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Comparative Genetic Structure and Demographic History in Endemic Galápagos Weevils

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Abstract

The challenge of maintaining genetic diversity within populations can be exacerbated for island endemics if they display population dynamics and behavioral attributes that expose them to genetic drift without the benefits of gene flow. We assess patterns of the genetic structure and demographic history in 27 populations of 9 species of flightless endemic Galápagos weevils from 9 of the islands and 1 winged introduced close relative. Analysis of mitochondrial DNA reveals a significant population structure and moderately variable, though demographically stable, populations for lowland endemics (F_{ST} = 0.094–0.541; π: 0.014–0.042; Mismatch P = 0.003–0.026; and $D_{(\text{Tajima})}$ = −0.601 to 1.203), in contrast to signals of past contractions and expansions in highland specialists on 2 islands (Mismatch P = 0.003–0.026 and $D_{(\text{Tajima})}$ = −0.601 to 1.203). We interpret this series of variable and highly structured population groups as a system of long-established, independently founded island units, where structuring could be a signal of microallopatric differentiation due to patchy host plant distribution and poor dispersal abilities. We suggest that the severe reduction and subsequent increase of a suitably moist habitat that accompanied past climatic variation could have contributed to the observed population fluctuations in highland specialists. We propose the future exploration of hybridization between the introduced and highland endemic species on Santa Cruz, especially given the expansion of the introduced species into the highlands, the sensitivity to past climatic variation detected in highland populations, and the potentially threatened state of single-island endemics.

Key words: *flightlessness,* Galapaganus, *gene flow, islands, mitochondrial DNA, population structure*

The challenge of maintenance of genetic diversity within populations can be exacerbated for island endemics if they display population dynamics strongly influenced by ecological factors and behavioral attributes—such as reduced vagility—that can expose them to the effects of genetic drift without the benefits of gene flow. Studies of insular populations of highly mobile species have demonstrated that populations may remain genetically connected with the mainland in spite of significant differentiation (Agudo et al. 2011) and can even display high levels of gene flow between island subpopulations, albeit with low overall levels of genetic diversity (Nims et al. 2008). In contrast, studies of flightless beetles on islands have found that genetic subdivision may be high, revealing restricted gene flow between populations on different islands (Finston and Peck 1995). However, the link between dispersal abilities and ensuing gene flow or differentiation of island populations is not entirely clear. Although low vagility and behavioral philopatry are suggested as limiting dispersal in the absence of physical barriers among genetically differentiated colonies of flightless cormorants in the Galápagos archipelago (Duffie et al. 2009), wider ranges have been linked with patchier distributions and more opportunities for genetic differentiation among populations of South American gastropod species (Donald et al. 2011).

Island populations can also bear the signature of past bottlenecks and the action of genetic drift either at the original founding event (Woolfit and Bromham 2005; Balmer et al. 2011) or during later climatic change, such as interglacial periods (Jordan and Snell 2008). If historical fragmentation of the habitat has contributed to diminished connectivity between suitable patches within islands, then island populations could also have smaller sizes and potentially harbor less variation (Frankham 1996, 1997).

Additionally, island populations are more vulnerable to species invasions than those in continental habitats because they are seemingly less saturated or the native island species are less competitive than their continental counterparts (Simberloff 1986). Island populations also have inherent but poorly understood properties as evidenced by the higher extinction rates of island endemics compared with those of their nonendemic relatives (Frankham 1998).

Although oceanic islands such as those in the Galápagos archipelago are natural models for studying the processes of generation and maintenance of species diversity (Gillespie and Roderick 2002; Emerson and Kolm 2005), such endemic diversity may be threatened by the introduction of exotic species and by fragmentation of the habitat (Peck et al. 1998; Snell, Powell, et al. 2002; Snell, Tye, et al. 2002). Phylogeny-based studies of island groups have provided a framework for testing hypotheses on both the mode and timing of colonization of archipelagos (Emerson 2002). Still, only population-level studies will provide information on the processes underlying the early stages of diversification (Beheregaray et al. 2004; Ciofi et al. 2006; De Busscherre et al. 2010) and the impact of shorter term events on the population dynamics of island groups (Moya et al. 2004; Holland and Cowie 2007; Jordan and Snell 2008).

The weevil genus *Galapaganus* Lanteri (Coleoptera: Curculionidae) presents a suitable system to study species radiations on islands (Sequeira, Lanteri, et al. 2008; Sequeira, Sijapati, et al. 2008) and could also shed light on the genetic composition, structure, and demographic stability of populations of flightless endemics. This genus contains a total of 15 species; within the 13 that are flightless and fairly heavy-bodied, 10 are endemic to the islands (Lanteri 1992) (Figure 1). As opposed to its flightless endemic counterparts, a recent island invader from lowland Ecuador and Perú (*Galapaganus howdenae howdenae*) has well-developed wings (Lanteri 2004; Causton et al. 2005; Peck 2005). Even though *G. h. howdenae* is thought to have been introduced into lowland agricultural zones in Santa Cruz, we have now repeatedly found it outside of the boundaries of the disturbed agricultural zone feeding on introduced and endemic vegetation side by side with its endemic close relatives, *G. conwayensis* and *G. ashlocki*.

In addition to a complex and steep topography generated by a history of volcanic activity (White et al. 1993; Geist 1996; Reynolds 1996), the islands' climatic patterns have produced vegetation zones that change along an elevational gradient. Six ecological zones are recognized in the largest islands of the archipelago ranging from a littoral zone at sea level containing salt-tolerant vegetation, through arid, transition, *Miconia*, *Scalesia*, and pampa zones, to include high-altitude cloud forest (Wiggins and Porter 1971; Peck 1991, 1996, 2005). Complexity of the landscape increases further for many of the less vagile groups, where each volcano within an island can be considered as an independent platform (Finston and Peck 1995, 1997, 2004; Parent and Crespi 2006).

Endemic *Galapaganus* weevil species are usually restricted to 1 or 2 contiguous ecological zones with characteristic vegetation where adults feed preferentially on host plants restricted to those particular zones. One such "'habitat specialist" is *G. collaris*, known exclusively from the moist highlands of Floreana and San Cristóbal (Lanteri 1992; Peck 2005). When 2 or more species occupy an island, as is the case of *G. galapagoensis*, *G. vandykei*, and *G. collaris* on San Cristóbal and *G. conwayensis* and *G. ashlocki* on Santa Cruz, one is usually confined to the moist highlands, whereas its counterpart/s inhabit the lowlands (Lanteri 1992). The genus also contains a few "habitat generalists" whose adults feed on plant hosts across most ecological zones (*G. conwayensis* and *G. williamsi* on Northern and Southern Isabela, respectively, and *G. caroli* on Floreana) and some species whose ecological preferences have not been determined due to the small number of localities available (*G. blairi* on Santiago) or the paucity of ecological diversity available on the islands they inhabit (*G. darwini* on Darwin and Wolf). Through maximum likelihood optimization of habitat preferences on a well-supported phylogeny for *Galapaganus*, we proposed multiple parallel shifts toward the more restricted highland preferences in different islands and suggested that those habitat shifts could be potentially linked to species formation (Sequeira, Lanteri, et al. 2008).

At least one other Galápagos endemic group, the *Hogna* wolf spiders, appears to have diverged and specialized in parallel forming similar morphological and ecological sets of species in different islands (De Busscherre et al. 2010). Furthermore, the *Hogna* specialization within the island of Santa Cruz appears to have occurred under low levels of historical gene flow (De Busscherre et al. 2010). In *Galapaganus*, no significant conflict between phylogenies obtained from mitochondrial and nuclear data sets, combined with concordance between groupings in the mitochondrial derived genealogy and morphologically defined species, appears to rule out the possibility of recent interspecies hybridization in this radiation (Sequeira, Sijapati, et al. 2008). In the case of *Galapaganus*, where genealogical reconstructions have provided details of the general geographical context in which populations were founded (Sequeira, Lanteri, et al. 2008), a large combined nuclear and mitochondrial data set could aid in the quantification of gene exchange during early divergence under the isolation with migration model (Nielsen and Wakeley 2001) to inform the particular circumstances of those putatively habitat-promoted divergences. However, the limited amount of variation found within and between *Galapaganus* populations and species through the analysis of 6 nuclear regions ranging between 0% and 0.1% average pairwise sequence divergence between species (Sequeira, Sijapati, et al. 2008) precludes the exploration of those questions in the present study using a multilocus approach.

Due to the potential limitations of arguments regarding gene flow between species derived from the analysis of a single locus, the focus of this analysis using a comprehensive mitochondrial data set (4 gene regions for multiple localities in all but one of the endemic species in the archipelago) is to make inferences about the historical processes and biological attributes that have shaped the structure of populations within endemic species across the genus. Specifically, we assess the potential effects of 3

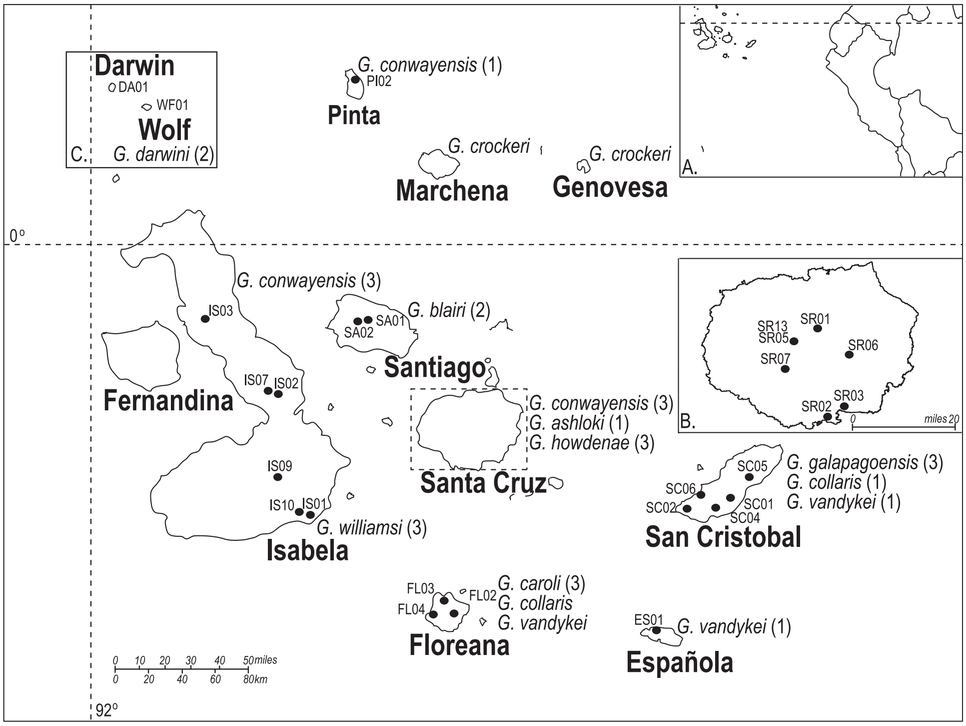

Figure 1. Map of the Galápagos archipelago indicating distribution per island of *Galapaganus* species, and in parentheses, the number of collecting sites per species included from each island. Inset A: relative position of the archipelago, inset B: detailed view of Santa Cruz island, and inset C: 2 northernmost islands.

processes on the genetic structure and historical demography of weevil populations: 1) colonization of highland habitats, 2) population differentiation facilitated by ecological zonation and/or volcanic activity in those species with more generalized habitat preferences, and 3) microallopatric differentiation possibly facilitated by flightlessness or host plant dependence for all *Galapaganus* species.

Materials and Methods

Sampling

We obtained samples from 9 of the islands including 9 of the 10 endemic species occurring in the archipelago (Figure 1, Table 1). For comparison purposes, we included *G. h. howdenae*, widespread in coastal Ecuador, and introduced to the agricultural area of Santa Cruz (Lanteri 1992, 2004; Peck et al. 1998; Causton et al. 2005). *Galapaganus conwayensis* was previously described as a single-island endemic for the island of Santa Cruz (Lanteri 1992; Sequeira et al. 2000; Peck 2005); however, we are adding recent collections from Northern Isabela and Pinta (Sequeira, Lanteri, et al. 2008).

DNA Preparation, PCR Amplification, and Sequencing

We used 3 legs from each specimen to isolate DNA according to the protocol of Normark (1996) or alternatively used the DNeasy Tissue Kit (Qiagen, Valencia, CA). We amplified genomic DNA to produce double-stranded products for 4 mitochondrial regions partially covering: 12S, cytochrome c oxidase subunit I, cytochrome c oxidase subunit II, and cytochrome b (primers and amplification conditions described in Sequeira, Lanteri, et al. (2008)). We purified amplification products using a MinElute PCR purification kit (Qiagen) and sequenced with PCR primers following the BigDye v3.1 terminator protocol (Applied Biosystems) in an ABI3100 capillary sequencer. We compiled, edited, and aligned sequences in Sequencher v. 4.5 (GeneCodes corporation, Ann Arbor, MI). For non-protein-coding regions (12S), we performed alignments in Clustal X (Thompson et al. 1997).

We compiled a combined matrix of 2579 characters from all 4 mitochondrial DNA (mtDNA) regions, 397 for 12S, 800 for CO I, 631 for CO II, and 749 for Cyt b for 166 individuals from 27 localities of 10 *Galapaganus* species (GenBank and accession numbers in Table 1, Dryad doi number:10.5061/dryad.01n56147).

Diversity within Populations and Population Demographic Parameters (1)

We compiled standard descriptive polymorphism statistics including number of haplotypes, haplotype diversity (*h*), nucleotide diversity (π), the average number of pairwise differences per collecting locality under Kimura 2 parameter model, and population-specific F_{ST} per locality and per species when relevant (Table 2).

Table 1 Population codes, locality details, and accession numbers for 4 mitochondrial gene regions for 27 populations of introduced and endemic *Galapaganus* species

Accession number							
Species	Island	Population code and locality	*N*	12S	COI	COII	CytB
Galapaganus ashlocki$^{\text{SIE}}$	Santa Cruz (SR)	SR05, Los gemelos, 614 m	11	EU264748–757	EU264913–923	EU265090–099	EU265275–282
Galapaganus blairi$^{\text{SIE}}$	Santiago (SA)	SA01, Las Pampas, 556 m	6	EU748834–837, JN899859-60	EU748847–848, JN899918-19	EU748820–823, JN899979-80	EU748805–808, JN900040-41
		SA02, Camino a La Central, 663 m	5	JN899861-65	JN899920-24	JN899981-85	JN900042-46
Galapaganus caroli$^{\text{SIE}}$	Floreana (FL)	FL02, Casa Cruz, 130 m	10	EU264609–618	EU264815–824	EU264975–981	EU265158–167
		FL03, Cerro Pajas, 320 m	7	EU264619–624	EU264825–831	EU264982–985	EU265168–174
		FL04, Bahia Las Cuevas, 0 m	10	EU264625–634	EU264832–841	EU264987–994	EU265177–185
Galapaganus collaris	San Cristobal (SC)	SC01, El junco, 620 m	10	EU264652–661	EU264860–867	EU265012–019	EU265208–216
Galapaganus conwayensis	Isabela (IS)	IS02, VA, Los Guayabillos, 850 m	5	EU264641–644	EU264849–853	EU265002–004	EU265197–200
		IS07, VA, Los Pega-Pega, 493 m	6	EU748832–833, JN899834-37	EU748845–846, JN899892-95	EU748818–819, JN899953-56	EU748803–804, JN900014-17
		IS03, VD, Camp 760 m, 768 m	9	EU748827–831, JN899830-33	EU748840–844, JN899888-91	EU748813–817, JN899949-52	EU748798–802, JN900010-13
	Santa Cruz (SR)	SR03, Charles Darwin Research Station, 0 m	11	EU264737–747	EU264906–912	EU265078–088	EU265263–273
		SR02, Tortuga Bay trail, 0 m	7	EU264732–734, 36, EU748838, JN899866-67	EU264905, EU748851, JN899926-27	EU265073–075, 77, EU748825, JN899988-89	EU265259–262, EU748809, JN900047-48
		SR13, Los gemelos, 614 m	11	EU264778–788	EU264941–949	EU265120–122, 24–30	EU265300–302, 04–10
	Pinta (PI)	PI02, Pinta	10	EU264645–651, JN899856-58	EU264854–859, JN899915-17	EU265006–011, JN899976-78	EU265201–207, JN900037-39
Galapaganus galapagoensis$^{\text{SIE}}$	San Cristobal (SC)	SC02, SE Wreck Bay, 0 m	6	EU264662–663, 65, 67, 70–71	EU264868–871, 73–74	EU265020–021, 23–24	EU265217–218, 21–22
		SC04, E Wreck Bay, 100 m	9	EU264673–674, 78–80, 82–83, 85–86	EU264875–880, EU748849–850, xxx	EU265027, 31–32, 34–35, 37–38	EU265224–25, 27–29
		SC06, N Wreck Bay, 0 m	6	EU264702–704, 06–07, 10	EU264887, 90, 92–93	EU265051, 53, EU748824 JN899986-87	EU265241–242, 44–46, 48
Galapaganus darwini	Darwin (DA)	DA01, Darwin, 14 m	15	EU264599–603, JN899817-26	EU264804–808, JN899876-85	EU264965–969, JN899937-46	EU265148–150, JN899998- 900007
	Wolf (WF)	WF01, Wolf, 20 m	17	EU264789–796, EU748839, JN899868-75	EU264950–956, EU748852, JN899928-36	EU265131–138, JN899990-97	EU265311–316, EU748812, JN900050-57
Galapaganus vandykei	San Cristobal (SC)	SC05, Rosa Blanca Bay, 0 m	10	EU264687–690, 92–96, 700	EU264881–886	EU265039–046, 50	EU265230–237, 39
	Espanola (ES)	ES01, Punta Suarez, 0 m	6	EU264604–608	EU264809–814	EU264970–974	EU265152–157

Table 1 Continued

				Accession number			
Species	Island	Population code and locality	N	12S	COI	COII	CytB
Galapaganus williamsi$^{\mathrm{SIE}}$	Isabela (IS)	IS01, VSN, Puerto Villamil, 0 m	9	EU264636–638, 40, JN899827-29	EU264842–848, JN899886-87	EU264995–998, 00–01, JN899947-48	EU265188–191, 93, 95–96, JN900008-09
		IS09, VSN, Volcan Chico Trail, 995 m	9	JN899838-46	JN899896-904	JN899957-65	JN900018-26
		IS10, VSN, Trail Centro de Crianza, 0 m	10	JN899847-55	JN899905-14	JN899966-75	JN900027-36
Galapaganus bowdenae bowdenae	Santa Cruz, introduced (SR)	SR01, Trail to Cerro Croker, 400–800 m	15	EU264717–731	EU264895–904	EU265058–072	EU265250–258
		SR06, 2 km. El Cascajo, 240 m	10	EU264758–767	EU264924–931	EU265100–109	EU265283–289, EU748810–811, JN900049
		SR07, F. Steve Devine, 351 m	10	EU264768–777	EU264932–940	EU265110–119	EU265290–299

N indicates the number of individuals analyzed, and SIE indicates a single-island endemic. VA, Alcedo volcano, VD, Darwin volcano, and VSN, Sierra Negra Volcano.

In order to assess the likelihood of a history of demographic expansion, we contrasted patterns across endemic populations with differing ecological preferences (highland vs. lowland specialists and all altitude endemics as defined in Table 2) employing 2 lines of evidence. Using ARLEQUIN v 3.1 (Schneider et al. 2000), we calculated Tajima's D statistic because this metric is expected to be negative after a population expansion and adjusted the statistical significance of Tajima's D statistic for multiple tests using a sequential Bonferroni correction (Table 2). In order to examine the mismatch distribution, we assessed the number of pairwise differences between haplotypes for island population groups (Figure 3). Because it reflects the highly stochastic nature of the coalescent process, this distribution is expected to be multimodal in a population at demographic equilibrium and unimodal in a population that has recently experienced a demographic expansion (Rogers and Harpending 1992). Estimates of time since the population expansion can be derived from the slope of the leading edge of the curve (inversely related to the time since expansion and the minimum population size during the bottleneck), and the vertical intercept of the wave reflects the increase in population size during recovery so that the larger the recovery the smaller the intercept (Rogers and Harpending 1992). Given the uncertainties associated with the bionomics of some of these endemic species (i.e., generation time) and the concatenated nature of the data set (spanning multiple gene regions with potentially differing mutation rates), we did not attempt precise estimation of the timing of the expansion events or of the size of the population recoveries and only compared the general shapes of the distributions across islands and species. Additionally, using LAMARC v.2.0.2 (Kuhner 2006), we calculated maximum likelihood estimates of effective population sizes in populations of introduced and endemic weevils. For species with localities on more than one island (*G. darwini*, *G. vandykei*, and *G. conwayensis*), estimates were calculated for each island population group (Table 4). Details of the runs and validation of the results are listed below.

Population Connectivity, Migration Estimates, and Genetic Structuring (2 and 3)

We also used LAMARC v.2.0.2 (Kuhner 2006) to calculate maximum likelihood estimates of intra- and interisland migration rates between localities of introduced and all endemic species where more than one locality was available using models of equilibrium gene flow and considering that, in general, within islands, lowland endemic populations are likely to have long-term stable structure (Kuhner 2009) (Table 4). All 4 gene regions were analyzed as a single locus given their common mitochondrial origin. The rates are reported as $M = m/m\mu$, where m is the immigration rate per generation (the chance of immigration per individual per generation) and $m\mu$ is the neutral mutation rate per site per generation (the chance of mutation per site per generation). The estimation process uses an expansion of the coalescent theory that includes migration. Three runs were

Table 2 Genetic diversity and population statistic measures of Galápagos weevils per locality and species

Population	Number of u. h./l. s	Number of haplotypes	h	K2P	π	PS F_{ST}	D
Galapaganus ashlocki$^{\mathrm{HS}}$							
SR05	_9/1(2)_	_10_	_0.909_	_16.094_	_0.012 ± 0.0066_	_N/A_	_−1.641*_
Galapaganus blairi$^{\mathrm{ND}}$							
SA01	3/1(3)	4	0.667	4.400	0.0030 ± 0.001	_N/A_	−0.524
SA02	4/1(2)	4	0.800	12.600	0.0061 ± 0.0039	_N/A_	−2.439*
All *G. blairi*		_8_		_20.0545_	_0.0137 ± 0.007_		_−0.479_
Galapaganus caroli$^{\mathrm{AA}}$							
FL02	10	10	1.000	11.633	0.0073 ± 0.0041	0.4194	−0.977
FL03	7	7	1.000	31.033	0.0196 ± 0.0112	0.3489	−0.585
FL04	10	10	1.000	21.320	0.0129 ± 0.0071	0.3911	−1.313
All *G. caroli*		_27_	_1.000_	_26.117_	_0.017 ± 0.008_		_−1.726*_
G. collaris$^{\mathrm{HS}}$							
SC01	10	10	1.000	_21.920_	_0.0143 ± 0.0078_	_N/A_	−1.663*
Galapaganus conwayensis$^{\mathrm{LS,\ AA}}$							
SR03	11	11	1.000	13.954	0.0119 ± 0.0065	0.6979	−1.494
SR02	7	7	1.000	8.491	0.0068 ± 0.0041	0.7277	−1.709*
SR13	11	11	1.000	12.139	0.0071 ± 0.0039	0.7241	−1.602*
PI02	7/1(3)	8	0.800	11.433	0.0067 ± 0.0038	0.7429	−2.102*
IS02	3/1(2)+	4	0.800	7.997	0.0069 ± 0.0045	0.7563	−1.237
IS07	2/1(4)+	3	0.333	7.800	0.0055 ± 0.0035	0.7575	−1.063
IS03	5/1(4)	6	0.667	13.566	0.0064 ± 0.0036	0.7463	−2.014*
All *G. conwayensis* SR		_29_	_1.000_	_21.022_	_0.0168 ± 0.0085_		−1.203
All *G. conwayensis*		_49/1_	_0.8448_	_37.765_	_0.0235 ± 0.0115_		[_−1.493*_]
Galapaganus darwini$^{\mathrm{ND}}$							
DA01	11/2(2, 2)	13	0.866	14.7	0.0074 ± 0.0039	_N/A_	−1.909*
WF01	13/2(2, 2)	15	0.882	17.77	0.0097 ± 0.0051	_N/A_	−1.515
All *G. darwini*		_28_	_0.8750_	_22.5181_	_0.0119 ± 0.006_		[_−1.463_]
Galapaganus galapagoensis$^{\mathrm{LS}}$							
SC02	6	6	1.000	12.156	0.0174 ± 0.0106	0.0526	−1.401
SC04	9	9	1.000	10.414	0.0174 ± 0.0098	0.0514	−1.238
SC06	6	6	1.000	30.519	0.0262 ± 0.0015	0.1989	−0.406
All *G. galapagoensis*		_21_	_1.000_	_6.7857_	_0.014 ± 0.007_		_−2.645*_
Galapaganus vandykei$^{\mathrm{LS}}$							
ES01	4/1(2)	5	0.833	10.280	0.0081 ± 0.0049	_N/A_	−0.657
SC05	10	10	1.000	20.288	0.0157 ± 0.0086	_N/A_	−1.926*
All *G. vandykei*		_15_	_0.937_	_22.35_	_0.042 ± 0.006_		[_−0.601_]
Galapaganus williamsi$^{\mathrm{AA}}$							
IS01	3/2(3, 3)+	5	0.555	1.6701	0.00149 ± 0.001	0.5571	−0.398
IS09	2/2(5, 2)	4	0.444	0.8339	0.0004 ± 0.0003	0.5672	−0.936
IS10	1/3(4, 2, 2)+	5	0.500	25.5761	0.0124 ± 0.007	0.4444	0.471
All *G. williamsi*		_13/1_		_17.0211_	_0.0865 ± 0.0044_		_−0.885_
Galapaganus howdenae howdenae$^{\mathrm{INT}}$							
SR01	7/1(8)+	_8_	0.533	_10.431_	_0.0122 ± 0.0066_	0.0225	_−2.402*_
SR06	2/1(8)+	_3_	0.300	_0.808_	_0.0004 ± 0.0064_	0.1398	_−1.667*_
SR07	1/1(9)+	_2_	0.200	_1.011_	_0.0005 ± 0.0004_	0.1509	_−1.741*_
All *G. h. howdenae*		_11/2_	0.440	_4.0979_	_0.005 ± 0.0002_		_−2.568*_

Superscripts after species names indicate ecological preferences: HS, highland specialist; LS, lowland specialist; AA, all altitude endemic; INT, introduced; ND, not determined. u.h. : unique haplotypes/l.s. : locally shared (in parenthesis, the number of individuals sharing that haplotype). +indicates haplotypes shared between localities within species, h: haplotype diversity, K2P: average number of pairwise differences per population under Kimura 2 parameter model, π: nucleotide diversity, PS F_{ST}: population specific F_{ST}, and D: Tajima's statistic. N/A, not applicable. Values in italics correspond to calculations for each species as a single population unit when applicable.

*$P < 0.05$.

started for each subsample of the data set to be analyzed starting with different random number seeds. The length of the runs was considered adequate when the confidence intervals of the point estimates for multiple runs did not exclude one another (Kuhner 2009), and the acceptance rate was not below 15%. Runs consisted of 10 short chains of 1000 steps each and 2 long chains of 15 000 steps using an F_{ST}-based estimator as a starting value and a sampling interval of 20. Reported values are the point estimates of the longer chains (Table 4). For comparison purposes, we also scaled the estimates by the distance between collecting localities (Table 4). As a complement to the migration rate estimates obtained using genealogy samplers, we used ARLEQUIN v 3.1 (Schneider et al. 2000) to calculate M, the absolute number of migrants between populations (Nm) based on the summary statistic F_{ST} (Table 4).

In order to visualize the pattern of haplotype sharing across all endemic populations and/or species and the links between haplotypes present in different ecological zones, we constructed a minimum spanning network illustrating minimum number of mutational steps between haplotypes in endemic populations, using the algorithm of Rohlf (1973) (Figure 2).

To explore the effects of microallopatric differentiation across populations of all flightless endemic species (with introduced populations as a comparison) and the role of islands, ecological zones, and volcanoes on the current genetic structure of the more widely distributed species (*G. conwayensis*), we assessed hierarchical partitioning of genetic variation at multiple levels (Table 3). We performed analyses of molecular variance (AMOVAs) between islands and between morphologically defined species for the data set containing all endemic species, between and within localities (populations) for all endemics with more than 2 localities (*G. caroli*, *G. galapagoensis*, and *G. williamsi*—pairwise F_{ST} values are reported in Table 4 for those species with 2 localities, *G. blairi*, *G. darwini*, and *G. vandykei*), between volcanoes for *G. conwayensis* populations from Northern Isabela, and between ecological zones for those from Santa Cruz (using a simplified ecological zonation scheme of littoral, arid, and highland). The effect of ecological zonation on the hierarchical partitioning of genetic variability within a species could only be tested for *G. conwayensis* in Santa Cruz, given that sampling was insufficient for tests on endemics with wider ecological preferences within Floreana, Isabela, or San Cristobal. We performed AMOVAs as described by Weir and Cockerham (1984) and implemented in ARLEQUIN v 3.1 (Schneider et al. 2000) estimating variance components and F-statistics analogs (Φ-statistics) for haplotype diversity at each level of the hierarchical levels requested (Table 3). Significance of Φ-statistics was tested by 10 000 permutations of haplotypes among and within populations.

Results

Demographic History, Genetic Diversity, and Effective Population Sizes in Endemic Populations (1)

Lowland endemic populations of *G. conwayensis* that share the island of Santa Cruz with the introduced *G. h. howdenae* (SR02 and SR03), either display nonsignificant neutrality test estimates indicating stable populations or show indications of expansions (Table 2).

The mismatch distribution for pooled Santa Cruz populations of *G. conwayensis* as well as for the San Cristobal lowland endemics *G. vandykei* and *G. galapagoensis* and the widely distributed Southern Isabela endemic *G. williamsi* are multimodal, many with nonsignificant neutrality tests, suggesting that those populations have been stable over a longtime period (P = 0.003, 0.015, 0.026, and 0.008, respectively: Table 2, Figure 3). Mismatch distributions for localities spanning a wide altitudinal range pooled by species and by island such as *G. caroli* from Floreana and *G. conwayensis* from Northern Isabela or for species with undetermined ecological preferences as *G. blairi* from Santiago and *G. darwini* from Darwin, also display multimodal patterns (P = 0.012, 0.09, 0.046, and 0.002, respectively), yet in the case of the Isabela *G. conwayensis* pattern, the multimodality could be due to reduced gene flow between localities from different volcanoes. However, not all endemic patterns are similar, populations of highland endemics, such as *G. ashlocki* and *G. collaris*, display mismatch distribution patterns, and negative and significant neutrality tests estimates compatible with a history of demographic expansion (Table 2, Figure 3). Despite the compatibility with an expansion model, mismatch patterns differ between highland endemics. In particular, differing features are the slope of the leading edge of the wave and the vertical intercept of the peak of the wave, suggesting differing initial populations and recoveries (Rogers and Harpending 1992). Additionally, the τ values that can be derived from fitting the crest of the wave as an x intercept also differ suggesting different time estimates for when the population expansions could have taken place.

In general, within-population nucleotide diversity indexes are moderate across endemic populations (Table 2). Overall haplotype diversity is higher for endemic populations than for introduced ones, with many endemic populations (19 of 24) displaying haplotype diversity equal to or higher than 0.8 (Table 2). An overall pattern of high haplotype diversity (h) and moderate nucleotide diversity is observed for many endemic populations. Haplotypes are not shared among species nor are they shared within species among islands or volcanoes in Isabela. Shared haplotypes among localities are only observed in 3 instances: among *G. conwayensis* localities from Volcan Alcedo in Northern Isabela, among *G. williamsi* localities from Volcan Sierra Negra in Southern Isabela, and among *G. h. howdenae* localities in the agricultural zone of Santa Cruz (Table 2, Figure 2).

Comparing LAMARC'S maximum likelihood estimates of $\Theta_f(2N_{ef}\mu)$, as an approximation of long-term historical female effective population sizes for mitochondrial genes assuming equal mitochondrial mutation rate across species, similar Θ_f is found for many endemics and the Santa Cruz introduced species (Table 4). Populations of the lowland endemic, sharing hosts with the introduced *G. h. howdenae* in Santa Cruz, display higher Θ_f compared with that of introduced populations, however, notably lower than lowland endemics in San Cristobal (Table 4).

Population Structure and Migration Rates in Endemic Populations (2 and 3)

Hierarchical analysis of molecular variance performed using the set of concatenated mtDNA sequences reveals

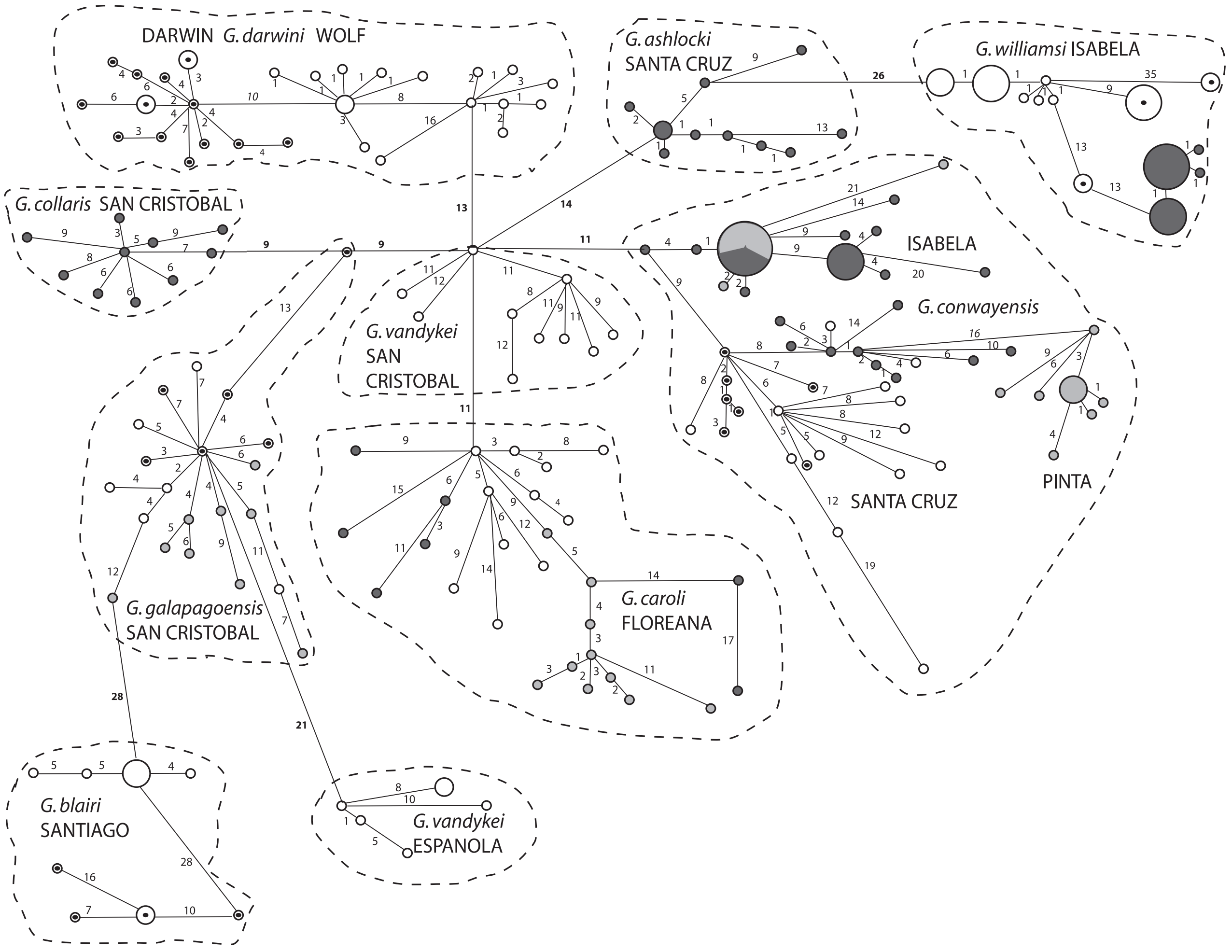

Figure 2. Minimum spanning network of all combined COI, COII, CytB, and 12S haplotypes for the 9 island endemic species. Haplotypes belonging to the same species are surrounded by dashed lines, and circle sizes are proportional to the number of individuals sharing that haplotype. Numbers on the lines indicate the number of mutational steps between haplotypes, italic indicates steps between haplotypes from different islands, and bold indicates steps between haplotypes from different species. Shaded circles correspond to the simplified ecological zonation scheme used in the AMOVA analysis. White: lowland habitats, light gray: midelevation habitats, and dark gray: highland habitats; when there are 2 or more populations of the same species from a certain habitat, haplotypes belonging to one of the populations are marked with a dot.

significant Φ_{CT} indexes for the effect of islands and morphologically defined species on the partitioning of genetic variation on all the pooled endemic populations (Table 3). This is not surprising given that previous phylogenetic estimates revealed well-supported and generally monophyletic morphologically defined species and proposed a single colonization with subsequent within-island lineage splitting for 2 of the 3 islands harboring more than one species (Sequeira, Lanteri, et al. 2008). Mitochondrial haplotypes are not only not-shared between species but are also generally clustered by species in the minimum spanning network with one exception: *G. vandykei* (Figure 2).

Partitioning of molecular variance among populations (within islands) is significant for all the endemic species analyzed (*G. caroli* in Floreana, *G. conwayensis* in Isabela and Santa Cruz, *G. galapagoensis* in San Cristobal, and *G. williamsi* in Isabela) indicating considerable structure within each of the islands even among localities within very narrow geographical ranges (1.5–6 km) indicating significant differentiation at a microallopatric scale (Table 3). The effects of volcanoes on the partitioning of genetic diversity of *G. conwayensis* on Northern Isabela populations are not clear, given that relatively high Φ_{CT} statistics (42.15% of the variation) are nonsignificant, possibly due to the large proportion of the variation existing within those Isabela populations (Table 3). In contrast to the overall pattern of structured endemic populations, hierarchical partitioning of molecular diversity in the introduced *G. h. howdenae* populations shows that the majority of the genetic variance is explained by differences within the populations, with a much lower percentage of the variation observed among the localities (Table 3).

Table 3 Hierarchical AMOVAs for endemic and introduced *Galapaganus* species grouped by morphologically defined species, by island, and within species by ecological zone or volcano (when applicable)

Species	Source of variation	% of variation	Fixation indices
All endemics	Among species	57.92	$\Phi_{CT} = 0.57918**$
	Among populations within species and total among populations	25.22	$\Phi_{SC} = 0.59923**$, $\Phi_{ST} = 0.83135**$
	Within populations	16.87	
	Among islands	37.18	$\Phi_{CT} = 0.37183**$
	Among populations within islands and total among populations	45.54	$\Phi_{SC} = 0.72493**$, $\Phi_{ST} = 0.82721**$
	Within populations	17.28	
Galapaganus caroli$^{\mathrm{AA}}$	Among populations	39.07	$\Phi_{ST} = 0.3907**$
	Within populations	60.93	
Galapaganus conwayensis$^{\mathrm{LS,\ AA}}$	Among populations	74.89	$\Phi_{ST} = 0.748**$
	Within populations	25.11	
(IS02 + IS03 + IS07) (PI02)(SR02 + SR03 + SR13)	Among islands	56.07	$\Phi_{CT} = 0.561**$
	Among populations in islands and total among populations	22.76	$\Phi_{SC} = 0.518**$, $\Phi_{ST} = 0.788**$
	Within populations	21.17	
Santa Cruz only	Among populations SR	54.13	$\Phi_{ST} =0.5416**$
	Within populations SR	45.87	
(SR02 + SR03) (SR13)	Among eco. zones SR	48.65	$\Phi_{CT} = 0.486$
	Among populations in eco. zones SR and total among populations	11.73	$\Phi_{SC} = 0.228**$, $\Phi_{ST} = 0.603**$
	Within populations SR	39.62	
Isabela only	Among populations IS	36.17	$\Phi_{ST} = 0.3617**$
	Within populations IS	63.83	
(IS02 + IS07) (IS03)	Among volcanoes IS	42.15	$\Phi_{CT} = 0.421$
	Among populations in volcanoes IS and total among populations	0.28	$\Phi_{SC} = 0.005**$, $\Phi_{ST} = 0.424**$
	Within populations IS	57.57	
Galapaganus galapagoensis$^{\mathrm{LS}}$	Among populations	9.39	$\Phi_{ST} = 0.094*$
	Within populations	90.61	
Galapaganus williamsi$^{\mathrm{AA}}$	Among populations	52.01	$\Phi_{ST} = 0.5201**$
	Within populations	47.99	
Galapaganus howdenae howdenae$^{\mathrm{INT}}$	Among populations	2.84	$\Phi_{ST} = 0.028$
	Within populations	97.16	

$*P < 0.05$, $**P < 0.01$.

Migration and immigration rate estimates averaged across populations within islands, as well as rates scaled by distance, are low for endemic populations, even for those comparisons that clearly, despite significant genetic structure and some significant pairwise Φ_{ST} between populations, could be pooled into a single unit due to very small geographic distances between localities (*G. galapagoensis* in San Cristobal and *G. blairi* in Santiago) (Table 4). In contrast, introduced populations display larger migration estimates, suggesting either substantial connectivity across locations or recently established units from a single source that have not yet differentiated.

LAMARC estimates of immigration rates averaged across some endemic populations, which show definite signs of geographic structure, such as for *G. conwayensis* and *G. williamsi* populations in Northern and Southern Isabela, respectively, and *G. galapagoensis* in San Cristobal are considerably high (Table 3), some of those accompanied by the only nonsignificant pairwise Φ_{ST} values (*G. conwayensis* IS and *G. galapagoensis*, Table 4). However, LAMARC estimates of movement of breeding individuals into a population averaged over other equally structured endemic populations within one island are notably lower for *G. caroli* on Floreana, *G. blairi* on Santiago, and *G. conwayensis* on Santa Cruz, comparable with immigration estimates between populations located on different islands (Table 4).

Despite the significant structuring of *G. conwayensis* populations in Santa Cruz, partitioning of genetic variability by ecological zone is high but not significant ($\Phi_{CT} = 0.486$, Table 3). Concordantly, for most of the endemics with wider ecological ranges, the minimum spanning network reveals that haplotypes from different ecological zones are interspersed and do not cluster together (Figure 2), suggesting that the pattern of accumulation of variation among haplotypes is independent of the rather simple ecological scheme used here.

Discussion

Common Demographic Signatures of Past Population Bottlenecks and Expansions in Endemic Highland Specialists

Galapaganus ashlocki and *G. collaris* are endemic to the archipelago and found exclusively in highland habitats

Table 4 Maximum likelihood estimates of migration rates and effective population sizes from LAMARC analyses

Species	Average migration (immigration) rates ($M = m/m\mu$) ± SE	Migration rates/km	Average pairwise F_{ST} ± SD/M (Nm)	$\Theta = 2N_{ef}\mu$ (99% CI)
Galapaganus ashlocki^{HS} SR	—	—	—	0.0421 (0.02–0.11)
Galapaganus blairi^{NA} SA	30.74	20.63	0.76657* 0.15226	0.0172 (0.008–0.05)
Galapaganus caroli^{AA} FL	10.08 ± 3.24	1.31	0.3503* ± 0.1571 1.1931	0.1432 (0.08–0.28)
Galapaganus collaris^{HS} SC	—	—	—	0.0823 (0.03–0.22)
Galapagansu conwayensis^{LS} SR	17.69 ± 6.71	1.49	0.4881* ± 0.2525 0.8650	0.0720 (0.04–0.13)
G. conwayensis^{AA} IS	124.59 ± 89.94	5.03	0.2610∧ ± 0.2225 38.8295	0.0312 (0.02–0.06)
G. conwayensisND PI	—	—	—	0.0246 (0.01–0.07)
G. conwayensis II	7.18 ± 3.16	0.063	0.7903* ± 0.051 0.1352	—
Galapaganus darwini^{NA} II	28.94	0.76	0.4687* 0.5667	0.0335_{DA} (0.02–0.22) 0.0244_{WF} (0.01–0.05)
Galapaganus galapagoensis^{LS} SC	106.78 ± 11.05	25.49	0.0695∧ ± 0.0609 4.3304	0.1454 (0.08–0.29)
Galapaganus vandykei^{LS} II	6.88	0.09	0.7930* 0.1305	0.0414_{SC} (0.03–0.08) 0.0150_{ES} (0.004–0.06)
Galapaganus williamsi^{AA} IS	138.13 ± 39.51	8.74	0.5257 ± 0.3585 0.9013	0.0129 (0.007–0.024)
Galapaganus howdenae howdenae^{INT}	360.23 ± 143.9	33.97	0.3839* ± 0.3777 1.7282	0.0426 (0.02–0.07)

Average immigration rates across populations and scaled by geographic distance between populations, average pairwise F_{ST} among populations within 8 *Galapaganus* species grouped by species and within species by island. Superscripts after species names indicate ecological preferences as in Table 2. II: interisland, additional 2 letter codes after species names or as subscripts after estimates indicate island of origin following codes in Table 1. SE, standard error; CI, confidence interval.

∧ indicates significant values for 1/3 pairwise F_{ST} for *G. galapagoensis* and for 2/3 for *G. conwayensis* IS.

$^*P < 0.05$.

(Lanteri 1992; Peck 2005). Additionally, *G. ashlocki* is a single-island endemic on Santa Cruz, whereas *G. collaris* has been described from the highlands of San Cristobal and Floreana. Previous phylogenetic studies suggest independent colonizations of the highland habitats on Santa Cruz and San Cristobal by these 2 endemic species (Sequeira, Lanteri, et al. 2008). Mismatch distributions and Tajima's *D* indexes suggest past population size changes including expansions following bottlenecks for both of these highland specialists. Details of the shape of the mismatch distribution plots potentially suggest different timing and scenarios for the inferred population recoveries (or expansions) after more and less severe population bottlenecks; however, both distributions would be concordant with bottlenecks and expansions largely postdating colonization times estimated for highland habitats in both islands (Sequeira, Lanteri, et al. 2008).

Multiple forces have been proposed as causes of population bottlenecks in other volcanic islands (Holland and Cowie 2007) and most are also true for Galápagos. Forces that could reduce and fragment island populations include volcanic eruptions, Pleistocene sea-level rise and climate variation, colonization by new predators or competitors and expansions into new habitats (Carson and Templeton 1984; Gillespie and Roderick 2002).

Paleoclimate data summarized by Peck (2005) indicate that the Galápagos islands have not been appreciably wetter than they are today. Indeed, evidence shows that in the past glacial cycle, and probably earlier ones, islands were without the present significant rainy season, and much of the vegetated area of the wetter upland forests was thus diminished or even absent (Colinvaux 1972; Colinvaux and Schofield 1976a, 1976b; Peck 2005). Additionally, the increased wet conditions later induced by interglacial events have been proposed as favoring expansion of the pampa vegetation toward lower altitudes and underlying large historical population sizes for highland dwellers (De Busscherre et al. 2010). In this context, and given that populations of lowland species show signals of long-term stability, one potential underlying cause of the population fluctuations described here for highland specialists in both islands could be the severe reduction and subsequent increase of suitably moist habitat that accompanied the Pleistocene climatic variations.

Population Structuring for Flightless Endemics in Volcanic Landscapes

Poor dispersal capabilities do not necessarily preclude organisms from inhabiting wide ranges nor from having connected populations because the effects of flightlessness could be circumvented by behavioral attributes that allow organisms to survive across fragmented landscapes

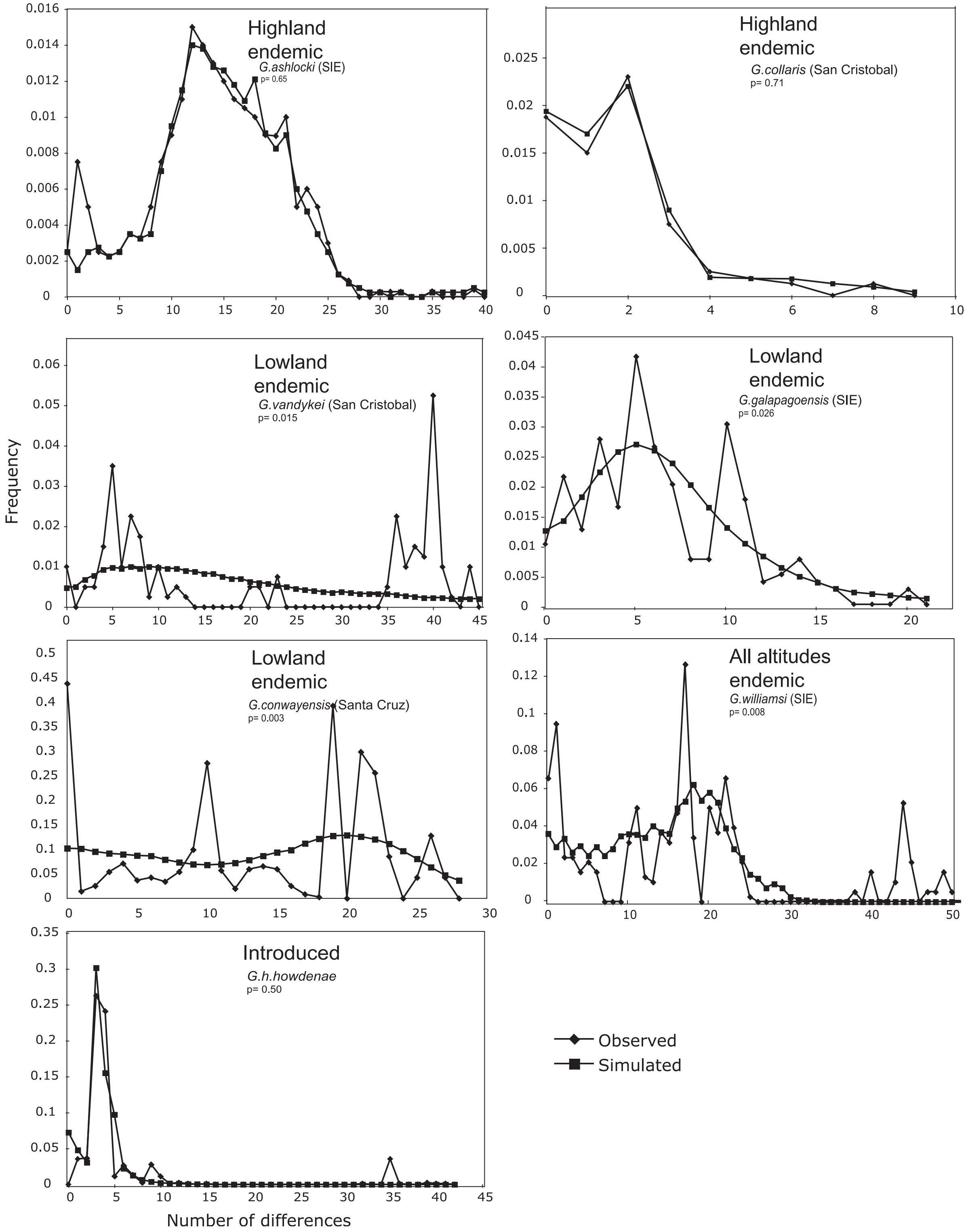

Figure 3. Mismatch distributions for the concatenated mitochondrial data set for some of the endemics and the introduced species. When a species' range spans more than one island, mismatch distributions are presented for population groups within one island as indicated in parenthesis after species names. *Galapaganus ashlocki*, *G. galapagoensis*, and *G. williamsi* are single-island endemics as indicated by SIE. Curves of expected values were obtained by simulating 10 000 data sets under a coalescent algorithm by implementing parameter estimates based on a sudden demographic expansion (Schneider and Excoffier 1999). *P* values represent the probability that the variance of the simulated data set is equal or greater than the observed one.

(Diekotter et al. 2010). However, highly significant genetic differentiation across the range is common in flightless populations with island-like distributions (Keller et al. 2004; Vandergast et al. 2007, 2009; Matern et al. 2009) and in flightless island endemics (Finston and Peck 1995; Duffie et al. 2009).

The history of volcanic activity of many oceanic islands has been invoked as an explanation for vicariance and fragmentation of island populations, for divergent lineages within islands (Juan et al. 1996; Moya et al. 2004; Holland and Cowie 2007), and for population reductions and reduced variability within populations (Beheregaray et al. 2003; Gubitz et al. 2005). We have found significant population differentiation between and within islands across 6 of the endemic *Galapaganus* species studied. However, in the rather limited testing performed here, we do not find evidence of structuring determined by volcanoes in Isabela nor by the ecological zone inhabited in Santa Cruz, not allowing us to link elevation and its correlate, habitat, with differentiation within these endemic species, as reported for other Galápagos insects (Schmitz et al. 2008). Differentiation between localities within islands was significant across a range of geographic distances between localities, across 5 islands with differing area, geological age estimates, and presumably volcanic histories—ranging from the oldest to the youngest—(Peck 2005) and across species whose origins and colonization times differ by millions of years (Sequeira, Lanteri, et al. 2008; Sequeira, Sijapati, et al. 2008). Again, this precludes an association of the degree of within-species variation with island geological youth—and its correlate of habitat heterogeneity—as proposed for endemic Galápagos snails (Parent and Crespi 2009).

Population-level studies of other invertebrate groups with limited dispersal abilities and narrow or discontinuous distributions within this island system show patterns with some, though not all, of the features reported here for most species of flightless *Galapaganus* (Finston and Peck 1995, 1997; Verdyck and Desender 1999). Specifically, the pattern detected is one of highly structured populations groups with low within-population variability. For example, a metapopulation structure with recurrent extinctions and recolonizations has been proposed to explain the considerable genetic variation found between populations of Alticine beetles that occur on the same island within Galápagos but that bear low within-population heterozygosity (Verdyck and Desender 1999). Alternatively, the relatively young age of the genus and an allopatric mode of speciation have been used to explain high levels of intra- and interspecies genetic identities (Finston and Peck 1997) and signals of restricted gene flow between islands in *Stomion* beetles (Finston and Peck 1995).

In general for *Galapaganus*, haplotype diversity is high across all endemic collecting localities with moderate nucleotide diversity. Island population groups harboring high haplotype variation can result from multiple colonizations of each island with multiple founder events favoring haplotype fixation in each subgroup (Holland and Cowie 2007). However, in *Galapaganus*, clustering of island populations in the haplotype network and previous phylogenetic evidence of single colonizations for each island/species combination (Sequeira, Lanteri, et al. 2008; Sequeira, Sijapati, et al. 2008) are not compatible with such a scenario. Additionally, the pattern of variable and highly structured populations is repeated in 5 species endemic to different islands with heterogeneous history of volcanic activity (Geist et al. 1985, 1994; Geist 1996; Harpp and Geist 2002; Chadwick et al. 2006; Haymon et al. 2008) precluding the use of volcanism as the underlying cause of the observed population structure. Alternatively, this series of species with variable and highly structured population groups can be interpreted as long-established independently founded island units, where structuring could be a signal of microallopatric differentiation due to patchy host plant distribution and poor dispersal abilities.

The potential effects of more recent habitat degradation on the genetic makeup of these island populations should still not be underestimated. Preliminary analyses of variation at 13 microsatellite loci on *G. conwayensis* populations across volcanoes on Northern Isabela (Sequeira AS, Stepien CC, Tran CN, Stuckert A, Guo W, in preparation) show a pattern of reduced variation within populations and increased genetic structure consistent with those of populations on poorly connected and fragmented habitats (Frankham et al. 2002; Frankham 2003).

Shared Hosts and Habitats for Endemic and Introduced Species on the Lowlands of Santa Cruz Island

The genetic effects of introduced populations on their local or endemic counterparts can range from increased vigor for introduced native hybrids (Fitzpatrick and Shaffer 2007) or accelerated contemporary evolution in native populations (Carroll 2007; Fisk et al. 2007) to complete loss of fitness in the interspecific hybrids (Ayres et al. 2008). No hybrids have been found to date between *G. h. howdenae* and *G. conwayensis*, and phylogenetic evidence points to well-defined species boundaries across the genus (Sequeira, Sijapati, et al. 2008).

The ecological effects of introduced species, possibly influencing the genetic composition of native species, are harder to unravel because they have been shown to not only reduce survivorship but also alter the dynamics of dispersal and habitat use by native species (Evans 2004). In the absence of samples and genetic patterns that predate the introduction of *G. h. howdenae* into Santa Cruz, inferences on the state of *G. conwayensis* endemic populations can only be made at this time through comparisons with populations of close relatives with similar habits. As for other lowland endemics, genetic evidence for Santa Cruz populations of *G. conwayensis* indicates demographically stable populations with reduced gene flow across localities and substantial effective population sizes, albeit lower compared with that of San Cristobal endemics. In general, this could signify that there is no immediately detectable effect of this introduction on variation patterns of native populations.

The degree to which the introduced and the lowland endemic *Galapaganus* compete for resources is still unclear; however as with other oligophagous insects (Berenbaum and Zangerl 1991), field observations indicate that *G. h. howdenae* has shifted to feeding on the same native and endemic host plants as their endemic counterparts. In the case of native widespread species, if the 2 species were to compete it does not follow that the native species would necessarily be immediately displaced (Leger and Espeland 2010), even though introduced species have been shown to pose significant competitive threats to native populations (Stokes et al. 2009). For endemic species with restricted populations or those such as *G. conwayensis* that effectively function as isolated populations due to the extremely unlikely transport across islands, intervention, protection from introduced and invasive counterparts, and monitoring have been proposed as important components for the island native's continued survival (Cole et al. 2005; Garrett et al. 2007; Simbana and Tye 2009).

Conservation Implications

Highly structured and stable populations of flightless lowland endemics are contrasted with signals of more labile demographic histories for highland specialists. Currently, there is no evidence of past or present genetic exchange between introduced and native *Galapaganus*. However, we suggest that future studies incorporating nuclear markers should explore the possibility of hybridization between the introduced and highland endemic species on Santa Cruz, especially given the expansion of the introduced species into the highlands, the sensitivity to past climatic variation detected in highland populations, and the potentially threatened state of single-island endemics.

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