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Date Defended/Approved: July 28, 2008
ABSTRACT

Adaptive radiation is one of the main processes involved in the formation of the world’s organic diversity. Thus, understanding the processes involved in adaptive radiation becomes a key component of the study of evolutionary biology. The study of adaptive radiation entails a wide range of questions involving various aspects of phylogeny, biogeography, adaptation, and speciation. One of many approaches to a problem as broad as this is to seek an understanding of a small and simplified part of it. Islands provide this simplicity, naturally. The aim of the present work is to reach a better understanding of diversification in adaptive radiations, using Galápagos endemic bulimulid land snails as model system. First, I combined phylogenetics and biogeographical analyses to partition island species diversity by its originating processes: between-island colonization and within-island speciation. The results show that 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. I then tested the classic evolutionary theory of ecological opportunity, which proposes that the combination of increase in wealth of resources and decrease of potential enemies promotes diversification in lineage colonizing previously empty adaptive zones. I show that ecological opportunity predicts the extent of intraspecific phenotypic variation, explicitly linking the role of competition from congeners and
the heterogeneity of resources to the extent of intraspecific phenotypic divergence as adaptive radiation proceeds. Finally, I tested for a fit between the phenotypes of diversifying species and their environments. I tested for such a phenotype-environment association (1) across the adaptive radiation of Galápagos bulimulid land snail species, and (2) within one species, Bulimulus reibischi. I found that shell morphology has evolved across species repeatedly on different islands in response to moisture gradient in the same direction as within-species morphological variation among populations of B. reibischi. The parallel association between environment and phenotype both above and below the species level suggests that ecologically-based selection is involved in diversification at both population and species levels in this adaptive radiation.

**Keywords:** adaptive radiation, diversification, ecological opportunity, Galápagos Islands, island biogeography, phenotypic variation, species richness.
ACKNOWLEDGEMENTS

This work would not have been possible without the support and encouragement of a long list of people.

Dr. Bernard Crespi, my supervisor, for his endless patience and constant sound advice.

The other members of my committee: Arne, there from the start, and Mike and Felix, welcomed additions later on. Together with Bernie, they provided an incredible source of support and inspiration.

All members of the FAB*-lab at SFU, simply the best research group a graduate student in evolutionary biology could wish to be part of. In particular, former and current members of the Crespi Lab, for providing a stimulating and fun environment in which to learn and to live. My time at SFU would not have been as enjoyable without you.

The staff of the Charles Darwin Research Station and Parque Nacional de Galápagos for their continued support and collaboration. Particularly Charlotte Causton and Lazaro Roque, as well as the team of Terrestrial Invertebrates at the Charles Darwin Research Station.

My collaborators for chapter 1, Ken Petren and Gisella Caccone. The numerous people that helped gathering field or lab data: Sampson Wu, Clair Keays, Deborah Austin, Eugénie Parent, Louis de Redon, MarieLou Poisson,
Carole Bender, Novalino Castillo, Pedro Castillo, and Tom Poulsom. Shannon Wood and Heather Robertson from the Archaeology Department for help with the shell radiographs.

I would also like to thank Guy, for the very special person he is, and for the incredible amount of understanding he had with me over the past few years.

And last but not least, my parents, for creating an environment in which following this path seemed so natural. I would like to dedicate this work to my dad, who taught me that asking the question can often be more important than finding an answer.
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CHAPTER 1: COLONIZATION AND DIVERSIFICATION OF GALÁPAGOS TERRESTRIAL FAUNA: A PHYLOGENETIC AND BIOGEOGRAPHICAL SYNTHESIS

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ABSTRACT
Remote oceanic islands have long been recognized as natural models for the study of evolutionary processes involved in diversification. Their remoteness provides opportunities for isolation and divergence of populations, which make islands remarkable settings for the study of diversification. Groups of islands may share a relatively similar geological history and comparable climate, but their species experience subtly different environments and have distinct evolutionary histories, offering the potential for comparative studies. A range of organisms have colonized the Galápagos Islands, and various lineages have radiated throughout the archipelago to form unique assemblages. This review pays particular attention to molecular phylogenetic studies of Galápagos terrestrial fauna. I find that most of the Galápagos terrestrial fauna has diversified in parallel to the geological formation of the islands. Lineages have occasionally diversified within islands, and the clearest cases occur in taxa with very low vagility and on large islands with diverse habitats. Ecology and habitat specialization appear to be critical in speciation both within and between islands. Although the number of phylogenetic studies is continuously increasing, studies of natural history,
ecology, evolution and behaviour are essential to completely reveal how diversification proceeded on these islands.

**Keywords**: adaptive radiation, diversification, evolution, phylogeny, phylogeography, speciation

“The distribution of the tenants of this archipelago would not be nearly so wonderful, if, for instance, one island had a mocking-thrush, and a second island some other quite distinct genus […]. But it is the circumstance, that several of the islands possess their own species of the tortoise, mocking-thrush, finches, and numerous plants, these species having the same general habits, occupying analogous situations, and obviously filling the same place in the natural economy of this archipelago, that strikes me with wonder.”

(Darwin 1845)

**INTRODUCTION**

Islands have always attracted professional and amateur students of biology alike. By their very nature, islands are biologically simpler than continental regions and therefore provide ideal geographical and historical settings for the study of colonization and diversification of species. If the timing of their geological formation is known, such study can be conducted within a defined time frame. Moreover, if an island is part of an archipelago, then each island represents a replicate natural experiment and therefore provides increased statistical power to test ideas related to patterns observed and processes inferred about diversification.
The Galápagos archipelago occupies a unique position in the history of evolutionary studies, mainly because of its importance as a conceptual landmark (Darwin 1859) and because it continues to shape our understanding of evolutionary biology (Grant and Grant 2008). Notwithstanding its tropical climate, the Galápagos archipelago has been the stage of surprisingly few animal diversifications compared with other Pacific tropical island groups. With only one known exception, evolutionary radiations on Galápagos comprise fewer than 20 species, and include mainly lineages of land birds, reptiles and terrestrial invertebrates. Among vertebrates, the absence of amphibians and the virtual absence of mammals are particularly striking and nearly unique among terrestrial island ecosystems.

The goal of this review is three fold: (1) to review literature on Galápagos animal groups that have speciated on the islands; (2) to synthesize the available evidence in light of classic hypotheses related to colonization and diversification on islands; and (3) to suggest future avenues in the study of Galápagos faunal diversification and how they can be used to aid in conservation efforts.

THE GALÁPAGOS ARCHIPELAGO

Geographic setting
The Galápagos Islands are located in the Pacific Ocean, about 960 km west of the South American coast, straddling the equator at the 90th meridian west (Figure 1A). The Galápagos archipelago is composed of 13 major islands larger
Figure 1 Map of the Galápagos Islands (A) and current hypotheses for the phylogenetic relationships of three Galápagos lineages: giant Galápagos tortoises (B), Darwin’s finches (C) and terrestrial snails (D).

A. Estimated minimum and maximum geological ages for each island are in parentheses (D. Geist unpublished). Solid triangles indicate the summits of the six Isabela volcanos. The star indicates the location of the hotspot, currently between Fernandina and volcano Cerro Azul of Isabela (Hooft et al. 2003). B. Bayesian tree based on mtDNA control region sequences of extant and extinct Galápagos tortoises. Extinct taxa are asterisked. Placement of the three lineages on Santa Cruz is indicated with an arrow and a grey box, and the pictures exemplify their different carapace morphologies. Redrawn from Russello et al. (2005). C. Phylogenetic tree of Darwin’s finches estimated from cyt B sequences and neighbour joining (Petren et al. 2005). There is significant genetic structure and paraphyly among populations of sharp-beaked ground finches and warbler finches but lack of resolution among the phenotypically distinct species of ground finches and tree finches. Photographs are proportional to actual size. D. Best maximum likelihood phylogenetic tree based on combined mtDNA COI and nDNA ITS1 sequence data for bulimulid snails. Two long branches were shortened and their actual lengths are reported in brackets below them. The snail outlines are roughly proportional to actual size. Species on older islands connect at deeper nodes, with a significant negative relationship between the depth of the clade encompassing an islands’ species and the island’s age. For each tree numbers above branches are Bayesian posterior probabilities. Maximum likelihood bootstrap proportions > 50% are given below branches for B.
than 10 km$^2$, six smaller islands, over 40 islets with official names, and many smaller unnamed islets and rocks, for a total of about 8000 km$^2$ of land spread over 45 000 km$^2$ of water (Snell et al. 1996). Of these islands, Isabela is the largest (more than the total area of all the other islands combined) with an area of 4588 km$^2$ and the highest, with a maximum elevation of just over 1700 m. Isabela is formed by six volcanos that are interconnected mostly by barren lava flows, which may represent a geographical barrier to dispersal analogous to open water for terrestrial animal species with low dispersal capacity such as flightless terrestrial invertebrates.

**Geological history**

The Galápagos Islands are a young, oceanic, volcanic archipelago. Their geological history is relatively well understood (Bailey 1976; Cox 1983; Geist 1996; Hall 1983; Nordlie 1973; Swanson et al. 1974; White et al. 1993), with individual islands being formed as the Nazca plate has moved over a hotspot. However, in contrast to the roughly linear arrangement by age of the Hawaiian Islands (Price and Clague 2002), the Galápagos Islands are clustered into groups of similar age (White et al. 1993). The age of the islands increases moving eastward along the plate, with the oldest islands located towards the southeast of the archipelago. K-Ar age determinations and marine fossils indicate a maximum age of the oldest land on the order of 3 million years (Myr), whereas geological plate motion models set a maximum age of emergence around 4 Myr ago, depending on the velocity of the Nazca plate (Geist 1996; Hickman and Lipps 1985; White et al. 1993; Geist unpublished data; Figure 1A). The discovery
of drowned seamounts east of San Cristobal extend the time the Galápagos have been available for colonization to at least 14 Myr ago (Werner et al. 1999). However, the history of island production over this hotspot could extend back to 80-90 Myr ago, which is the estimated age of the birth of the Galápagos hotspot (Christie et al. 1992; Hoernle et al. 2002).

**Biogeography and ecology**

The climate of the Galápagos Islands is unusually dry for the tropics and has marked seasonality. Since the archipelago is so isolated, ocean and wind currents mostly influence its climate. There are two main seasons: the warm season, typically January through May, and the cool season from June to December. The warm season is caused by warm ocean currents sweeping southward from the direction of Panama, which cause both sea and air temperatures around the islands to rise. During this time the skies are normally clear, with occasional heavy showers. During the cool season the weather is influenced by the Humboldt Current, which brings cold water north from the Antarctic along the west coast of South America and then westward through the archipelago, which results in cooler air temperatures. During the cool season, the skies are usually overcast, but with little precipitation in the lowlands. However, many parts of the highlands are constantly wet at this time because of a mixture of light rain and mist. The higher islands have increasingly more rain at progressively higher elevations and a humid forest zone exists at 300-600 m on their windward (eastern) sides. Precipitation is variable in occurrence and quantity, even in the wet season. The direction of the wind and oceanic currents
change in response to a seasonal north-south shift in the Intertropical
Convergence Zone (ICZ). On an irregular basis, but typically once every three to
six years (Allan et al. 1996), this change in direction becomes more dramatic and
warmer and wetter seasons occur with potentially 10 times more than the normal
annual rainfall (referred to as El Niño events). In the cool season during these
years, the ICZ moves greatly to the north of the islands, and the archipelago falls
under the influence of the cool, dry wind and cool ocean currents (termed La
Niña events). These cyclic changes in climate can have a pronounced effect on
Galápagos vegetation (McMullen 1999) and fauna (Grant and Grant 1993; Grant
et al. 2004; Steinfartz et al. 2007).

Vegetation on Galápagos can be separated into six (or seven) altitudinal
zones (van der Werff 1979; Wiggins and Porter 1971) and 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 Galápagos flora
comprises 749 angiosperm species, of which 216 are endemic to the archipelago
(just under 30%), and seven of the 192 genera of flowering plants listed by
McMullen (1999) are endemic to the islands.

**Endemism and diversification**

Like that of many other isolated oceanic islands, the Galápagos fauna is
impoverished and taxonomically unbalanced when compared to Neotropical
source areas. Because of their isolation and arid climate, the Galápagos Islands
have been successfully colonized by only a subset of the diverse flora and fauna
of the closest continent. Some lineages that have colonized Galápagos have not
differentiated from their continental ancestor (and thus are considered native) or have differentiated without radiating into multiple lineages. Both of these scenarios apply to all sea birds, more than one third of land bird diversity, and the great majority of terrestrial invertebrates (Peck 2001, 2006).

Although relatively few lineages of Galápagos terrestrial fauna have diversified within the archipelago (associated with adaptation or not), the resulting species constitute a large proportion of the terrestrial vertebrate fauna of the islands (Table 1). Bats (two species) and rats are the only terrestrial mammals that have naturally colonized the islands, and among them only the rice rats have diversified within the archipelago with 10 recognized species (three extant) representing three lineages (Clark 1984). Amphibians have not naturally colonized the islands. Seven reptile lineages have colonized the Galápagos and at least four diversified after their arrival, such that 34 of the 37 recognized reptile species are part of multi-species lineages. Of the 30 land bird species 25 are endemic and 60% of the endemic species belong to two lineages that have speciated within the archipelago: Darwin’s finches (14 species) and Galápagos mockingbirds (4 species). In contrast, the Galápagos Islands have approximately 1500 species of native insects of which only about 47% are endemic. Very few terrestrial insect lineages have led to multiple species: only about 5% of the approximately 1000 insect genera that have successfully colonized the islands have diversified (Peck 2006) and those that have diversified have given rise to lineages of 20 species or fewer. With 71 described species, the radiation of Galápagos bulimulid land snails is the most spectacular in the fauna in terms of
Table 1. Species diversity of Galápagos terrestrial animals.

For each taxonomic group, the number of native (undifferentiated from non-Galápagos relative), the number of species that have differentiated from their non-Galápagos relative but have not diversified within Galápagos (single species endemic), the number of species resulting from within-Galápagos diversification (multiple species endemic), and the total number of recognized species. The number of lineages leading within-Galápagos diversification is indicated in parentheses next to the number of multiple species endemics. Numbers are not available for all terrestrial invertebrate fauna, therefore only data for beetles and land snails are reported.

<table>
<thead>
<tr>
<th>taxonomic group</th>
<th>native</th>
<th>single species endemic</th>
<th>multiple species endemic (number of lineages)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>mammals</td>
<td>1</td>
<td>1</td>
<td>10 (1)</td>
<td>12</td>
</tr>
<tr>
<td>land birds</td>
<td>5</td>
<td>7</td>
<td>18 (2)</td>
<td>30</td>
</tr>
<tr>
<td>reptiles</td>
<td>2</td>
<td>1</td>
<td>34 (4)</td>
<td>37</td>
</tr>
<tr>
<td>beetles</td>
<td>108</td>
<td>108</td>
<td>164 (42)</td>
<td>380</td>
</tr>
<tr>
<td>land snails</td>
<td>0</td>
<td>5</td>
<td>82 (5)</td>
<td>87</td>
</tr>
</tbody>
</table>
species number (Chambers 1991; Parent and Crespi 2006). None of the other nine land snail genera that have colonized the islands has diversified into more than four species (Smith 1966) and preliminary phylogenetic evidence suggests that the four species of the genus Succinea are descendants of at least two independent colonizations (B. S. Holland personal communication). Table 2 summarizes the within-archipelago diversification of Galápagos terrestrial fauna, and in the following sections I will describe the main diversification patterns.

**ORIGINS OF ENDEMICS AND AGE OF GALÁPAGOS FAUNA**

**Source of the fauna**

The terrestrial Galápagos fauna that has diversified on the islands generally has close relatives on the South American continent. This is the case for Galápagos tortoises, which are closely related to the smaller bodied Geochelone chilensis, or Chaco tortoise, from South America (Caccone et al. 1999). The diversity of Galápagos lava lizards (Microlophus spp.) is the result of two independent colonizations, both from the west coast of South America (Benavides et al. 2007; Kizirian et al. 2004; Wright 1983). Likewise, Wright (1983) suggested that the leaf-toed gecko (Phyllodactylus) diversity stems from more than one colonization from South America; however, this suggestion has not been tested with phylogenetic analyses including possible mainland relatives.

The two land bird lineages that have diversified on Galápagos do not necessarily bear a strong relationship to relatives on the adjacent Ecuadorian mainland: Darwin’s finches appear to be most closely related to grassquits
Table 2. Summary of diversification of the terrestrial fauna of Galápagos.

The table includes the number of species per lineage that evolved on the archipelago, the number of colonizing species from which they evolved, their probable geographical origin, as well as molecular estimates (millions of years) of the initial split within the lineage and the temporal window of divergence from their ancestor. Also indicated is if the lineage follows the pattern of progression rule (see text), if multiple species are found within island or volcano, and if there is evidence that the lineage is an adaptive radiation. For terrestrial invertebrates, only lineages with 10 species or more have been listed.

<table>
<thead>
<tr>
<th>class</th>
<th>taxa</th>
<th>number of species or sub-species</th>
<th>phylogeny available?</th>
<th>number of colonization events</th>
<th>geographical origin</th>
<th>time of initial split within Galápagos (myrs)</th>
<th>divergence time with closest extant lineage (myrs)</th>
<th>progression rule?</th>
<th>multiple species per island or volcano?</th>
<th>adaptive radiation?</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalia</td>
<td>rice rats (Oryomys, Nesoryzomys, Megaoryzomys)</td>
<td>at least 8</td>
<td>no</td>
<td>3?</td>
<td>North, Central, and South America</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>on Santa Cruz, Isabela, and Fernandina</td>
<td>no</td>
<td>1</td>
</tr>
<tr>
<td>Reptilia</td>
<td>Galápagos giant tortoise (Geochelone nigra)</td>
<td>15 (11 extant)</td>
<td>yes</td>
<td>1</td>
<td>South America</td>
<td>1.5–2.0</td>
<td>6.0–12.0</td>
<td>yes</td>
<td>on Santa Cruz and Isabela</td>
<td>maybe</td>
<td>2-6</td>
</tr>
<tr>
<td></td>
<td>lava lizards (Microlophus)</td>
<td>9</td>
<td>yes</td>
<td>2</td>
<td>South America</td>
<td>1.4⁹</td>
<td>10.2⁷, 2.0–2.7⁸, 3.6–4.0⁹</td>
<td>yes</td>
<td>no, one island-one species</td>
<td>no</td>
<td>7-9</td>
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12
<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Order/Species</th>
<th>Number</th>
<th>Introduced?</th>
<th>Introduction Location</th>
<th>Age (Myrs)</th>
<th>Dispersal Ability</th>
<th>Dispersal Location</th>
<th>Age (Myrs)</th>
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<tbody>
<tr>
<td>leaf-toed Geckos (<em>Phyllodactylus</em>)</td>
<td>6</td>
<td>yes</td>
<td>3?</td>
<td>South America</td>
<td>?</td>
<td>8.9</td>
<td>yes</td>
<td>on San Cristóbal only</td>
</tr>
<tr>
<td>marine (Amblyrhynchus cristatus) and land (Conolophus spp.) iguanas</td>
<td>1 (marine) 2 (land)</td>
<td>yes</td>
<td>1?</td>
<td>South America</td>
<td>&gt;10 myrs</td>
<td>&lt;2 myrs</td>
<td>yes</td>
<td>land iguana: on v. Wolf (Isabela)</td>
</tr>
<tr>
<td>Aves</td>
<td>Darwin's finches (<em>Geospiza</em>, <em>Camarhynchus</em>, <em>Cactospiza</em>, <em>Platyspiza</em>, <em>Certhidea</em>)</td>
<td>14</td>
<td>yes</td>
<td>1</td>
<td>South America/Caribbean</td>
<td>1.6</td>
<td>2.0–2.3</td>
<td>no</td>
</tr>
<tr>
<td>Insecta</td>
<td><em>Galagete microlepidoptera</em></td>
<td>12</td>
<td>yes</td>
<td>1</td>
<td>South America</td>
<td>2.9–3.7</td>
<td>?</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td><em>Galapaganus weevils</em></td>
<td>10</td>
<td>yes</td>
<td>1</td>
<td>South America</td>
<td>10.7–12.1</td>
<td>7.2</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td><em>Stomion darklings</em></td>
<td>12</td>
<td>yes</td>
<td>1</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td><em>Philatis</em></td>
<td>20</td>
<td>no</td>
<td>1</td>
<td>South America?</td>
<td>?</td>
<td>?</td>
<td>yes</td>
</tr>
<tr>
<td>Genus</td>
<td>Specimens</td>
<td>Median Elevation</td>
<td>Minimum Elevation</td>
<td>Maximum Elevation</td>
<td>Presence in South America?</td>
<td>Presence on Santa Cruz, Santiago, and Isabela</td>
<td>Reference</td>
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<tr>
<td>Oliarus</td>
<td>12</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Pterostichus</td>
<td>12</td>
<td>no</td>
<td>2?</td>
<td>South America?</td>
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<td>? on Santa Cruz, Santiago, and Isabela</td>
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<tr>
<td>Dagbertus</td>
<td>12</td>
<td>no</td>
<td>2?</td>
<td>South America?</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Blapstinus</td>
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<td>South America?</td>
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<td>? yes</td>
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<tr>
<td>Bulimulus</td>
<td>82</td>
<td>yes</td>
<td>1</td>
<td>South America</td>
<td>?</td>
<td>? yes</td>
<td></td>
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\[A\] Microlophus 'Eastern radiation'; \[B\] Microlophus 'Western Radiation'

(Tiaris) and their allies, a group with a wide distribution throughout Central and South America that has undergone a similar radiation in the Caribbean (Burns et al. 2002; Sato et al. 2001). The closest living relatives of Galápagos mockingbirds appear to be those currently found in North America, northern South America and the Caribbean, rather than the geographically nearest species in continental Ecuador (Arbogast et al. 2006). The endemic subspecies of the yellow warbler (Dendroica petechia aureola) probably migrated from Latin America not North America but shows little evidence of divergence within Galápagos (Browne et al. in review). Several other taxa in Galápagos (e.g. flamingos, isopods, snakes, moths) show biogeographical affinities with populations in the Caribbean (Grehan 2001; Thornton 1971) but these proposed affinities have not been tested with molecular phylogenetic analyses.

The geographic origin of very few terrestrial invertebrate groups has been inferred with certainty. This gap in our knowledge is mostly because few detailed data exist on the continental distributions of possible sister species of Galápagos endemics. Schmitz et al. (2007) suggested that the source of the Galápagos microlepidopteran genus Galagete is probably coastal South America but this remains to be tested. Likewise, based on morphological affinities, Galápagos bulimulid snails seem to be most closely related to continental South American bulimulids (Breure 1979). One must be cautious though, as the South American fauna is rather poorly known, especially the invertebrate fauna, and it is possible that sister clades of Galápagos lineages have recently become extinct, undergone range shifts, or simply have yet to be identified. A striking example of
this lack of knowledge is the genus of flightless beetles *Stomion*, whose non-Galápagos closest relative is unknown (Finston and Peck 2004).

**Age of the fauna**

Colonization events have occurred over the last 3-4 Myr during the existence of the present islands, and might have happened earlier when now sunken islands were above sea level. The presence of several drowned seamounts on the Carnegie Ridge east of the Galápagos (Christie et al. 1992) suggests that earlier volcanic islands may have served as stepping-stones for colonization by some of the terrestrial fauna. Because divergence times estimated directly from molecular data require independent calibration, all current dating methods require that specific assumptions be made before inferring any divergence time. These assumptions typically are related either to rates of molecular evolution (molecular clock hypothesis, local clocks models) or to both rates and times (penalized likelihood, Bayesian methods). Among the few Galápagos taxa for which such estimations have been attempted, the divergence time between the mainland ancestor and island relatives seems in some cases to be older than the age of the existing emerged islands. The divergence between *G. chilensis* and the Galápagos tortoise lineage probably occurred 6-12 Myr ago, before the origin of the oldest extant Galápagos Island (Caccone et al. 1999). However the closest extant relative does not necessarily represent the direct ancestor, which could have been, and perhaps was, a now extinct lineage of mainland giant tortoises. Thus, the date of the oldest divergence within the Galápagos lineage probably provides a more accurate estimate of the minimum time since colonization. The
oldest node within the Galápagos tortoise radiation is a little more recent than the oldest islands and the molecular divergence time for the southern Isabela clade is less than 500 000 years, which agrees with estimates of the island’s age (Caccone et al. 2002; Beheregaray et al. 2003b; Beheregaray et al. 2004; Naumann and Geist 2000).

Data from cytochrome b mitochondrial DNA (mtDNA) sequences indicate a time for the divergence of the land and marine iguana lineages of < 10 Myr ago (Rassmann et al. 1997) that precedes the origin of the emerged islands. This suggests that they first colonized now submerged islands and then colonized the current islands as they emerged. Genetic work on both land and marine iguanas is revisiting this dating (Glaberman et al. unpublished; Gentile et al. unpublished), which was based on an overall mtDNA rate of 2% Myr\(^{-1}\) extrapolated from bovid data.

Colonization of the Galápagos by ancestors of Darwin’s finches has been estimated at 1.6-2.3 Myr ago (Sato et al. 2001), well within the age of the present islands.

*Galapaganus* beetles are estimated to have diverged from their closest living, mainland relative over 7 Myr ago, based on both an intrinsic (Sequeira et al. 2008) and extrinsic clock (Sequeira et al. 2000). However, most DNA derived estimates of the timing of inter-island colonization and within-island diversification are consistent with the geological formation of the islands (Sequeira et al. 2008). The earliest divergence time within the endemic Galápagos lepidopteran
Galagete radiation was estimated at 3.3 Myr ago, within the time that the present islands were available for colonization (Schmitz et al. 2007).

For other Galápagos lineages it is not possible to estimate the time of divergence from their closest relatives because there is no reliable molecular clock for the taxonomic group and/or genes in question (Parent and Crespi 2006), or the relevant continental biota is so poorly known that their close relatives cannot be identified (Table 2). Another possibility is that the closest extant relative outside the archipelago does not necessarily represent the direct sister-species of the insular lineage, which could be extinct (Emerson 2002).

**TIMING AND SEQUENCE OF COLONIZATION AND SPECIATION**

The sequence of colonization and speciation within the archipelago can be inferred by comparing phylogenetic information on a lineage with the geological ages of the islands. The most commonly predicted and tested colonization scenario is a movement from old to young islands suggesting that successful intra-archipelago colonization is more likely to occur on recently formed islands with unoccupied niches (Brooks and McLennan 1991). This pattern, often referred to as the progression rule (Wagner and Funk 1995), has been observed in a range of lineages and island systems (Fleischer et al. 1998; Juan et al. 2000). On Galápagos, this implies a diversification sequence from southeast to northwest, paralleling the geological formation of the islands. The Galápagos archipelago is formed by geographical clusters of islands of similar age (White et al. 1993) and therefore if the progression rule applies, species formation should
also occur in “clusters” and not necessarily match perfectly the sequence from old to young islands.

The historical biogeographical scenarios proposed for Galápagos endemic organisms based on phylogenetic hypotheses suggest inconsistent relationships between population diversification and island formation across lineages. The Galápagos giant tortoises lineage is among the best-studied cases of diversification on the islands, with species-level phylogenetic analyses based on multiple mtDNA markers (Caccone et al. 1999, 2002), as well as population-level demographic history and population structure analyses based on both mtDNA (Beheregaray et al. 2004; Russello et al. 2007a) and several microsatellite markers (Beheregaray et al. 2003a,b; Ciofi et al. 2006). Although the lineage of giant tortoises does not follow a strict linear migration from older to younger islands, the species-level phylogenetic pattern uncovered from mtDNA data suggest that giant tortoises diversified approximately in parallel to island geological formation (Caccone et al. 2002; Figure 1B). Furthermore, estimation of population-level genetic diversity, population divergence, and recent demographic history of Galápagos giant tortoises based on mtDNA revealed a pattern of lineage sorting consistent with the temporal formation of the archipelago (Beheregaray et al. 2004). Indeed, tortoise species from older islands have exclusively endemic haplotypes that define divergent monophyletic clades, whereas taxa from younger islands (Isabela, in particular) have haplotypes shared among lineages on different volcanos and that exhibit a recent history of coalescence (Beheregaray et al. 2004). One of the two lineages of
Galápagos lava lizards has also diversified in concordance with the geological formation of island clusters of similar age (Kizirian et al. 2004).

In a population genetic study of 22 populations of marine iguanas (*Amblyrhynchus cristatus*) Rassmann et al. (1997) found that the mtDNA cytochrome b marker had a signature of population differentiation concordant with geographical isolation of populations across the archipelago. However, data from three nuclear microsatellite loci indicated relatively high levels of gene exchange among populations (mean $F_{ST} = 0.1$), contradicting the mtDNA pattern. This discrepancy was suggested as being due to sex-biased dispersal, with higher interpopulation dispersal in males than females (Rassmann et al. 1997). A current analysis of the population structure of marine iguanas based on 13 microsatellite loci, however, depicts a very different picture in which most island populations represent different genetic clusters (Steinfartz et al., 2007, unpublished). A companion analysis using mtDNA control region data reveals three main mtDNA lineages: one on the eastern and central islands, one restricted to the northern and western islands, and one that spans from east to west (Glaberman et al. unpublished). The evolutionary relationships among these groups are unclear because of shallow levels of divergence, as in the cytochrome b data. Thus the patterns of island colonization are not as well defined as for the tortoises. It is clear, however, that the lineage occupying the northern and western regions of the archipelago is the product of a recent expansion based on a unimodal distribution of pairwise differences between
individuals as well as a high abundance of low frequency haplotypes. This conclusion fits with the overall younger age of the western islands.

Galápagos mockingbirds were inferred to follow the progression rule (Arbogast et al. 2006). In contrast, Darwin’s finches show limited evidence of diversification closely associated with the geological formation of the islands. Grant and Grant (2008) suggested that the radiation of finches unfolded with an increase of types of species with change in climate and increased habitat diversity, associated with an increased number of islands. However, there is no clear evidence suggesting that the number of islands has increased through time. Nevertheless, divergence time within this lineage is not associated with age of the islands where the species are found, perhaps a result of considerable inter-island dispersal.

A progression rule pattern has been inferred for Galápagos bulimulid land snails (Parent and Crespi 2006; Figure 1C). By contrast, Galápagos flightless weevils (genus Galapaganus) do not follow the progression rule. Phylogenies based on mtDNA (Sequeira et al. 2008) or mtDNA combined with nDNA (Sequeira et al. 2008) indicate that colonization and diversification in this lineage are linked to geological history in a more complex scenario. For example, G. williamsi, which occurs only on Isabela Island, coalesces at the deepest node of the phylogeny, while species from the oldest islands of Española and San Cristobal are shallower in the phylogenetic tree. The microlepidoptera genus Galagete (12 species) is another example where the progression rule fails to explain phylogeographical patterns of diversification. Species connecting at the
deepest node are not found on the oldest islands and the sequence of colonization and speciation does not follow the geological formation of the islands, but rather follows a stochastic colonization scenario (Schmitz et al. 2007).

In general, the diversification sequence in Galápagos terrestrial faunal groups seems to follow the progression rule, with the more vagile lineages constituting the exceptions (Galápagos finches, Galagete lepidopterans, Galapaganus weevils).

THE GEOGRAPHIC MODE OF SPECIATION

In an insular context, speciation can happen between or within islands. If colonization rate between islands is high and individuals interbreed among island populations, these populations will effectively be part of the same gene pool. However, ongoing but low levels of gene flow do not constrain local adaptations of Darwin’s finch (Petren et al. 2005) or of lava lizard (Jordan et al. 2005) populations on islets peripheral to a main island. Lineages that have recently colonized the islands might not have had the time to differentiate within the archipelago. The Galápagos hawk is thought to have diverged from its continental sister clade less than 300 000 years ago, and populations from different islands are genetically very similar (Bollmer et al. 2006). However, mitochondrial and minisatellite data indicate a general pattern of rapid population expansion followed by genetic isolation of hawk populations suggesting that this lineage is in the earliest stages of further divergence (Bollmer et al. 2006).
Genetic analysis of the Galápagos hawks’ ischnoceran louse may shed further light on the cryptic evolutionary history of its host (Whiteman et al. 2007).

**Speciation between islands**

If colonization rate between islands is low, one would expect to find a pattern of genetic structuring among island populations, potentially leading to differentiated species that are single-island endemics. Rassmann et al. (1997) initially proposed that marine iguana populations across the archipelago showed very little divergence but ongoing work suggests that most island populations have considerable levels of genetic differentiation (Steinfartz et al. unpublished). Galápagos giant tortoises have diverged to form 15 taxa, 11 of which are extant. Their taxonomic status is still in flux. Some authors consider them different subspecies (Pritchard 1996), while others, including me, refer to them as species because of their genetic distinctiveness (Powell and Caccone 2006; Russello et al. 2007a). The islands of Española, San Cristóbal, Pinzón, Santiago and Pinta each have only one named species; San Cristóbal had two distinct populations but one is now extinct (Caccone et al. 2002, Russello et al., 2007a). Isolation among islands has promoted speciation in the two independent lineages of lava lizards (Benavides et al. 2007; Kizirian et al. 2004). Using microsatellite markers, Jordan and Snell (2008) have tested the hypothesis that genetic drift in isolation has caused the differentiation of Galápagos lava lizards on 12 islets that were probably connected to the larger island of Santa Cruz during the late Pleistocene. They found strong genetic differentiation among island populations, a positive correlation of genetic diversity with island size, no relationship between genetic
and geographical distance and a strong negative correlation between heterozygosity and measures of genetic differentiation. They concluded that overwater dispersal is rare and further differentiation of lava lizards may be prevented by island fusion during periods of lower sea level. The land iguanas (Rassmann 1997; Snell et al. 1984) and the mockingbirds (Arbogast et al. 2006) also have at most one species per island but the relative contributions of selection and drift to differentiation remain unclear. Recent work on the two Galápagos land iguanas, Conolophus subcristatus and C. pallidus (Gentile et al. unpublished), based on mtDNA sequence data, confirms the genetic discontinuities between the populations of C. subcristatus from the western (Fernandina and Isabela) and central (Santa Cruz, Baltra, Seymour and Plaza Sur) islands. This pattern seems to parallel that of their sister taxon, the marine iguanas, in which there is a clear genetic differentiation between eastern and western islands (Glaberman et al. unpublished; Steinfartz et al. unpublished). The level of differentiation between the two C. subcristatus groups is only slightly lower than that between the two species (C. subcristatus and C. pallidus).

Darwin’s finches show limited evidence of speciation on different islands. The larger central islands contain largely the same suite of 9-10 species, many of which also occur on several smaller and peripheral islands (Grant 1999). Several phylogenetic analyses have revealed little resolution among six species of tree finches and five species of ground finches (Freeland and Boag 1999; Petren et al. 2005; Sato et al. 2001; Figure 1D). A combination of factors is probably responsible for this, including the recent origin of many species, the
homogenizing effects of hybridization (Grant et al. 2004) and inter-island dispersal (Petren et al. 2005). Allopatric speciation has been inferred between the small (G. scandens) and large (G. conirostris) cactus finches, although both populations of large cactus finches are more closely related genetically to different populations of small cactus finches than they are to one another. The warbler finches (C. olivacea and C. fusca) were believed to be the same species until studies showed different subsets of islands harbour two different species that are more genetically distinct than any other species in the entire group (Petren et al. 1999; Tonnis et al. 2005). There is pronounced morphological divergence between island populations of several species of Darwin’s finches. Some of these differences are paralleled by substantial genetic differences (G. difficilis, G. scandens/G. conirostris), while in other cases, genetic differences are minimal or are not correlated with morphological or geographical divergence (G. fortis/G. magnirostris; Petren et al. 1999, 2005). Recent studies suggest inter-island movements of Darwin's finches may not be as rare as previously suspected (Petren et al. 2005; Tonnis et al. 2005). Morphological differences among island populations may therefore not be as much evidence of isolation from gene flow as previously thought, but rather evidence of differing selective regimes and local adaptation in the presence of gene flow. Theoretically, low levels of gene exchange may promote local adaptation under some conditions (Whitlock and Phillips 2000). The warbler finches (Certhidea fusca and C. olivacea) appear to conform to an allopatric mode of speciation, being clearly separated on different subsets of islands. However, the occurrence of some very
widely distributed C. fusca haplotypes suggests that speciation was not driven by geographic isolation and limited dispersal. Other factors, such as habitat selection or natural selection, appear to have limited genetic exchange and allowed speciation to occur (Tonnis et al. 2005).

Terrestrial invertebrates often have low dispersal ability (especially flightless groups), so single-island endemics would be expected. The flightless beetle genus Neoryctes includes four single-island endemic species, each restricted to the moist highland zone of the island on which it occurs (Cook et al. 1995). The microlepidopteran genus Galagete has a complex pattern of diversification. Some of the species are single-island endemics, others are found on multiple islands, and two occur on 10 and 11 islands (Schmitz et al. 2007). The inference of the geographical mode of speciation from the phylogeny is problematic, mainly because of the wide distribution of most of its taxa. Little is known of the ecology of Galagete (Schmitz et al. 2007), which makes it difficult to infer ecological or habitat shifts.

In sum, patterns of speciation between islands tend largely to reflect a combination of dispersal capability and habitat suitability. Less vagile taxa show more pronounced genetic structure among island populations. They are more prone to longer periods of absence from suitable islands as a result of extinction. More vagile taxa show little differentiation among subsets of islands, but may be absent from islands with apparent suitable habitat. Several taxa show non-overlapping distributions among subsets of islands. In these cases, co-occurrence may be limited by dispersal, but also by the presence of other similar
species. The possibility of occasional gene exchange between islands that would create loose evolutionary connections among differentiated populations remains largely unexplored.

**Speciation within islands**

In addition to island isolation, other physical features of islands can influence the mode of diversification in an archipelago. Island area and elevation are positively correlated in many island groups and are prime determinants of moisture and island habitat diversity. Greater habitat diversity provides the potential for within-island diversification, but also increases the chances that a new colonist will find a suitable habitat. Once a particular lineage has colonized an island, it can speciate within that island either by adapting to different habitats, commonly referred to as ecological speciation (Schluter 2000), or by allopatric speciation if an island is large enough for populations to become isolated by geographical or topological means (volcanos, barren lava, inhospitable habitat, etc.). The strength of the effect of island area depends not only on geography and topology but also on the dispersal ability of the organisms, being strongest for animals with low dispersal ability and specialized ecological requirements. The low dispersal ability of the giant tortoises is a good example, with four species on Isabela, and one on each major volcano. Isabela probably started as separate island volcanos that then merged. The volcanos are now separated by lava fields, which are a formidable obstacle to tortoise movement. It is therefore possible that their populations were isolated for 400,000 years, then experienced limited mixing (over the lava fields) for the past 100,000 years once the volcanos were
connected. MtDNA and nDNA data support the genetic distinctiveness of the three species living on the central and northern volcanos (Alcedo, Darwin, Wolf) (Beheregaray et al. 2004; Caccone et al. 2002; Ciofi et al. 2006, in press; Russello et al. 2007a). However, genetic divergence and similarities among populations on the southern volcanos (Cerro Azul, Sierra Negra) do not support the taxonomic subdivisions (Ciofi et al. 2006) that were previously suggested based on morphology (Fritts 1984; Pritchard 1996). The taxonomic assignment of tortoise populations on Santa Cruz has been contentious. There are at least three lineages that are as genetically distinct from each other as from lineages on other islands, being connected only through the deepest nodes in the molecular-based tree (Russello et al. 2005). Shell shape (domed vs. saddlebacked carapace) is the most distinctive phenotypic difference among giant tortoise populations, and has been associated with habitat differences. The ancestral state of this character in Galápagos is most probably domed (Caccone et al. 2002, Russello et al. 2005) and recent unpublished DNA data from extinct taxa suggest that the saddleback morphology arose independently more than once (Russello et al., 2005; Poulakakis et al. unpublished). However, the role of selection and developmental constraints in the diversification of Galápagos giant tortoises remains to be clearly determined. Research using novel field-friendly 3D imaging techniques and morphometric methods is addressing such issues (Chiari et al. in press).

Within-island divergence has been suggested as a mode of speciation for Darwin’s finches. Divergence occurs along an elevational gradient in small
ground finches on Santa Cruz (Kleindorfer et al. 2006). *Geospiza conirostris* on Genovesa showed an ephemeral tendency to mate assortatively according to similarities in beak shape and song (Grant and Grant 1989). Assortative mating according to beak size occurs within populations of medium ground finches on Santa Cruz, although this tendency appears to be significantly reduced by human activity (Hendry et al. 2006) and wet conditions, when competition is also presumably reduced (Huber et al. 2007). Sympatric speciation has yet to be convincingly demonstrated in birds on islands such as the Galápagos that are smaller than 10 000 km\(^2\) (Coyne and Price 2000), and so it seems unlikely that any of these situations will result in speciation. Testing for sympatric speciation in Darwin’s finches is hindered by a lack of phylogenetic resolution, which is likely the result of inter-island movements (Petren et al. 2005).

Lack (1947) proposed a model for within-island species buildup of Darwin’s finches that relied on limited dispersal between islands to create initial differences in allopatry, followed by rare colonization, establishment of secondary contact and further differentiation in sympathy as a result of resource competition and character displacement. This model has in principle been adapted to account for faunal community buildup in a wide variety of systems including Galápagos taxa. Despite the apparent lack of isolation between finch populations on different islands (Petren et al. 2005), Lack’s model of faunal buildup may still apply. Immigrants that normally interbreed with residents may on rare occasions form distinct, reproductively isolated populations, depending on environmental circumstances and the combination of traits possessed by immigrants and
residents (e.g. Grant and Grant 2008). Some degree of geographic separation is probably more likely to establish initial correlations between ecological and mate preference traits among incipient species.

In the beetle genus *Galapaganus*, diversification was within island on San Cristobal, one of the oldest islands, and within-island speciation could not be rejected for the Santa Cruz clade; in both cases, shifts to highland habitats seem to have evolved independently (Sequeira et al. 2008). Although this habitat shift is consistent with selection contributing to within-island diversification, it is not known if it is associated with a corresponding adaptive shift in phenotype. Notably, Sequeira et al. (2008) inferred that speciation did not occur within the younger island of Isabela, where diversity is the result of between island colonization. Similarly, Parent and Crespi (2006) found a combination of within and between-island speciation in Galápagos bulimulid snails. Polyphyletic land snail assemblages were found on islands centrally located in space and time (Isabela, Santa Cruz, Pinzón, Santiago), whereas the more isolated islands of Española, San Cristobal and Floreana (again in both space and time) were occupied mostly by monophyletic assemblages resulting from within-island diversification. Some bulimulid species occur in sympatry and may have adapted to the different habitats where they occur (Coppois and Glowacki 1983; Parent and Crespi unpublished). However, the relative importance of drift and selection in within-island diversification of this group is not known.

The beetle genus *Stomion* has also diversified both within and between islands (Finston and Peck 2004). Each population contains only one morphi-
species, and even on large islands such as Santa Cruz and Isabela where two or three species occur, they never occur in sympatry. Finston and Peck (2004) therefore suggested that speciation happened in allopatry. *Stomion* species have low vagility, even at very small local scales (Finston et al. 1997), which should promote micro-allopatric speciation in agreement with the scenario proposed by Finston and Peck (2004). No evidence of ecological or morphological adaptation has been found in the genus (Finston and Peck 1997, 2004), strengthening the idea that diversification happens via micro-allopatric speciation within islands following between-island colonization. Peck (1990) has suggested that troglobites (cave-dwelling invertebrates) might provide good examples of diversification within islands. There are several examples of a cave-dwelling form that has a sister species living nearby outside the cave (Peck 1990; Peck and Finston 1993) but without a molecular phylogeny I cannot tell how these species were formed.

In sum, the clearest cases of within-island speciation occur in taxa with low vagility and on large islands with diverse habitats. Isabela, with several volcanos separated by recent lava flows, is especially conducive to within-island diversification in the case that a lineage reached the island when the volcanos were connected. However, it is difficult In some cases, subsets of taxa have found their way to other islands, making it more difficult to distinguish within-island speciation from multiple between-island speciation events. For more vagile taxa, speciation between islands seems more likely, given the extreme habitat
differences among islands; but within-island processes are often difficult to rule out, and in either case, ecological specialization can play an important role.

**Dynamics of species diversification**

The number of species on an island depends on speciation, colonization and extinction rates. The three processes are affected by biogeography (island area, elevation, insularity) and ecology (habitat diversity). However, the species diversity equilibrium is dynamic and changes through time because islands themselves change (Whittaker et al. 2008). Moreover, a delay is expected between the time an island becomes available for colonization and speciation, and the time equilibrium is reached. For example, Parent and Crespi (2006) found that the younger islands of Fernandina and Isabela had fewer land snail species than expected based on their area, elevation and insularity. Similarly, there are only two species of flightless *Galapaganus* beetles on Isabela, whereas San Cristobal, Santa Cruz and Floreana each have three species (Sequeira et al. 2008). In contrast, there are more species of the moth genus *Galagete* on Isabela and Fernandina (eight each) than any of the older islands (Schmitz et al. 2007). This contrast among lineages might be a reflection of their different dispersal capacity or the stochasticity of inter-island dispersal in some lineages.

Furthermore, the geographical configuration of the whole archipelago is dynamic, and there have been changes in island number, size and isolation due to sea level variation on various time scales. On a short time scale (from now back to the Pleistocene), it appears that changes in sea levels created terrestrial avenues for gene flow during glacial periods that disappeared when sea levels
rose. The effect this sea level change can have on species diversity on Galápagos has been relatively unexplored, but Jordan and Snell (2008) suggested that lava lizard diversity might have increased if islands had been isolated for longer periods. Environmental changes over the short term can influence the evolutionary trajectory of species (Grant 1999; Grant and Grant 2004), so it is also likely that longer-term climate changes have had an important effect on the evolution and extinction of species that remains largely unknown. A sobering example comes from a recent study on El Niño effects on levels of genetic diversity in Galápagos marine iguanas (Steinfartz et al. 2007), in which even species with large populations sizes may suffer dramatic collapses in genetic diversity when populations are subject to frequent and possibly recurrent environmentally devastating events.

**FUTURE PROSPECTS FOR EVOLUTIONARY RESEARCH ON GALÁPAGOS ISLANDS**

Despite the large volume of evolutionary research that has been undertaken in Galápagos, several species groups remain relatively unstudied in terms of diversification within Galápagos and their relationship to possible sources of colonists (Table 2). Most phylogenetic studies of Galápagos organisms have been based on mtDNA, sometimes in combination with one or a few nuclear markers. MtDNA has desirable properties for phylogenetic inference (Avise 2000) but the likelihood that a single locus reflects the history of species and populations decreases when divergence occurs rapidly and recently (Hudson and Coyne 2002), as for much of the diversification in the Galápagos. Multi-locus
studies of sequence variation, such as intron locus variation (Edwards et al. 2007), offers some promise for providing more robust estimates of phylogenetic and phylogeographic divergence. However, in the only case in which they have been used, giant tortoises, they were not helpful (Caccone et al. 2004). For more vagile taxa such as winged insects and birds, multilocus microsatellites now offer a powerful means to estimate even low levels of gene flow, admixture and population divergence.

Ecology and habitat specialization can be critical factors in speciation both within and between islands. Phylogenetic studies can be broad but are limited in their ability to reveal speciation processes. The number of studies of natural history, ecology, evolution and behaviour is small and limited in scope. Field studies are limited spatially and temporally by logistics and funding. Thus a great deal remains to be revealed about speciation in Galápagos animals. One need only consider a single field research program (Grant 1999; Grant and Grant 2008) to see the magnitude of contributions to the study of evolution and speciation that are possible.

CONSERVATION AND THE ROLE OF EVOLUTIONARY GENETICS IN GALÁPAGOS RESEARCH

While providing ecologists and evolutionary biologist with “natural laboratories”, island biotas are also very often in danger of going extinct. This is due both to their generally small population sizes associated with small geographic ranges, as well as to the relatively simple ecosystems they present (which is why they are so valued by biologists). Island ecosystems are therefore particularly
susceptible to catastrophic disturbances by invasive species. Understanding the phylogenetics and biogeography of island species allows greater insights into setting conservation priorities and enacting rational conservation policies, as illustrated in the examples below on Galápagos tortoises (Powell and Caccone 2008).

Phylogenetic analysis detected previously unrecognized taxa that deserve special conservation attention (Russello et al. 2005) and assignment of individuals of unknown origin (zoo animals) to their correct location (Burns et al. 2003; Russello et al. 2007b). Such studies also allowed identification of genetic lineages in natural populations that were previously thought to be extinct or nearly extinct (Russello et al. 2007a; Poulakakis et al. unpublished). Understanding the genetics of endangered island biota may also aid in designing more rational recovery efforts such as captive breeding/repatriation programs (Milinkovitch et al. 2004, 2007).

Over the last century, the human population of Galápagos has risen dramatically. Prior to 1900 fewer than three hundred people lived in the islands (Lundh 2001). There are now nearly 30 000 residents on four inhabited islands and more than 130 000 visitors each year (Watkins and Cruz 2007). The human population boom has caused a marked increase in introduced species and diseases (Fessl and Tebbich 2002; Gottdenker et al. 2005; Wikelski et al. 2004). Several populations of birds have recently become extinct (Grant 1999; Grant et al. 2005) and several species are now threatened or on the brink of extinction (e.g. Dvorak et al. 2004).
The near future will determine whether the Galápagos will provide a model for the deterioration of an island fauna in our time, or whether the brighter prospect of effective management and persistence will be realized so that future generations can continue to study the processes of evolution in action.
CHAPTER 2: SEQUENTIAL COLONIZATION AND DIVERSIFICATION OF GALÁPAGOS’ ENDEMIC LAND SNAIL GENUS *Bulimulus* (GASTROPODA, STYLOMMATOPHORA)

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ABSTRACT

Species richness on island or island-like systems is a function of colonization, within-island speciation, and extinction. Here I 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. I use molecular phylogenetics (based on COI and ITS1 sequence data) to infer the diversification patterns of extant species of *Bulimulus*, and multiple regression analyses 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 I 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 inter-island 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 harbouring 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.

**Keywords**: Adaptive radiation, colonization, Galápagos, land snails, speciation, species richness.

**INTRODUCTION**

The theory of island biogeography as initially proposed by MacArthur and Wilson (1963, 1967) stipulated that variation in rates of inter-island colonization and extinction generates and maintains 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 inter-island 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 such islands (MacArthur and Wilson 1963, 1967; 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 since older islands have had more time to accumulate species through within-island speciation and colonization, but it also might reduce species diversity since 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 inter-island 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 inter-island speciation. In this study, I first evaluate the relative importance of biogeographical and ecological factors in generating overall island species richness. I then use a phylogeny to partition species diversity that presumably results from inter-island colonization, and species diversity resulting from within-island speciation, and I evaluate the relative importance of biogeographical and ecological factors in these two different processes. I predict that island area, elevation, and habitat diversity will promote overall island species diversity through their direct and indirect positive effects on inter-island colonization and within-island speciation. Furthermore, I 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; Grant and Grant 2002; Lack 1947). 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. Finston and Peck 2004; Grant 1986; Jackson 1993; Rassmann 1997). 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, behaviours, and phylogenetic relationships can reliably be inferred.

The geological history of the Galápagos archipelago is relatively well understood (Cox 1983; Geist 1996; Hall 1983; Nordlie 1973; Swanson et al. 1974; White et al. 1993), 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 [myrs] old); Santa Cruz, Floreana, Pinzon and Santiago islands form a middle-aged group (0.77-1.52 myrs old), and finally the most recent group of islands includes Isabela and Fernandina islands (less than 0.5 myrs old), with the volcanically active Fernandina Island presumably located over the hot spot (Fig. 2 this chapter; White et al. 1993).

Well-studied radiations on Galápagos comprise relatively few species (16 or fewer), which has constrained analyses of the biogeographical and ecological processes involved in species diversification. By contrast, more than 80 endemic species and subspecies of land snails in the genus *Bulimus* have been described from Galápagos (Broderip 1832; Chambers 1986; Coppoïs 1985; Dall 1896; Dall and Ochsner 1928; Pfeiffer 1846; Sowerby 1833; Sowerby 1833-1939;
The different species vary in shell size, shape, colour, and colour pattern, and this 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 (van der Werff 1979; Wiggins and Porter 1971), 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 (species with slender shells being found at lower elevations) or microhabitat (species with slender shells are found in more exposed open habitats) 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 (chapter 4), 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 belong 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). I 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 are the only group for which there is evidence for more than one colonization event. This is not too surprising since 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, I assume that the same is true for Galápagos bulimulid 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: FA=Fernandina, AL=Alcuedo, 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 SH likelihood ratio tests (Shimodeira and Hasegawa, 1999). Three successively more constrained colonization scenarios (represented as A, B, and C; see text) are tested against the unconstrained (best) ML tree.
In this chapter I generated molecular-systematic data to reconstruct a phylogeny for the bulimulid land snails of the Galápagos. I 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, I 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). I tested specifically for monophyly of island assemblages, and infer the number of species on each island that are the result of inter-island colonization vs. within-island speciation based on our molecular phylogeny. I then investigated the relative contribution of the biogeographical and ecological factors to each of these two diversifying processes. I 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, I provide evidence that different biogeographical factors have distinct relative importance depending on their effects on these processes. I 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 on island systems in general.

MATERIALS AND METHODS

Field sampling
Snail specimens were sampled from as many different locations as possible throughout all major islands on the archipelago that are known to harbour extant populations of *Bulimulus* snails between September 2000 and April 2005. Of the 71 described Galápagos bulimulid land snail species (Chambers 1991; Coppois 1985), 26 described species (including two likely extinct species) and 9 possibly new species (currently being described) were included in our study. I 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 2003), so despite being
incomplete, I 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 A.

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, I followed a CTAB/phenol/chloroform extraction protocol (Richard 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 I (COI) primers 1718 and 2191 (Simon et al. 1994), and the combinations of internal transcribed spacer region 1 (ITS1) primers SN-18S-F11 (5' -
TTCCGTAGTGAACCTGCGG-3') or SN-18S-F18 (5'-
TAACAAAGTTTCCGTATGTGAA-3') with SN-5.8S-R28 (5'-
GCGTTTCTTCATCGATGC-3') or SN-5.8S-R87 (5'-
GTGCGTTGAAATGTGAGTTCAA-3') designed for this study. PCR 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 of 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. I 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. I 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. I 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 $5^{th}$ character. Gaps that spanned more than one base pair position were re-coded 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 ML phylogenetic reconstructions. ML 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 maximum-likelihood 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 ($\gamma = 0.9599$) for COI. Again, support for branches was assessed by bootstrap analyses (200 replicates). I 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 4 chains running simultaneously, three heated at the default temperature and one cold, and a sampling frequency of 100 generations. I 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 volcanos 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 volcanos forming Isabela. 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, I considered bootstrap support and the posterior probabilities from the Bayesian MCMC 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 I 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 data sets 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 non-constrained 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 (towards 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 from and 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.

As this method is obviously sensitive to the degree of taxon sampling and the topology of the tree, I used it only to infer the overall colonization direction in this group. I 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), Geist (1996), and White et al. (1993). 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, I 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. 2) were tested using SH LRTs against the best (unconstrained) ML tree. In scenario A, I 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 four islands are partly overlapping.

To further evaluate the general colonization sequence from old to young islands, I 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. I used the average depth for each island assemblage that comprised multiple clades, such that each island represented an independent data point. I 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.

**Biogeographical and ecological correlates of species diversity**

I 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 fieldwork as well as from other sources (Chambers 1986; Chambers 1991; Coppois 1985; Dall 1896; Dall and Ochsner 1928; Odhner 1951; Smith 1972; Smith 1974; Vagvolgyi 1977). 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 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 I 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 3).

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, since 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 intercorrelation among the predictor variables. Multiple regression analysis was first run on a dataset including all islands and volcanos for which data were available.
(n = 26). I 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). I used the best ML phylogenetic tree based on the combined datasets to determine the number of within- and between-island speciation events. I followed Losos and Schluter (2000) and assumed that the presence of sister taxa on an island resulted from within-island speciation, and I 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 I need a phylogenetic hypothesis to infer the presumed process of speciation of species, I included only species that are represented in our phylogeny for this part of the analysis. I also included species that are sole inhabitant of islands, since they had to arise by between-island colonization. However, since I 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, I ran this part of the analysis with a reduced dataset (n=14 islands). In order to take into account the uncertainty in estimating the number of species resulting from within-island speciation and inter-island colonization due to the uncertainty of the phylogeny, I calculated the number of within-island speciation and inter-island colonization events for each of
the 200 best trees (with highest log likelihood scores) obtained from the Bayesian search. I 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.
Table 3. 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 = inter-island colonization; E = extinction), along with the direction of the predicted relationship with an increase in the value of the predictor (+ for positive; - for negative). §This effect is likely to become less significant as niches on islands are filled by colonists and newly formed species.

<table>
<thead>
<tr>
<th>Species Richness Predictor</th>
<th>Description</th>
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<td>Direct Effect(s)</td>
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| 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 colonization rate by plants (S-, C-) |
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. 3A, 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, I used the number of 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. 3) 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 since 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. 3A, B), so I used the best ML tree resulting from the combined datasets in the rest of our analysis.
Figure 3. 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 labelled with both the species designation and the island or volcano where it comes from, island abbreviations are the same as in Figure 2. 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).
Tests of island monophyly

SH 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 tests for the remaining island clades for which ML and MP bootstrap values did not (strongly) support monophyly (Fig. 3, Table 4). 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. SH tests do not provide any support for the monophyly hypothesis for the other volcanos and islands (Table 4).

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 non-monophyletic 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 4).

The posteriori probability of monophyly for the bulimulid assemblages of Wolf and Sierra Negra volcanos on Isabela Island, as well as for the assemblage
Table 4. 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 SH 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 *. N/a=not applicable because group is monophyletic; n.s.=non significant.

<table>
<thead>
<tr>
<th>Island or volcano</th>
<th>ML</th>
<th>MP</th>
<th>MCMCMC</th>
<th>PB</th>
<th>SH test</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Cristobal (SL)*</td>
<td>&lt;50</td>
<td>67</td>
<td>79</td>
<td>n.s.</td>
<td>n/a</td>
</tr>
<tr>
<td>Española (ES)*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Floreana (FL)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.02</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Santa Cruz (SC)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Pinzon (PI)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Santiago (SA)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Wolf (WF)*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Darwin (DA)*</td>
<td>&lt;50</td>
<td>88</td>
<td>94</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Alcedo (AL)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Sierra Negra (SN)*</td>
<td>82</td>
<td>95</td>
<td>100</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Fernandina (FA)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
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 less than 50%. These results support the results of the SH and parametric tests (Table 4).

**Colonization sequence**

I 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. 2). 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.**——I 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. 2). 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 follow colonization scenario B was worse than the best unconstrained tree, I did not test the significance level of, and rejected the more constrained scenario C. I 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 scenarios I tested were found to be significantly worse than the best unconstrained tree.

Therefore, if I 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 age of the islands.

I 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 Fig. 4. 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. 4). Island area had a significant positive indirect effect (indirect coefficient, $i = 0.103$, standard error of $i$, s.e. = 0.056) through its positive (but non significant) 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 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 ($i = -0.531$, s.e. = 0.173, $i = -0.314$ s.e. = 0.138, respectively).

Island elevation has a significant positive indirect effect ($i = 0.350$, s.e. = 0.175) through its positive significant direct effect on plant diversity on islands ($d = 0.727$, $P < 0.01$) (Fig. 4).

**Multiple regression analyses.**---Island elevation, island area, and habitat diversity, three predictors of diversity which are indirect measures of habitat complexity or niche space, are inter-correlated ($r > 0.53$). 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 was 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 5). When island elevation is replaced
by island area or island habitat diversity (keeping island age in the model), the models explain lower or 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 5 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).

I 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 results are not biased by the presence of several zero values in our dataset. 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
Figure 4. 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.
significant positive effect on the number of species per island, and island
insularity has a significant negative effect (Table 5). 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 5).

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
Figure 5. 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.
Table 5. 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: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

<table>
<thead>
<tr>
<th>Model</th>
<th>Independent variables</th>
<th>n</th>
<th>β</th>
<th>s.e. of β</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall species diversity</td>
<td>Island elevation***</td>
<td>26</td>
<td>0.642</td>
<td>0.144</td>
<td>4.48</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.440***</td>
<td>Island age**</td>
<td></td>
<td>0.762</td>
<td>0.221</td>
<td>3.46</td>
</tr>
<tr>
<td>Overall species diversity</td>
<td>Island area*</td>
<td>26</td>
<td>0.136</td>
<td>0.056</td>
<td>2.46</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.169*$</td>
<td>Island age*</td>
<td></td>
<td>0.559</td>
<td>0.263</td>
<td>2.13</td>
</tr>
<tr>
<td>Overall species diversity</td>
<td>Island habitat diversity***</td>
<td>26</td>
<td>0.539</td>
<td>0.122</td>
<td>4.42</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.433***</td>
<td>Island age</td>
<td></td>
<td>0.256</td>
<td>0.187</td>
<td>1.36</td>
</tr>
<tr>
<td>Between-island speciation</td>
<td>Island insularity*</td>
<td>14</td>
<td>-0.747</td>
<td>0.307</td>
<td>-2.43</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.307*$</td>
<td>Island area*</td>
<td></td>
<td>0.306</td>
<td>0.104</td>
<td>2.94</td>
</tr>
<tr>
<td>Within-island speciation</td>
<td>Island habitat diversity*</td>
<td>14</td>
<td>0.289</td>
<td>0.118</td>
<td>2.44</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.276*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
overall island land snail species richness, I found that it does contribute to that 
fraction of the species richness presumed to result from inter-island colonization. 
Notably, speciation due to colonization (between-island speciation) is mainly a 
function of island area and island insularity whereas species richness resulting 
from within-island speciation is largely determined by island habitat diversity.

DISCUSSION

I 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, which 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. 3). These two groups still hold when I 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. *B. 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 on 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 inter-island 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 volcanos where inter-island colonization is rare. Very young islands have had less time for repetitive colonization events to occur, and isolated islands might have lower inter-island colonization rate. Thus, I 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 volcanos), and Sierra Negra (two species) volcanos on Isabela Island, among the youngest volcanos 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 harbour 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 harbouring 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 volcanos referred to in this study), two islands well isolated north-west of the rest of the archipelago (located over 250 km from the centre of the archipelago, therefore falling outside of the Galápagos map depicted in Fig.2) 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 non-random 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, (Beheregaray et al. 2004; Caccone et al. 2002)), 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 non-monophyletic island assemblages if niche space is broad enough to support multiple species. The importance of intra-island 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 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 (Cox 1983; Geist 1996; Nordlie 1973; White et al. 1993). 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 island-like systems (Chiba 1999a; Holland and Hadfield 2004; Roderick and Gillespie 1998).

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 (Kizirian et al. 2004; Wright 1983) 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 (Beheregaray et al. 2004; Caccone et al. 2002) found that inter-island 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 neighbour 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 micro-allopatric speciation (Arnaud et al. 2001; Davison 2000; Douris et al. 1998; Giokas and Mylonas 2004; Guiller et al. 2001; Pfenninger et al. 1996; Pfenninger and Posada 2002). However, they can potentially achieve long distance colonization (such as inter-island colonization) by passive dispersal (Carlquist 1974). I can only speculate about mechanisms of dispersal, but among the most likely ones there is transport by birds travelling 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 inter-island movements seem to be influenced, at least partly, by the distance between islands. Indeed, bulimulid land snail inter-island movements are apparently much more common on islands that have close neighbours, 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 volcanos, 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, I spent considerable time on Isabela and Fernandina islands (more than doubling the collecting effort on these islands if I 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. I did not include collecting effort as an independent variable in our regression analysis because I 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 volcanos 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 Fig. 5. Since 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 provide 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 (Karr 1971; MacArthur 1965; MacArthur 1969; Murdoch et al. 1972; Pianka 1966).

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.

I analyzed the roles of colonization and within-island speciation in bulimulid diversity by partitioning the overall species diversity into species resulting from inter-island 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 inter-island 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 inter-island colonization, followed by intra-island 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 intra-island speciation whereas half or more of species diversity on mid-aged and young islands is the result of inter-island 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 1996a], or Cerion land snails [Goodfriend and Gould 1996; Gould and Woodruff 1990]), 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 and 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.
CHAPTER 3: ECOLOGICAL OPPORTUNITY IN ADAPTIVE RADIATION OF GALÁPAGOS ENDEMIC SNAILS

ABSTRACT

The classic evolutionary hypothesis of ecological opportunity proposes that both heterogeneity of resources and freedom from enemies promote phenotypic divergence as a response to increased niche availability. Although phenotypic divergence and speciation have often been inferred to be the primary consequences of the release from competition and predation that accompanies a shift to a new adaptive zone, increased phenotypic variation within species is expected to represent the first stage resulting from such a shift. Subsequently, a lineage diversifies, speciates, and evolves to utilize a broader range of resources, and interspecific competition increasingly constrains within-species phenotypic variation. Using measures of intraspecific morphological variation of 30 species of Galápagos endemic land snails, I show that the number of local congener (an index of stronger interspecific competition) is associated with reduced intraspecific phenotypic variation, and that the number of local plant species (an index of resource heterogeneity) is linked with higher intraspecific phenotypic variation in a phylogenetically-controlled framework. Ecological opportunity (the combination of decreased competition and increased resource heterogeneity) therefore predicts the extent of intraspecific phenotypic variation, explicitly linking the role of competition from congeners and the heterogeneity of
resources to the extent of intraspecific phenotypic divergence as adaptive radiation proceeds.

**Keywords**: Adaptation, diversification, ecological release, intraspecific variation, shell morphology

**INTRODUCTION**

Adaptive radiation, the evolution of ecological diversity in a rapidly-multiplying clade (Schluter 2000), is often characterized by species using a broader range of resources than their less-diversified sister lineages (Lack 1947; Mayr 1963; Schluter 1988). This observation has been interpreted as the result of ecological release, whereby a lineage invading a new adaptive zone encounters a wealth of unexploited resources and relief from competition and predation, and this ecological opportunity is thought to promote phenotypic divergence and speciation in the lineage colonizing the empty or under-exploited adaptive zone (Cox and Ricklefs 1977; Lack 1969; MacArthur and Wilson 1967; Schluter 1988; Schluter 2000; Simpson 1953; Van Valen 1965). If a species is released from interspecific competition by colonizing a vacant adaptive zone, competition among individuals for limiting resources should promote divergence in phenotype and resource use, so that intraspecific phenotypic variation accumulates (Robinson and Wilson 1994; Svanback and Bolnick 2007). As diversification proceeds and the lineage splits into multiple species increasingly specializing on and utilizing more of the available resources, both interspecific and intraspecific competition should constrain phenotypic divergence between individuals, so that overall diversification slows down as species accumulate and specialize on
available resources (Arthur 1987). Under this scenario, species should exhibit a maximum degree of intraspecific phenotypic variation due to diversifying selection when they first invade an empty adaptive zone, when the phenotypic consequences of intraspecific competition are not yet constrained by interspecific competition. As the lineage diversifies into multiple species, the total range of resources use expands, and interspecific competition between newly formed species promotes phenotypic divergence among species but constrains it within species. Species therefore decrease in phenotypic diversity due to stabilizing selection as species accumulates in the same zone.

Previous studies of ecological opportunity have generally focused on how interspecific competition affects phenotypic divergence in pairs of species found in sympatry versus allopatry (Bolnick 2004; Brown and Wilson 1956; Schluter 2000). There are two potential outcomes to competition between closely-related species for limiting resources: the competitively-superior species excludes the other species, or the species diverge in phenotype and resource use (character displacement), which reduces the intensity of competition and facilitates coexistence (Brown and Wilson 1956). The extent of resource heterogeneity or diversity is sometimes also considered as an explanatory variable mediating such shifts, although its role remains unclear (Bolnick 2004; Losos 2000; Robinson et al. 2000). The relative contributions of both competition and resource heterogeneity in promoting phenotypic divergence within species as a lineage undergoes adaptive radiation have yet to be explicitly evaluated in the
context of adaptive radiation, although both mechanisms are key to phenotypic shifts in adaptive radiation.

Adaptive radiations on oceanic archipelagos provide ideal systems to test the predictions of the ecological opportunity hypothesis, since colonization of each new island corresponds to the invasion of an empty but similar adaptive zone. Moreover, given the ecological simplicity of insular systems, competition and resource heterogeneity can be readily estimated for each island assemblage. In the present study I use the radiation of Galápagos endemic land snails of the genus *Bulimulus* to test for a role of ecological opportunity in adaptive radiation.

The native land snail fauna of Galápagos includes 88 described species, and the genus *Bulimulus* comprises approximately 80% of this diversity (Chambers 1991). Galápagos bulimulids represent the most-species rich radiation of any animals or plants on this archipelago. Bulimulids colonized all major islands roughly in the same sequence as their geological formation, and successful colonization was followed by independent radiation within islands so that island assemblages are composed of one or very few independent lineages (Parent and Crespi 2006). Species are found in all described vegetation zones, save the lava boulder and sandy coastal zones. Although vegetation on Galápagos has been traditionally organized into up to seven vegetation zones (Wiggins and Porter 1971), in this study I follow the simple three-level zonation (coastal zone, dry lowland zone, and humid highland zone) that has been proposed recently (McMullen 1999), and classify the snail species and plant
species accordingly (snail and plant species geographical ranges rarely include more than one of these three zones). Galápagos land snails are mostly found on vegetation or on the ground near vegetation, and therefore plant species diversity may be a good proxy of local resource heterogeneity. Although species are not host specific, when found in sympatry, species typically partition the area among habitat, vegetation type and resting elevation and angle (e.g. under rocks versus particular heights on particular plant species). Increased plant species diversity is likely associated with habitat complexity, providing increased ecological heterogeneity for snails to exploit.

There is evidence that Galápagos bulimulid land snails have adapted to the different habitat and vegetation zones they inhabit, and shell phenotypes are associated with moisture levels and microhabitats (Chapter 4). Other studies of ecologically-comparable land snails have demonstrated that species compete for microhabitat, such that interactions within and between species drive diversification in ecological and morphological traits (Baur and Baur 1990; Chiba 1996b; Chiba 1999b; Chiba 2004; Lee and Silliman 2006). The only known predators of bulimulid land snails on Galápagos are mockingbirds, and possibly rats (Smith 1966). Mockingbirds are found on all major islands, and are not likely the source of differential selection. Coppois (2000) has suggested that introduced rats prey on bulimulid snails, and it is possible that endemic rats, where they still occur, do so as well. Although once found on several major islands, Galápagos endemic rats have suffered extinction and the four species that remain are distributed on only 3 islands. Undoubtedly some of the other land birds prey on
bulimulids occasionally, but given the shell thickness of most species, as well as their size, birds are probably more likely to prey on the smaller-sized snail species in other genera. Snail predators were never observed in action in the field, and I follow Smith (1966) in concluding that Galápagos bulimulids are not subject to appreciable predation.

I quantified the effect of ecological opportunity on intraspecific variation in phenotype for each bulimulid species on a given island and in a given vegetation zone as (1) the number of congeners (interspecific competition) and (2) the number of native plant species (proxy for resource heterogeneity) found in the same vegetation zone. I use multiple regression analyses to test for the relative contribution of number of congeners and habitat heterogeneity in a given vegetation zone on bulimulid land snail intraspecific variation in shell shape and shell size.

MATERIAL AND METHODS

Shell radiography and geometric morphometric analysis

Between 11 and 133 adult specimens of each of 30 bulimulid land snail species (median=20, total= 816) were sampled across their respective distribution range on 8 Galápagos islands, Ecuador.

All specimens were put in 75% ETOH for preservation. Snail specimens were radiographed using a H.G. Fischer X-ray machine (Model FP200) at 70 kV, 20mA exposed for 0.9 second at 60 cm focus-film distance. Shells were held in place with mounting putty, aperture up, on a sheet of photographic emulsion
(Kodak ULTRASPEED DF-58 dental film). The shells were always positioned in the same orientation, with the axis of the shell on the y-axis and the aperture parallel to the photographic film. After radiograph development, each individual radiograph was mounted on a slide frame, scanned, and saved as Tag Image File (TIF) format. The shell images obtained were digitized by recording the x and y coordinates of 6 morphological landmarks on the shells in TPSDIG2 (Rohlf 2006). The geometric morphometric approach that I used partitions morphological variation into components of size and shape. Shape includes all features of a configuration of landmarks that are invariant to scaling (variation in size), translation (variation in the position of the shell on the radiograph), and rotation (variation in the orientation of the specimen on the plane of the radiograph). I used Procrustes superimposition to characterize shape variation of each individual within each species. This method extracts the shape information from the original landmark configurations by scaling to unit centroid size, superimposing the centroids of all configurations, and rotating the configurations to a position of optimal fit according to a least-squares criterion. The scaling, superimposition, and rotation steps are performed for all specimens of a given species simultaneously (I used a full Procrustes fit and projection onto a tangent space) in TPSSUPER (Rohlf 2004). Following this method it is possible to describe each specimen by a size component (unidimensional) and a shape component (multidimensional), which can then be used to describe intraspecific shell morphological variation. Intraspecific variation in shell size was measured as the variance in centroid size for each species, whereas intraspecific variation
in shell shape was measured as the mean euclidean distance between each specimen’s shape vector and the species’ mean shape vector.

**Multiple regression analyses**

Six out of the 30 species included in this study had a sample size smaller than 20, the median. To make sure that I did not introduce a bias by including these species in our analysis, I tested for an association between sample size and the number of congeners a species was found with in a given vegetation zone. I found no significance for such a correlation ($r = -0.11, p > 0.05$), and I therefore included all species in the multiple regression analyses.

Some bulimulid species had no congener in the same vegetation zone, so I added 1 to the number of congeners to enable data transformation. Data were transformed to meet assumptions of normality: number of congeners, number of plant species, and variation in shell size data were transformed using Box-Cox transformation ($\lambda = 0.2, \lambda = 2, \lambda = -0.3$, respectively). I then computed phylogenetic generalized least square (GLS) models (Garland and Ives 2000), an approach that is functionally identical to independent contrasts regressions (Garland and Ives 2000). These models allow the inclusion of detailed information on the taxonomic relations among the species investigated, but have the restriction of assuming linear relations between response and all predictor variables. I used both topology and branch lengths of a published phylogeny (Parent and Crespi 2006) to generate a phylogenetic distance matrix among species included in this study. This phylogeny is the best Maximum Likelihood tree obtained from combined nuclear (ITS) and mitochondrial (COI)
sequence datasets. It is a well-resolved phylogeny with good nodal support. Species diversification generally parallels the geological formation of the islands, and seems to have occurred particularly rapidly on the mid-aged and younger islands (Parent and Crespi 2006). Multiple linear GLS models with shell shape variation and shell size variation as the dependent variables, and Box-Cox transformed values of habitat heterogeneity and numbers of congeners as predictor variables were computed with the R-library PHYLOGR (Díaz-Uriarte and Garland 2006). Because collinearity between independent variables might confound the analyses I checked for redundancy by investigating tolerance levels for resource heterogeneity and competition intensity. Tolerance value for resource heterogeneity regressed on competition intensity (tolerance = 1-R² = 0.6) was adequately high (Quinn and Keough 2002), and both variables could therefore be included in the regression models.

Intraspecific variation in shell morphology could be a function of mean shell size through allometric scaling relationship, with larger shells potentially being more variable, and I therefore tested for an association between mean shell size and the variation in shell morphology within species. Furthermore, since vegetation zones on different islands vary in number of species for which morphological data were available, I also used the mean intraspecific morphological variation per vegetation zone and further investigated the relationship between intraspecific phenotypic variation and competition and resource heterogeneity. Because the results did not change using this procedure,
it is highly unlikely that my results are confounded by the number of species for which I have been able to collect morphological data.

**RESULTS**

I found that intraspecific variation in shell shape was not significantly associated with shell size, therefore ruling out the possibility that the pattern of ecological release observed is a reflection of allometric scaling. The number of congeners inhabiting the same vegetation zone was strongly negatively correlated with the degree of intraspecific variation in shell shape (Table 6). Furthermore, the best regression model determined by forward stepwise multiple regression analyses also included resource heterogeneity, measured as native plant species richness of the vegetation zone, as a determinant of intraspecific variation in shell shape (Table 6). Dividing number of plant species by number of congeners yields an index of ecological opportunity, and this metric was strongly positively correlated with the degree of intraspecific variation in shell shape (Figure 6). By contrast, intraspecific variation in shell size was not significantly explained by competition from congeners or resource heterogeneity (Table 6). A higher degree of intraspecific variation in shell shape was associated with vegetation zones that exhibit a combination of high resource heterogeneity and low intensity of interspecific competition (Figure 7).
Table 6. Regression results (intraspecific phenotypic variation as the dependent variable).
Values of $t$ and associated $P$ values are presented for standard and phylogenetically controlled ($t_{PIC}$) multiple regression models.

<table>
<thead>
<tr>
<th>phenotypic component (standardized contrast)</th>
<th>independent variables (standardized contrasts)</th>
<th>$n$</th>
<th>$\beta$</th>
<th>s.e. of $\beta$</th>
<th>$t$</th>
<th>$t_{PIC}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>shell shape</td>
<td>number of congeners</td>
<td>30</td>
<td>-0.00366</td>
<td>0.0012</td>
<td>-3.05**</td>
<td>-10.21***</td>
</tr>
<tr>
<td></td>
<td>number of plant species</td>
<td></td>
<td>0.000000134</td>
<td>0.000000142</td>
<td>0.943</td>
<td>4.84***</td>
</tr>
<tr>
<td>shell size</td>
<td>number of congeners</td>
<td>30</td>
<td>0.0439</td>
<td>0.0391</td>
<td>1.12</td>
<td>2.44*</td>
</tr>
<tr>
<td></td>
<td>number of plant species</td>
<td></td>
<td>-0.00000392</td>
<td>0.00000471</td>
<td>-0.83</td>
<td>0.690</td>
</tr>
</tbody>
</table>

Separate multiple regression analyses were performed for each component of intraspecific morphological variation (size and shape), with number of congeners and number of plant species included as separate independent variables included in the final model. Shell shape: $r^2 = 0.349$, $F_{(2, 27)} = 7.23$, $P = 0.003$; shell size: $r^2 = 0.045$, $F_{(2, 27)} = 0.640$, $P = 0.54$. $P$ values are indicated in the table as follow: *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. 

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Figure 6. Positive relationship between intraspecific morphological variation in shell shape and ecological opportunity (measured as the number of plant species divided by the number of congeneres found in a given vegetation zone): \( n = 30, r^2 = 0.188, P < 0.05 \).

The relationship becomes much stronger by excluding *Bulimulus pinzonopsis* and an undescribed species from the volcano Sierra Negra on Isabela Island \( (n = 28, r^2 = 0.548, P < 0.001) \). Comparable results are obtained by regressing the mean intraspecific morphological variation in shell shape for all species for which morphological data are available within a given vegetation zone against ecological opportunity: \( n = 14, r^2 = 0.369, P < 0.05 \) (see Methods). Two species represent apparent outliers on this plot, and it is unclear why they show considerably higher (for *B. pinzonopsis*) and considerably lower (for the undescribed species on Sierra Negra) intraspecific morphological variation than the ecological opportunity on these islands predicts.
Figure 7. Geographic distribution of the association between the intraspecific variation in shell shape and ecological opportunity (number of plant species divided by number of congeners) across major Galápagos Islands.

Intraspecific variation in shell shape for each bulimulid species is indicated by the filled circles on the map (proportional to the radius), and the level of ecological opportunity is depicted by shades of grey: dark indicates high whereas light indicates low ecological opportunity. No bulimulid land snails were found in vegetation zones in white, and hatched areas are covered with barren lava flows. Island age (in millions of years) is indicated in parentheses for the islands included in this study.
DISCUSSION

The results of the present study support a combined role for competition and resource heterogeneity in generating ecological release in the initial stages of adaptive radiation, and support niche-filling models of diversification (Freckleton and Harvey 2006; Simpson 1953).

Older islands tend to have vegetation zones with more bulimulid land snail diversity, although this is not a significant relationship ($R^2 = 0.21$, $P = 0.09$). In addition, larger islands have vegetation zones with significantly more resource heterogeneity ($R^2 = 0.48$, $P < 0.01$). The combined effects of island age on number of competitors and island area on resource heterogeneity results in a distribution of ecological opportunity across the archipelago where younger and larger islands have vegetation zones presenting greater ecological opportunity, whereas older and smaller islands have vegetation zones with lower ecological opportunity (Fig. 2). Consequently, I find that species show high levels of intraspecific morphological variation in vegetation zones that present a combination of few competitors and considerable resource heterogeneity (mainly on younger, larger islands). Conversely, species that are found in vegetation zones where there is high land snail diversity (mainly on older islands) and relatively low habitat heterogeneity (mainly on smaller islands), are generally less variable in intraspecific morphology. This predictable variation in intraspecific morphology is consistent with the evolutionary process of ecological opportunity, leaving this morphological signature in contemporary species associated with the
ecological context where they occur, a signature that can be found across the
different stages of this adaptive radiation.

Release from interspecific competition has been suggested to result in expansion of resource use in lineages invading depauperate environments such as islands (Schluter 2000). For example, Hawaiian silverswords and *Bidens* are two plant groups that are more variable than their mainland relatives (Schluter 2000). Similarly, Schluter (1988) found accelerated morphological divergence in Hawaiian and Galápagos finches compared to their mainland relatives. These patterns are consistent with the hypothesis that adaptive radiation on islands is mediated in part by release from competition.

Another potential explanation for the increased phenotypic variability in environments with fewer competitors is competitive displacement, which has the causality reversed, such that morphologically more variable species preclude the accumulation of new species in the same habitat (Arthur 1978; Arthur 1982; Gause 1934). This scenario involves the process of interspecific competition in determining community assembly, and in the present study it implies that phenotypically more variable species would outcompete less variable species. Both competitive exclusion and ecological opportunity imply competition between species and are not necessarily mutually exclusive scenarios. It will not be possible to clearly differentiate the potential role of these two processes until further information about the possible association between direction and strength of competition and intraspecific phenotypic variation is available.
Other studies have provided evidence that snails compete within and between species, in the laboratory and in nature (Baur and Baur 1990; Cowie and Jones 1987; Cross and Benke 2002; Fenchel and Kofoed 1976). Furthermore, interspecific and intraspecific competition in land snails has been inferred to result in both character displacement (Barker and Mayhill 1999; Chiba 1999b; Cowie 1992; Davison and Chiba 2006; De Weerd et al. 2006) and competitive displacement (Arthur 1982). Although the limiting factor(s) over which species or individuals compete is often not clear in these studies, authors usually point to food and suitable resting or feeding microsites, although predator free space could also be important. The ecology of Galápagos land snails is not well known, but bulimulids seem to feed on lichen growing on the vegetation, as well as decaying matter on the ground, and individuals may compete for suitable microhabitat to feed and to avoid dessication.

The mechanistic basis linking shell shape with competition and resource use in this study has yet to be investigated in detail, but associations between shell shape (but not size) and vegetation zone or microhabitat have been described for Galápagos bulimulids (Coppois and Glowacki 1983). Shell shape can mediate habitat use via effects on mobility, exploitation of different food sources, utilization of different types of shelter, and resistance to dessication (Cook and Jaffar 1984; Goodfriend 1986; Heller 1987). Similarly, interspecific and intraspecific populations of Mandarina land snails on the Bonin Islands appeared to differ more markedly in shell shape (compared to shell size) in association to the habitat where the populations were found (Chiba 1996b).
The genetic basis of the intraspecific morphological variation I quantified in bulimulid land snails is unknown at present. It is possible that the morphological variation I observed is a plastic response to ecological opportunity. Release from competition presumably increases niche availability, and this release following the colonization of a new vegetation zone could potentially induce a plastic response that would increase the morphological variation within land snail species faced with an increased wealth of resources.

Competition between closely related species is often considered to be an important factor in driving phenotypic diversification and speciation (Schluter 2000). Our results are consistent with competition between closely related species limiting phenotypic variation within species. Although generally overlooked, this constraining role of competition between species is particularly important in adaptive radiation since the competing species are likely to be phenotypically very similar, given their recent common ancestry.
CHAPTER 4: INTRASPECIFIC PHENOTYPIC VARIATION IN BULIMULUS REIBISCHI PARALLELS THE ADAPTIVE RADIATION OF GALÁPAGOS ENDEMIC BULIMULID LAND SNAILS

ABSTRACT

One defining feature of adaptive radiation is a fit between the phenotypes of diversifying species and their environments. In the present study I test for such a phenotype-environment correlation (1) across the adaptive radiation of Galápagos bulimulid land snail species, and (2) within Bulimulus reibischi, a Galápagos endemic land snail species. I first consider the distribution of shell morphology across 31 species of Galápagos bulimulids in a phylogenetically-controlled framework and test for a phenotype-environment association across this lineage. I then test if the patterns of interspecific morphological differentiation are congruent with morphological variation observed within species. Population differentiation in shell shape of B. reibischi is associated with elevation difference, but not with genetic distance. I find that shell morphology has evolved among species repeatedly on different islands in response to moisture gradient in the same direction as within-species morphological variation among populations of B. reibischi. Species with more globose-shaped shells are found at higher elevations, and a similar pattern of morphological distribution was found for B. reibischi populations, over a smaller geographical scale. Associations of morphology with elevation, both above and below the species level, may be the
result of tradeoffs between dessication resistance, which may be lower for individuals and species with more globose shells, and energetics or fecundity, which may be higher for shells with this shape. Taken together, these results suggest that ecologically-based selection is involved in diversification at the levels of both population and species in this adaptive radiation.

**Keywords**: Adaptation, geometric morphometrics, population differentiation, shell shape, species divergence.

**INTRODUCTION**

A persistent debate in evolutionary biology is one over the continuity of microevolution and macroevolution scales (Hendry and Kinnison 2001), in particular whether macroevolutionary patterns are governed by the principles of microevolution. Some paleobiologists have argued that long-term evolutionary phenomena such as stasis or adaptive radiations cannot be predicted from population-genetic processes, arguing that additional macroevolutionary rules applicable over much longer-term timescales are needed (Gould 1985; Jablonski 1986). Several approaches have been proposed to bridge the gap between these scales (Hendry and Kinnison 2001): for example, one approach is to use theoretical models for asking how microevolutionary processes can generate large morphological changes or reproductive isolation (e.g., Gavrilets 2000; Hansen and Martins 1996; Kirkpatrick 1982; Kondrashov and Kondrashov 1999; Lynch 1990; Orr and Orr 1996). Another approach is to ask whether rates of evolution in contemporary populations are consistent with rates in the fossil record (e.g., Losos et al. 1997; Reznick et al. 1997). One can also test whether
divergence between species is consistent with patterns of genetic variation within species and whether microevolutionary processes are responsible for reproductive isolation in natural systems (e.g., Schluter 1996). Empirically, a potential first step to link the two evolutionary scales is to determine the significant predictors of phenotypic variation both above and below the species level, and to assess the degree of similarity between them. The relation between patterns of intraspecific and interspecific differentiation can thus potentially be informative in understanding the nature of diversification (Emerson and Arnold 1989, Emerson 1991, Moreno et al. 1997).

With over 70 described species, Galápagos bulimulid land snails form the most species-rich taxonomic group of these islands. The species have colonized all major islands, and their diversification is the result of a combination of inter-island colonization and within-island speciation (Parent and Crespi 2006). Species are found at all island elevations, except for the littoral zone, which is primarily composed of sandy beaches and lava rocks. When more than one species are found in the same habitat, 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 or in the leaf litter. The different species vary remarkably in shell morphology, mainly in size, shape, colour, and colour pattern. The adaptive significance of land snail shell variation has been the subject of considerable debate (e.g., Gittenberger 1991), but previous studies have noted that the variation in shell shape among Bulimus snail species on Galápagos might appear to be related to aspects of ecological variation, including vegetation.
zones, related moisture levels, and microhabitat (Coppois 1984; Coppois and Glowacki 1983, Chapter 3).

In this study I tested for the role of environment (in particular, altitude, as a proxy for a humidity-aridity gradient) in driving both intra- and interspecific phenotypic differentiation in Galápagos endemic bulimulid land snails. I first test for a phenotype-environment association across 31 species of Galápagos endemic bulimulid land snails within a phylogenetically-controlled framework. I then focus on the intraspecific phenotypic variation among populations *Bulimulus reibischi* distributed across an environmental gradient, and test for a phenotype-environment association controlling for genetic population structure. Presumably, a globose shell is the most cost-effective shell shape, thereby maximizing shell volume for surface area. Shell shape can mediate habitat use via effects on mobility, exploitation of different food sources, utilization of different types of shelter, and resistance to dessication (Cook and Jaffar 1984; Goodfriend 1986; Heller 1987). Land snails might suffer from considerable humidity loss through their shell aperture in dry environments. A morphological solution to this problem is to keep the shell aperture as small as possible, which involves building a shell with a more slender shape.

The prediction that intraspecific and interspecific patterns of morphological divergence should be concordant is based on the assumption that species divergence is ultimately the result of local natural selection on habitat use. I predict that if the processes leading to intraspecific divergence are the same processes leading to interspecific divergence, then intraspecific variation in
morphological traits should mirror interspecific differences in habitat use. Dissimilar patterns of interspecific and intraspecific divergence would suggest that morphological diversification is not constrained by moisture gradient associated with elevation at one or both taxonomic levels.

MATERIAL AND METHODS

Study area and data collection
To characterize interspecific phenotypic diversification in Galápagos bulimulids, I sampled adult specimens of 31 bulimulid land snail species across their respective distribution range on eight Galápagos islands (Fig. 8). Between 2-10 live specimens of each species, and large numbers of adult empty shells were sampled at each collecting site as available. To characterize intraspecific divergence, I sampled 15 populations of *Bulimulus reibischi* across its entire known distribution range on Santa Cruz Island (Fig. 8). I collected between 10 and 27 live specimens as well as a large number of empty adult shells at population site. Here I define a population as a distinct collecting site isolated by at least 40m from any other such site. GPS coordinates and elevation data were recorded at all collecting sites for all species and all populations. A small sample of snail foot tissue was taken from each live specimen for preservation in 20% DMSO/saturated NaCl for subsequent DNA extraction, and specimens were preserved in 75% ETOH. All collections were made in 2000-2005.

In this study I use elevation as a proxy for moisture level at collecting sites. Although moisture levels also vary with topography, maximum
Figure 8. Map of Galápagos Islands.
The number of species sampled on each island (or major volcano, marked with a filled triangle) is indicated on or by the island. Sampling localities of *B. reibischi* are indicated by filled circles.
height and area of island, exposure, and angle of slope, the amount of rain and moisture on Galápagos islands generally increases with island elevation (Jackson 1991). This moisture gradient contributes to the vegetation zonation on the islands with desert-like climate at low elevations up to almost tropical-like climate at higher elevations (McMullen 1999; Wiggins and Porter 1971). All but three of the 31 species sampled were collected at multiple sites, and I used the average island elevation of collecting sites to determine these species' island elevation.

To quantify intraspecific and interspecific variation in shell morphology, I took radiographs of an average of 27 adult specimens (median=21, range=11-133, total=866) per bulimulid species, and an average of 20 specimens (median=20, range=20-27, total=347) per population of B. reibischii. Snail specimens were radiographed using a H.G. Fischer X-ray machine (Model FP200) at 70 kV, 20mA, exposed for 0.9 second at 60 cm focus-film distance. Shells were held in place with mounting putty, aperture up, on a sheet of photographic emulsion (Kodak Ultraspeed DF-58 dental film). The shells were all positioned in the same orientation, with the axis of the shell on the y-axis and the aperture parallel to the photographic film. After radiograph development, each individual radiograph was mounted on a slide frame, scanned at 900 dpi resolution, and saved as Tag Image File (TIF) format. The shell images obtained were digitized by recording the x and y coordinates of a set of six morphological landmarks on the shells for the interspecific morphological variation, and 14 landmarks and 14 semi-landmarks to characterize the intraspecific morphological
variation (Fig. 9). Shell images were digitized in TPSDIG2 (Rohlf 2006). The size of each specimen was estimated using its centroid size, the square root of the sum of the squares of the distances of each landmark from the centroid (Bookstein 1991). The original landmark configurations were scaled to unit centroid size, and then specimens were aligned with respect to the average configuration of landmarks (the consensus configuration) using Generalized Procrustes Analysis (GPA, Rohlf and Slice 1990). The closeness of the approximation of the tangent space to shape space was estimated using the program TPSSMALL (ver. 1.02, Rohlf 1997), which compares Procrustes distances between individuals in the shape space with the Euclidean distances in the tangent space. The scaling, superimposition, and rotation steps were performed simultaneously for all specimens of all populations of _B. reibischi_ for the intraspecific dataset and for all specimens of all species for the interspecific dataset, in TPSSUPER (Rohlf 2004). Each specimen is thus described by a size component (unidimensional) and a shape component (multidimensional), which can then be used independently to describe intra- and interspecific shell morphological variation, and to test for differences in shape and size among populations of _B. reibischi_, and among species, among species.

**Data analyses**

To adjust for patterns of phylogenetic covariance in the interspecific morphological data, I used the best Maximum Likelihood species-level molecular phylogeny presented in Parent and Crespi (2006) as description of species phylogenetic relationships. This phylogeny is the best Maximum
Figure 9. Landmarks on the shell radiographs.

(A) 31 Bulimulus spp. (B. olla is presented here), and (B) for 15 populations of B. reibischii. Regular landmarks at shell suture points are in red and semi-landmarks (only for intraspecific variation in shell morphology of B. reibischii) along the outer margin of the aperture are coloured in blue.
Likelihood tree obtained from combined nuclear (ITS) and mitochondrial (COI) sequence datasets. It is a well-resolved phylogeny with good nodal support. Species diversification generally parallels the geological formation of the islands, and seems to have occurred particularly rapidly on the mid-aged and younger islands (Parent and Crespi 2006). I pruned out the species not included in this study, to keep only the 31 species for which I have morphological data. I then used the pruned phylogeny and the matrix of mean aligned coordinates for each species to calculate a phylogenetically corrected variance-covariance (VCV) matrix. This phylogenetically-corrected VCV matrix was obtained using the package GEIGER (Harmon et al. 2007) in the R statistical software (R development Core Team 2005). The interspecific morphological variability was then quantified with a principal component analysis (PCA) performed on the phylogenetically-corrected VCV matrix. The first principal axis obtained explained 99% of the variation, and it was transformed to meet assumptions of normality using Box-Cox transformation (Box-Cox parameter, λ=10). Elevational data was log transformed. The relationship between elevation and shell shape across species was tested with regression analysis using the transformed data.

Similarly to closely related species, populations within a species share both historical and demographic connections, and they may not be statistically independent. To take this shared history into account, I quantified population structure among populations of *B. reibischi* by genotyping an average of 19 specimens per populations (median=19, range=10-20, total=315) for four microsatellite loci (A114, B6, B110, B118) developed from a *Bulimulus akamatus*
library (Parent et al. 2008b, Appendix B). Genetic differentiation among populations was estimated using the genetic measure \( F_{ST} \) (Weir and Cockerham 1984) in the software ARLEQUIN version 2.0 package (Schneider et al. 2000), hierarchically partitioning molecular variation into within- and among-population components to estimate genetic structure in predefined groups of populations. I then tested for demographic and historic effects by conducting a Mantel test (Mantel 1967) of the morphological matrix against the genetic distance matrix (Böhning-Gaese et al. 2003) using the program Passage (Rosenberg 2001). Significance was assessed by comparing the \( z \)-statistic of the actual matrices to the \( z \)-statistics from 9999 random permutations. This test was significant, so to factor out the confounding effects of these genetic relationships, I used three-way Mantel tests controlling for genetic distance in the remainder of the matrix correlation tests (Thorpe 2002; Thorpe and Stenson 2003). Significance of relationships was evaluated using a partial Mantel test in the PASSAGE analysis package (Rosenberg 2001).

**RESULTS**

Mean elevation ranged from 85m to 1330m across the 31 species used in this study, and from 134m to 261m for *B. reibischi* populations on Santa Cruz Island alone. Once adjusted for phylogenetic relatedness, interspecific variation in shell shape was significantly associated with elevation across the 31 bulimulid species included in this study \( (r^2 =0.23, P<0.01; \text{Fig. 10}) \). The shell shape deformation with increased elevation is represented with a vector on each landmark of the average shell shape configuration for all species (Fig. 10).
Figure 10. Shell shape deformation represented by the first principal component (PC) (explaining 99.0% of the variation) as a function of island elevation.

The range of shell shape deformation along the first PC axis is represented by the species with the highest and the species with the lowest PC score. The average landmark configuration and the shape deformation vectors among 31 bulimulid species associated with the maximum increase in elevation are depicted in the shaded area.

linear model:
$R^2=0.23, \ P < 0.01$
I found no correlation between differences in geographic and genetic distances among population sites across the distribution of *B. reibischi* (Mantel test, \( r_M = 0.013, p > 0.05; \) Table 7), indicating that these two datasets did not vary similarly among population sites of *B. reibischi*. Elevation differences were also not correlated with genetic distance (\( r_M = -0.089, P > 0.05; \) Table 7). However, differences in shell shape among sites were correlated with genetic differentiation among sites (\( r_M = 0.19, P < 0.05; \) Table 7). I therefore tested for a correlation between morphological differences and elevational differences controlling for genetic differences among sites, and this correlation was found to be highly significant (\( r_M = 0.30, P < 0.01; \) Table 7). The correlation between elevation and shell shape among populations of *B. reibischi* can be visualized with vectors of shell shape deformation from the average shell shape configuration for all individuals of all populations. The mean shell shape of *B. reibischi* populations becomes more globose (the shell becomes wider and shorter) with increasing elevation (Fig. 11).

**DISCUSSION**

Adaptive radiations provide unique opportunities to study patterns of diversity at different taxonomic levels. Here, I show that studying adaptive divergence at the population level may allow for a better understanding of the processes that lead to adaptive radiation. Specifically, I have shown that intraspecific morphological divergence in phenotypic characters is similar to the interspecific patterns of divergence exhibited by species in the adaptive radiation of bulimulid snails. In particular, species that are found at higher elevations tend to have more globose
Table 7. Results of Mantel tests for correlations between population genetic, geographical, morphological, and elevational distances.

(1) population genetic and geographic distances (test for isolation by distance); (2) genetic and morphological distances (genetic autocorrelation); (3) morphological and geographic distances, controlling for genetic distance; and (4) morphological and elevational distances, controlling for genetic distance between populations. Significance levels, as determined by 9999 matrix permutations, are indicated with asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

<table>
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<tr>
<th>Matrix 1</th>
<th>Matrix 2</th>
<th>Control matrix</th>
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<tr>
<td>Genetic</td>
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<td>6.4382</td>
<td>0.0125</td>
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<tr>
<td>Genetic</td>
<td>Morphological</td>
<td>-</td>
<td>0.5494*</td>
<td>0.188</td>
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<tr>
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<td>Geographic</td>
<td>Genetic</td>
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<td>0.335</td>
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<tr>
<td>Morphological</td>
<td>Elevational</td>
<td>Genetic</td>
<td>72.3270**</td>
<td>0.302</td>
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Figure 11. The average landmark configuration and the shape deformation vectors among population of *Bulimulus reibischi* associated with the maximum increase in elevation.

The vectors illustrate the magnitude and the direction of the deformation of the landmark configuration of populations of *B. reibischi* from low to high elevation across the distribution of the species.
(rounder) shells compared with species found at lower elevations. Similarly, intraspecific shell shape variation of *B. reibischi* was significantly associated with elevation gradient across its distribution range so that populations with more globose shell shape are found at higher elevation end of the distribution of this species. The genetic information in the present study indicates that this morphological differentiation among population of *B. reibischi* occurs despite levels of gene flow between populations on Santa Cruz Island. Together, these intraspecific results support the idea that ecologically-based selection drives morphological divergence among populations of Galápagos bulimulids. Despite considerable levels of gene flow among populations, strong natural selection apparently generates population-level adaptive divergence that is similar to the interspecific differentiation characterizing the adaptive radiation. This supports our assertion that the mechanisms of diversification acting within populations are the comparable to those that have given rise to species level diversity. Furthermore, the parallel I found between the phenotype-environment association above and below the species level demonstrates that studies of intraspecific variation can be useful for better understanding the processes that lead to diversification.

The relative contributions of genes and environments to the observed within and among species variation is unclear and remain to be tested. It is possible, for example, that the intraspecific phenotypic variation is a plastic response to variation in environmental conditions (in this case moisture), and that the similar phenotypic-environment association among species results from
genetic assimilation (Pigliucci and Murren 2003). More work is needed to establish the contribution of genetics to this phenotypic-environment association both above and below the species level.

Associations between shell shape (but not size) and vegetation zone or microhabitat have been previously described for Galápagos bulimulids (Coppoiss and Glowacki 1983). Shell shape can determine habitat use by constraining mobility, exploitation of different food sources, use of different types of shelter, and resistance to dessication (Cook and Jaffar 1984; Goodfriend 1986; Heller 1987). Interspecific and intraspecific populations of Mandarina land snails on the Bonin Islands appeared to differ more markedly in shell shape (compared to shell size) in association to the habitat where the populations where found (Chiba 1996b). More globose shells maximize volume/surface area ratio, and are therefore presumably less costly to build than slender shells, which may result in energetic and fecundity benefits. However, slender shells have a smaller aperture relative to overall shell size, and are therefore less susceptible to moisture loss than rounder shells. The results of the present study indicate that populations within a species or species with slender shells tend to occur at lower elevations than populations or species with more globose shells. The mechanistic link between shell shape and habitat use (more specifically moisture levels) needs to be investigated further, possibly through transplant experiments, and direct quantification of moisture loss for different shell shape. Furthermore, shell colour and thickness might also be adaptive morphological components that need to be considered.
Despite the longstanding controversy surrounding the importance of allopatri to the speciation process (Mayr 1963), there is a growing appreciation for cases of population-level divergence in the face of ongoing gene flow (Lu and Bernatchez 1999; Ogden and Thorpe 2002; Schneider et al. 1999; Smith et al. 2001). I have shown that morphological divergence within islands has occurred despite ongoing gene flow among populations of *B. reibischii*. The divergence pattern in shell shape observed among populations of *B. reibischii* is parallel to the divergence across the adaptive radiation of the genus. The role of geographical isolation in the evolution of reproductive isolation among bulimulid species remains unclear. Closely related species are sometimes found in sympaty, and up to 11 species have been recorded at a single site (Coppois 1985). When found in sympaty, species usually partition the habitat so that some are found on the ground or under rocks, some on low vegetation and grass, and some are found on trunks or higher up on trees, and this habitat partition is associated with shell morphology (Parent and Crespi, unpublished). Although other mechanisms of diversification may also be present, our results suggest that the model of phenotypic divergence associated with the environment is likely to be an important process in the diversification of Galápagos bulimulid land snails above and below the species level.
CHAPTER 5: SYNTHESIS

Adaptive radiation is thought to be one of the main processes involved in the formation of the world’s organic diversity (Schluter 2000). Thus, understanding the processes involved in adaptive radiation becomes a key component of the study of evolutionary biology. The aim of this thesis has been to reach a better understanding of diversification in adaptive radiations, using Galápagos endemic bulimulid land snails as model system.

In this synthesis chapter I highlight the unique contributions each chapter of the present work offers to the advancement of the field, especially as related to island biogeography and the study of species multiplication and diversification, and I discuss how the findings of the different chapters are interrelated.

Diversification of Galápagos terrestrial fauna

The first chapter of this thesis represents the first phylogenetics and biogeographical review of Galápagos terrestrial fauna. Despite the large volume of evolutionary research that has been undertaken in Galápagos, several species groups remain relatively unstudied in terms of diversification within Galápagos and their relationship to possible sources of colonists (Table 2). In this review, I find that most of the Galápagos terrestrial fauna has diversified in parallel to the geological formation of the islands. Lineages have occasionally diversified within islands, and the clearest cases occur in taxa with very low vagility and on large
islands with diverse habitats. Ecology and habitat specialization appear to be critical in speciation both within and between islands. Although the number of phylogenetic studies is continuously increasing, studies of natural history, ecology, evolution and behaviour are essential to completely reveal how diversification proceeded on these islands.

**The speciation-area relationship**

The species-area relationship is often referred to as the only rule in ecology (Schoener 1976), and the pattern seems to be so common that it would be simpler to list the few exceptions rather than the thousands of studies reporting this pattern (Lomolino 2000). Inspired by the species-area relationship, MacArthur and Wilson (1967) proposed the equilibrium theory of island biogeography, which was based on the processes of colonization and extinction as determinants of species richness of islands. The MacArthur and Wilson theory has dominated thinking about island species diversity since its publication. However, islands are also widely recognized as natural laboratories of evolution, ideal localities in which to study evolutionary processes, and the scene for remarkable adaptive radiations. Indeed, many of the most famous cases of adaptive radiations—Darwin’s finches, Hawaiian silverswords, African Rift Lake cichlids—occur on islands or island-like settings. One consequence of adaptive radiation, if it occurs in situ, is that the diversity of an island is a result not just of colonization and extinction, but also of the evolutionary input of species resulting from within-island speciation. For example, the small island of Rapa in the South
Pacific (size = 40 km$^2$) harbours 67 species of *Miocalles* weevils, all the presumed descendants of a single ancestral colonist (Paulay 1985).

The goal of chapter 2 of my thesis was to combine phylogenetics and biogeographical analyses to partition island species diversity by its originating processes: between-island colonization and within-island speciation, and to determine what biogeographical and ecological factors are important in driving these two processes. Very few studies have explicitly used phylogenetics to determine the processes involved in island diversification (Losos and Parent, in press), and chapter 2 is the first of such studies to evaluate a combination of biogeographical and ecological factors determining diversification resulting from both between-island colonization and within-island speciation. The results obtained show that diversification in Galápagos bulimulid land snails is 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. This study 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. Overall, these results beg for a more dynamic model of island biogeography that would include island age (see for example Whittaker 2008),
and a model that would be based on diversification processes rather than resulting patterns.

**Ecological opportunity in adaptive radiation**

In chapter 3, I tested the classic evolutionary theory of ecological opportunity, which proposes that the combination of increase in wealth of resources and decrease of potential enemies promotes diversification in lineage colonizing previously empty adaptive zones. This theory was first formulated by Simpson (1953), inspired by the general observation that species on islands would often use a wider range of resources than their mainland relatives (Schluter 2000). Although the link between competition and intraspecific phenotypic variation has been demonstrated in several studies (mostly pairwise comparisons of populations that differ in competition intensity), chapter 3 represents the first study where an association between intraspecific variation and both factors presumed to drive ecological opportunity, that is competition among closely related species and resource diversity, is tested. I show that ecological opportunity predicts the extent of intraspecific phenotypic variation, explicitly linking the role of competition from congeners and the heterogeneity of resources to the extent of intraspecific phenotypic divergence as adaptive radiation proceeds. The results of the present study support a combined role for competition and resource heterogeneity in generating ecological release in the initial stages of adaptive radiation, and support niche-filling models of diversification (Freckleton and Harvey 2006; Simpson 1953).
Competition between closely related species is often considered to be an important factor in driving phenotypic diversification and speciation (Schluter 2000). Our results are consistent with competition between closely related species limiting phenotypic variation within species. Although generally overlooked, this constraining role of competition between species is particularly important in adaptive radiation since the competing species are likely to be phenotypically very similar, given their recent common ancestry.

Parallel between intra- and interspecific phenotypic variation
A persistent debate in evolutionary biology is one over the continuity of microevolution and macroevolution scales (Hendry and Kinnison 2001), in particular whether macroevolutionary patterns are governed by the principles of microevolution. Adaptive radiations provide unique opportunities to study patterns of diversity at different taxonomic levels. Here, I show that studying adaptive divergence at the population level may allow for a better understanding of the processes that lead to adaptive radiation. Specifically, I have shown that intraspecific morphological divergence in phenotypic characters is similar to the interspecific patterns of divergence exhibited by species in the adaptive radiation of bulimulid snails. I found that shell morphology has evolved across species repeatedly on different islands in response to moisture gradient in the same direction as within-species morphological variation among populations of *Bulimulus reibischi*. These results support the assertion that the mechanisms of diversification acting within populations are comparable to those that have given rise to species-level diversity. Furthermore, the parallel I found between the
phenotype-environment association above and below the species level demonstrates that studies of intraspecific variation can be useful to reach a better understanding of the processes that lead to diversification.

Although other mechanisms of diversification may also be present, the results of chapter 4 suggest that the model of phenotypic divergence associated with the environment is likely to be an important process in the diversification of Galápagos bulimulid land snails above and below the species level.

**Diversification in adaptive radiation**

Adaptive radiations are defined by the accumulation of species within a lineage accompanied with ecological and phenotypic variation. In chapter 1, I find that the species accumulation in adaptive radiations on Galápagos Islands is the result of the combination of geographical isolation and ecological speciation. The relative importance of these two processes seems to be determined at least in part by the dispersal ability of the lineage. In chapter 2, I show that species accumulation in adaptive radiation can be better understood when considering the processes that are driving it. I find that, as the classic theory of island biogeography predicts, species resulting from between-island colonization is the result of island area and insularity. However, when considering species resulting from within-island speciation alone, then island habitat is the only significant predictor of species diversity. The importance of these two processes is likely to be dependent on the dispersal ability of the diversifying lineage and the geographical settings where it is evolving. Together these two first chapters
illustrate the potential for changing island biogeography from a field of natural history into a field of predictive science.

Phenotypic diversification in adaptive radiation is the main focus of chapters 3 and 4. Chapter 3 highlights the important role of both resource diversity and competition in determining intraspecific variation as an adaptive radiation proceeds. When a new lineage colonizes an island system, it will likely encounter a wealth of resources and very few potential enemies. This will open new ecological opportunities for this species, and will soon be followed by ecological and phenotypic diversification. In chapter 3 I show that intraspecific phenotypic variation in adaptive radiations is both constrained by interspecific competition from closely related species and promoted by resource diversity. Furthermore, the results of chapter 4 show that intraspecific phenotypic variation is associated with environmental gradient in a very similar way (but at a smaller scale) that interspecific variation is. This finding suggests that processes that are causing phenotypic variation at the population level might be at least in part responsible for the patterns of phenotypic variation observed across an adaptive radiation. Furthermore, the fact that interspecific competition from closely related species is an important factor in determining intraspecific phenotypic variation suggests that there might be an association between the processes of phenotypic diversification and species multiplication in adaptive radiation.

Unanswered questions

The relative contributions of genes and environments to the observed within and among species variation (chapters 3 and 4) is unclear and remain to be tested. It
is possible, for example, that the intraspecific phenotypic variation is a plastic response to variation in environmental conditions (in this case moisture), and that the similar phenotypic-environment association among species results from genetic assimilation (Pigliucci and Murren, 2003). More work is needed to establish the contribution of genetics to this phenotypic-environment association both above and below the species level.

The mechanistic basis linking shell shape with competition and resource use (chapter 3), and intraspecific shell shape variation and moisture gradient (chapter 4) has yet to be investigated in detail, but associations between shell shape (but not size) and vegetation zone or microhabitat have been described for Galápagos bulimulids (Coppois and Glowacki 1983). Shell shape can mediate habitat use via effects on mobility, exploitation of different food sources, utilization of different types of shelter, and resistance to dessication (Cook and Jaffar 1984; Goodfriend 1986; Heller 1987). Similarly, interspecific and intraspecific populations of Mandarina land snails on the Bonin Islands appeared to differ more markedly in shell shape (compared to shell size) in association to the habitat where the populations were found (Chiba 1996b).

Associations between shell shape (but not size) and vegetation zone or microhabitat have been previously described for Galápagos bulimulids (Coppois and Glowacki 1983). Shell shape can determine habitat use by constraining mobility, exploitation of different food sources, use of different types of shelter, and resistance to dessication (Cook and Jaffar 1984; Goodfriend 1986; Heller 1987). Interspecific and intraspecific populations of Mandarina land snails on the
Bonin Islands appeared to differ more markedly in shell shape (compared to shell size) in association to the habitat where the populations where found (Chiba 1996). More globose shells maximize volume/surface area ratio, and are therefore presumably less costly to build than slender shells, which may result in energetic and fecundity benefits. However, slender shells have a smaller aperture relative to overall shell size, and are therefore less susceptible to moisture loss than rounder shells. The results of the present study indicate that populations within a species or species with slender shells tend to occur at lower elevations than populations or species with more globose shells. The mechanistic link between shell shape and habitat use (more specifically moisture levels) needs to be investigated further, possibly through transplant experiments, and direct quantification of moisture loss for different shell shape. Furthermore, shell colour and thickness might also be adaptive morphological components that need to be considered.

**Concluding remarks**

These are exciting times to be a student of adaptive radiations: new population genetic and phylogenetic tools are constantly developed or perfected, tools that can open windows into the past more clearly than limited fossils have allowed us before. At the same time, the world’s undisturbed environments are shrinking, and it becomes increasingly difficult to study the ecology of organisms in the environments where they naturally evolved. Ultimately, I hope that the study of Galápagos bulimulid land snail radiation will add to a growing list of island system
studies that will provide more answers to the question of how and why do species diversify and multiply.
APPENDICES
Appendix A: Collection sites of individuals included in chapter 2.

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<th>Location</th>
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(IS) denotes volcano on Isabela Island
Appendix B: Characterization of eight microsatellite loci in the Galápagos endemic land snail *Bulimulus reibischi*, and their cross-species amplification.

Modified from Parent et al. (2008) by permission of Blackwell Publishing

ABSTRACT

Variation in and amplification conditions for eight polymorphic microsatellite loci initially identified from *Bulimulus akamatus*, a pulmonate land snail from Galápagos, are described. Intraspecific polymorphism and heterozygosity of the eight markers were studied in 19 populations of *B. reibischi*, a closely related species of *B. akamatus*. Furthermore, the eight loci were also cross amplified in six other closely related bulimulid species. The number of alleles across populations of *B. reibischi* at six loci is moderate (three to 10), but considerable for two other loci (19 and 20). There is no strong evidence for linkage among any of the loci examined.

With more than 70 described species, Galápagos endemic land snails of the genus *Bulimulus* form the most species rich genus on these islands. The species have colonized all of the major Galápagos islands, and can be found in all vegetation zones except for the littoral zone which is mainly composed of sandy beaches and barren lava flows (Parent and Crespi 2006). Fifty-seven of the species are listed as endangered or threatened according to the IUCN Redlist (Parent 2003), suffering from restricted distribution ranges and population declines principally due to habitat destruction by humans and introduced species. Genetic characterization of populations holds important implication for the
understanding of the diversification of this group as well as its conservation. To further genetic population analysis of natural populations, I designed microsatellite primers for *B. akamatus* and tested their application in related species. I examined variation at eight microsatellite loci in 44 populations of 8 bulimulid species from Galápagos islands. This note describes the microsatellite loci and the amplification conditions used to assess their variation.

Eight microsatellite loci and corresponding primer sites for their amplification (Table 7) were identified from a genomic library of *B. akamatus*, enriched for different microsatellite motifs (AAT, ATG, and TACA) by Genetic Identification Services (GIS, Chatsworth, CA, http://www.genetic-id-services.com/). I optimized amplification reaction conditions for each locus. All reactions were performed in a total volume of 25µL with the following components: 0.2µM dNTP (GE Healthcare), 1x ThermoPol reaction buffer with 2mM MgCl₂ (New England Biolabs), 0.1µM IR labelled forward primer LI-COR Biosciences), 0.1µM forward primer and 0.2µM reverse primer (Invitrogen), 0.5mM MgCl₂, 0.1U Thermostable DNA polymerase (Biotools, and about 100 ng of template genomic DNA. All reactions were performed following a touchdown thermocycling protocol: denaturation at 94°C for 3 min, 3 cycles of 92°C for 40 s, 60°C for 40 s, 72°C for 35 s; 3 cycles of 92°C for 40 s, 57°C for 40 s, 72°C for 35 s; 3 cycles of 92°C for 35 s, 54°C for 40 s, 72°C for 35 s, 30 cycles of 92°C for 40 s, 51°C for 35 s, and a final extension at 72°C for 4 min. Amplification products were separated in a 4% acrylamide: TBE gel using a LiCor 4200 DNAnalyser, and analysed using Gene ImageIR 4.05 software (Scanalytics).
I genotyped a total of 338 *B. reibischi* specimens sampled across 19 populations on Santa Cruz Island, and 76 bulimulid specimens from 7 other species, including 25 distinct populations (on average 3 populations and 10 individuals per species) from Santa Cruz, Santiago, and Fernandina islands, Galápagos, Ecuador. The failure rate of cross-species amplification has been shown to be associated with the evolutionary distance between the species for which the microsatellite loci have been developed and the species that are used for amplification (Primmer et al. 2005), and references therein). I tested for such a correlation using the proportion of individuals that failed to amplify at any one locus for each species included in this study and phylogenetic distance of each species to *B. akamatus* (obtained from the best maximum likelihood tree presented elsewhere [Parent and Crespi 2006]). Although the correlation between phylogenetic distance and amplification failure is not significant (*r* = 0.364, *P* > 0.05, *n* = 7), it is in the predicted direction.

Number of alleles per locus varied. If I consider allelic diversity for *B. reibischi* populations alone, I find a range of 5 to ten different alleles for the “B” loci and the A115 locus, and 19 and 20 distinct alleles for A116 and A114 respectively (Table 7). Similar allelic diversity is found when all other species genotyped are considered. All loci were polymorphic across all specimens genotyped, except for one species that was monomorphic at one locus (Table 8). All the loci genotyped across *B. reibischi* populations were polymorphic (Table 7). I did a Blast search for the clone sequences against the GenBank nucleotide database to determine if loci with relatively low allelic richness are impoverished
because of selection on a gene of well-known function. I found no significant similarity between our clone sequences and any sequence from the GenBank database.

Observed and expected heterozygosities for loci over all *B. reibischi* genotyped individuals and for four of the more polymorphic populations were calculated using the software Popgene version 1.32 (Yeh et al. 1997) (Table 7). Hardy-Weinberg equilibrium (HWE) was tested across all loci including all *B. reibischi* individuals genotyped, and also for the four most polymorphic populations using exact P-values from the Genepop software version 3.4 (Raymond and Rousset 1995) (Table 7). There is marked differentiation among several of the populations of *B. reibischi* studied, inflating the difference between expected and observed heterozygosity calculated over all individuals. Deviations from HWE in direction of heterozygote deficiency for some populations for some loci might have resulted from genetic drift, Wahlund effect, or other effects. However, for none of the loci was there significant deviation from Hardy–Weinberg equilibrium for all populations, indicating that the presence of null alleles is unlikely. A test for genotypic disequilibrium in each population performed in Popgene did indicate significant association of genotypes for a few pairs of loci: A114 and B110 in three populations, A114 and B118 in one population, and A114 and B123 in one population out of the 48 populations tested. However, since no locus association was consistent across all or even several populations, the few significant associations observed might be the result of small sample size for some of the populations genotyped.
The eight microsatellite loci described here are expected to be useful markers for elucidation of population structure and differentiation in this unique taxonomic group of endangered land snails.
Table 8. Characteristics of eight polymorphic microsatellite loci amplified in *B. reibischi*.

For each, locus name, repetitive sequence, primer sequences (the forward primers were labeled), number of individuals ($N_{ind}$), and number of populations ($N_{pop}$) genotyped are indicated. The resulting number of alleles ($N_a$), allele size range ($A_{range}$), observed ($H_O$) and expected ($H_E$) heterozygosity, and results of exact P-values for Hardy-Weinberg equilibrium (HWE) for all *B. reibischi* individuals genotyped, and for the four most polymorphic populations of *B. reibischi* are also indicated (n.s. = non significant; * = $P < 0.05$).

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<th>$N_{pop}$</th>
<th>$N_a$</th>
<th>$A_{range}$</th>
<th>$H_O$</th>
<th>$H_E$</th>
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<td>180-237</td>
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Table 9. Cross-amplification data of 7 Galápagos bulimulid species using the primers developed for *B. akamatus*.

Number of individuals (N$_{ind}$), number of populations (N$_{pop}$) for each species, as well as number of alleles genotyped (N$_a$), and the allele size range (A$_{range}$) for each marker and each species are reported. - : not amplified.

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