen for geckos and lizards. Applying a mitochondrial DNA clock for a subset of the endemic species, the timing of the initial split separating the basal lineage on the oldest island from the remaining species in the Galápagos exceeded the geological ages of the extant emerged islands (Sequeira *et al*. 2000). This genus contains a total of 15 species placed within the *femoratus* and *darwini* species groups (Lanteri 1992). The *darwini* group includes 10 species endemic to the islands that are flightless and fairly heavy-bodied. Five of the 10 endemic species are single island endemics while the rest inhabit several neighbouring islands (Table 1, Fig. 1). Weevils of particular species are usually restricted to one or two contiguous ecological zones with characteristic vegetation where adults feed preferentially on host-plants restricted to those particular ecological zones. One such 'habitat specialist' is *Galapaganus collaris*, known exclusively from the moist highlands of Floreana and San Cristóbal (Lanteri 1992; Peck 2005). When two or more species occupy an island, as is the case of *Galapaganus vandykei* and *Galapaganus collaris* in San Cristóbal, one is usually confined to the moist highlands while its counterpart will inhabit the lowlands (Lanteri 1992). The genus also contains a few species that could be considered 'habitat generalists' whose adults feed on a variety of hosts across all the altitude range and therefore all ecological zones (*Galapaganus williamsi* in Isabela). The distribution of *Galapaganus* weevils across islands and ecological zones provides a replicated system for examining within- and between-island patterns of colonization and speciation across similar environmental extremes (Roderick & Gillespie 1998) and investigating the relative roles of habitat shifts vs. dispersal in leading to founder-induced divergence in this radiation.

Perhaps the most dominant ecological features of the Galápagos Islands are the archipelago's arid, harsh environment and active volcanic history. In the last 200 years, approximately 50 eruptions have occurred almost exclusively in the younger islands (Colinvaux *et al*. 1968; Eliasson 1972; Shen *et al*. 1991; Beheregaray *et al*. 2003). Volcanism has been invoked for Hawaiian and Canarian biota to explain genetic structure by recurrent isolation and/or bottlenecking populations (Carson *et al*. 1990; Desalle & Templeton 1992; Pestano & Brown 1999). Once an island has been colonized, within island differentiation can be facilitated by vicariant events produced by lava flows and by local extinctions (Carson *et al*. 1990; Roderick & Gillespie 1998; Pestano & Brown 1999; Holland & Cowie 2007).

Several phylogenetic efforts have been directed towards making inferences about timing and tempo of diversification (Mooers & Heard 1997; Pybus & Harvey 2000; Lieberman 2001; McKenna & Farrell 2006). Methods to investigate the fit to different models of variation of diversification rates can be tested for clades within phylogenies (Paradis 1997; Paradis 1998a, b). For example, models for constant diversification, gradual change in diversification or sudden change can be tested. Even though this approach was primarily developed to examine the phylogenies of recent species, it is equally suited and has been successfully applied to intraspecific gene genealogies (Emerson *et al*. 2000a, c). Trends in diversification rates can be estimated for each clade recovered from the phylogenetic tree where monophyletic island groupings allow comparisons between island populations or close relatives in different islands.

If we test the idea that volcanic activity, either through shifting barriers to gene flow or through causing constant extinction and recolonization, influences the magnitude and pattern of diversification rate, we could predict that diversification rate should be greatest on islands that undergo disturbance, such as periodic volcanic activity, compared with islands that are volcanically inactive. Populations in volcanically active islands will experience periodic disjunction and reductions in population size; both events fostering haplotype divergence and acquisition of new alleles by random drift (Kimura & Ohta 1969). Studies of phylogeography and diversification of *Brachyderes* weevils and colyiid beetles in the Canary Islands have found that diversification rates were not dissimilar among islands (i.e. no increased rates on volcanically active islands) (Emerson *et al*. 2000a, c). For both groups, lineages on each of the four islands also exhibited similar patterns of decreasing rate of mtDNA diversification over time, sometimes marked by sudden, periodic changes (Emerson *et al*. 2000a, c).

Studies of a variety of arthropod groups have successfully combined mtDNA derived phylogenies with geological evidence to elucidate the timing and sequence of colonization of the Canary Islands (Juan *et al*. 1996; Juan *et al*. 1998; Emerson *et al*. 2000a, b, c; Arnedo *et al*. 2001; Emerson & Oromi 2005) and Hawaii (Holland & Hadfield 2004; Shaw 1996). Mitochondrial DNA sequence data were collected from a variety of locations, islands and species to study the colonization history and modes of diversification in *Galapaganus* (Table 2). A well-resolved phylogeny allows testing of the following explicit predictions: (i) if the colonization history of the archipelago follows the chronological ages of the islands, then the phylogeny should follow the progression rule; (ii) if islands were colonized soon after they emerged, then estimates of divergence time in each island should be consistent with island age estimations; (iii) if intra-island speciation plays a role in *Galapaganus* diversification, then species from a single island should be more closely related than species from different islands; (iv) if habitat shifts have contributed to diversity in *Galapaganus*, then such shifts should be linked to species formation across the phylogeny; (v) if volcanic activity plays a role in shaping the diversification rates of endemic populations, then the diversification rate and patterns of diversification rate for endemics on older volcanically inactive islands (San Cristóbal, Floreana, Santa Cruz) should be significantly different from those of endemics to younger, equally ecologically complex but more volcanically active islands (Isabela and Pinta).

Materials and methods

Sampling

Samples were obtained from eight of the islands in the archipelago (Fig. 1, Table 2). We attempted to sample across multiple locations on all islands; collecting a minimum of five specimens per location whenever possible. Eight of the 10 endemic species occurring in the archipelago and *Galapaganus howdenae howdenae*, widespread in coastal Ecuador and recently introduced in the agricultural area of Santa Cruz, were sampled (Peck *et al*. 1998; Lanteri 2004; Causton *et al*. 2005). *Galapaganus h. howdenae* is one of the two members of the *femoratus* species group while all endemics belong to the *darwini* species group (Lanteri 1992) (Table 1). Half of the endemic species analysed are single-island endemics (four out of eight). The material available includes conspecifics from different islands for *Galapaganus darwini, G. vandykei* and *G. conwayensis* allowing the analysis of interisland divergence and colonization below the species level only for these three species. *Galapaganus conwayensis* was previously described as a single island endemic for the island of Santa Cruz (Lanteri 1992; Sequeira *et al*. 2000; Peck 2005), until we recently collected it in Northern Isabela and on Pinta. Outgroups include seven species also within the tribe Naupactini in the weevil subfamily Entiminae, six belonging to the species-rich South American genus *Naupactus* and one to the genus *Aramigus*.

DNA preparation, polymerase chain reaction amplification and sequencing

DNA was isolated from three legs from each specimen according to the protocol of Normark (1996) or alternatively using the DNeasy Tissue Kit (QIAGEN). One microlitre of a 1/10 dilution of genomic DNA was amplified to produce double-stranded products for four mitochondrial regions:

Table 2 Islands sampled, locality codes, locality names and number of specimens per locality for all *Galapaganus* and *Naupactus* species included in our analysis (ingroup and outgroup). The number of ecological zones on each island (a measure of ecological complexity; following Peck 2005) is indicated in brackets after island names. *N* indicates the number of specimens analysed per locality

Island	Species	Locality code	Locality	*N*
Santa Cruz (6)	*Galapaganus h. howdenae*	SR01	Trail to Cerro Croker	12
Santa Cruz	*G. h. howdenae*	SR06	2 km. N of El Cascajo	8
Santa Cruz	*G. h. howdenae*	SR07	Finca Steve Devine	10
Santa Cruz	*G. ashlocki*	SR05	Los Gemelos	11
Santa Cruz	*G. conwayensis*	SR03	CDRS	12
Santa Cruz	*G. conwayensis*	SR02	Tortuga Bay trail	4
Santa Cruz	*G. conwayensis*	SR13	Los Gemelos	11
Isabela (6)	*G. conwayensis*	IS02	Volcan Alcedo	5
Pinta (4)	*G. conwayensis*	PI02		7
Floreana (4)	*G. caroli*	FL02	Casa Cruz	10
Floreana	*G. caroli*	FL03	Cerro Pajas	7
Floreana	*G. caroli*	FL04	Bahia Las Cuevas	10
San Cristóbal (6)	*G. galapagoensis*	SC02	SE Wreck Bay	6
San Cristóbal	*G. galapagoensis*	SC04	E Wreck Bay	3
San Cristóbal	*G. galapagoensis*	SC06	N Wreck Bay	6
San Cristóbal	*G. collaris*	SC01	El Junco	10
Española (2)	*G. vandykei*	ES01	Punta Suarez	6
San Cristóbal	*G. vandykei*	SC05	Rosa Blanca Bay	10
Isabela	*G. williamsi*	IS01	Puerto Villamil	10
Darwin (1/2)	*G. darwini*	DA01		5
Wolf (1/2)	*G. darwini*	WF01		6
South America	*Aramigus tesselatus*	AT03		1
	Naupactus cinerosus	NC01		2
	N. cervinus	NE01		1
	N. xanthographus	NX01		1
	N. dissimulator	ND01		1
	N. verecundus	NV01		1
	N. leucoloma	NL01		1

12S (primers: J14233 5′-AAGAGCGACGGGCGATGTGT-3′ and N14588 5′-AAACTAGGATTAGATACCCTATTAT-3′ from Simon *et al*. 1994), partial sequence of cytochrome *c* oxidase subunit I (COI primers: S2183 5′-CAACATTTATTTTGATTTTTTGG-3′ and A2771 5′-GGATARTCAGARTAACGTCGWGGTATWC-3′ from Farrell laboratory), cytochrome *c* oxidase subunit II (COII primers: J3038 5′-TAATATGGCAGATTAGTGCATTGGA-3′ and N3668 5′-GCTCCACAAATTTCTGAGCA-3′ from C. Linnen, Farrell Laboratory) and cytochrome *b* (cyt *b* primers: cyt *b* b.1 5′-TTAATTATTCAAATTGCAACAGGATTATTT-3′, CB1 5′-TATGTACTACCATGAGGACAAATATC-3′ and CB2 5′-AATACACCTCCTAATTTATTAGGAAT-3′ modified from Crozier & Crozier (1992), Cryan *et al*. (2001) and Vogler & Welsh 1997). Amplification reactions contained 0.2 mM of each primer, 0.2 mM of each dNTP, between 1.5 and 3 mM $MgCl_2$, buffer supplied by the manufacturer (TaKaRa) and 1.25 U Ex*Taq* polymerase (TaKaRa), in a total volume of 50 μL. A typical temperature profile consisted of 35 cycles: four cycles at 94 °C for 1 min, 47 °C for 30 s and 72 °C for 1 min 30 s, followed by 31 cycles at 94 °C for 1 min, 50 °C for 30 s and 72 °C for 1 min 30 s. A final extension step held the temperature at 72 °C for 5 min. The product was purified using a MinElute PCR purification kit (QIAGEN). Purified products were sequenced using the BigDye version 3.1 terminator protocol (Applied Biosystems) using polymerase chain reaction (PCR) primers in an ABI 3100 capillary sequencer. Sequences were compiled, edited and aligned in SEQUENCHER version 4.5 (Gene Codes corporation) for protein-coding regions. For non-protein-coding regions (12S), alignments were performed in CLUSTAL_X (Thompson *et al*. 1997).

A combined matrix of 2579 characters was compiled from all four mtDNA regions, 397 for 12S, 800 for COI, 631 for COII and 749 for cyt *b* for 166 individuals from 21 populations of nine *Galapaganus* species and seven outgroup species within the Naupactini tribe. The complete set of sequences has been submitted to GenBank under accession nos EU264599–EU265325.

Phylogenetic analysis

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) were used to reconstruct phylogenetic relationships from mitochondrial sequence data. Maximum parsimony analysis was performed in PAUP* version 4.0b10 (Swofford 1998) including all nucleotide sites. Searches were performed using the heuristic search algorithm with the random addition sequences option, 1000 replicates and the tree-bisection–reconnection branch-swapping (TBR) algorithm. One thousand bootstrap replicates each with 100 random addition replicates were performed to assess the support of individual branches.

Maximum likelihood topologies were first constructed using the model selected by MODELTEST (general time reversible, GTR) (Posada & Crandall 1998) in PHYML (Guindon *et al*. 2004) and further optimized in PAUP* using the same model. Bayesian inference analysis was performed with MRBAYES version 3.1.1 (Huelsenbeck & Ronquist 2001). The model selected by MODELTEST for each gene region and incorporated in Bayesian searches was a site-specific model applying the GTR model estimating the proportion of invariable sites and the gamma distribution (GTR + I + G) for all protein-coding regions and a simpler GTR for 12S. Each search in MRBAYES started from a random tree and was continued for 2 000 000 generations with four simultaneous chains, each sampled every 100 generations. All trees below the stationarity level were discarded. A slightly more complex GTR + I + G model for the whole data set yielded an identical topology and comparable support. Slightly simpler models (GTR) did not yield higher support across the topology. Branch lengths were optimized on this topology using PAUP* through maximum likelihood under the GTR + I + G model as selected with MODELTEST.

Tests of alternative colonization scenarios, island assemblage monophyly and single origin of habitat preferences

Alternative colonization scenarios concordant with the sequence of island ages given by geological dating were tested against the ML topology (identical to the Bayesian topology shown in Fig. 2). In the first scenario, we constrained the first divergence to be between the lineage on San Cristóbal (SC), the oldest island according to the plate movement models (Geist 1996), and the rest of the ingroup (SC-others). A second scenario established the splitting of the lineage on Española (ES), right after the first split in San Cristóbal with the rest of the ingroup derived from the Española clade (SC-ES-others). Two more scenarios were tested that followed the chronological order proposed by K-Ar dating (Geist *et al*. 1985; Hickman & Lipps 1985): ES-others and ES-SC-others. Finally a scenario (old–young) constrained the lineages from geologically younger islands (Pinta, Isabela, Darwin and Wolf) to derive from a non-resolved assemblage of weevils on the geologically older islands [ES-(SC,SR,FL)-(PI,IS,DA,WF)].

To test for monophyly of different island assemblages and habitat preferences, we applied hypothesis-specific tests. Likelihood-ratio tests were performed comparing the unconstrained ML tree with trees that were constrained for each of the hypotheses to be tested [one tailed SH log-likelihood test (Shimodaira & Hasegawa 1999) as implemented in PAUP*]. We tested the island assemblages of San Cristóbal, Santa Cruz, Isabela and Wolf for monophyly. Three of the states scored as ecological preferences were constrained to have evolved only once and evaluated the same way (Table 3).

Table 3 Island and ecological/habitat preference monophyly tests results. For each island, the number of endemic species present on the island and included in this study are indicated by *me*, multiple endemics or se, single endemic. Under the heading SH are the results of the Shimodaira–Hasegawa test, and under PB are the probability values under parametric bootstrapping of the MP tree length score differences between constrained and non-constrained topologies for those monophyletic island and ecological-preference groupings not present in the analysis. Post prob. lists the posterior probabilities of those same groupings. *indicates significant differences between hypothesis trees (constrained) and empirical topologies suggesting that the hypothesis of monophyly of either islands or ecological zones is rejected in favour of the alternative hypothesis of multiple colonizations

Island or ecological zone tested	SH	PB	Post prob.
Isabela (*me*)	$P < 0.01$*	$P < 0.01$*	0.01
San Cristóbal (*me*)	$P > 0.05$	$P > 0.05$	0.47
Santa Cruz (*me*)	$P > 0.05$	$P > 0.05$	0.33
Wolf (*se*)	$P > 0.05$	$P > 0.05$	0.45
Lowlands	$P < 0.01$*	$P < 0.01$*	0.0001
Mid-elevations	$P < 0.01$*	$P < 0.01$*	0.0008
Highlands	$P < 0.01$*	$P < 0.01$*	0.0001

We evaluated the effect of constraining the monophyly of the three islands and ecological preferences not represented as a single origin in our analyses via parametric bootstrapping. Sequences were simulated on a constrained tree for a given hypothesis constructed with PAUP* using maximum likelihood distances with parameter estimates derived from the MODELTEST analysis (Posada & Crandall 1998). Simulated sequences (100 data sets) were generated in SEQGEN (Rambaut & Grassly 1997) using the same model of sequence evolution and parameter estimates used to construct the hypothetical tree. The resulting distribution of differences was then compared with the MP tree length differences for the empirical constraint and nonconstraint trees (Ruedi *et al*. 1998; Emerson *et al*. 2000b). All tree searches were performed in PAUP* 4.0 with 1000 random addition sequences and TBR with no MaxTrees limit (Table 3). Posterior probabilities were also determined for island groupings and ecological preferences not supported by our analyses (Table 3).

Estimation of divergence times

A previous estimate of the ages of island colonization events by *Galapaganus* was achieved using an extrinsic molecular-clock calibration (Sequeira *et al*. 2000). This approach was useful for a reduced data set, but when intraspecific level

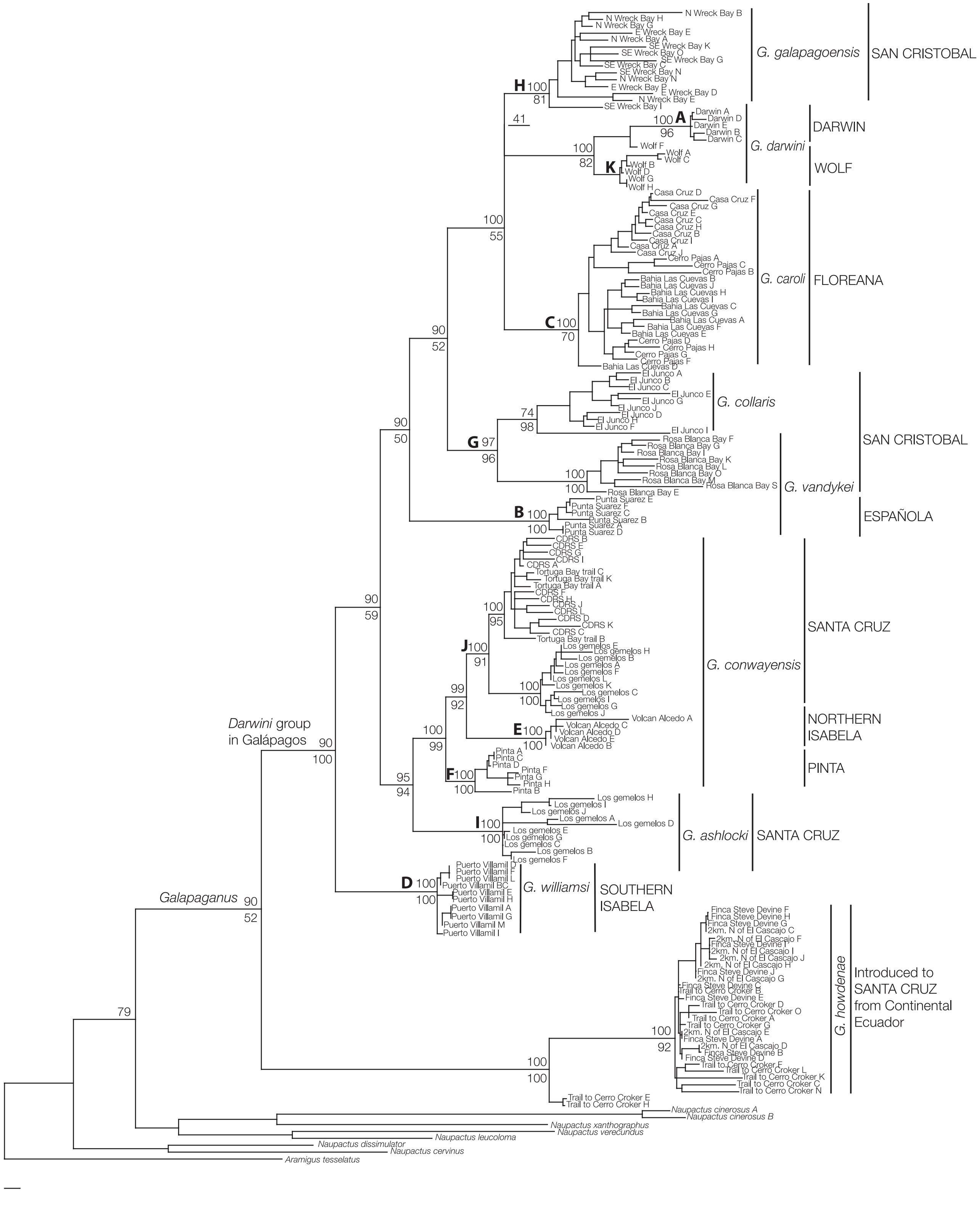

Fig. 2 Bayesian majority rule consensus (site-specific model with GTR + I + G for all protein-coding regions and GTR for 12S, 17802 trees, two million generations, burn-in = 1000, 4 chains, *T* = 0.5). Branch lengths derived from ML optimization with GTR + I + G model. Numbers above the branches indicate posterior probabilities expressed as percentages. Numbers below the branches indicate bootstrap values. Letters correspond to island clades used in diversification rate analyses detailed in Table 5.

lineage evolution is also evaluated, it may be more appropriate to use an intrinsic clock calibration and a method that allows some branch length variation (Emerson *et al*. 2000a). Geological ages for the islands are available and can be used to calibrate and estimate divergence ages and to infer the timing of colonization.

Penalized likelihood (PL) (Sanderson 2002) was implemented in the program R8S 1.71 (Sanderson 2002) to generate an ultrametric tree and estimate divergence times from the Bayesian topology with maximum-likelihood branch lengths optimized in PAUP* under the model selected by MODELTEST. This method replaces the constraint of a constant rate across a tree with a weaker constraint on rate variation that is still sufficient to allow the estimation of divergence times. The PL method combines likelihood and the nonparametric penalty function used in nonparametric rate smoothing (NPRS) (Sanderson 1998), allowing for the specification of the relative contribution of the rate smoothing and data fitting parts of the estimation procedure. The optimal smoothing parameter was obtained by cross validation in R8S (Sanderson 2002). Analyses were then performed with optimizations rerun 10 times with a perturbation factor of 0.05. We used the divtime function in R8S to calculate the ages of nodes in the tree relative to nodes with constrained maximum ages. Three different analyses were performed. Geological estimations of island age were used as maximum age constraints for divergences within each of the islands (see Fig. 3 for constraints). These nine constraints were distributed across the topology, but did not include the node originating the *darwini* group (which was unconstrained). The first analysis (AI) applied all nine constraints together. A second set of analysis (nine in total: AII) was completed individually removing each of those constraints, therefore allowing the age for each node to be estimated unconstrained. A final analysis was performed where we stipulated a maximum age for the *darwini* group, including all island endemics, as the age of the oldest sunken seamount located closest to the archipelago which could have been available for colonization 11 million years ago (Geist 1996) and estimated the ages of all other nodes which were then unconstrained (AIII). In addition to the age estimation ranges provided by the results of AII, the profile command in R8S was used to obtain confidence intervals for node times from a set of phylograms constructed from 1000 bootstrapped sequence data sets on a fixed topology using PHYLIP (Felsenstein 1989). The 95% confidence intervals for node times for each node are indicated as error bars for the minimum and maximum ages in Fig. 3.

The upper and lower bounds of the ranges of estimated colonization and within-island diversification times derived through PL were compared to the geological age ranges of the islands (Geist 1996; Peck 2005) and depicted in the inset in Fig. 3. Additionally, the relationship between PL within-island diversification times (obtained with the single constraint for the origin of the *darwini* group and all other nodes unconstrained, AIII) and geological ages was studied via simple correlation analysis.

Scoring and optimization of ecological/habitat preferences

Ecological or habitat preferences were scored as one summary trait for each species rather than particular host preferences per se, as detailed below. Habitat preferences were compiled from Lanteri (1992), Peck (2005) and host records from field observations. When habitat/ecological preference assignations were derived from host records, host distributions and relative abundance in neighbouring ecological zones were considered, and the ecological zones occupied by the vast majority of the hosts were recorded as the ecological preference for each species and expanded to include the published distributions (Lanteri 1992; Peck 2005). The derivation of habitat preferences from host distributions attempts to avoid bias introduced by the inaccessibility of some collecting localities. When host records were not available, only published distributions were considered. In the case of *G. conwayensis*, abundant host records, collecting dates and broad geographical sampling are available and the habitat preference derived from those records differs from that established by the literature. Analyses were performed alternatively assigning both states for this species, since the broader ecological preference assignation is possibly the result of rare climatic conditions (extremely dry years) that have temporarily shifted the species upwards generating an artificially wide habitat range. Ecological zone delimitations and groupings followed a slightly modified classification described by Johnson & Raven (1973) and detailed by McMullen (1999). The habitat preference coding devised for endemic *Galapaganus* uses a simplified interpretation of the six main vegetation/ecological zones defined for the largest and highest Galápagos islands. We scored each species as preferentially inhabiting and feeding in the lowlands (0), which includes littoral and arid zone plants; the lowlands and mid-elevations (1), which includes littoral, arid and transition zone plants; the moist highlands (2) which includes *Scalesia, Miconia* and Fern-Sedge (Pampa) zone plants; and finally all altitudes and therefore all ecological zones (3) (Table 1). The issue of resource availability, especially geographical availability (Thompson 1994) becomes particularly relevant when considering the niche/habitat preferences of endemics to the smaller and less ecologically complex islands. Members of *G. darwini* will never encounter highland plant hosts since the islands they inhabit are not high enough to develop humid forest that would harbour those plants (Peck 2005).

Likelihood reconstructions of ancestral ecological preferences were applied as implemented in MESQUITE

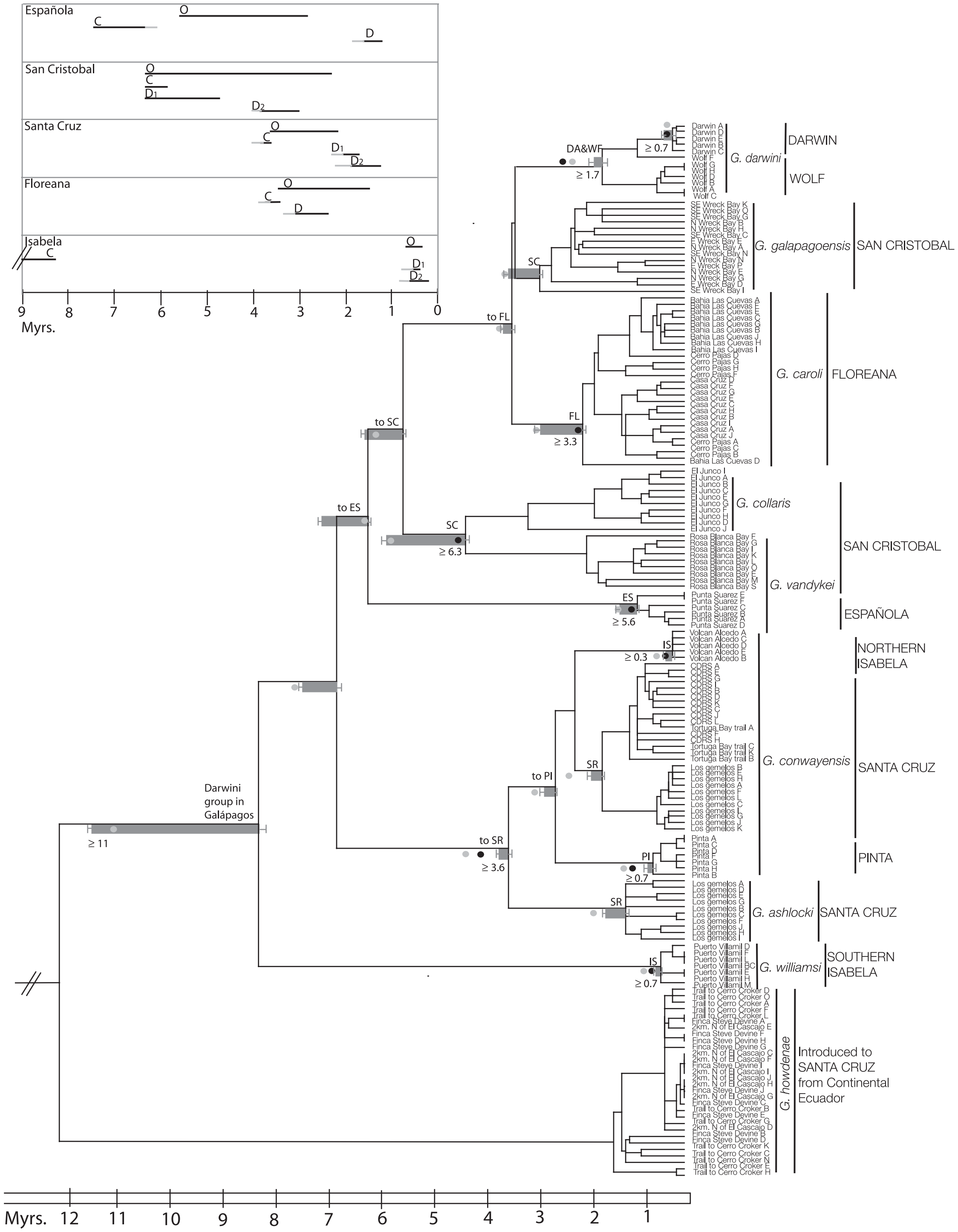

Fig. 3 Chronogram showing colonization and within-island diversification time range estimations obtained with three different analyses. Constrained nodes are marked with black dots and the relative position of the dot on the time scale indicates the point estimate obtained for that node when the constraint was removed. Gray bars indicate PL estimated time ranges for each node obtained during the analysis sequentially removing each of the nine constraints. Gray dots indicate point estimates when only the origin of island endemics was constrained to be less or equal to 11 Myr. Two-letter codes on the PL time bars indicate island colonized and diversification within that island (ES, Española; SR, Santa Cruz; SC, San Cristóbal; FL, Floreana; IS, Isabela; and WF & DA, Wolf and Darwin). The inset displays the comparison of geological estimations of island origin (O) with the timing of colonization (C) and within island divergence (D) for five of the islands (multiple clades in one island are indicated as D1 and D2). Gray line projections of these ranges in the inset show the widening of the range when applying the 11 Myr constraint and unconstraining all other nodes.

Table 4 Proportional likelihood (PL) values for the ancestral ecological preferences reconstructed on the Bayesian topology using maximum-likelihood optimized branch lengths (nodes marked in Fig. 4). Likelihoods for those states judged to be the best according to a decision threshold $T = 2$ for each node are marked with *. Character states used in the text for the four-state scheme are (0) for species preferentially inhabiting and feeding in the lowlands; (1) the lowlands and mid-elevations; (2) the moist highlands and (3) all altitudes (and therefore all ecological zones). The designations 1 and 3 refer to the alternative coding for *Galapaganus conwayensis*

Node number	PL (1)	PL (3)
1	0: 0.214	—
	3: 0.635	3: 0.970*
2	0: 0.427	—
	1: 0.143	—
	2: 0.149	—
	3: 0.281	3: 0.940*
3	1: 0.383	—
	2: 0.385	2: 0.033
	—	3: 0.948*
4	1: 0.994*	1: 0.995*
5	0: 0.995*	0: 0.984*
6	0: 0.991*	0: 0.991*
7	0: 0.710	0: 0.986*
	2: 0.101	—
8	0: 0.985*	0: 0.986*
9	0: 0.329	0: 0.329
	1: 0.327	1: 0.330
	3: 0.334	3: 0.334

(Maddison & Maddison 2006) to find the ancestral states that maximize the probability that the observed states would evolve under a stochastic model of evolution (Schluter *et al*. 1997; Pagel 1999). The model applied was the Markov k-state 1 parameter model (Lewis 2001) where the single parameter is the rate of change. Four different optimizations were performed for this four-state scheme (alternatively with two possible states for *G. conwayensis* and assigning the lowland or unknown state to *G. darwini*). Maximum-likelihood optimized branch lengths were incorporated into the Bayesian phylogeny used in MESQUITE for the calculations of proportional likelihoods. Proportional likelihoods for each state are shown graphically in Fig. 4 and those judged to be the best states according to a decision threshold $T = 2$ are marked in Table 4.

Comparisons of clade/island specific diversification rates

We used the program APE version 1.8 (Paradis 1997; Paradis *et al*. 2004) to evaluate the fit of three alternative models of diversification to the patterns derived from island clades in the phylogenetic tree using a maximum-likelihood approach. Three general models of diversification were evaluated: model A which specifies a constant diversification rate (δ), model B which specifies a gradually decreasing or increasing diversification rate and model C which specifies two different diversification rates separated by an abrupt rate shift. Models A and B are not time dependent, and are constant over time. Under model B, values of the parameter $\beta > 1$ indicate that diversification rates have gradually decreased over time, while values of $\beta < 1$ indicate that diversification rates have gradually increased over time (Paradis 1997; Paradis *et al*. 2004). No age constraints were implemented for the comparisons of island specific diversification rates for *Galapaganus* and only relative times were obtained by fixing the age of the root to an arbitrary value of 1.0. Model C depends on the timing of a hypothesized shift in diversification rate, and was evaluated from APE analyses modelling a hypothesized shift every 1/100 intervals of the relative time established over the evolutionary history of each island. The fit of the data to each model is assessed by either (i) likelihood-ratio test for nested models (in this case model A is nested within both models B and C), or (ii) Akaike information criterion (AIC) where there is no nesting relationship (as is the case of models B and C) (Table 5). Specifically defined models of diversification were constructed for analysis of between clade variation, comparing the null model where all clades have the same δ and a model where all clades have a different δ. All likelihood-ratio tests and AIC tests were performed at the 5% probability level.

	Model A		Model B			
Island clade	LL	AIC	LL	AIC	*P*	β
San Cristóbal I (G: 19)	0.622	0.765	4.25	–4.5	0.007	1.728
San Cristóbal II (H: 15)	–7.977	17.953	2.845	–1.691	3e-06	3.736
Santa Cruz end I (I: 10)	–3.38	8.76	–1.069	6.139	0.032	1.942
Santa Cruz end II (J: 27)	–12.788	27.577	13.437	–22.873	4e-09	4.389
Floreana (C: 27)	–2.777	7.555	4.013	–4.025	2e-04	1.883
Darwin (A) and Wolf (K: 12)	1.806	–1.612	1.922	0.157	0.907	0.885
Isabela I (D: 10)	0.666	0.668	2.734	–1.468	0.042	0.528
Pinta (F: 7)	0.502	0.995	1.122	1.756	0.265	0.703

Table 5 Likelihood-ratio test (LL), Akaike Information criteria (AIC) values, and probabilities (*P*) for diversification rate variation model selection in island clades. β parameter for model B indicates increasing ($\beta < 1$) or decreasing ($\beta > 1$) diversification rates. Parentheses after each island clade contain clade identification letters as in Fig. 2, followed by the sample size for each clade

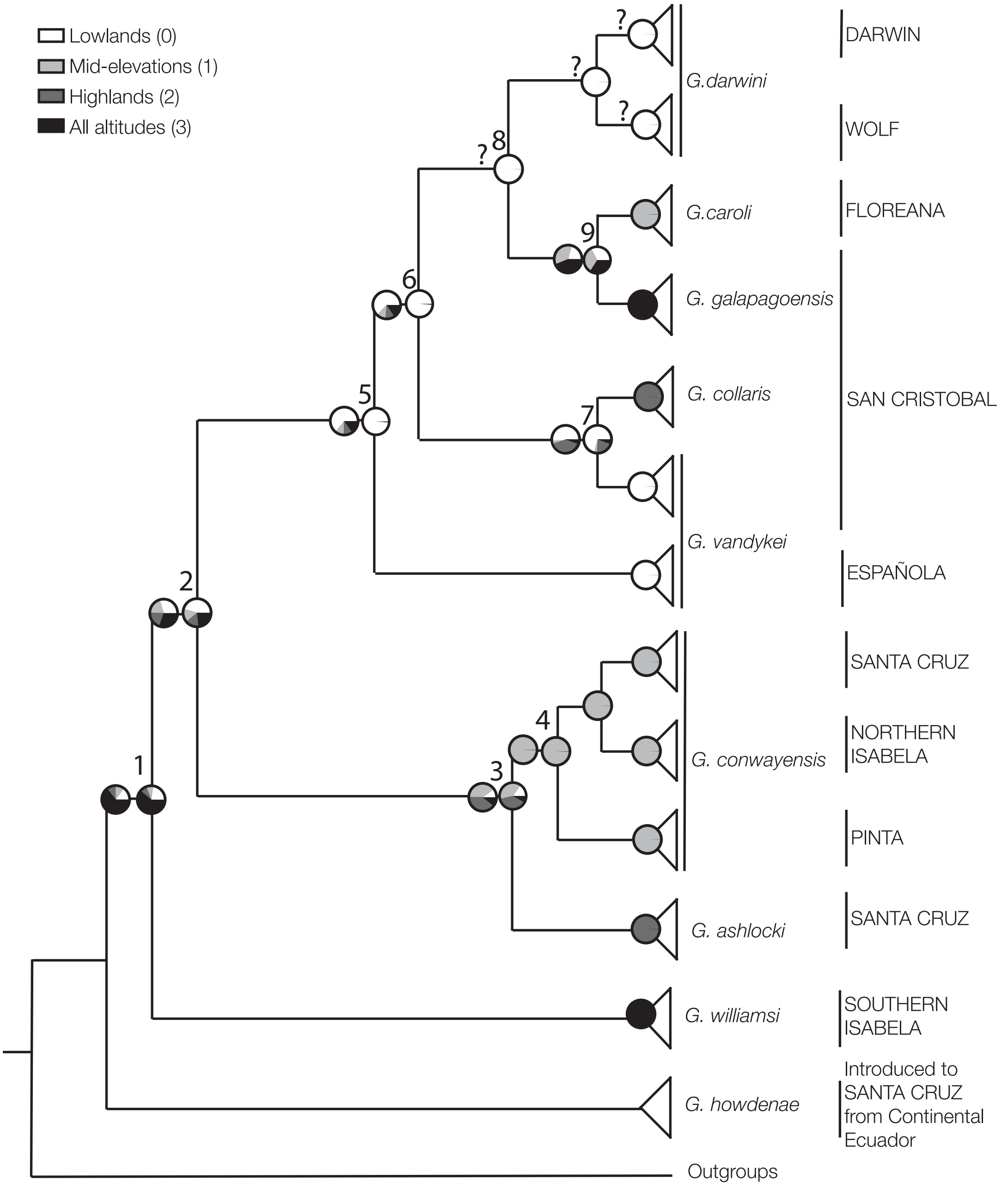

Fig. 4 Maximum likelihood reconstruction of ancestral ecological preferences on the Bayesian topology with maximum-likelihood optimized branch-lengths as in Fig. 2 following the four-state scheme assigning state 1 to *Galapaganus conwayensis*. Current preferences are indicated in the terminal circles and listed in Table 1. The relative proportion of colours in each pie indicates the probability of each ancestral ecological preference (white, lowlands; light grey, mid-elevations; dark grey, highlands and black, all altitudes/all ecological preferences). Circles or? to the left of each node correspond to optimizations where the character state for *Galapaganus darwini* was an unknown state (see text), while circles to the right of each node correspond to optimizations where the character state for *G. darwini* was assigned as a known condition. Node numbers correspond to values in Table 5.

Results

Phylogeny and colonization sequence

The same overall topology was obtained from the analysis through maximum parsimony, maximum likelihood and Bayesian inference (Fig. 2). The Bayesian estimate of the phylogeny shows that all except one of the morphologically defined species are monophyletic, displaying posterior probability values (in the form of percentages) from 74 to 100 and bootstrap support values from 70 to 100. The exception is a paraphyletic *Galapaganus vandykei* in Española and San Cristóbal. Monophyly of the genus is moderately well-supported while the monophyly of the members of the *darwini* group in the archipelago is very well-supported (Fig. 2).

It is not obvious from the branching order of island endemics proposed by the phylogeny that colonization could be inferred as following the chronological order of the islands (Fig. 2). The most notable deviation from a colonization following the progression rule is the basal position of the ancestor of *Galapaganus williamsi*, endemic to the young island of Isabela. Despite this discordance, further investigation of the colonization sequence through the Shimodaira–Hasegawa (1999) test yielded different results. Results of the SH test indicate that the best ML tree among those compatible with the scenario proposing the colonization of Española as a first step and a split of all other lineages (ES-others), is not significantly worse than the best unconstrained ML tree ($-\ln L$ diff = 2.56, $P > 0.05$) suggesting that this scenario cannot be rejected. The colonization scenario that proposes colonization of San Cristóbal following that of Española (ES-SC-others) also yields trees that are not significantly worse than the best unconstrained ML tree ($-\ln L$ diff = 3.78, $P > 0.05$), as does the older-younger scenario ($-\ln L$ diff = 6.29, $P > 0.05$). On the other hand, both scenarios that propose splitting of the San Cristóbal lineage first (SC-others and SC-ES-others) yield significantly worse ML trees than the unconstrained ML tree ($-\ln L$ diff = 35.32, $P < 0.01$; $-\ln L$ diff = 98.26, $P < 0.01$) suggesting that those scenarios can be rejected. Therefore, we cannot completely disregard a colonization sequence that loosely follows the chronological order of island ages.

Intra-island speciation

Evidence of intra-island speciation, where species sharing an island are placed in the phylogenetic tree as each other's closest relatives, is observed only once within this genus. On San Cristóbal, the oldest island of the archipelago, the highland inhabitant *Galapaganus collaris* appears to have split from lowland populations of *G. vandykei* within the same island. On the other hand, geographical isolation and multiple colonizations from neighbouring islands best explain the pattern in the slightly younger island of Santa Cruz where the differentiation of *Galapaganus conwayensis* from *Galapaganus ashlocki* appears to have occurred through colonization of the Northern Island of Pinta with subsequent dispersal to Northern Isabela and Santa Cruz.

Monophyly tests fail to reject monophyly for San Cristóbal, Santa Cruz and Wolf but not for Isabela (Table 3). MP length differences between constrained and unconstrained trees (generated from simulated data) used for the parametric bootstrapping comparisons were 2, 5 and 3, respectively, for these three island groupings, rendering the differences between the single colonization scenario and the unconstrained best tree nonsignificant. On the other hand, both tests significantly favour multiple colonizations for the species sampled from Isabela (length difference for PB test = 25). The older islands of Santa Cruz and San Cristóbal harbour two and three *Galapaganus* species, respectively. According to these analyses, the younger island of Isabela would be the only case of multiple species sharing an island where our data significantly supports multiple colonizations vs. *in-situ* differentiation as a plausible explanation for that island's origin of *Galapaganus* fauna.

Estimation of divergence times

In order to explore the timing of colonization of the archipelago and diversification within each island, penalized likelihood (PL) ages were estimated. The chronogram obtained with all nine constraints (AI) is shown in Fig. 3. The ages calculated with the set of analyses sequentially removing each constraint compose the age ranges shown in the same topology as grey bars (AII). The time estimates obtained when each of the nodes was unconstrained are shown as black dots. The results of the analysis with the constraint only for the origin of the *darwini* species group are shown with grey dots also in Fig. 3 (AIII). The inset in Fig. 3 compares ranges of time estimates of both island-specific colonization (C) and within-island divergence (D and D1 and D2 for several independent clades in the same island) with the geological age ranges of those islands when available (O).

There is variation in the age estimations depending on the node constrained in the PL analysis. The prevailing pattern is one of colonization and, in most cases, within-island differentiation soon after the emergence of the island. This is especially evident when comparing the higher geological age estimates with PL estimated ages of colonization and divergence within San Cristóbal and Florena, colonization of Santa Cruz and divergence in Isabela (SC, FL, SR and IS in Fig. 3). All time estimates of within-island diversification must be considered lower bound estimates since further sampling of divergent populations within those same islands could render higher estimates. Despite this caveat, there is a significant correlation between the geological ages of the islands derived from plate movement models and the within island time estimations derived via PL with a single age constraint (AIII: $r = 0.749$, $P = 0.015$).

The time estimate for the origin of the ancestor of all island endemics (marked as *darwini* group in Fig. 3) is between 8.6 and 11.5 million years, predating the geological time estimates for the origin of all extant islands regardless of the constraints applied across the tree. The PL calculated time estimate for potential colonization and the geological estimate of an island's age do not overlap for two of the islands studied: Española and Isabela. In those cases, we might have to invoke the colonization of now sunken seamounts, or of older available islands followed by local extinctions as a prelude to colonization of the soon emerging Española and the significantly younger Isabela.

Ecological/habitat preferences

Proportional likelihoods for ancestral character states under two different optimizations are detailed in Table 4. In general, proportional likelihoods were not significantly different when performing the analysis assigning an unknown character state to *Galapaganus darwini*, except for the increase in the likelihood for character state 2 in node 7 and the undetermined ancestral state for node 8 (see Fig. 4 for both optimizations). Likelihood reconstruction of ancestral ecological preferences suggests a scenario of ancestral generalist preferences (node 1 in Fig. 4 originating the *darwini* group) where larger proportional likelihoods are assigned to the broader ecological preferences (character state 3) in all analyses (Table 4). Subsequent ecological preferences as established by the likelihood reconstruction are for lowlands (character state 0) and mid-elevations (character state 1). Highland habitats (character state 2) only contribute to the proportional likelihoods with the origin of the ancestors of *G. ashlocki* and *G. collaris* in nodes 3 and 7, respectively (Table 4). According to this optimization, preferences for feeding on hosts found in highland habitats appear to have evolved independently in San Cristóbal and Santa Cruz as opposed to species being grouped by habitat affinity (in this case for highland hosts) and later colonizing different islands. In addition, the presence of *G. vandykei* lineages in the lowlands of Española and San Cristobal (albeit unclear due to the paraphyly of the grouping) also indicates the evolution of alternative ecologies to the ancestral lowland state in San Cristobal and Floreana (for *G. collaris* and *G. caroli*, respectively).

Shimodaira–Hasegawa and parametric bootstrapping topology tests of the monophyly of the scored ecological/habitat preferences significantly reject the hypothesis of a single colonization of each of these habitats (Table 3). The scenario favoured by both the ML reconstruction and the topology tests is one where these preferences have evolved, or habitats have been colonized independently, multiple times in the different islands.

Temporal variations in diversification rates

The results of the evaluation of the fit of models of diversification to the patterns derived from island clades are summarized in Table 5. Even though topology tests cannot reject the monophyly of San Cristóbal and Santa Cruz, the clade diversification analysis was performed on each of the two clades proposed by the phylogeny for island. The best fitting model for endemic lineages on the older islands of San Cristóbal, Santa Cruz and Floreana is one of gradual change in rate (model B) with a decreasing diversification rate ($\beta > 1$) since colonization. Despite their smaller sample sizes, a different pattern still emerges from the models best fitting the younger islands (Isabela, Pinta, Darwin and Wolf) encouraging further investigation. Given the paraphyly of the Wolf endemics, both island clades that harbour *G. darwini* (Darwin and Wolf) were analysed together. The diversification rate variation in this young island grouping and the also young island of Pinta best fits a model proposing a drastic shift in diversification rates later in the history of the island (Darwin and Wolf, model C, $P = 0.046$ for shift at 0.3 of the relative time scale; Pinta, model C, $P = 0.036$ for shift at 0.25 of the relative time scale). Only the clade containing endemics of Southern Isabela were used for clade diversification analysis given the smaller sample size of the Northern Isabela clade. The analysis for the Southern Isabela clade also best fits a model displaying a drastic diversification rate shift over a model of rate constancy (model C, $P = 4 * 10^{-9}$ for shift at 0.2 of the relative time scale). However, a model with a gradually increasing rate (model B, $\beta = 0.258$, $P = 0.042$) fits this clade significantly better than a constant rate model for most of the evolutionary history of the sampled *G. williamsi* in Isabela.

Through analysis of the among-clade variation in diversification rate, we compared a model where all islands have the same rate with one where each island is allowed to have a different rate. Through a likelihood-ratio test, the hypothesis of homogeneous diversification rates among islands was rejected (chi 7.895, $P = 0.005$) favouring an alternative where lineages on different islands display different diversification rates.

Discussion

Origin of endemics and colonization sequence

The inferred original colonization of the Galápagos Archipelago by *Galapaganus* based on the mitochondrial DNA sequences presented here can be interpreted in different ways: either as the result of a single colonization event, or, given the divergence times of all endemics and the absence of continental members of the *darwini* group, as the result of multiple colonizations of already divergent continental forms. *Galapaganus* weevils endemic to the archipelago are flightless and fairly heavy bodied (Lanteri 1992; Lanteri 2004). Because of their lack of wings and large body size (5–10 mm), aerial transport would have been unlikely (Peck 1994a). Transport to the Galápagos Archipelago from the Pacific coastal areas of South America may be facilitated by the Humboldt and South Equatorial currents that carry along great quantities of flood debris and pleuston passively transporting terrestrial animals (Finston & Peck 2004). One potential scenario would then suggest that oceanic currents aided in the rafting transport of the ancestor of the *Galapaganus* endemics to the archipelago from Coastal Ecuador and Perú where other flightless members of the *darwini* group and all members of the *femoratus* species group reside (Wright 1983; Peck & Kukalova-Peck 1990; Lopez *et al*. 1992).

The current phylogenetic estimate does not include three members of the *darwini* species group from Puna Island and continental Ecuador and Perú; *Galapaganus lacertosus*, *G.* propinquus and *G. squamosus*. The flightless nature of these continental congeners (Lanteri 2004) also fits a scenario of colonization of the archipelago by rafting of a wingless ancestor after divergence from the winged *femoratus* species group and the continental wingless forms. The alternative scenario of multiple colonizations and establishment in the archipelago of already divergent wingless lineages through rafting seems unlikely; however, only the inclusion of all members of the *darwini* group could refute this possibility. A third potential pattern of back colonization from the islands to the continent by these *darwini* group members after loss of flight in the islands also cannot be disregarded a priori; however, such events are apparently uncommon for isolated oceanic islands. In addition, the direction of the main oceanic currents would not facilitate back colonization of the continent by wingless *Galapaganus* weevils (Wyrtki 1966; Graham 1975). Nonetheless, the Galápagos Archipelago can be considered as a potential source of colonists for the Cocos Islands for some bark beetle groups (Kirkendall & Jordal 2006). However, the overall direction of colonization of both island systems (Cocos and Galápagos) for these proficient flyers has not yet been elucidated and it could follow the main route of aerial overseas dispersal westward from the Americas (Peck 1994a). Affinities to South American groups in Ecuador and Perú for Galápagos biota are not uncommon; they have also been established for Galápagos land reptiles (Bisconti *et al*. 2001, but see Rassmann 1997), for endemic land snails (Parent & Crespi 2006) and for tenebrioid beetles (Finston & Peck 2004).

A stepwise colonization pattern following the progression rule is prevalent in Hawaii and also present in the Canary and South Pacific Islands (Funk & Wagner 1995; Juan *et al*. 2000; Hormiga *et al*. 2003; Spironello & Brooks 2003; Holland & Hadfield 2004). This movement of colonists from older to younger islands has been observed in the Galápagos, at least in part, for bulimulid snails (Parent & Crespi 2006), giant tortoises (Caccone *et al*. 2002; Beheregaray *et al*. 2004) and flightless *Stomion* beetles (Finston & Peck 2004; Finston *et al*. 1997). A previous estimate of *Galapaganus* phylogeny placed *Galapaganus galapagoensis* on the old island of San Cristóbal as basal to the rest of the *darwini* group (Sequeira *et al*. 2000). This is also the pattern in the flightless scarab genus *Neoryctes* (Cook *et al*. 1995) and in the lizard genera *Tropidurus* and *Phyllodactylus* (Wright 1983). The sequence of events implied by the branching order of the current, more comprehensive phylogeny of *Galapaganus* does not clearly display a pattern consistent with the progression rule, mostly because of the basal position of the ancestor of *Galapaganus williamsi*, the endemic to the young island of Isabela not included in the previous study. Even though a simple colonization model with formation of species in the oldest islands (Española or San Cristóbal) and colonization of the nearest island as it emerges cannot be disregarded, our results point towards a more complex pattern of colonization and speciation for *Galapaganus*.

Colonization and divergence times older than the extant emerged islands

Analysis of divergence times derived from our previous phylogenetic estimate using an extrinsic molecular clock illustrated that *Galapaganus* showed molecular divergence in apparent excess of even the oldest geological age estimate of the extant emerged islands (Sequeira *et al*. 2000). With the age estimation method performed in this analysis (PL, Sanderson 2002) again, the age of island endemics and presumably colonization of the archipelago by the ancestor of all island endemics (8.6–12 Myr) exceeds the geological estimates of the extant emerged islands (Cox 1983; Geist *et al*. 1985; Hickman & Lipps 1985; White *et al*. 1993; Geist 1996). This repeated discordance between molecular-based colonization time estimates and geological age estimates of the emerged islands continues to underscore the potential use of then emerged, but now sunken, seamounts located in the vicinity of the Galápagos Archipelago (Christie *et al*. 1992; Werner *et al*. 1999) for the early evolution of groups such as *Galapaganus*. As for geckos and lizards (Wright 1983; Lopez *et al*. 1992), divergence time estimates suggest that the initial colonizations could have occurred on substrates which were above water before the remaining younger islands had emerged. Interestingly, submerged features are not necessary to explain the divergence times of the vast majority of Hawaiian or Canarian taxa since they appear to have colonized existing, emerged high islands (Price & Clague 2002). Potential reasons for differing patterns among biotas inhabiting archipelagos of similar volcanic origins could rely on differences among archipelagos in their island subsidence rates (Ludwig *et al*. 1991; Carracedo 1999), their relative locations of the older now-submerged islands with respect to currents transporting colonists from the continent or their palaeo-climatic conditions.

Most DNA derived estimates colonization times and all estimates of within-island divergence after the initial colonization are in line with the geological ages of the islands. Moreover, the overlap of colonization and divergence estimates derived from molecular data and geological ages and the significant correlation between geological dating (White *et al*. 1993; Geist 1996) and within-island divergence time estimates support a scenario of islands colonized soon after they became available. Despite the need to invoke sunken seamounts for the landing of the older colonists, some of the species divergence is within the age-range of the present islands, as seen for other Galápagos endemics (Grant 1994; Sato *et al*. 2001) and most of the Hawaiian biota (Price & Clague 2002).

Intra-island speciation and island age

There is high concordance between mtDNA clades in the phylogeny estimate and species designations for *Galapaganus* (Lanteri 1992; Lanteri 2004). A comparison of nuclear and mitochondrial-based phylogenies in Hawaiian crickets suggested that interspecific hybridization can be a persistent feature in the evolution of recent groups and has prompted authors to caution against interpretations based solely on mtDNA phylogenies in young species radiations (Shaw 1996, 2002). The high correspondence with morphological species definitions, the large number of individuals and populations included for each species and the level of resolution and support for monophyletic species displayed by the mtDNA phylogeny of *Galapaganus* lead us to believe that interspecies hybridization is not prevalent in this group, contrary to that observed in other island beetles (Jordal *et al*. 2006) and flies (Pestano *et al*. 2003). Interestingly, the exception is a paraphyletic *Galapaganus vandykei* in Española and San Cristobal where the possibility of hybridization with *Galapaganus collaris* in San Cristobal should be investigated further using nuclear markers.

The present phylogeny suggests only one instance of *in-situ* or intra-island origin for *Galapaganus* endemics in the older island of San Cristóbal. However, based on results of monophyly tests, we cannot reject the possibility of a larger contribution of *in-situ* speciation to *Galapaganus* diversity in both San Cristóbal and Santa Cruz, but not in Isabela. Both San Cristóbal and Santa Cruz can be considered older islands when compared to the younger, though equally ecologically complex, Isabela. It is relevant to point out that intra-island speciation can be masked by subsequent successful colonizations of other islands, rendering the original species in the source island a paraphyletic assembly (Emerson 2002; Jordal *et al*. 2006). The importance of intra-island speciation in generating diversity has been made explicit for terrestrial reptiles as related to island area and habitat diversity (Losos & Schluter 2000). A recent comparison of molecular phylogenetic studies for 12 arthropod groups on the Canary Islands revealed that intra-island speciation has had a much greater influence on species diversity on older islands than on younger ones (Emerson & Oromi 2005).

There has not been an equally thorough compilation of speciation modes in Galápagos to date. Within-island speciation has recently been proposed as playing an important role in the generation of diversity of bulimulid land snails (Parent & Crespi 2006); interestingly it appears to be occurring with equal frequency on young and older substrates. It is speciation through between-island dispersal that best explains the patterns found in groups with multiple species per island, as the Darwin finches and other flightless beetles (Finston *et al*. 1997; Sato *et al*. 1999). Our current sampling of *Galapaganus* does not effectively cover all those islands that harbour multiple endemic species. Even though preliminary, it appears that island age, and not island ecological complexity, is influencing speciation modes in *Galapaganus* allowing more time for in-situ speciation in older islands in line with the pattern observed for other arthropods in the Canary Islands (Enghoff & Baez 1993; Emerson & Oromi 2005).

Evolution of habitat preferences

Diversification of ecological affinities is a common feature of taxa that have proliferated within islands (Roderick & Gillespie 1998). Habitat shifts and adaptive shifts have been linked to species formation for cave endemics and *Tetragnatha* spiders in Hawaii (Howarth 1981; Howarth 1993; Gillespie *et al*. 1997; Gillespie 2004). However, species formation is not always associated with change in ecological role, since species with the same preferences have dispersed between islands (Gillespie 2004).

The maximum-likelihood optimization of habitat preferences performed in *Galapaganus* displays multiple shifts towards the more restricted highland preferences in different islands which would suggest that habitat shifts could be potentially linked to species formation. Based on the branching order and PL age estimations, preferences for lowland habitats appear to have evolved before those for moister highland environments. This is also consistent with colonization by rafting of a flightless ancestor, as a winged arrival would have as likely colonized the uplands. Generation of several species that colonized higher altitudes from coastal species has also been the pattern unveiled for the species-rich millipede genus *Dolichoiulus* in the Canary Islands (Enghoff & Baez 1993).

Volcanic activity, island age and temporal variation of diversification rates

Volcanic activity usually results in the burial of surfaces where local populations are continually being exterminated and are required to colonize younger lava flows (Carson *et al*. 1990). Canary island beetles *Brachyderes rugatus* and *Tarphius canariensis* display no apparent differences in pattern or magnitude of diversification rates between lineages on volcanically inactive and active islands (Emerson *et al*. 2000a, c). The authors suggest that the remaining volcanic activity present in even the oldest islands (Hoernle *et al*. 1991) might account for the similarity in rates among younger and older islands. The explanation for the prevailing patterns of a decrease in rate observed across all islands is that following an initial rate reduction, due to colonization by a few founders, diversification of mtDNA haplotypes further decreases because of volcanic activity (Emerson *et al*. 2000a, c).

Younger islands in the Galápagos have displayed significant volcanic activity very recently (Eliasson 1972; Shen *et al*. 1991; Reynolds 1996; Chadwick *et al*. 2006). However, there are scattered reports of some activity for one of the older islands in Galápagos, Floreana (Bow & Geist 1992). Diversification rates as well as patterns of diversification rate differ between *Galapaganus* endemic to the older and younger of the Galápagos Islands. Differing diversification rates, or the variation of such rates between older and younger islands, does not necessarily contradict the pattern of haplotype variation found in Hawaiian land snails (Holland & Cowie 2007), where older islands bear more divergent haplotypes than younger islands. Indeed, in *Galapaganus* average uncorrected sequence divergences calculated over island haplotypes were also generally higher in older than in younger islands.

The most meaningful contrasts in this study are those between lineages on islands with comparable levels of ecological complexity but differing histories of volcanic activity (San Cristóbal, Santa Cruz and Floreana as less volcanically active vs. Isabela and Pinta as more volcanically active). An even more relevant comparison would be that of populations of the same species on islands with contrasting volcanic activity, for example *G. conwayensis* populations on Santa Cruz vs. those on Pinta. The consistent trend of decreasing diversification rate present for lineages on older islands could be due to remnant volcanic activity depleting some of the long existing variation, extending the time and increasing the probability of lineage extinction. Whereas, the increase in diversification rate found for lineages on Isabela and the drastic changes in rate that best fit the patterns for lineages on Pinta, could be assigned to more active and harsher volcanic regimes that are effectively generating lineage extinction and recolonization due to changing barriers to gene flow. Further analyses with larger sample sizes for the younger islands should be performed to confirm this trend. The underlying causes for the pattern found in the younger and less ecologically complex Darwin and Wolf are harder to tease apart since these islands display more complex geological and tectonic scenarios than those suggested for other islands (Harpp & Geist 2002).

Conclusion

There is high concordance between mtDNA clades and species designations in our analyses of *Galapaganus*. Future analysis of patterns derived from nuclear genes will be useful to explore the possibility of interspecies hybridization, especially for the paraphyletic *Galapaganus vandykei* in San Cristobal. The penalized likelihood age estimates for colonization of the archipelago exceed the age of the emerged islands and underscore the potential role of now sunken seamounts for the early evolution of *Galapaganus*. The phylogeny suggests one intra-island origin for *Galapaganus* endemics, but monophyly tests suggest a larger contribution of *in-situ* speciation on older islands. The colonization sequence and timing of colonization derived from our data present a complex scenario where, even though most islands appear to have been colonized soon after their emergence, there are notable deviations from the sequential colonization expected under the progression rule if we consider only the emerged islands. Patterns of diversification rates on older and younger islands correspond to the volcanic activity or remnants of such activity, while the pattern of independent evolution of restricted habitat preferences in different islands suggests that habitat shifts could also have contributed to species diversity in *Galapaganus*.

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