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SEQUENTIAL COLONIZATION AND DIVERSIFICATION OF GALÁPAGOS ENDEMIC LAND SNAIL GENUS *BULIMULUS* (GASTROPODA, STYLOMMATOPHORA)

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Abstract.—Species richness on island or islandlike systems is a function of colonization, within-island speciation, and extinction. Here we evaluate the relative importance of the first two of these processes as a function of the biogeographical and ecological attributes of islands using the Galápagos endemic land snails of the genus *Bulimulus*, the most species-rich radiation of these islands. Species in this clade have colonized almost all major islands and are found in five of the six described vegetation zones. We use molecular phylogenetics (based on COI and ITS1 sequence data) to infer the diversification patterns of extant species of *Bulimulus*, and multiple regression to investigate the causes of variation among islands in species richness. Maximum-likelihood, Bayesian, and maximum-parsimony analyses yield well-resolved trees with similar topologies. The phylogeny obtained supports the progression rule hypothesis, with species found on older emerged islands connecting at deeper nodes. For all but two island species assemblages we find support for only one or two colonization events, indicating that within-island speciation has an important role in the formation of species on these islands. Even though speciation through colonization is not common, island insularity (distance to nearest major island) is a significant predictor of species richness resulting from interisland colonization alone. However, island insularity has no effect on the overall bulimulid species richness per island. Habitat diversity (measured as plant species diversity), island elevation, and island area, all of which are indirect measures of niche space, are strong predictors of overall bulimulid land snail species richness. Island age is also an important independent predictor of overall species richness, with older islands harboring more species than younger islands. Taken together, our results demonstrate that the diversification of Galápagos bulimulid land snails has been driven by a combination of geographic factors (island age, size, and location), which affect colonization patterns, and ecological factors, such as plant species diversity, that foster within-island speciation.

Key words.—Adaptive radiation, colonization, Galápagos, land snails, speciation, species richness.

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The theory of island biogeography as initially proposed by MacArthur and Wilson (1963, 1967) stipulated that variation in rates of interisland colonization and extinction generate and maintain island species diversity. This theory was recently expanded by Losos and Schluter (2000) to include within-island speciation as an additional process increasing species diversity on islands above a certain size. Island species diversity is thus the result of the accumulation of species through time by interisland colonization and within-island speciation, and loss of species via extinction. Various island-biogeographical factors including area, elevation, age, insularity, and habitat diversity might influence the relative importance of these processes in generating overall species diversity (Cowie 1995; Peck et al. 1999; Price 2004). Although it is clear that factors such as island area, elevation, and habitat diversity are all factors promoting species diversity, other factors such as island age have potentially counteracting effects, so that they might promote or reduce species richness depending on what diversifying processes they act upon or their indirect effects.

Island species diversity may be decreased on more-isolated islands because fewer species will be able to successfully colonize more isolated islands (Ricklefs and Bermingham 2004), however island insularity (measured here as the distance to nearest major island) should not affect the rate of within-island speciation. Likewise, island age has the potential to exert a direct positive effect on species diversity because older islands have had more time to accumulate species through within-island speciation and colonization, but it also might reduce species diversity because species on older islands have had more time to go extinct. Furthermore, this negative direct effect of island age on species diversity is indirectly inflated by the fact that volcanic islands also become smaller and lower with time, and island area and elevation are expected to have a positive relationship with species diversity.

The fact that some biogeographical factors presumably have distinct (possibly opposing) direct and indirect effects on species diversity depending on if they affect interisland colonization or within-island speciation has been generally overlooked, and such effects have never been formally evaluated. This is important because species diversity on islands that vary in their biogeographical attributes might be driven by different processes, so that some island assemblages are primarily the result of within-island speciation and others are driven by interisland speciation. In this study, we first evaluate the relative importance of biogeographical and ecological factors in generating overall island species richness. We then use a phylogeny to partition species diversity that presumably strictly results from interisland colonization, and species diversity strictly resulting from within-island speciation, and we evaluate the relative importance of biogeographical and ecological factors in these two different processes. We predict that island area, elevation, and habitat diversity will promote overall island species diversity through their direct and indirect positive effects on interisland colonization and within-island speciation. Furthermore, we predict that more isolated islands will have lower species diversity resulting from between-island colonization, but island insularity should not have an effect on within-island speciation. It is more difficult to predict the direction and magnitude of the overall influence that island age has on overall island species diversity given its opposing effects.

The Galápagos Archipelago stands out among island ecosystems for the study of insular adaptive radiations largely owing to its historical importance in shaping our present understanding of evolution through natural selection (Darwin 1859; Lack 1947; Grant and Grant 2002). A range of organisms has colonized the Galápagos Islands, and many of them have radiated throughout the islands to form unique assemblages of diversified forms (e.g., Grant 1986; Jackson 1993; Rassmann 1997; Finston and Peck 2004). Furthermore, the Galápagos Archipelago is one of the best protected and preserved oceanic island systems in the world, so its fauna and flora are still sufficiently intact that their distributions, ecology, behaviors, and phylogenetic relationships can reliably be inferred.

The geological history of the Galápagos Archipelago is relatively well understood (Nordlie 1973; Swanson et al. 1974; Hall 1983; Cox 1983; White et al. 1993; Geist 1996), with individual islands formed as the Nazca plate moves over a single active hotspot. However, in contrast to the roughly linear arrangement of islands by age in the Hawaiian Archipelago, the Galápagos Islands are clustered into groups of similar ages (White et al. 1993). Española, Santa Fe, and San Cristobal are the oldest islands of the archipelago (2.35–3.90 million years [myr] old); Santa Cruz, Floreana, Pinzon, and Santiago islands form a middle-aged group (0.77–1.52 myr old), and finally the most recent group of islands includes Isabela and Fernandina islands (less than 0.5 myr old), with the volcanically active Fernandina Island presumably located over the hot spot (White et al. 1993; Fig. 1).

Well-studied radiations on Galápagos comprise relatively few species (16 or fewer), which has constrained analyses of the biogeographic and ecological processes involved in species diversification. By contrast, more than 80 endemic species and subspecies of land snails in the genus *Bulimulus* have been described from Galápagos (Broderip 1832; Sowerby 1833; Pfeiffer 1846; Dall 1896; Dall and Ochsner 1928; Vagvolgyi 1977; Coppois 1985; Chambers 1986). The different species vary in shell size, shape, color, and color pattern, and this morphological variation in shell morphology has formed the basis for most of the early systematic studies in the group. Vegetation on Galápagos can be separated into six (or seven) altitudinal zones (Wiggins and Porter 1971; van der Werff 1979), and bulimulid land snails have adapted to all of them except for the littoral (or coastal) zone, which comprises the shoreline and is composed mainly of lava boulders and sandy beaches. The plant-species composition of each zone is a reflection of the humidity level of the zone, with moisture level increasing with elevation (McMullen 1999). The distribution of bulimulid species on Santa Cruz Island follows a distinct zonation related to the vegetation zones, the substrate and the climatic gradient, so that different species predominate at different elevations (Coppois 1984). When more than one species are found in the same area, they usually partition it, and either live in the open on tree trunks and vegetation (some can be found predominantly on specific plants), or live under rocks. Coppois and Glowacki (1983) noted that there appear to be some correlations between the shell shape and the vegetation zone or microhabitat where species are found on Santa Cruz Island, although these associations were not tested statistically. A significant positive correlation between shell shape (degree of shell roundness) and elevation exists for 24 of the species included in the present study (C. E. Parent, unpubl. data), which suggests that snail species have adapted morphologically to the varying moisture levels. These considerations indicate that the variation in shell shape among *Bulimulus* snail species on Galápagos is related to aspects of ecological variation, including vegetation zones, related moisture levels, and microhabitat.

All bulimulid land snails of Galápagos are described as pertaining to the *Naesiotus* group. *Naesiotus* has been previously classified as a synonym or a subgenus of the genus *Bulimulus*, or as a distinct genus; and there is no clear consensus as to its taxonomic rank (Richardson 1995). We follow Smith (1966), Coppois and Glowacki (1983), among others, and adopt the most conservative view treating *Naesiotus* as a subgenus of *Bulimulus*. The entire group *Bulimulus* (*Naesiotus*) includes 162 known species (Breure 1979), and it is defined by unique shell morphology, anatomy, and radula; and a distribution limited to South America, from Venezuela to Argentina (mostly in the Andean region) and covering the southern half of Brazil (Breure 1979). Based on this distribution and morphological resemblance, the most plausible source for the species on the Galápagos is from South America (Dall and Ochsner 1928; Smith 1966). Monophyly of the Galápagos bulimulid group has never been formally tested. Of all the groups of organisms that colonized the Galápagos, lava lizards is the only group for which there is evidence for more than one colonization event. This is not too surprising because the Galápagos are located about 700 km off the South American Coast. Given that no other reptile, bird, invertebrate, plant, or other group of organisms has been showed to be the result of multiple colonization events, and pending the collection of appropriate data, we assume that the same is true for Galápagos bulimulid snails.

In this paper we generated molecular-systematic data to reconstruct a phylogeny for the bulimulid land snails of the Galápagos. We then used the phylogeny to infer the spatial and temporal patterns of colonization and speciation on the archipelago, and to test alternative hypotheses for the geographic pattern of diversification and the role of history in the evolution of Galápagos *Bulimulus* species diversity. Next, we used data on numbers of extant and extinct species on each island to test for associations between overall species diversity on each island and five putatively causal factors: (1) island age, inferred from geological data; (2) island habitat diversity measured as the number of native plant species per island; (3) maximum island elevation; (4) island area (measured as island area not covered by barren lava flow); and (5) island insularity (distance to the nearest older major island). We tested specifically for island monophyly of island assemblages, and infer the number of species on each island that are the result of interisland colonization versus within-island speciation based on our molecular phylogeny. We then investigated the relative contribution of the biogeographical

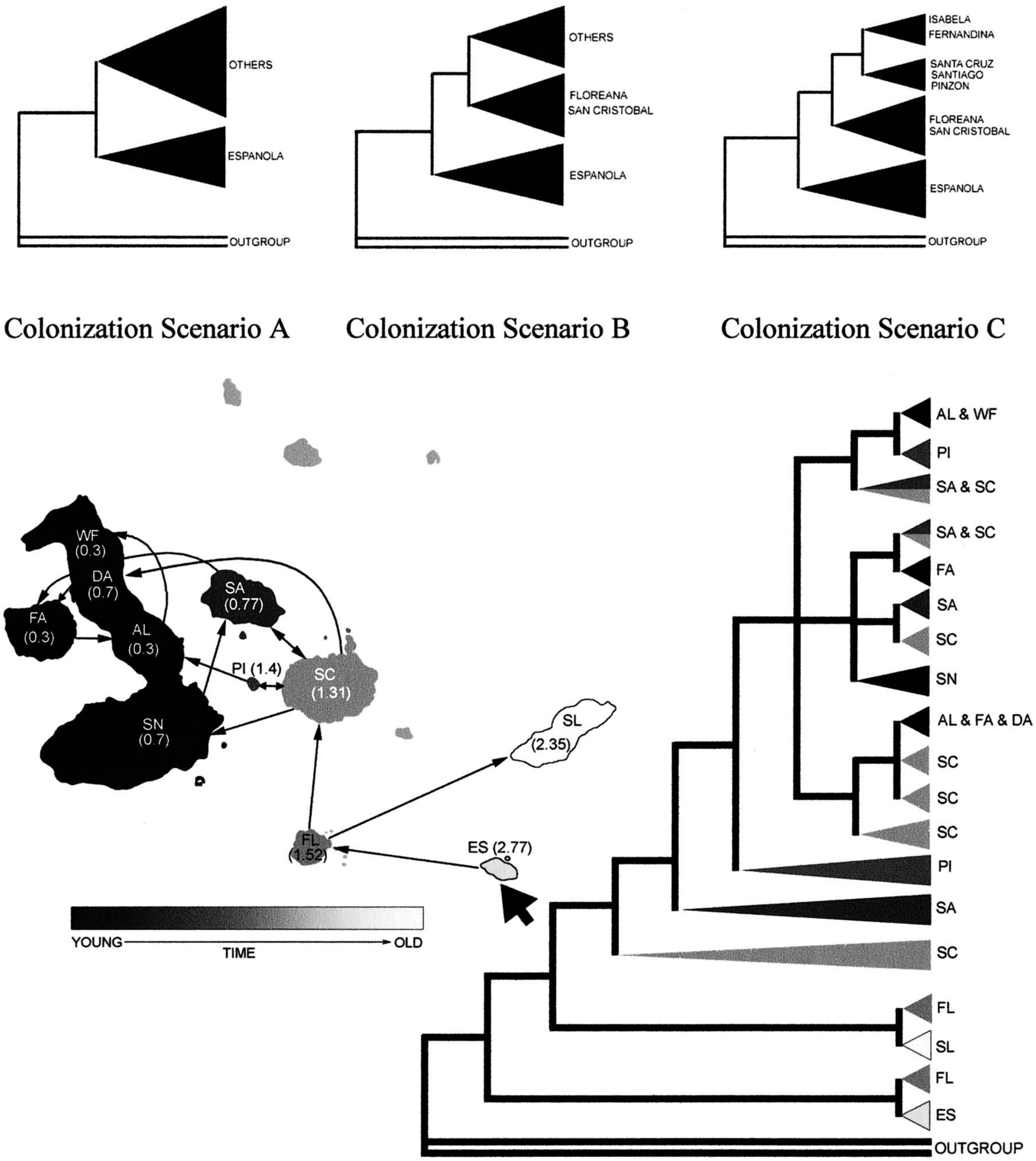

FIG. 1. Schematic of proposed colonization sequence of Galápagos bulimulid land snails. Española Island, one of the oldest islands of the archipelago, is hypothesized to be the first island to be colonized from a mainland ancestor. The arrows represent colonization events within Galápagos. Island abbreviations are used as follows: AL, Alcedo; SN, Sierra Negra; DA, Darwin; WF, Wolf; SA, Santiago; SC, Santa Cruz; PI, Pinzon; SL, San Cristobal; ES, Española; FL, Floreana. Approximate age (in million of years) is given in parentheses for each island or volcano. The colonization sequence roughly parallels the order of the geological formation of the Galápagos Islands. This sequence is tested against the order of formation of the islands (derived from geological dating) using Shimodaira and Hasegawa (1999) likelihood ratio tests. Three successively more constrained colonization scenarios (represented as A, B, and C; see text) are tested against the unconstrained (best) maximum-likelihood tree.

and ecological factors to each of these two diversifying processes. We found a role for island area and insularity in determining species diversity inferred to be due to colonization; thus, the number of species on each island may be constrained or directed by numbers of successful colonization events (Brown and Lomolino 1998). Effects of ecology may also influence local species diversity, both directly if diversification rates within-island are functionally linked to habitat diversity (Gillespie 2004) or indirectly if influenced by interspecific interactions such as competition. Island size may also affect local diversity; at least to the extent that it influences extinction rates (MacArthur and Wilson 1963, 1967) and the likelihood of within-island speciation (Losos and Schluter 2000).

By partitioning species diversity according to the processes that are presumably responsible for their formation, we provide evidence that different biogeographical factors have distinct relative importance depending on their effects on these processes. We believe that a detailed consideration of the biogeographical factors and their varying effects on within-island and between-island speciation is crucial to reach a better understanding of the formation and maintenance of species diversity in island systems in general.

MATERIAL AND METHODS

Sampling

Snail specimens were sampled from as many different locations as possible throughout all major islands on the archipelago that are known to harbor extant populations of *Bulimulus* snails between September 2000 and April 2005. Of the 71 described Galápagos bulimulid land snail species (Coppois 1985; Chambers 1991), 26 described species (including two likely extinct species) and nine possibly new species (currently being described) were included in our study. We were unable to find live specimens for additional species despite intensive fieldwork focused near the type localities (when available) of these species and other apparently suitable sites. These missing species are probably extinct or extremely rare (Coppois and Wells 1987; Parent 2004), thus despite being incomplete, we believe that our sampling is likely the best possible. A detailed list of the collection sites with precise geographical location for all the taxa included in this study is presented in Appendix 1 available online only at: http://dx.doi.org/10.1554/06-366.1.s1.

A small sample of snail foot tissue was taken from each specimen for preservation in 20% DMSO/saturated NaCl, and specimens were preserved in 75% ethanol. Specimens of *B. cavagnaroi* and *B. blombergi* (presumably extinct today) were kindly provided by Guy Coppois; these specimens were collected on Santa Cruz Island in the 1970s. The specimens used as outgroup (*B. quitensis*) were collected in November 2000 in the Ecuadorian Andes, about 5 km south of the city of Guayllabamba. Sampling of foot tissue and preservation of the outgroup specimens were performed as described above. Except for *B. cavagnaroi* and *B. blombergi*, voucher specimens have been deposited at the terrestrial invertebrate museum of the Charles Darwin Research Station, as well as at the Museum of Comparative Zoology, Harvard University.

DNA Preparation, Amplification, and Sequencing

Total genomic DNA was isolated from 87 individual land snails following DNA extraction protocol for molluscan tissues described by Sokolov (2000). For the *B. cavagnaroi* and *B. blombergi* specimens, we followed a CTAB/phenol/chloroform extraction protocol (Grosberg et al. 1996). A small portion (about 0.01 gram) of the foot tissue of each specimen was used for DNA extraction. Polymerase chain reaction (PCR) was performed using the mitochondrial cytochrome oxydase subunit 1 (COI) primers 1718 and 2191 (Simon et al. 1994), and the combinations of internal transcribed spacer region 1 (ITS1) primers SN-18S-F11 (5′-TTCCGTAGTGA ACCTGCGG-3′) or SN-18S-F18 (5′-TAACAAGGTTTCC GTATGTGAA-3′) with SN-5.8S-R28 (5′-GCGTTCTTCAT CGATGC-3′) or SN-5.8S-R87 (5′-GTGCGTTCGAAATGT CGATGTTCAA-3′) designed for this study. Polymerase chain reaction product was processed using exonuclease I and shrimp alkaline phosphatase, and Big Dye Cycle Sequencing (Applied Biosystems, Foster City, CA) was used to sequence fragments about 500 bp and 725 bp long for COI and ITS1, respectively (see Appendix 1 for Genbank accession numbers). Fifteen of the taxa were sequenced in both directions for the ITS1 marker to ascertain that the fragments amplified are single copies (see Vollmer and Palumbi 2004).

Phylogenetic Analyses

DNA sequences were aligned by eye in Se-Al (Rambaut 1996), and also checked using Clustal X (Thompson et al. 1997). Ambiguous sequence alignment positions of the ITS1 sequences (first 55 bp and last 240 bp) were not included in subsequent analysis. We partitioned the sequence data into mitochondrial and nuclear subsets. Phylogenetic analyses were performed using maximum parsimony (MP) and maximum likelihood (ML) in PAUP* (Swofford 2002) and Bayesian analysis in MrBayes (Huelsenbeck and Ronquist 2001). For each inference method (MP, ML, and Bayesian), mitochondrial and nuclear sequence datasets were analyzed both separately, and combined. We employed the incongruence length (ILD) test as implemented in PAUP* (using TBR, 1000 replicates) (Huelsenbeck and Bull 1996; Swofford 2002), to help evaluate the congruence of the trees inferred from the two different genetic markers under MP. We ran the ILD test on a reduced dataset of 20 taxa including representatives from all the islands because of the prohibitive computing power required to include the complete dataset. For the MP analyses, searches were performed using heuristic search methods. A starting tree was obtained by stepwise random addition of sequences with 10 trees held per addition. Optimization was performed by TBR branch-swapping, equal weight was assigned to each substitution, and gaps were treated as 5th character. Gaps that spanned more than one base pair position were recoded as one character for the whole gap rather than one character for each base pair position deleted. Support for branches under parsimony was assessed by bootstrap analyses with 500 replicates.

Prior to ML phylogenetic reconstructions, the program MODELTEST (Posada and Crandall 1998) was used to determine the appropriate model of sequence evolution for the COI and the ITS1 datasets. The optimal models defined by MODELTEST (TVM + I + G for COI and HKY for ITS1) were then used in subsequent phylogenetic reconstructions. Maximum-likelihood analyses were carried out using the empirically determined Ti/Tv ratio for ITS1 sequence data (Ti/Tv = 1.6098) and six-parameter instantaneous rate matrix estimated using ML for COI sequence data (0.6707, 12.3635, 1.5485, 2.1877, 12.3635). Among-site rates were assumed to be equal for ITS1 and to follow a gamma distribution (γ = 0.9599) for COI. Again, support for branches was assessed by bootstrap analyses (200 replicates). We used Metropolis-Hastings Coupled Markov Chain Monte Carlo (MCMCMC) methods within a Bayesian framework to estimate the posterior probability of phylogenetic trees as implemented in the program MrBayes 3.0vb4 (Huelsenbeck and Ronquist 2001). Bayesian analysis was performed on the combined and separate COI and ITS1 datasets using the general time reversible (GTR) model of sequence evolution, combined with gamma rate heterogeneity and rate variation partitioned by gene and by codon positions. The program was run for 10 million generations with four chains running simultaneously, three heated at the default temperature and one cold, and a sampling frequency of 100 generations. We checked that chains had converged and that the log likelihood was stationary, and a 50% majority-rule consensus tree was constructed from the resulting trees using PAUP*. All phylogenetic trees were rooted on *B. quitensis* from mainland Ecuador and *Rhabdotus alternatus* (Bulimulidae; partial COI sequence obtained from Genbank, accession number AY148561).

Test of Island Assemblage Monophyly

Isabela Island is formed by six major volcanoes that are separated by extensive barren lava flows. Bulimulid land snails cannot survive without a minimum of vegetation for food and shelter, and thus they are not found at low elevations between the volcanoes forming Isabela Island. Therefore, each volcano can be considered as a separate island as regards bulimulid land snail distribution.

To test for monophyly of the different island assemblages, we considered bootstrap support and the posterior probabilities from the Bayesian MCMCMC searches (Lewis 2001), as well as two hypothesis-specific tests. In the first, likelihood-ratio tests (LRTs) were performed comparing the unconstrained ML tree with trees that were constrained to be monophyletic for each island clade (one-tailed SH log-likelihood test as implemented in PAUP* (Shimodaira and Hasegawa 1999). Parametric bootstrapping was also used to evaluate hypotheses of island assemblage monophyly when classical (nonparametric) bootstrap values indicated weakly supported monophyletic island clades. Parametric bootstrapping provides the opportunity for testing a priori hypotheses about the phylogeny of a group (Hillis and Bull 1993). Even if a tree has weakly supported branches by nonparametric bootstrap support values, the overall tree structure, through the cumulative effects of many small branches, may contain enough phylogenetic signal to reject a null hypothesis (Hillis et al. 1996). Huelsenbeck et al. (1996) suggest a ML approach for obtaining score estimates, but we have followed the MP approach outlined by Ruedi and colleagues (Ruedi et al. 1998) owing to prohibitive computation time of ML searches. For a given hypothesis of island monophyly, a ML search in PAUP* was performed to find the best tree under the constraint of island monophyly. Seq-Gen version 1.2.5 (Rambaut and Grassly 1997) was then used to simulate DNA sequences along each constrained phylogeny corresponding to each hypothesis of island monophyly. Sequences were generated under the defined model of substitution process obtained with MODELTEST from the sequence data. One hundred new datasets were thus simulated for each of the constrained topologies. For each dataset thus obtained, heuristic searches under MP were carried out first with, and then without, the specified constraints. The resulting distribution of tree score differences was then compared with the tree length differences for the empirical constrained and nonconstrained trees obtained by MP.

Colonization Sequence Analysis

The colonization sequence and relative timing of diversification of the bulimulid land snails was inferred based on the phylogenetic tree topology and geographical setting following methods in Thorpe et al. (1994). By this approach, sister taxa are joined on a geographical map based on their phylogenetic and geographical proximity. Moving down the tree (toward the root), pairs of sister taxa are joined on the map by the shortest geographical distance. The nodes joining these pairs are then allocated a geographical locality based on the geographical proximity between the location of the population from which they are derived and from the geographically closest member of the pair. The colonization sequence is thus obtained by joining the node and population localities on a map, with the direction of colonization being away from the root of the tree toward its terminal branches.

Because this method is obviously sensitive to the degree of taxon sampling and the topology of the tree, we used it only to infer the overall colonization direction in this group. We then used this general colonization sequence to propose more specific and alternatively more constrained colonization scenarios to test against the order of geological formation of the islands using Shimodaira-Hasegawa (SH) likelihood ratio tests (Shimodaira and Hasegawa 1999). The geological ages of the islands were obtained from Cox (1983), White et al. (1993), and Geist (1996). Because island age is determined by K-Ar dating of lava rock, different rocks on a same island can yield different ages. If more than one estimate is available for a given island, we assumed that the oldest age estimate corresponds more closely to the actual age of this island. Three successively more constrained colonization scenarios (presented in Fig. 1) were tested using SH LRTs against the best (unconstrained) ML tree. In scenario A, we constrained the first divergence event to be between the snail assemblage found on Española Island, the oldest island, and the rest of the ingroup. In scenario B, the Española group was still constrained to split from the deepest ingroup node, and Floreana and San Cristobal lineages were constrained to split from the rest of the ingroup next. Finally, the colonization sequence represented by scenario C constrained snail assemblages found on Fernandina and Isabela islands, the geologically youngest islands, to split from snail assemblages of Santa Cruz, Santiago, and Pinzon islands on the most shallow part of the phylogeny, while keeping Española, and San Cristobal and Floreana snail assemblages to sequentially split from deeper nodes as in scenarios A and B. The rationale for grouping Santa Cruz, Santiago, and Pinzon islands together in scenario C is that age estimates for these three islands are partly overlapping.

To further evaluate the general colonization sequence from old to young islands, we regressed the phylogenetic depth of island clades against the age of the island where the clade is found, where clade depth is the number of nodes between the ingroup node and the root of the tree. We used the average depth for each island assemblage that comprised multiple clades, such that each island represented an independent data point. We predicted that island clades that are found on younger islands should be farther from the root than those found on older islands, since they diversified more recently.

TABLE 1. Predictors of land snail species richness and the description of their predicted direct and indirect effects. The process(es) of species formation or maintenance each predictor is affecting is indicated in parentheses (S, within-island speciation; C, interisland colonization; E, extinction), along with the direction of the predicted relationship with an increase in the value of the predictor (+ for positive; − for negative).

Species richness predictor	Description	
	Direct effect(s)	Indirect effect(s)
Island area	larger target for colonists (C+) increased opportunities for isolation by distance (S+) reduced extinction risk by potentially sustaining larger population size (E−)	increased habitat diversity (S+, C+)
Island elevation	better target for colonists (C+)	increased habitat diversity (S+, C+)
Island habitat diversity	increased potential for adaptation (S+) increased probability that colonists will find suitable habitat (C+)	none
Island age	more time to be colonized (C+)[§] more time to speciate (S+)[§] more time to go extinct (E+)	increased habitat diversity (S+, C+) decreased island elevation and area due to erosion and subsidence (S−, C−, E+)
Island insularity	target harder to reach for colonists (C−)	decreased habitat diversity because of lower colonisation rate by plants (S−, C−)

[§] This effect is likely to become less significant as niches on islands are filled by colonists and newly formed species.

Biogeographical and Ecological Correlates of Species Diversity

We tested for the relative importance of five biogeographical and ecological factors on (1) overall island species diversity, (2) species diversity strictly resulting from within-island speciation, and (3) species diversity resulting from between-island colonization. Data on the distributions of extant and extinct bulimulid land snail species by islands were obtained from recent field work as well as from other sources (Dall 1896; Dall and Ochsner 1928; Odhner 1951; Smith 1972, 1974; Vagvolgyi 1977; Coppois 1985; Chambers 1986, 1991). The total number of bulimulid land snail species per island (extant and extinct) was analyzed in relation to island area, maximum elevation, age, insularity (measured as distance from the nearest older major island), and the number of native plant species which was used as a surrogate for habitat diversity. Most land snails use plants as food and shelter (Boycott 1934), so increased plant diversity is expected to provide higher habitat diversity for land snails, which may allow more species to coexist. Maximum elevation was obtained from Wiggins and Porter (1971), and Alan Tye (department of Botany, Charles Darwin Foundation, Galápagos) provided the most recent data available on number of native and endemic plant species per island. Island area was measured as the area of an island that was not covered with barren lava flows, which was estimated from NASA satellite images of the archipelago imported into the image processing software ImageJ 1.30v. Island insularity, the distance from the nearest older island, was obtained from Snell et al. (1996), and estimated directly from satellite images for each volcano forming Isabela Island in ImageJ 1.30v.

Some of the biogeographical and ecological variables included were intercorrelated, making multiple regression analysis problematic (Connor and Simberloff 1978; Kleinbaum et al. 1988; Rosenzweig 1995). Thus, investigative path analysis was used to explore the relative contribution of each variable to variation in species numbers (see, e.g., Cowie 1995). The program Piste (Vaudor 2000) was used to estimate the magnitude and significance level of the direct effects, and we estimated the magnitude and tested the significance of the indirect effects in the model with a bootstrap approach (10,000 resamples) as described by Preacher and Hayes (2004). Each variable was assumed to have a direct causal effect on bulimulid land snail species numbers, and some variables were also considered to have indirect effects (Table 1).

All variables were log-transformed to meet the assumptions of parametric statistics. The significance levels of the direct and indirect effects of each predictor on the total number of species on each island might be difficult to assess in path analysis, because significance tests particularly for indirect coefficients are difficult to interpret (Cohen and Cohen 1983; Lewinsohn 1991). Thus, a multiple regression analysis, for which significance tests are better understood, was run to assess the significance level of a simplified model which retained the main direct effects from the path analysis yet minimized inter-correlation among the predictor variables. Multiple regression analysis was first run on a dataset including all islands for which data were available ($n = 26$).

We then evaluated the individual role that each of the biogeographical factors (island area, age, elevation, insularity, and habitat diversity) has had on both speciation within islands and speciation due to colonization between islands alternatively by assigning each species an inferred mode of speciation: either species have arisen purely through isolation as a result of colonization of one island from another (between-island speciation), or the species originated within a given island (within-island speciation). We used the best ML phylogenetic tree based on the combined datasets to determine the number of within- and between-island speciation events. We followed Losos and Schluter (2000) and assumed that the presence of sister taxa on an island resulted from within-island speciation, and we considered that the alternative scenario of an island being colonized multiple times accompanied by extinction of the source species on other islands is far less likely. Because we need a phylogenetic hypothesis to infer the presumed process of speciation of species, we included only species that are represented in our phylogeny for this part of the analysis. We also included species that are sole inhabitant of islands, because they had to arise by between-island colonization. However, since we could not infer the number of within-island speciation or between-island colonization events for islands with more than one species that had no phylogenetic information, we ran this part of the analysis with a reduced dataset ($n = 14$ islands). To take into account the uncertainty in estimating the number of species resulting from within-island speciation and interisland colonization due to the uncertainty of the phylogeny, we calculated the number of within-island speciation and interisland colonization events for each of the 200 best trees (with highest log likelihood scores) obtained from the Bayesian search. We then used these 200 datasets to run multiple regression analyses of species diversity due to within- and between-island speciation alternatively as described above.

RESULTS

Sequence Variation

A total of 451 bp were sequenced for the COI gene, and the nuclear ITS1 sequence was about 413 bp in length (after the exclusion of ambiguous alignment, see Methods), varying with the length of the indel. The COI dataset comprised 74 different haplotypes, whereas 29 different haplotypes were found for ITS1. All of the 15 ITS1 sequences that were obtained in both directions to ascertain that they were single copies were found to be identical. Levels of sequence variation and the number of phylogenetically informative sites varied substantially between both gene fragments. Thirty six percent (167 sites) of COI nucleotides were variable, of which 80.2% was variation at the 3rd codon position. Overall 91.0% (152 sites) of the variable COI nucleotide sites were parsimony informative. ITS1 displayed less variation with only 17.4% of nucleotide sites (74 sites) variable, and of these 60.8% (45 nucleotide sites) were parsimony informative. Across all sites adenine-thymine richness was 41.3% for ITS1 and 68.3% for COI.

Within the ingroup taxa, the maximum divergence was 7.6% for ITS1 and 15.9% for COI, and the divergences of ingroup taxa from outgroup ranged from 7.2 to 12.5% and 17.4 to 24.5% for ITS1 and COI, respectively.

Phylogenetic Analyses

The best maximum-likelihood (ML) tree based on the COI dataset was considerably better resolved and supported than the best ML tree based on the ITS1 dataset (Fig. 2A, B). The ITS1 tree presents support for two main clades: one including species from the Southeast located islands of Española, San Cristobal, and Floreana, and the other including species from the remaining islands of the archipelago. There is support for the grouping of the San Cristobal assemblage with *B. unifasciatus* from Floreana Island in both the COI tree and the combined tree, and *B. snodgrassi* from Española Island is a sister taxon to the *B. nux* species group from Floreana Island on the COI tree and the combined tree.

The bulimulid assemblage of Santa Cruz Island is composed of five independent lineages according to the COI and the combined dataset trees, and the Santiago Island land snail assemblage is divided into four independent lineages. Four of Santa Cruz lineages are sister clades to Santiago lineages on the COI tree, and this holds true for three of them on the combined dataset tree. The bulimulid assemblages on Pinzon and on Fernandina islands, as well as the bulimulids on Alcedo volcano on Isabela Island are each formed by at least two independent clades.

The phylogenetic results suggest a few problems with the taxonomy of Galápagos bulimulids. For example, *B. perrus* of Fernandina form clearly two independent lineages. Similarly, *B. nux* from Floreana Island does not group with *B. nux* of San Cristobal Island, and neither does *B. snodgrassi* from San Cristobal Island and *B. snodgrassi* from Española Island. When determining the number of species on a given island, we used the number independent clades such that *B. snodgrassi* was counted as one species on Española Island and as another species on San Cristobal Island. *Bulimulus perrus* was counted as two separate species (denoted *B. perrus* 1 and *B. perrus* 2 on Fig. 2) on Fernandina Island. Some of the younger island assemblages comprise species that have very similar haplotypes (for example *B. spp.* 1–5 on Alcedo). This is not too surprising given that these species are likely to be very young because Alcedo experienced an explosive eruption that certainly destroyed most of the snail populations about 100,000 years ago (Geist et al. 1994). The fact that a few species seem to share similar or same haplotypes might also be due to recent recurrent gene flow, although more data will be needed to test this idea.

The ITS1 and COI datasets are not significantly different based on the results of the ILD test ($P > 0.05$) on a reduced dataset of 20 taxa including representatives of all the islands. Furthermore, the best ML ITS1 and COI trees do not have well supported nodes that are conflicting (Fig. 2A, B), so we used the best ML tree resulting from the combined datasets in the rest of our analysis.

Tests of Island Monophyly

Shimodaira-Hasegawa tests.—Maximum-likelihood and maximum-parsimony bootstrap values of the phylogenetic hypotheses built using the combined or the partitioned datasets strongly support monophyly for Darwin (one species), Wolf (one species) and Sierra Negra (two species) volcano-clades (on Isabela Island), as well as Española Island (one species). Island clade monophyly was tested using SH likelihood ratio test for the remaining island clades for which ML and MP bootstrap values did not (strongly) support monophyly (Fig. 2, Table 2). For Santa Cruz and Santiago islands, the unconstrained (best) tree was significantly better than the monophyletic island clade constrained trees. Indeed, the snail assemblages on Santa Cruz and Santiago islands appear to be the result of at least four independent colonization events. The likelihood value of the constrained tree for Floreana Island assemblage monophyly was also significantly worse than the unconstrained (best) tree: the snail assemblage on Floreana Island seems to have been the result of at least two colonization events.

Shimodaira-Hasegawa tests do not provide any support for

TABLE 2. Summary of tests of island monophyly. Support for the monophyly hypothesis of each island assemblage is provided: ML, maximum likelihood bootstrap support; MP, maximum parsimony bootstrap support; MCMCMC, Bayesian posterior probability; PB, significance level for the parametric bootstrap test; SH test, significance level for the Shimodaira Hasegawa test as implemented in PAUP*. A significant result for the PB or SH tests implies that the hypothesis of monophyly can be rejected. The island clades for which there is good support for monophyly are indicated with *. na, not applicable; ns, nonsignificant.

Island or volcano	ML	MP	MCMCMC	PB test	SH test
San Cristobal (SL)*	<50	67	79	ns	na
Española (ES)*	100	100	100	na	na
Floreana (FL)	na	na	na	$P < 0.02$	$P < 0.05$
Santa Cruz (SC)	na	na	na	$P < 0.01$	$P < 0.05$
Pinzon (PI)	na	na	na	$P < 0.01$	$P < 0.05$
Santiago (SA)	na	na	na	$P < 0.01$	$P < 0.05$
Wolf (WF)*	100	100	100	na	na
Darwin (DA)*	<50	88	94	na	na
Alcedo (AL)	na	na	na	$P < 0.01$	$P < 0.05$
Sierra Negra (SN)*	82	95	100	na	na
Fernandina (FA)	na	na	na	$P < 0.01$	$P < 0.05$

the monophyly hypothesis for the other volcanoes and islands (Table 2).

Parametric bootstrap.—The constrained trees for island clade monophyly were 4, 6, 7, 13, 7, and 11 steps longer (for Santiago, Santa Cruz, Pinzon, Floreana, Alcedo and Fernandina clades respectively) than the shortest unconstrained tree. The shortest tree obtained under the constraint of San Cristobal clade monophyly is no longer than the shortest unconstrained tree. The differences between Santiago, Santa Cruz, Pinzon, Floreana, Alcedo, and Fernandina clade monophyly hypotheses and the alternative nonmonophyletic hypothesis can be evaluated against parametric bootstrap simulations of the null hypotheses of monophyly for the different clades.

The largest observed difference between the null and alternative hypotheses generated from the simulated data was 3 steps for Santiago Island, 2 steps for Santa Cruz and Alcedo clades, and 5 steps for Fernandina assemblage. The probability of observing a difference of 4, 6, 7, and 11 steps for Santiago, Santa Cruz, Alcedo, and Fernandina clades respectively ($P < 0.01$) permits us to reject the null hypothesis of monophyly for these clades. The largest difference between the observed difference between the null and alternative hypotheses generated from the simulated data for Floreana Island was 16 steps, and 98% of the parametric bootstraps reached a difference of 12 or less. Therefore, the probability of observing a difference of 13 steps due to some stochastic errors along the branches is low enough ($P < 0.02$) to reject the null hypothesis of Floreana clade monophyly. Likewise, in the expected difference distribution under the null hypothesis of Pinzon clade monophyly, up to 99% of the parametric bootstraps reached a difference of less than 7 steps, so that the hypothesis of monophyly of this clade can be rejected ($P < 0.01$). Finally, no difference in step length was found between the constrained tree for San Cristobal clade monophyly and the unconstrained tree, so that the null hypothesis of monophyly cannot be rejected for that island (Table 2).

The posteriori probability of monophyly for the bulimulid assemblages of Wolf and Sierra Negra volcanoes on Isabela Island, as well as for the assemblage of Española Island is 100% for the combined dataset. The probability of monophyly of Darwin volcano on Isabela Island is 94%, and is of 75% for San Cristobal bulimulid assemblage. The probability of monophyly for Alcedo volcano on Isabela, Fernandina, Santiago, Santa Cruz, and Pinzon islands is 0%. These results support the results of the SH and parametric tests (Table 2).

Colonization Sequence

We combined information about the geographical setting of individual Galápagos islands with the topology of the best ML tree based on the combined COI and ITS1 dataset to infer a hypothetical colonization sequence of Galápagos bulimulid land snails (Fig. 1). Although this scenario is certainly not the only possible one, and it does not take into account the uncertainty of the tree topology, it should represent the most probable scenario based on the combined phylogenetic and geographical information that is now available.

SH tests.—We evaluated the overall order of colonization from old to young islands by testing the best ML tree obtained from the combined COI and ITS1 datasets against tree topologies reflecting progressively more constrained colonization scenarios (scenarios A, B, and C, Fig. 1). Results of the SH test indicate that the best ML tree obtained among trees that were compatible with hypothesis A was not significantly worse than the unconstrained (best) ML tree ($-\ln L$ difference = 3.07, $P > 0.05$). For colonization scenario B, which constrains the deepest node to the splitting of Española lineage and the rest of the ingroup followed by a split of the ingroup into species found on San Cristobal and Floreana islands and the remaining of the ingroup, the constrained tree was significantly worse than the unconstrained (best) tree ($-\ln L$ difference = 109.42, $P < 0.01$), suggesting that this colonization hypothesis can be rejected. Given that the best tree constrained to followed colonization scenario B was worse than the best unconstrained tree, we did not test the significance level of, and rejected the more constrained scenario C. We investigated the colonization sequence further by testing a colonization scenario in which San Cristobal lineage was constrained to split from the ingroup at the deepest node, and an alternative scenario in which Floreana lineage was constrained to split from the rest of the ingroup at the deepest node (scenarios not shown). The best tree obtained among trees compatible with the former scenario was not significantly worse than the best unconstrained tree ($-\ln L$ difference = 0.15. $P > 0.05$); however, the latter scenario constraining the deepest node to split Floreana and the rest of the ingroup resulted in a best constrained tree that was significantly worse than the best unconstrained tree ($-\ln L$ difference = 24.28, $P < 0.05$). Finally, a scenario in which the deepest split was between San Cristobal lineage and the rest of the ingroup, which was then followed by a split between a group including Floreana and Española lineages versus the remaining of lineages (scenario not shown) was supported by a best constrained tree not significantly worse than the best unconstrained tree ($-\ln L$ difference = 6.49, $P > 0.05$). All other more constrained

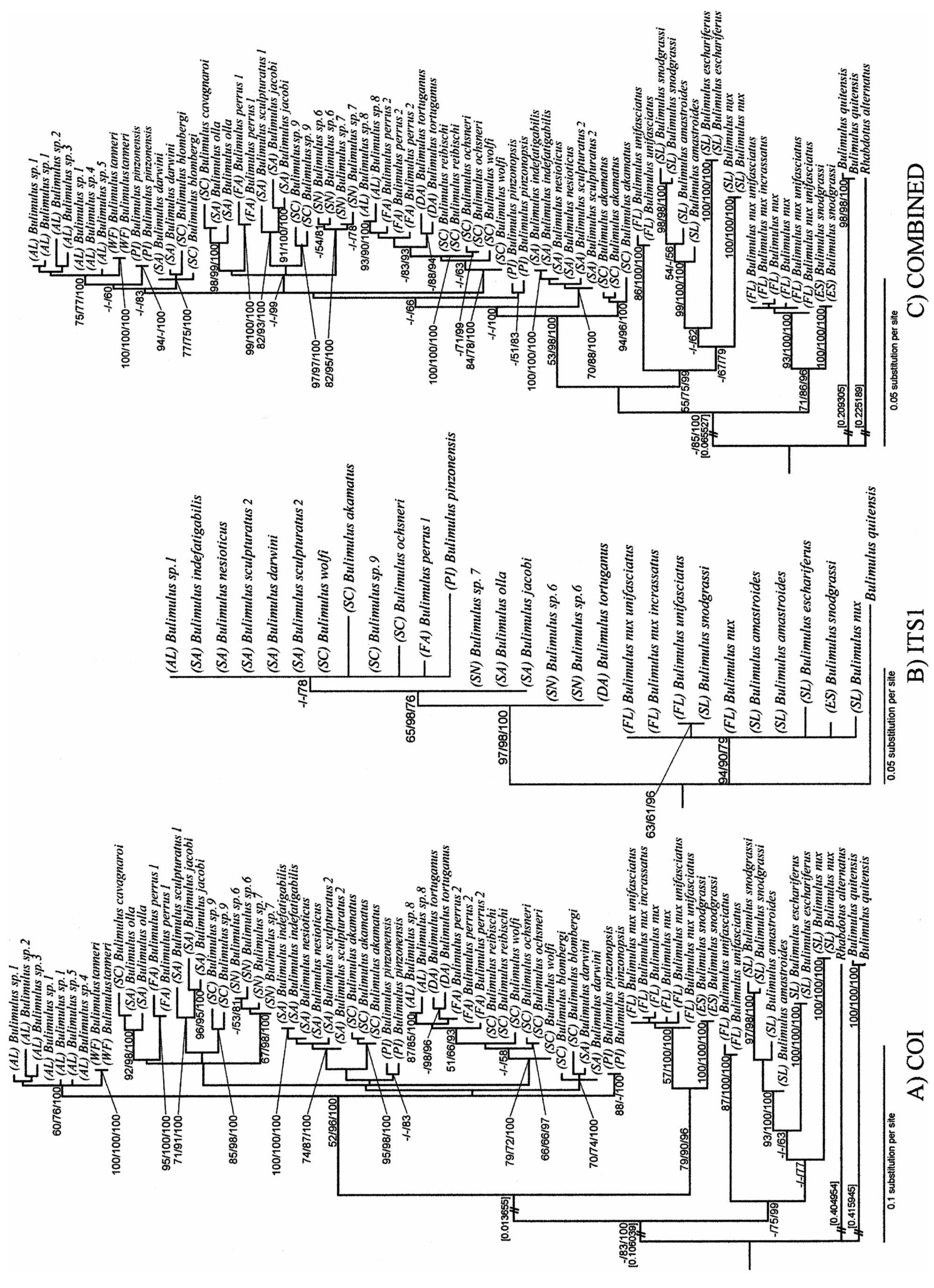

FIG. 2. Best maximum-likelihood trees based on the (A) mtDNA COI dataset, (B) ITS1, and (C) combined dataset for 35 species of Galápagos bulimulid land snails and two outgroups. The tips of the tree are labeled with both the species designation and the island or volcano where it comes from, island abbreviations are the same as in Figure 1. Branch lengths are proportional to the number of substitutions per site. Some very long branches were shortened for ease of presentation, and their actual branch lengths have been bracketed and reported above them. Bootstrap values for maximum-likelihood (200 replicates), maximum-parsimony (500 replicates), and Bayesian posterior probability are reported for well-supported branches (see Methods).

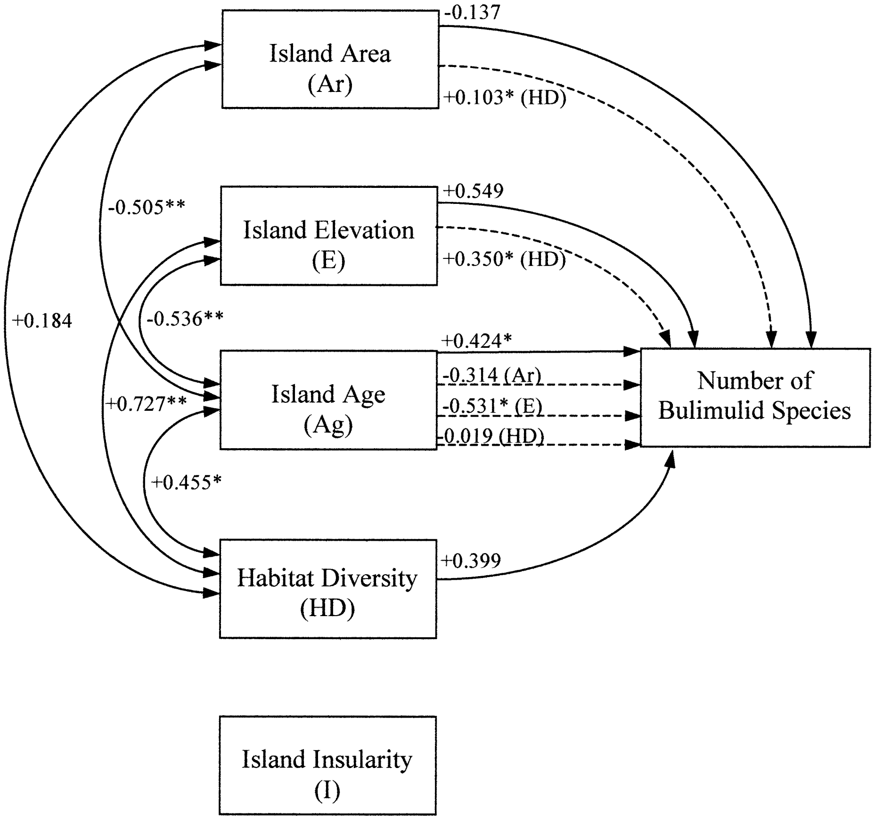

FIG. 3. Path diagram of biogeographical and ecological predictors of bulimulid land snail diversity per island on Galápagos. All effects in model are shown as arrows; continuous lines indicate direct effects, and dotted lines indicate indirect effects. Coefficient values are provided, and significant relationships are indicated (*$P < 0.05$, **$P < 0.01$). The predictor causing the indirect effect is indicated by its abbreviation in parentheses after the indirect coefficient. Island insularity was left out of the model because it did not contribute significantly to the overall variation in species diversity on islands. Island age has a significant positive direct effect on bulimulid species diversity. Island age has a significant negative direct effect on island elevation and area and has a significant positive effect on island habitat diversity. Island area has a significant positive indirect effect on bulimulid species diversity. Likewise, island age has a significant negative indirect effect on the number of bulimulid species found of each island because of the significant direct effects island age has on island elevation and area.

scenarios we tested were found to be significantly worse than the best unconstrained tree.

Therefore, if we only consider the emerged islands and the species included in the present study, the data support the first colonization event being to Española or San Cristobal Island. However, the best ML tree supports a more constrained colonization scenario if the first colonization event is constrained to be on San Cristobal, which is then followed by colonization of Española or Floreana (in any order), and then on to the remaining islands (for which the specific colonization order cannot be determined with statistical confidence). This approximate colonization sequence (San Cristobal first, then Floreana or Española, followed by the remaining islands) roughly parallels the geological order of the islands.

We also found a strong significant negative relationship (R^2 adjusted for the number of independent variables included in the model = 0.709, $P < 0.001$) between the depth of the clade encompassing an island's species and the island's age, consistent with the hypothesis that the colonization and speciation sequence of bulimulid land snails on Galápagos parallels its geological formation.

Biogeographical and Ecological Correlates of Species Diversity

Path analysis.—The path analysis model that best describes the potential direct and indirect effects of the predictor variables on island total species number is presented in Figure 3. This model includes number of plant species (as a proxy of habitat diversity), island area, and island elevation, and island age (overall $R^2 = 0.559$, $P < 0.01$). Island insularity (distance to nearest older major island) did not contribute significantly to the variation in total bulimulid species number directly or indirectly.

Island age had a significant positive direct effect on number of bulimulid land snail species found on islands (direct path coefficients d = 0.424, $P < 0.05$); thus, older islands have more bulimulid land snail species (Fig. 3). Island area had a significant positive indirect effect (indirect coefficient, i = 0.103, standard error of i, SE = 0.056) through its positive (but nonsignificant) direct effect on number of endemic and native plant species (habitat diversity). Island age had a significant positive direct effect (d = 0.455, $P < 0.05$) on the number of plant species found on island. Island age also has

TABLE 3. Multiple regression analysis results, with the number of bulimulid land snail species used as dependent variable for all models considered. The sample size (n) is provided for each model, as well as the standardized regression coefficient (β) and the test statistic (t) for each independent variable entered in each model. P values for adjusted R^2 and β values are indicated as follow: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Model	Independent variables	n	β	SE of β	t
Overall species diversity	Island elevation***	26	0.642	0.144	4.48
Adjusted $R^2 = 0.440$***	Island age**		0.762	0.221	3.46
Overall species diversity	Island area*	26	0.136	0.056	2.46
Adjusted $R^2 = 0.169$*	Island age*		0.559	0.263	2.13
Overall species diversity	Island habitat diversity***	26	0.539	0.122	4.42
Adjusted $R^2 = 0.433$***	Island age		0.256	0.187	1.36
Between-island speciation	Island insularity*	14	−0.747	0.307	−2.43
Adjusted $R^2 = 0.307$*	Island area*		0.306	0.104	2.94
Within-island speciation	Island habitat diversity*	14	0.289	0.118	2.44
Adjusted $R^2 = 0.276$*					

a significant negative direct effect (d = −0.536, $P < 0.01$) on island elevation, and on island area (d = −0.505, $P < 0.01$), which are both translated into significant negative indirect effects on number of bulimulid species (i = −0.531, SE = 0.173, i = −0.314 SE = 0.138, respectively). Island elevation has a significant positive indirect effect (i = 0.350, SE = 0.175) through its positive significant direct effect on plant diversity on islands (d = 0.727, $P < 0.01$) (Fig. 3).

Multiple regression analyses.—Island elevation, island area, and habitat diversity, three predictors of diversity that are indirect measures of habitat complexity or niche space, are intercorrelated ($r > 0.53$). Three linear regression analyses were run with a model including island age, and successively one of these three predictors. Island insularity was excluded here because it did not contribute significantly to the overall variation in bulimulid species number per island, either alone or in combination with other factors. The linear regression model that explains the most variation in bulimulid species number per island is a model that includes island age and island elevation (overall adjusted $R^2 = 0.\ 440$, $P < 0.001$). Under this model, island age has a significant positive effect on the number of species per island (Table 3). When island elevation is replaced by island area or island habitat diversity (keeping island age in the model), the models explain similar level of variation in bulimulid diversity, with overall adjusted $R^2 = 0.169$, $P < 0.05$ and $R^2 = 0.433$, $P < 0.001$, respectively. Island area has a significant positive effect on the number of bulimulid species per island (standardized partial regression coefficient $\beta = 0.136$, $P < 0.05$), as has island habitat diversity ($\beta = 0.539$, $P < 0.001$). Island age contributes significantly to the variation under the model including island area ($\beta = 0.559$, $P < 0.05$), but not in the model including island habitat diversity ($\beta = 0.256$, $P > 0.05$). Figure 4 presents island species diversity corrected for island age (by using the standardized residuals of island species diversity against age) against each of three measures of niche space (island elevation, area, and habitat diversity) representing the best models, as well as against island insularity (which has no effect on overall species island diversity).

We ran the same regression analyses (using alternatively different surrogates for niche space) excluding eight islands that have no bulimulid land snail species, to ascertain that our dataset is not zero inflated. This does not change the relative strength and the significance of the different models and the partial regression coefficient of the independent variables in the models.

Variation in bulimulid species diversity per island strictly resulting from colonization (as inferred from the best ML phylogenetic tree using the combined datasets) was best explained by a model including island area and insularity (overall adjusted $R^2 = 0.307$, $P = 0.05$). Under this model, island area has a significant positive effect on the number of species per island, and island insularity has a significant negative effect (Table 3). By contrast, the best model describing the variation in bulimulid species diversity due to within-island speciation (as inferred from the best ML phylogenetic tree using the combined datasets) is a model including only habitat diversity as an explanatory variable (adjusted $R^2 = 0.276$, $P < 0.05$, Table 3).

Although the 200 trees with highest log likelihood scores obtained from Bayesian search differ slightly in topology, these differences do not translate into differences in the number of within- and between-island speciation events inferred from the trees. This is because branches can be connected at different nodes on the tree without changing the inference of speciation versus colonization events. To induce a change, branches would have to be swapped between island clades, and this does not occur among the best 200 trees obtained from Bayesian searches. Therefore, the uncertainty of the phylogeny does not change the results obtained from the multiple regression analyses.

Overall, the results of the path analysis and multiple regression analyses indicate that island area, habitat diversity, and island elevation, all indirect measures of habitat complexity or niche space, are each positively associated with overall island land snail species diversity. Among other correlates, island age also contributes significantly to species diversity, suggesting that younger islands have not yet reached their maximum expected land snail diversity given their area or elevation. Although island insularity does not have a significant effect on overall island land snail species richness, we found that it does contribute to that fraction of the species richness presumed to result from interisland colonization. Notably, speciation due to colonization (between-island speciation) is mainly a function of island area and island insularity whereas species richness resulting from

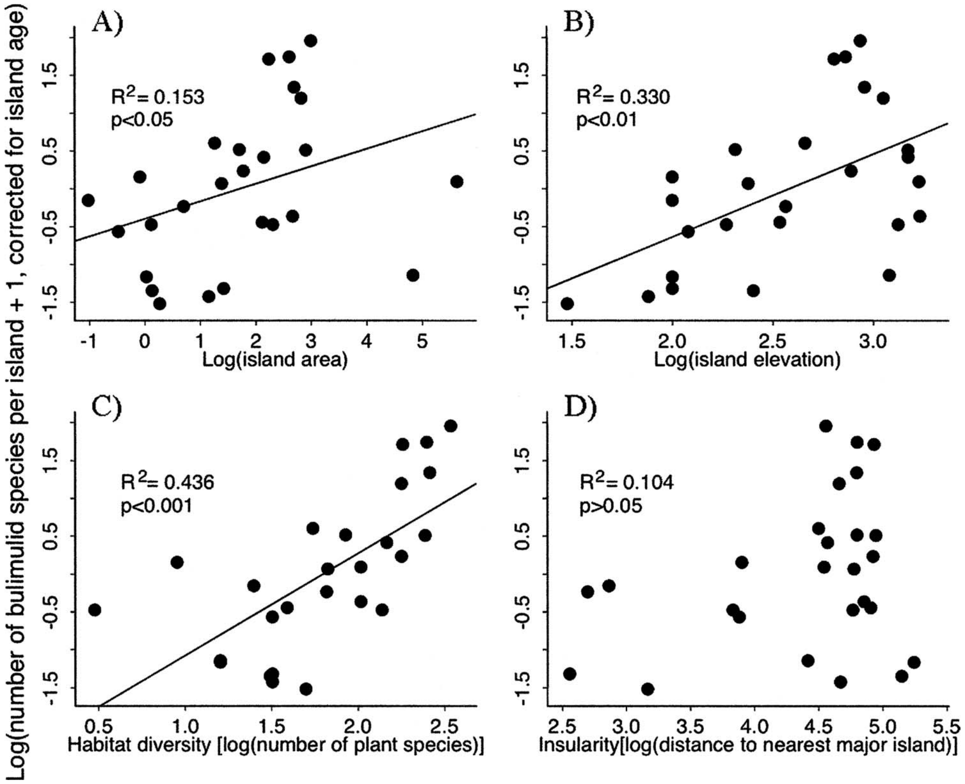

FIG. 4. Regression of the number of bulimulid land snail species on the Galápagos islands corrected for island age (using the standardized residuals) against (A) island area, (B) maximum island elevation, (C) habitat diversity measured as the number of native plant species, and (D) island insularity measured as the distance to nearest major island.

within-island speciation is largely determined by island habitat diversity.

DISCUSSION

We combined molecular phylogenetic and biogeographical analyses to better understand the processes involved in the formation and maintenance of bulimulid land snail species diversity on Galápagos islands. Phylogenetic work shows that the sequence of species formation approximates the pattern of geological formation of the islands. Our results demonstrate that the diversification of Galápagos *Bulimulus* land snails has been driven by a combination of geographic factors (island age, size, and location), which affect colonization patterns, and ecological factors, such as plant species diversity, that foster within-island speciation.

Phylogeny of Bulimulid Land Snails

Most species included in the phylogenetic analyses are monophyletic, but this is not surprising given that most species are represented by only two individuals, usually from one locality. However, a few exceptions suggesting the need of taxonomic revision are worth noting. First, individuals identified as *B. perrus* form two clearly distinct monophyletic groups based on the COI dataset, separated by 4.0–4.9% divergence at COI (uncorrected pairwise distances as computed in PAUP*, Fig. 2). These two groups still hold when we increase our sample size of *B. perrus* individuals to 10 (data not shown). Among the possible explanations for this pattern, *B. perrus* might be two cryptic species (with individuals that cannot be discriminated based on their external morphology) or the species might have retained two divergent mitochondrial lineages. *Bulimulus nux* is found on Floreana and San Cristobal islands, and each island assemblage forms a distinct monophyletic group. Again, there are multiple possible explanations for such a pattern; for example, it could be the result of convergent evolution or introgression. By contrast, specimens of ‘‘*B. snodgrassi*’’ found on Española and San Cristobal islands are morphological distinct, and will be the subject of future taxonomic revision. Although such taxonomic uncertainties as well as incomplete taxon sampling might make inference of species diversification problematic, there is no reason to believe that such uncertainties disproportionately affect taxa resulting from within-island speciation than those arising from interisland colonization, or certain island assemblages more than others.

All phylogenetic hypotheses resulting from the different search methods or datasets show the same general partition of the ingroup into three major well-supported clades: one formed by *B. snodgrassi* from Española Island and *B. nux* from Floreana Island, one by *B. unifasciatus* from Floreana and *B. nux*, *B. snodgrassi*, *B. eschariferus*, and *B. amastroides* from San Cristobal Island, and the last group formed by all remaining species on the other islands of the archipelago. This pattern is consistent with the geological formation of the archipelago in island clusters instead of the typical linear arrangement resulting from plate moving above a hotspot as seen for the Hawaiian Islands, and is also consistent with what Beheregaray et al. (2004) found for giant tortoises on Galápagos.

Bulimulid land snail assemblages found on islands forming the southeastern portion of the archipelago (San Cristobal, Española, and Floreana) are each composed of only one or at most two independent lineages. By contrast, assemblages on younger and more centrally located islands can be formed by up to five clades. This suggests that the southeastern islands have not been successfully colonized as often as the remaining snail-inhabited islands of the archipelago, or less likely, extinction wiped out all but species originating within these islands.

Island Monophyly

Monophyletic island assemblages of multiple species are the result of speciation within islands; thus, monophyly is more likely to occur on islands or volcanoes where interisland colonization is rare. Very young islands have had less time for repetitive colonization events to occur, and isolated islands might have lower interisland colonization rate. Thus, we expect monophyletic groups to occur on young islands and on very isolated islands. The phylogenetic results provide support for the monophyletic hypothesis for bulimulid assemblages on Darwin, Wolf (one species on each of these volcanoes), and Sierra Negra (two species) volcanoes on Isabela Island, among the youngest volcanoes of the islands. Monophyly is also supported for San Cristobal (four species) and Española (one species) islands, two of the most isolated of the islands that harbor bulimulid land snails.

Bulimulid assemblages found on five of the 11 Galápagos Islands or volcanoes included in the phylogenetic analysis form monophyletic groups, although for three of them only one species was available for this study. Land snail assemblages of Floreana, Pinzon, Fernandina islands, as well as the assemblage of bulimulids found on Alcedo volcano (Isabela Island) are each formed by at least two independent lineages. Santiago and Santa Cruz islands have been colonized by at least four and five independent lineages, respectively. These relatively large islands (second and fourth largest islands of the archipelago) are also centrally located both in space and in time. Pinzon Island is also centrally located but has only two bulimulid lineages; however Pinzon Island is considerably smaller than both Santa Cruz and Santiago, which probably reduces the chance of successful colonization events on this island.

Successful colonization can be limited by the rate of new potential immigrants or by the probability that a new immigrant will be able to survive and persist as a species on the newly colonized island. The survival and persistence of new colonists depends in turn mainly on the habitat they will encounter on the newly colonized islands. San Cristobal, Española, and Floreana islands have comparable habitat diversity to other islands such as Santa Cruz or Santiago islands, and it is therefore unlikely that successful colonization events were constrained by the availability of suitable habitat for colonists. On the other hand, San Cristobal, Española, and Floreana islands are more isolated from the rest of the archipelago than any other islands harboring bulimulid land snails, and it is likely that this increased isolation reduces the rate of new bulimulid colonization. Indeed, Darwin and Wolf islands (as opposed to Darwin and Wolf volcanoes referred to in this study), two islands well isolated northwest of the rest of the archipelago (located over 250 km from the center of the archipelago, therefore falling outside of the Galápagos map depicted in Fig.1) have no record of land snail fauna although they are larger and have greater habitat diversity than a few other islands that do maintain bulimulid land snails. Another possible explanation for the reduced number of independent lineages on Española, Floreana, and San Cristobal islands is extinction. Not only have these older islands had more time for extinction to occur, they also experience reduced area, elevation, and habitat diversity due to subsidence and erosion. For extinction to be a likely explanation however, it must have proceeded in a phylogenetically nonrandom way, such that entire clades were wiped out. Although there is no reason so far to believe this has been the case, data on the phylogenetic relationship of extinct taxa would be required to test this idea further.

Bulimulid land snails are one of the only Galápagos taxonomic groups that form island monophyletic assemblages. Most other taxa that have diversified on Galápagos have at most one representative species per island or volcano (giant tortoises for example, Caccone et al. 2002; Beheregaray et al. 2004), which suggests that these groups have not speciated within islands. The pattern for groups that have more than one species per island, such as Darwin's finches, is consistent with speciation primarily via between-island dispersal events rather than within-island diversification (Freeland and Boag 1999; Petren et al. 1999; Sato et al. 1999).

Traditionally, island systems have been regarded as outstanding situations for species formation, mainly due to isolation between the population found on a given island and its source (Mayr 1963). Under this scenario, a species colonizes an island and then eventually differentiates from its source population. Multiple species could co-occur on a single island if it was reinvaded by individuals from the source population after the first speciation event was well under way. Alternatively, species could co-occur on an island that has been colonized by multiple different species coming from different sources. This latter scenario is most appropriate for taxa with high dispersal rates, such as flighted birds, some reptiles, and mobile insects. The phylogeographic pattern expected for such taxa diversifying on islands would be either one species per island (for islands with low habitat diversity) or nonmonophyletic island assemblages if niche space is broad enough to support multiple species. The importance of intraisland speciation in generating diversity has been made explicit only recently (Losos and Schluter 2000). Larger islands (offering higher habitat diversity) should allow for within-island speciation in taxonomic groups that have low dispersal abilities and the potential to adapt to different habitats. Monophyly of total or partial island assemblages is the expected phylogeographical pattern resulting from this process, and this is a pattern that should be common for taxa with low dispersal abilities such as flightless arthropods, land snails, some reptiles, and plants with low dispersal ranges. Overall, species diversity should reach its maximum in lineages with low dispersal abilities (provided that they can colonized different islands, even if it is by passive dispersal) and with the potential for adaptation to varying habitats occurring on insular systems where habitat diversity is high.

Colonization and Speciation Sequence

All major islands of the Galápagos Islands are volcanic in origin, and they were formed as a result of an eastward displacement of the Nazca plate over a ''hotspot,'' so that easternmost islands are oldest with progressively younger islands to the west (Nordlie 1973; Cox 1983; White et al. 1993; Geist 1996). The most parsimonious colonization scenario is thus from east to west, paralleling the geological formation of the islands. This movement from old to young islands would suggest that successful colonization is more likely to occur on islands with unoccupied niches (Brooks and McLennan 1991), a pattern often referred to as progression rule (Wagner and Funk 1995). This pattern found for Galápagos bulimulid land snails is also seen in other organisms that have evolved on island or islandlike systems (Roderick and Gillespie 1998; Chiba 1999; Holland and Hadfield 2004).

Phylogenetic relationships within most other groups of Galápagos endemic organisms that have undergone diversification are just starting to be elucidated, and molecular studies of their historical biogeographical scenarios have detected inconsistent relationships between population diversification and island formation. Finch diversity might be partly the result of secondary contact between species (Grant and Grant 1996), lava lizard diversity is due to at least two colonization events from the mainland (Wright 1983; Kizirian et al. 2004) and the different marine iguana groups might be affected strongly by sex-biased dispersal (Rassmann 1997). By contrast, recent phylogeographical studies of Galápagos giant tortoises (Caccone et al. 2002; Beheregaray et al. 2004) found that interisland founding events generally occurred from geologically older to younger islands, and a similar pattern has been inferred for Galápagos endemic beetles of the genus *Stomion* (Finston and Peck 1997, 2004). This is consistent with the simplest hypothesis of species formation on archipelagos: formation of species following the serial colonization from the nearest neighbor island as each new island emerges, and is expected for taxonomic groups with relatively poor dispersal abilities.

Land snails are known for their low dispersal ability, which may predispose them to isolation by distance, and microallopatric speciation (Pfenninger et al. 1996; Douris et al. 1998; Davison 2000; Arnaud et al. 2001; Guiller et al. 2001; Pfenninger and Posada 2002; Giokas and Mylonas 2004). However, they can potentially achieve long distance colonization (such as interisland colonization) by passive dispersal (Carlquist 1974). We can only speculate about mechanisms of dispersal, but among the most likely ones there is transport by birds traveling from one island to the other, even though other agents such as winds have been suggested (Kirchner et al. 1997). Rafting on vegetation is also a possibility that has been previously invoked to account for the first colonization event of Galápagos by bulimulid land snails (Dall 1896; Dall and Ochsner 1928; Smith 1966). Bulimulid land snails are adapted to arid environments and harsh conditions, and can seal their shell aperture to prevent desiccation, which could be advantageous if rafting on salt water. However, bulimulid land snails have never been found in the littoral zone, the lowest of the vegetation zones described for Galápagos. Furthermore, bulimulid land snails can be found at high elevations on some young islands where vegetation at lower elevation is not suitable or not even present at all. These observations suggest that land snails have dispersed to these islands by other means than rafting (Vagvolgyi 1975). Given that vegetation tends to accumulate on new islands from the summit towards sea level (as suggested by the vegetation distribution on very young islands), and that land snails are found on young islands even when there is no suitable vegetation for food and shelter at lower elevation, it seems unlikely that passive dispersal by rafting would be the only or even the main dispersal mechanism. However, bulimulid interisland movements seem to be influenced, at least partly, by the distance between islands. Indeed, bulimulid land snail interisland movements are apparently much more common on islands that have close neighbors, such as the centrally located islands of Santa Cruz and Santiago. Land snails, as other terrestrial invertebrates with low local dispersal capacity, may be especially likely to speciate extensively in insular contexts because populations can be isolated locally but they also have some potential for passive long distance dispersal.

Biogeographical and Ecological Correlates of Bulimulid Species Diversity

Smith (1971) made the first attempt to evaluate the role of different biogeographical factors on bulimulid species diversity on islands; his study was later extended and published by Chambers (1991). The best multiple regression model explaining the number of bulimulid species found per island included the number of plant species as the only significant explanatory variable (Smith 1971). Smith acknowledged that his study was preliminary and it did not include island age. Furthermore, Smith (1971) did not divide Isabela Island into its different volcanoes, but instead, ran the same analysis with Isabela Island excluded from the dataset. By doing so, an important stage of the radiation process is excluded from the analysis, and this might bias the conclusions drawn from the analysis done on the reduced dataset. Chambers (1991) reanalyzed Smith's data, and included collecting effort as an explanatory variable in his regression model because it had been previously suggested that considerable more bulimulid species remained to be discovered, especially on the larger, young islands. Chambers found that collecting effort had the largest contribution to bulimulid island diversity, and concluded that the bulimulid fauna was too poorly known to be able to drawn any meaningful conclusions from a biogeographical analysis. However, we spent considerable time on Isabela and Fernandina islands (more than doubling the collecting effort on these islands if we consider time spent looking specifically for land snails), and found only a few potential new species, which allowed us to adjust the island species number accordingly. We did not include collecting effort as an independent variable in our regression analysis because we believe that the actual number of species per island is a close reflection of what is found on the islands, and because of the problems associated with estimating collecting effort accurately (Chambers 1991).

Island age has an obvious direct effect on island diversity: older islands should have more species because they have had more time for speciation and colonization to occur. However, this positive direct effect becomes less important as island niche space becomes saturated with colonists and newly formed species. Island age has also a negative direct effect; species on older islands have more time (thus more chance) to go extinct. Nevertheless, niche space left open by extinct species should eventually become occupied again by either newcomers from other islands or newly formed species. As they emerge, oceanic islands of volcanic origin rapidly reach their largest size and elevation. They then get smaller with age due to erosion and subsidence, and they eventually disappear below the water level to become seamounts (White et al. 1993). Therefore, age has an indirect negative effect on diversity because of its negative effect on island area and elevation. Ultimately, when islands reach speciation/extinction equilibrium, the direct effect of island age should become insignificant, and only a negative relationship should be detected between age and species diversity due to its indirect negative effect translated into a decrease in island elevation and size.

When all Galápagos islands for which data are available are considered, island age has an overall significant positive effect on bulimulid species number. This suggests that the direct positive effect of island age on land snail diversity is greater than its indirect negative effect, and at least some islands have not reached their speciation/extinction equilibrium. Younger islands and volcanoes have apparently not yet reached the same biodiversity equilibrium as the older islands of Galápagos (as suggested by the age effect found in multiple regression analyses). Even though together Fernandina, Isabela, and Tortuga islands represent over 60% of Galápagos total land area, they include only 12 of the 71 described bulimulid land snail species found on Galápagos. At equilibrium, diversity should mostly be a reflection of habitat diversity, measured as the number of native plant species, as suggested by the regressions of species diversity corrected for island age presented in Figure 4. Because plants provide food and shelter, land snails can potentially adapt to different plant species for feeding or hiding. An island with more plant diversity thus provides more potential for differentiation for land snails, so that land snail species diversity is promoted by within-island speciation. In addition, an island with higher plant diversity is more likely to offer a suitable habitat for a new coming colonizing species. Therefore, species diversity on a given island should be a reflection of the habitat diversity on that island (Gillespie 2004). Island area has traditionally been used as a proxy for habitat diversity, but number of plant species provides a more direct measure of habitat diversity for land snails or other animals whose ecology is directly related to plant diversity. Nevertheless, the relationship between the number of animal species in a community and habitat diversity or heterogeneity (measured as the number of plant species or the structural heterogeneity of the vegetation) has long been recognized (MacArthur 1965, 1969; Pianka 1966; Karr 1971; Murdoch et al. 1972).

Different faunal groups can differ in their responses to area and habitat diversity, so that area, habitat diversity, or a combination of both have a strong effects on species richness depending on the biological traits of the different taxonomic groups (Ricklefs and Lovette 1999). In a study examining the species-area relation of Aegean land snails, Welter-Schultes and Williams (1999) found that even after accounting for island area, species richness was still affected by habitat diversity. Galápagos bulimulid land snails seem to have adapted to the different vegetation zones, and most species are found on specific plants or defined microhabitats. Thus adaptation to specific vegetation types apparently provides the opportunity for bulimulid snails to differentiate within-island and partition the niche space to allow species to co-occur.

Islands that are more isolated are presumably more difficult to reach, so that land snail diversity would decrease with increasing distance to nearest island, thus reducing immigration rate. On the other hand, island insularity is not expected to affect within-island speciation rate.

We analyzed the roles of colonization and within-island speciation in bulimulid diversity by partitioning the overall species diversity into species resulting from interisland colonization and species resulting from within-island speciation. Variation in bulimulid land snail diversity on individual islands resulting from within-island speciation is mostly explained by habitat diversity of each island. By contrast, bulimulid diversity generated from speciation due to interisland colonization is explained by both island area and island insularity. This distinction is only possible by partitioning biodiversity among its different contributors, namely colonization, speciation, and extinction, and the present study is the first attempt to explicitly look at two of these processes independently. By comparing phylogenetic studies of arthropods on the Canary Islands, Emerson and Oromi (2005) found that the origin of endemic species on an island is first the result of interisland colonization, followed by intraisland speciation as the island matures. This is consistent with the results found here, where bulimulid species assemblages on old islands are mainly the result of intraisland speciation whereas half or more of species diversity on midaged and young islands is the result of interisland colonization. The same way that within-island speciation and colonization rates are determined at least partly by a combination of biogeographical and ecological factors, extinction is a process affecting biodiversity that could also be influenced by some of these factors. For example, Marui et al. (2004) demonstrated that temporal variation in island area caused change in species diversity in Kikai Island land snail fauna due to extinction. A more thorough test of the role of these biogeographical factors on extinction could be possible in lineages where the extinct snail taxa are known (e.g., *Mandarina* [Chiba 1996], or *Cerion* land snails [Gould and Woodruff 1990; Goodfriend and Gould 1996]), given sufficient information on their biogeography and inferred ecology.

Conclusions

The two main types of diversification processes involved in generating and maintaining species diversity on islands are between-island and within-island speciation. These processes are influenced by the islands' biogeographical setting as well as the biology of the diversifying lineages. The initial island biogeography theory established island area and insularity as determinants of diversity equilibrium through their effect on colonization and extinction (MacArthur and Wilson 1963, 1967). Losos and Schluter (2000) showed that within-island speciation was mostly determined by island area. The present study demonstrates that number of plant species, a measure of habitat diversity for land snails predicts the number of species on an island more accurately than island area. Plant species diversity is a good measure of habitat diversity, especially for taxonomic groups depending heavily on plants for food and/or shelter. More than island area, niche space, or ecological opportunity is important in generating and structuring species diversity on islands.

This study also highlights the importance of considering the two processes involved in generating and maintaining species diversity separately when trying to identify the importance of different biogeographical and ecological factors influencing species diversity. The importance of factors that have opposing or simply different effects on between and within-island speciation might go uncovered if only overall diversity is considered.

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