Biogeographic and Evolutionary Mechanisms Driving Diversification in Caribbean ground crickets (genus *Amphiacusta*)

by

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2009

For my husband

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Abstract

Amphiacusta is a species-rich genus of flightless ground crickets distributed throughout the Caribbean which exhibits substantial variation habitat use and male reproductive traits. This ecological and sexual diversity displayed by *Amphiacusta* furnishes a natural experiment to investigate driving population and species divergence because geographic isolation on islands can lead to the reduction or elimination of gene flow

This research addresses three questions. First, what biogeographic mechanisms have influenced the pattern of species diversification of *Amphiacusta*? Estimates of the phylogenetic relationships among *Amphiacusta* are used to test the predictions of two biogeographic models for the Caribbean: island-island vicariance and overwater dispersal. Both topological evidence and estimates of the timing of divergence events provide little evidence for a strict interpretation of the island-island vicariance model, suggesting rather that both vicariance and overwater dispersal, followed by intra-island diversification, have played a role in the history of this genus. Second, is morphological divergence in the Virgin Islands species *Amphiacusta sanctaecrucis* the result of neutral or selective divergence? A comparison of phenotypic differentiation in three morphological traits (body size and shape, mandible shape, and genitalia shape) with neutral gene differentiation, indicates that all three exhibit significant variation between populations and have diverged by natural or sexual selection. Finally, are these traits

evolving solely through local selection in isolation, or do extrinsic factors, such as the geographic configuration of islands and the ocean currents between them, influence morphological divergence between populations? An analysis of microsatellite loci finds that gene flow is very limited between islands, and furthermore, that there is no association between distance or gene flow and morphological divergence. This confirms that morphological divergence is the result of local adaptation and suggests that even weak selection over the course of their recent isolation could result in significant morphological divergence between populations. Overall, these results demonstrates that both the geological history of the Caribbean, as well as the evolutionary mechanisms operating within isolated populations are involved in the diversification of *Amphiacusta*.

Chapter 1

Testing for biogeographic mechanisms promoting divergence in Caribbean crickets (genus *Amphiacusta*)

INTRODUCTION

The Caribbean is characterized by a rich and diverse fauna that exhibits high levels of endemism. Numerous attempts have been made over the past several decades to elucidate the means by which this unique fauna emerged (Barbour, 1914; Darlington. 1938; Simpson, 1956; Rosen, 1975; Pregill, 1981; Iturralde-Vinent & MacPhee, 1999; Dávalos, 2007), but the region's complex geological history has made it difficult to draw general conclusions about the mechanisms responsible (Donnelly, 1989a). Both vicariance (Rosen, 1975; Iturralde-Vinent & MacPhee, 1999) and dispersal (Hedges et al., 1992; Hedges, 1996; Heinicke et al., 2007) have been proposed as the predominant forces behind the emergence and diversification of Caribbean taxa. This includes debate over possible connections between continental mainlands and the Caribbean islands, possibly through emergent islands between North and South America (Donnelly, 1989b, 1990; Pindell & Barrett, 1990), or a South American connection via a subaerial landspan centered on the Aves Ridge ("GAARlandia") (Iturralde-Vinent & MacPhee, 1999), the timing of emergence and connections between the Greater Antillean islands (Perfit & Williams, 1989), as well as the role of inter-island diversification in generating the diverse Caribbean fauna – the focus of this study.

Continual tectonic and volcanic activity in the Caribbean basin has resulted in a geological history that is dynamic and complex (Pindell, 1994; Iturralde-Vinent & MacPhee, 1999), making it difficult to distinguish between dispersal versus vicariant induced species diversification from geologic evidence (Hedges, 2001). Nevertheless,

various aspects of the geologic history generate explicit predictions about geographic patterns of taxonomic relationships, as well as the timing of diversification. The Lesser Antilles evolved independently from the Greater Antilles, and is composed of two arc systems, one of older islands originating in the late Eocene to Oligocene, and another of younger islands emerging in the late Miocene (MacDonald et al., 2000). The landforms comprising the current islands of Cuba, Hispaniola and Puerto Rico did not begin to emerge until the late Eocene (Pindell, 1994; Robinson, 1994; Iturralde-Vinent & MacPhee, 1999). Cuba and Hispaniola are both composites of three separate landblocks, and eastern Cuba was connected with northern Hispaniola until the early to mid-Miocene (25-20 mya) (Pindell & Barrett, 1990), while the connection between southern and northern Hispaniola was established in the mid-Miocene (Pindell & Barrett, 1990). The connection between Hispaniola and Puerto Rico was severed ~16-11 mya with the Mona Canyon (Iturralde-Vinent & MacPhee, 1999). During this time Jamaica experienced successive periods of emergence followed by submergence, becoming fully emergent only in the middle Miocene (~12 mya: Robinson, 1994). Changes in sea levels have also profoundly affected the connections between some islands – climatic cycles during the Pliocene and Pleistocene isolated the Virgin Islands from Puerto Rico and resulted in the repeated submergence/emergence of the Bahamas between ~3 mya and 118,000 ya (Hearty & Neumann, 2001; Reijmer et al., 2002). To the extent that the geologic history and associated vicariant events contributed to the species diversity of Caribbean faunas, the signature of this history will be evident in the biogeographic pattern and timing of species divergence.

The vicariant model of Iturralde-Vinent and MacPhee (1999) proposes that subsequent to the submergence of putative continental connections, formerly widespread taxa diverged allopatrically as islands moved apart during the Oligocene and Miocene, and thus predicts that the history of species divergence in the Greater Antilles will reflect the history of island separation. This vicariant model is distinct from that of Rosen (1975), which proposed that current- day species distributions are the result of vicariant fragmentation of island connections during the Cretaceous – a hypotheses that is refuted by molecular clock estimates of divergence times between Caribbean and mainland taxa

(Hedges et al., 1992; Heinicke et al., 2007), and phylogenies of Caribbean fauna and flora (Malone et al., 2000; Fritsch, 2003; Heinicke et al., 2007).

In contrast, the emergence and diversification of Caribbean taxa may be the result of overwater dispersal from South America, followed by inter-island dispersal and divergence (Hedges et al., 1992, Hedges, 1996). The extent to which island-island vicariance and inter-island dispersal are responsible for shaping the evolutionary history and modern distributions of island taxa remains uncertain (Dávalos, 2004). While it is clear that dispersal is the only mechanism by which Jamaican taxa could arise (because of its constant isolation from other islands), dispersal among the other islands might also play a role in speciation. However, since such dispersal events would not be constrained to any particular geologic period (i.e., the dispersal scenario does not make explicit predictions about the timing and pattern of diversification), the focus of this study is on whether the phylogeny of a group of flightless crickets (genus *Amphiacusta*) supports a vicariant model of diversification. Specifically, this study tests the timing and pattern of divergence among island groups predicted by island-island vicariance (Fig. 2) using the mitochondrial gene cytochrome oxidase I (COI) and the nuclear gene elongation factor 1-alpha (EF1-α).

The genus *Amphiacusta* (Saussure, 1874) is a genus of ground crickets restricted to the Caribbean and constitutes the largest portion of Neotropical diversity in the Phalangopsinae (Gryllidae, Orthoptera) – a subfamily distributed worldwide. There are more than 80 described species of *Amphiacusta*, most of which are found in the Greater Antilles and are endemic to one island (Fig. 1) (Desutter-Grandcolas & Otte, 1997; Otte & Perez-Gelabert, unpublished manuscript). The species are nocturnal and occur in a variety of habitats, including sandy beaches, wet and dry tropical forest, and caves. All species in this family are flightless, a feature which may hinder inter-island colonization by dispersal and make intra-island speciation more likely (e.g., Mendelson & Shaw, 2005). This reduced potential for dispersal coupled with the impressive diversity of *Amphiacusta* means that the genus may provide insights into how the complex geologic history may have contributed to species diversification in this biodiversity hotspot (Myers et al., 2000).

METHODS

Sampling

Twenty-eight species in the genus *Amphiacusta* and a co-distributed outgroup in the same family, *Yoyuteris phacodes*, were sampled (Table 1). This sampling constitutes approximately 35% of described species, with sampling particularly dense in Hispaniola, Puerto Rico, and the Virgin Islands. We discuss the implications of our sampling in the Discussion section. All specimens are located at the University of Michigan Museum of Zoology, Ann Arbor, MI, or the Academy of Natural Sciences, Philadelphia, PA. Specimens awaiting formal description (i.e., species 1, 2, and 3) exhibit significant differentiation in their genitalic morphology, the primary distinguishing feature among species in this genus.

Genomic DNA was extracted from the femur of each individual using a Qiagen DNeasy kit. A 1185 base pair (bp) fragment of the mitochondrial gene cytochrome oxidase I (COI) and a 900 bp fragment of the nuclear gene elongation factor I-α (EF1-α) were sequenced (see Table 2 for primers and Knowles (2000) for PCR protocol). Sequences can be found in GenBank under accession numbers EU939163-EU939220. and both strands of each product were sequenced on an ABI Model 3730 sequencer. All sequences were aligned using Sequencher 3.1 (Gene Codes, Ann Arbor, MI); regions with gaps in EF1-α were readily aligned by eye.

Phylogenetic Analysis

Gene trees were estimated separately for each locus using maximum likelihood (ML) in PAUP* 4.0 (Swofford, 2002), as well as a Bayesian analysis using MrBayes 3.0 (Ronquist & Huelsenbeck, 2003). For the ML-analyses, 25 random addition replicates and tree bisection reconnection (TBR) branch swapping were used for each data set with a maximum of 100 trees saved; branch support was evaluated with nonparametric ML bootstrapping with 100 random addition replicates limited to 10 trees each. Four Markov chains of 5.5 million generations were used in the Bayesian analyses, with trees sampled every 1000 generations; posterior branch probabilities were calculated after excluding the

first 0.5 million generations. A HKY + I + G model of sequence evolution for COI and a Timura-Nei + G model of evolution for EF1-α (details are given in Figures 3 and 4, respectively) were used in the phylogenetic analyses; these models were identified by the program DT ModSel (Minin et al., 2003) based on a comparison of likelihood scores among possible models of evolution.

Recent results have identified potential problems with concatenation of multiple genes for phylogenetic analysis, and in particular, the tree resulting from concatenated data may not accurately reflect the species history (Edwards et al., 2007, Kubatko & Degnan, 2007). To assess whether concatenation was appropriate, a parametric boostrap was employed to determine whether the topologies of the mitochondrial gene tree COI and the nuclear gene tree EF1-α were significantly different. To make the test more conservative, only differences in the topology corresponding to the branches relevant to the inter-island vicariance model were considered. Since both a parsimony and ML parametric bootstrap detected a significant difference in the trees of the two loci (parsimony: $p < 0.01$, ML: $p < 0.05$), each gene was analyzed separately in tests of the biogeographic hypotheses.

Test of Biogeographic Hypotheses

Given the difference in the estimated gene trees and the topology predicted under a model of inter-island vicariance, a parametric bootstrap of the log likelihood ratios (Huelsenbeck et al., 1996; Goldman et al., 2000) was used to determine whether the data was statistically consistent with inter-island vicariance model (Fig. 2). The likelihoods of the gene trees of COI and EF1-α were estimated with and without the topological constraints predicted by inter-island vicariance model (Fig. 2) and the differences in likelihood scores between the constrained and unconstrained trees were compared to a distribution generated from simulated data sets, where a significant departure from the inter-island vicariance model would be indicated if the log-likelihood ratio value in the empirical data was greater than or equal to the value observed in 95% of the simulated data sets (i.e., $P < 0.05$ of obtaining the log-likelihood ratio). Since inter-island vicariance makes predictions about the relationships among island groups, but not within islands, only the branching sequence among islands was considered (e.g., {Cuba (Hispaniola

(Puerto Rico, Virgin Islands)}) in this test; all other nodes remained unconstrained. The test was also performed using the difference in parsimony scores resulting from the topological constraints of the vicariance model with significance evaluated by a distribution generated by parametric bootstrapping as outlined in Goldman et al. (2000). Mesquite (Maddison & Maddison, 2004) was used to simulate the data sets, and PAUP* 4.0 (Swofford, 2002) to estimate trees and calculate ML and parsimony scores.

Dating Divergences

Because of rate heterogeneity in COI (a molecular clock was rejected based on a log-likelihood ratio test, $p < 0.01$), calculations of divergence times were based on a COI tree with rate homogeneity imposed under a penalized likelihood criterion, as computed with the program r8s (Sanderson, 2003). While ideally fossils would be used to calibrate the divergence times estimated from phylogenies (Smith & Peterson, 2002), such data are not available. In addition to the scarcity of orthopteran fossils (Poinar, 1993; Poinar, 1999), controversy surrounds the age of insect fossils collected in the region (Grimaldi, 1995). For example, dates range from 15-20 mya (Iturralde-Vinent & MacPhee, 1996) to 25-40 mya (Poinar, 1993) for fossils from amber in the Dominican Republic, depending on the method used and the source of amber. Given the lack of fossils for calibrating a rate of molecular divergence, two standard rates of divergence reported in the literature for insects and arthropods more generally – 1.5% (Farrell, 2001) and 2.3% sequence divergence per million years (Brower, 1994), respectively – were used to fix one node in the tree using uncorrected species pairwise distances, and r8s was used to calculate the relative divergence times of all other nodes. Confidence intervals for divergence times were determined by reapplying penalized likelihood to 100 bootstrapped data matrices, which we generated using PHYLIP (Felsenstein, 2005). The range of estimates given throughout the paper includes these confidence intervals.

RESULTS

Of 1185 bp of COI sequence, 470 were variable and of the 782 bp of EF1-α studied, 234 were variable. Sequence divergence within the ingroup ranged from 0.09% to 21.4% for COI, and from 0% to 9.1% for EF1-α. The divergence between the ingroup and outgroup taxa (*Yoyuteris phacodes*) for COI ranged from 18.7% to 20.7% and 16.2% to 20.4% for EF1-α.

Despite some topological incongruence, the gene trees estimated for COI and EF1-α exhibit broad similarities (Fig. 3 and 4). In both trees taxa from Puerto Rico, the Virgin Islands, and Jamaica form a clade with *A. tijicohniae* and *A.* species 3 basal to the rest of the more recently derived taxa, whose relationships differed between the COI and EF1-α trees. Species also tend to cosegregate by island in both the COI and EF1-α trees, although in no cases did the taxa from a particular island form a monophyletic clade (Fig. 3 and 4). For example, the 17 species from Hispaniola form two closely related clades. The placement of the Cuban species (*A. ruizi* and *A. sincerus*) and the Bahaman species *A. henrymorgani* within the clade of Hispaniola taxa in the tree estimated from COI may indicate a more complicated biogeographic history; however, this position of Cuban and Bahaman taxa was not recovered in the tree estimated from EF1-α. For both trees, the Jamaican species *A.* species 4 was derived from a clade comprised of Puerto Rican and Virgin Island taxa.

The primary incongruence between the COI and EF1-α trees, in terms of topological differences relevant to the biogeographic hypotheses of interests, is the placement of the Cuban and Bahaman taxa (Fig. 3 and 4). There are some differences in the relative relationships of taxa from Hispaniola, as well as among the taxa within the clade of taxa from Puerto Rico, Jamaica, and the Virgin Islands, between the trees estimated for the two loci. The topology of our COI tree differed significantly from that predicted by inter-island vicariance under a likelihood framework, but not under parsimony (Table 3); however, the topology of our EF1-α tree was not significantly incongruent with this model.

Using the two rates of evolution of cytochrome oxidase I (i.e., 1.5 and 2.3 % sequence divergence per million years), the date of divergence between the Puerto Rican-Virgin Island and Hispaniolan and Cuban groups is between 14.2 and 9.3 mya. Fixing this node and employing the penalized likelihood function of r8s (Sanderson, 2003), the

split between the Cuban/Bahaman clade and its sister Hispaniolan occurred between 12.3 and 6.4 mya. The split between the Bahaman species *A. henrymorgani* and the Cuban species *A. ruizi* and *A. sincerus* corresponds to a divergence of 8.4 and 4.2 mya, and that between the Virgin Island species *Amphiacusta hyperphobos* and the Puerto Rican species *A. tijicohniae* of 7.6 and 4.2 mya . The speciation events within the group of Virgin Island, Puerto Rican, and Jamaican taxa that are sister to *A. tijicohniae* all appear to have occurred recently and rapidly. For example, the divergence of *A. sanctaecrucis* from *A. hyperphobos* dates within a range of 1.4 mya and 600,000 and the split between *A. pronauta* and *A.* species 1 around 890,000 and 520,000 ya; however, the estimates for these recent divergences are all overestimates because the divergence that predates the species divergence (i.e., occurred within the ancestral species) will have a disproportionate affect on recently derived species (Edwards & Beerli, 2000).

DISCUSSION

Emerging evidence suggests roles for both vicariance and dispersal as mechanisms promoting diversification of Caribbean taxa. The importance of overwater dispersal has been demonstrated in a range of taxa including reptiles (Hedges et al., 1992; Malone et al., 2000), amphibians (Hedges et al., 1992; Heinicke et al., 2007), mammals (Dávalos, 2007), and plants (McDowell & Bremer, 1998; Lavin et al., 2003; McDowell et al., 2003; Negrón-Ortiz &Watson, 2003). Nevertheless, it is likely that vicariance has also played a role, and in some cases, support for both processes has been found (Lavin et al., 2003; McDowell et al., 2003; Brandley & de Quieroz, 2004). Similarly, a more complex interplay between these two mechanisms is evidenced by the phylogenetic relationships within *Amphiacusta*.

The timing of divergences between Puerto Rican and Virgin Island taxa suggests a history of vicariance resulting from the rising of sea levels during the Pleistocene, severing populations that then diverged in allopatry. At the same time, the derivation of the Jamaican *A.* species 4 from either Puerto Rico or the Virgin Islands is clear evidence of long distance dispersal. Despite their flightlessness, the ocean does not constitute a

complete barrier to dispersal for these crickets. While such dispersal events over long distances may seem counterintuitive, release and recapture experiments with buoys have demonstrated that flotsam can drift across large distances in the Caribbean and may take a meandering course affected by prevailing currents and storm conditions (Molinari et al., 1979). Furthermore, observations for a range of taxa, including insects, indicate that rafting on floating vegetation (Holzapfel & Harrell, 1968; Censky et al., 1998) may result in successful colonization events..

While the tests for topological congruence do not strongly support a history of vicariance between Cuba and the islands of Hispaniola and Puerto Rico, there is some ambiguity in the data. The placement of the Bahaman/Cuban group basal to all the other taxa in the EF1-α tree (Fig. 3) supports the inter-island vicariance model, whereas its placement within a Hispaniolan clade in the COI tree is consistent with dispersal – it is worth noting that this topology is not associated with strong support (Fig. 2). Assuming an evolutionary rate of 1.5% divergence per million years for mitochondrial genes (Farrell, 2001), these taxa could not have diverged prior to 13 mya, making the data inconsistent with that predicted by inter-island vicariance (i.e., a divergence of 25-20 mya). For the data to be consistent with a divergence time of 20 mya, the rate of molecular evolution would have to be 1.0% sequence divergence per million years (or less) – a rate that is slower than anything reported in the literature for invertebrates. The finding that *A. henrymorgani* diverged from *A. ruizi* and *A. sincerus*, a split of a Bahaman taxon from Cuban taxa, 8.4 and 4.2 mya, is older than expected given current models of Pleistocene fluctuations of sea level in that area (Hearty & Neumann, 2001), but is similar to other recent findings (Glor et al., 2005).

A close affinity between eastern Cuban and northern and central Hispaniolan taxa has been taken as evidence for vicariance (McDowell et al., 2003) because of the historical connections between these landmasses; however, we do not see this pattern in our data. The Hispaniolan clade sister to the Cuban/Bahaman clade in the COI tree is composed of species occurring in both the northern, southern, and central parts of Hispaniola; *Amphiacusta* from these regions do not cosegregate in either gene tree. This fact, together with the extent of sequence divergence, suggests a scenario of recent dispersal among islands accompanied by intra-island diversification on Hispaniola, which

may have occurred following the collision in the mid-Miocene (~15 mya) between the landmasses that now comprise the southern and northern portions of the island (Graham, 2003). It must be noted, however, that a possible limitation of our study is the low level of sampling of Cuban taxa. This is problematic, as Cuba is the largest island and likely hosts a diversity of *Amphiacusta* species. It is possible that the Cuban samples included here represent a secondary colonization from Hispaniola, and that other Cuban species did arise by vicariance. Further sampling will resolve this issue.

This is one of the few studies on the historical biogeography of terrestrial invertebrates in the Caribbean (see also Davies & Bermingham, 2002, Wilder & Hollocher, 2003, Brisson et al., 2006, Velez & Feder, 2006). Most work on biogeography of Caribbean fauna has focused on vertebrates (Ricklefs & Bermingham, 2004, Glor et al., 2005, Perdices et al., 2005, Dávalos, 2007, Heinicke et al., 2007). Furthermore, studies of Caribbean fauna have lent themselves well to an investigation of the ecological mechanisms and evolutionary dynamics that may have driven their diversification (Warheit et al., 1999, Wilder & Hollocher, 2003, Langerhans et al., 2006). Given that the analyses support a history of both vicariance and dispersal, as well as intra-island diversification (in the case of Hispaniola), it is worthwhile to investigate what factors other than historical biogeography have resulted in the great diversity of *Amphiacusta*. The substantial differences in male genital morphology among species raises the possibility that sexual selection on male genitalia, often suggested to promote the evolution of species diversity (Eberhard, 1985; Arnqvist, 1998), may play a role in the diversification in this group. For example, the divergence between *Amphiacusta hyperphobos* and *Amphiacusta sanctaecrucis* and between *Amphiacusta pronauta* and *A.* species 1 all appears to be very recent, occurring sometime between 300,000 to 900,000 years – a time recent enough that the species appear to retain ancestral polymorphism (i.e., there has not been a sufficient amount of time for the sorting of neutral variation by genetic drift) (e.g., Kliman et al., 2000; Brougton & Harrison, 2003; Carstens & Knowles, 2007). Yet, the species in this clade differ significantly in male genitalia shape at both the species and population level (Oneal, unpublished data), suggesting that selection has played a role in the diversification of the crickets (e.g., Masta & Maddison, 2002; Marquez & Knowles, 2007). With its island distribution and varied history of

divergence, *Amphiacusta* presents an opportunity to examine the role of sexual selection in promoting diversification that would complement the large number of studies focused on adaptive divergence in the diversification of Caribbean taxa.

CONCLUSIONS

Tests of the biogeographic history of *Amphiacusta* provides support for both overwater dispersal among islands, intra-island diversification, and a pattern of island-island vicariance paralleling the movements of the Greater Antilles islands since the Oligocene. Specifically, analyses of two independent molecular markers suggests a history of long distance dispersal from Puerto Rico/Virgin Islands to Jamaica, in situ diversification within Hispaniola, as well as vicariant separation between Virgin Island and Puerto Rican taxa resulting from Pleistocene changes in sea level. While the history of divergence among Cuban/Hispaniolan species remains unclear because of discordance in the gene tree topologies of the mitochondrial and nuclear locus, the low degree of mitochondrial sequence divergence observed suggests that recent dispersal is the more likely explanation. However, because of limited access (and sampling of taxa from Cuba) it is difficult to determine if the taxa sampled from Cuba represents a secondary colonization from Hispaniola, and that other Cuban species actually fit a vicariant model. As a clearer picture about the geographic and geologic context of species diversification emerges with additional sampling of species (and perhaps loci), these Caribbean crickets will provide an ideal opportunity to examine the implications of this biogeographic context for speciation, but also will provide an interesting comparative context with other Caribbean taxa undergoing adaptive divergence since the primary patterns of selective divergence in *Amphiacusta* relate to sexual selection, rather than ecological divergence.

FIGURE LEGENDS

Figure 1

Map of the Caribbean, with number of endemic taxa for the largest islands and the smaller island chains (n).

Figure 2

Topology and timing of divergence predicted by the island-island vicariance model (Iturralde-Vinent & MacPhee, 1999, MacPhee et al., 2003). Because Jamaica was emergent only by the mid-Miocene (~12 ya) (Robinson, 1994) its fauna must have arisen by dispersal. The model predicts the relationships among Cuban, Hispaniolan, Puerto Rican, and Virgin Island taxa; the position of Jamaica is not included here.

Figure 3

Phylogeny of *Amphiacusta* (Saussure, 1874) resulting from a maximum likelihood search of the COI data set, under an HKY+I+G with base frequencies A=0.3610, C=0.1966, G=0.0611, T=0.3818, transition/transversion ratio=8.09, α=0.81, and proportion invariant sites = 0.58. Maximum likelihood bootstrap proportions and Bayesian posterior probabilities above 50% respectively are indicated above and below the branches, respectively.

Figure 4

Phylogeny resulting from a maximum likelihood search of the EF1-α data set, under a Timura-Nei+G model with base frequencies A=0.2931, C=0.1670, G=0.2309, T=0.3090, rate matrix rAC=1, rAG=2.17, rAT=1, rCG=, rCT=3.81, rGT=1, and α=0.30. Maximum likelihood bootstrap proportions and Bayesian posterior probabilities above 50% respectively are indicated above and below the branches, respectively.

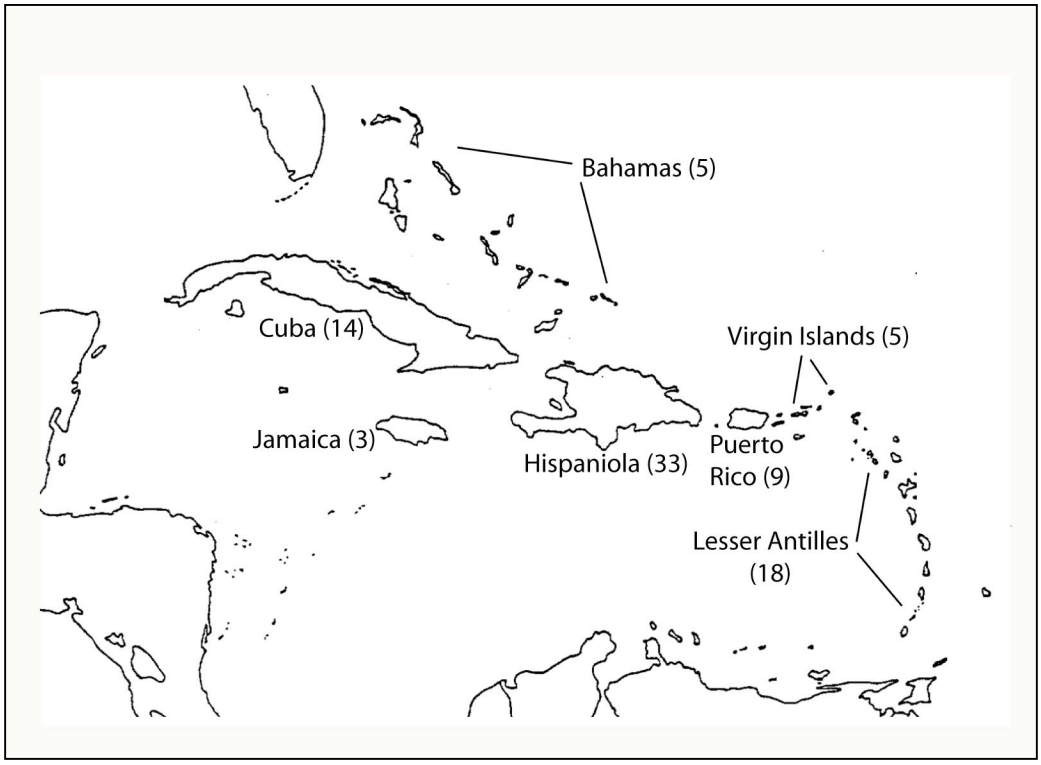

Bahamas (5)
Cuba (14)
Virgin Islands (5)
Jamaica (3)
Hispaniola (33)
Puerto
Rico (9)
Lesser Antilles
(18)

Figure 1.

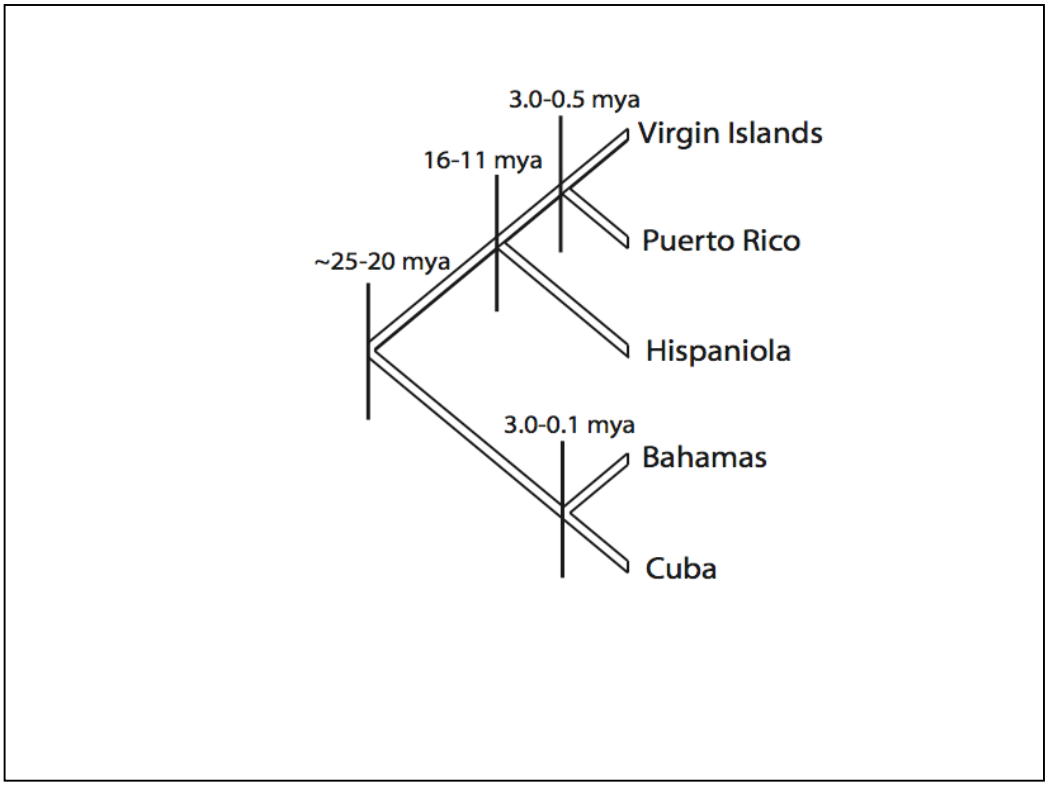
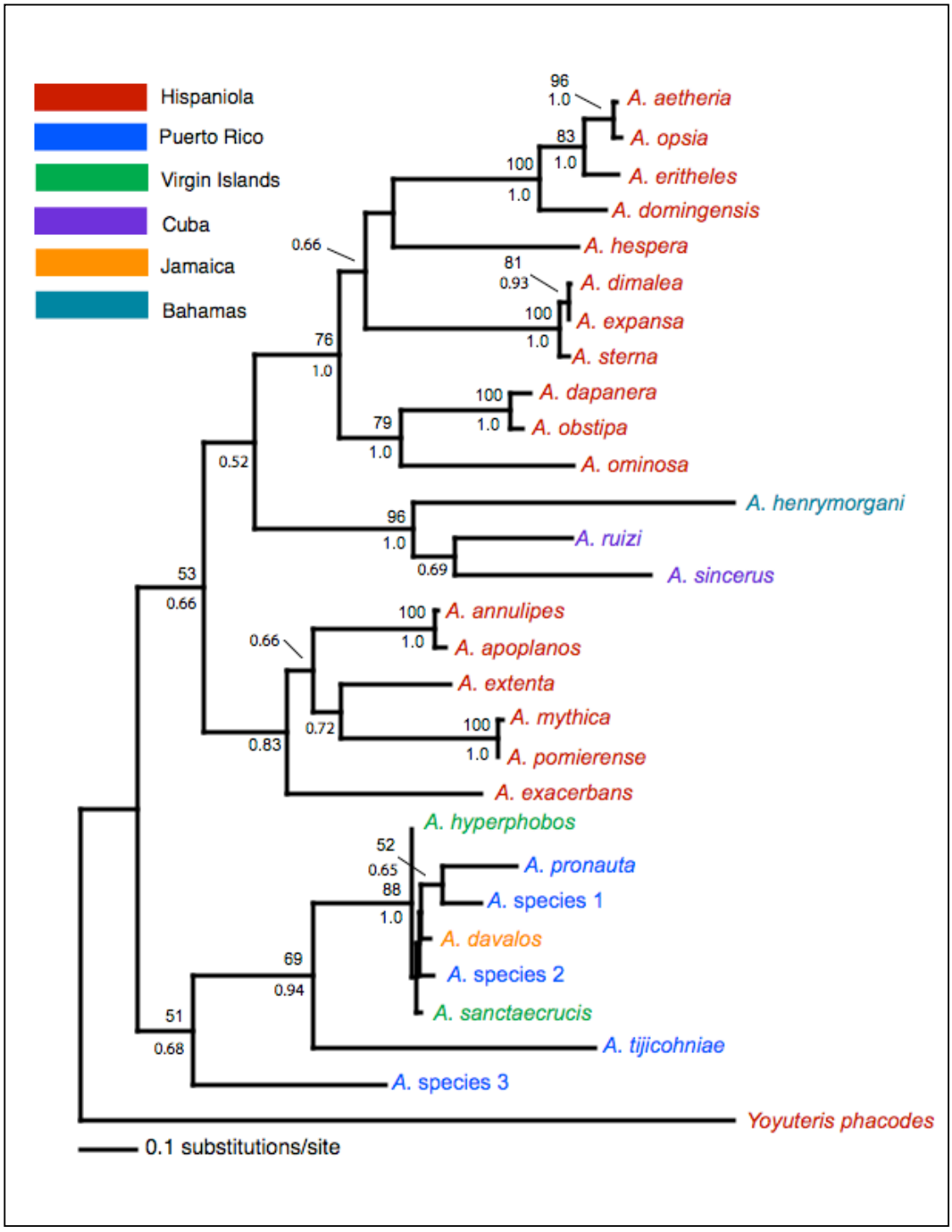

3.0-0.5 mya
Virgin Islands
16-11 mya
Puerto Rico
~25-20 mya
Hispaniola
3.0-0.1 mya
Bahamas
Cuba

Figure 2.


Hispaniola
Puerto Rico
Virgin Islands
Cuba
Jamaica
Bahamas
A. aetheria
A. opsia
A. eritheles
A. domingensis
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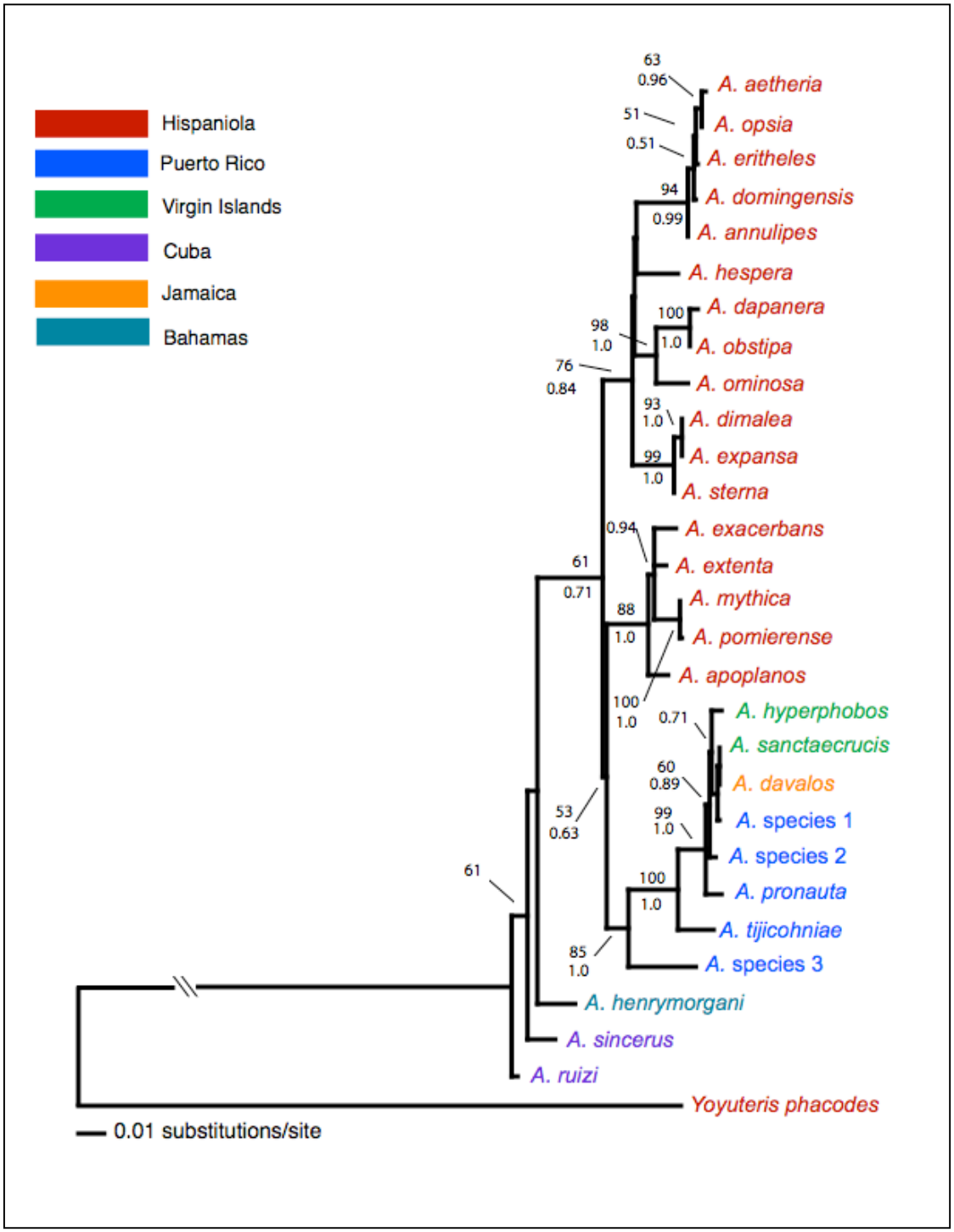
A. dimalea
A. expansa
A. sterna
A. dapanera
A. obstipa
A. ominosa
A. henrymorgani
A. ruizi
A. sincerus
A. annulipes
A. apoplanos
A. extenta
A. mythica
A. pomierense
A. exacerbans
A. hyperphobos
A. pronauta
A. species 1
A. davalos
A. species 2
A. sanctaecrucis
A. tijicohniae
A. species 3
Yoyuteris phacodes
0.1 substitutions/site

Figure 3.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A. davalos
A. species 1
A. species 2
A. pronauta
A. tijicohniae
A. species 3
A. henrymorgani
A. sincerus
A. ruizi
Yoyuteris phacodes
0.01 substitutions/site

Figure 4.

Table 1. Locality information for studied specimens. The undescribed species from Puerto Rico are referred to as *Amphiacusta* species 1, 2, and 3. The undescribed species from Jamaica is referred to as *Amphiacusta* species 4. [1]Collected by D. Otte, D. Perez, and R. Bastardo. [2]Collected by D. Perez, R. Bastardo, and B. Hierro. [3]Collected by D. Otte and D. Perez. [4]Collected by D. Perez and R. Bastardo. [5]Collected by D. Otte, L. L. Knowles, and E. Oneal. [6]Collected by D. Otte, L. L. Knowles, E. Oneal, and H. Huang. [7]Collected by D. Otte. [8]Collected by A. E. Ruiz and D. Otte. [9]Collected by A. Infante.

Species	Locality	Island
Amphiacusta aetheria[1]	Mano Matuey, San Cristobal Prov.	Hispaniola (Dominican Republic)
Amphiacusta annulipes[2]	Ebano Verde, La Vega Prov.	Hispaniola (Dominican Republic)
Amphiacusta apoplanos[4]	La Ciénaga, Barahona Prov.	Hispaniola (Dominican Republic)
Amphiacusta dapanera[2]	Sierra de Neiba, Bahoruco Prov.	Hispaniola (Dominican Republic)
Amphiacusta dimalea[3]	Parque Nacional Jaragua, Pedernales Prov.	Hispaniola (Dominican Republic)
Amphiacusta domingensis[2]	Sierra de Neiba, Bahoruco Prov.	Hispaniola (Dominican Republic)
Amphiacusta eritheles[3]	Padre Las Cases, Azua Prov.	Hispaniola (Dominican Republic)
Amphiacusta exacerbans[3]	Sierra de Neiba, Bahoruco Prov.	Hispaniola (Dominican Republic)
Amphiacusta expansa[2]	Sierra Martin Garcia, Azua Prov.	Hispaniola (Dominican Republic)
Amphiacusta extenta[2]	Parque Nacional Armando Bermúdez, La Vega Prov.	Hispaniola (Dominican Republic)
Amphiacusta henrymorgani[7]	Henry Morgan's Cave, Andros Town	Bahamas
Amphiacusta hespera[2]	Loma Guaconejo, Sanchez Prov.	Hispaniola (Dominican Republic)
Amphiacusta hyperphobos[3]	Guana Island	Virgin Islands
Amphiacusta mythica[2]	Ebano Verde, La Vega Prov.	Hispaniola (Dominican Republic)
Amphiacusta opsia[2]	Matadero, Peravia Prov.	Hispaniola (Dominican Republic)
Amphiacusta obstipa[4]	Puerto Escondido, Altagracia Prov.	Hispaniola (Dominican Republic)
Amphiacusta ominosa[2]	Monte Rio, Azua Prov.	Hispaniola (Dominican Republic)
Amphiacusta pomeriense[2]	La Ciénaga, Barahona Prov.	Hispaniola (Dominican Republic)
Amphiacusta pronauta[5]	El Yunque	Puerto Rico
Amphiacusta ruizi[9]	Los Morenos, Santiago Prov.	Cuba
Amphiacusta sanctaecrucis	Rainforest, Rte. 76, Saint Croix	Virgin Islands (US)

Species	Locality	Island
Amphiacusta sincerus[8]	Holquin Province	Cuba
Amphiacusta species 1[6]	Lares	Puerto Rico
Amphiacusta species 2[6]	Viequez Island	Puerto Rico
Amphiacusta species 3[5]	Cerro Las Piñas	Puerto Rico
Amphiacusta species 4[6]	Mandeville, Cockpit Country	Jamaica
Amphiacusta sterna[2]	Rojo-Aceitillar, Pedernales, Prov.	Hispaniola (Dominican Republic)
Amphiacusta tijicohniae[6]	Guanica	Puerto Rico
Yoyuteris phacodes[2]	Sierra Martin Garcia, Azua Prov.	Hispaniola (Dominican Republic)

Table 2. A list of primers for COI and EF1-α written 5' to 3'. PCR protocols are found in Knowles (2001).

Gene	Primer	Sequence
COI	C1-J-1718-2	GGA-GGA-TTY-GGA-AAT-TGA-TTA-GTW-CC
	L2-N-3014	TCC-ATT-GCA-CTA-ATC-TGC-CAT-ATT-A
	C1-J-2183	CAA-CAT-TTA-TTT-TGA-TTT-TTT-GG
	C1-N-2191	CCC-GGT-AAA-ATT-AAA-ATA-TAA-ACT-TC
EF1-α	EF1-F1	AGA-TGG-GYA-ARG-GTT-CCT-TCA-A
	EF1-R1	GAA-CAC-CAG-TCT-CCA-CAC-GA
	EF1-AF4	AGA-TGG-GYA-ARG-GTT-CCM-TCM-A
	EF1-BF2	TCG-TTT-CGA-GGA-AAT-MAA-GAA
	EF1-BR3	TGG-CAC-TGT-TCC-AAT-ACC-AC

Table 3. Probability of congruence between the COI and EF1-α trees and a vicariant history, as determined using a parametric bootstrap under a parsimony and likelihood framework.

Gene	Probability
Cytochrome oxidase I	
parsimony	$p > 0.10$
likelihood	$p = 0.05$*
Elongation factor 1-α	
parsimony	$p > 0.05$
likelihood	$p > 0.05$

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Chapter 2

Testing for selective divergence in ecological and sexual traits in a Caribbean cricket

INTRODUCTION

The relative importance of deterministic and stochastic processes for population divergence remains an intriguing question (Wright 1931, 1932; Fisher, 1958; Lande, 1976; Lande, 1977; Lynch, 1990; Wade & Goodnight, 1998, Turelli *et al*., 2001, Mitchell-Olds *et al*., 2007). Stochastic, non-selective processes can cause substantial differentiation in sexual and ecological traits, especially among small, isolated populations (Wright 1931, 1932; Lande, 1981). This effect can be particularly important in small populations in which drift can have a greater impact than selection on phenotypic evolution (Kimura, 1962), although random genetic drift may also be rapid in populations that are large (Lande, 1976).

Natural and sexual selection are also powerful forces that can cause rapid and significant population divergence (Grant & Grant, 1993, 1995; Badyaev *et al*., 2000; Nosil *et al*., 2002) and they are likely responsible for most population and species divergence. But the practice of attributing all phenotypic divergence in ecological or sexual traits to selection (Dale *et al.,* 1999; Wilder & Hollocher, 2003), however, ignores the potential for drift to have a large effect on the rate and extent of phenotypic differentiation (Lande, 1976). Rather, conclusions of selective divergence are best derived from the rejection of a null model of neutral divergence (Lynch, 1990).

A framework for identifying the forces driving divergence in a quantitative trait was first proposed by Wright (1931, 1932; see also Lande, 1977; Rogers & Harpending, 1983; Prout & Barker, 1993). Loci that are evolving neutrally, i.e., are not subject to

selection and are experiencing only genetic drift and migration, can be used to establish an expectation for the degree of differentiation for a neutrally evolving quantitative trait (Leinonen *et al.* 2008). Specifically, Wright proposed that a test for selective divergence in a quantitative trait can be performed by comparing its differentiation among populations (defined as Q_{ST}) with the degree of population differentiation at several neutral genetic loci (Wright's F_{ST}). If phenotypic differentiation is greater than neutral genetic differentiation, then phenotypic differences are the result of divergent selection between populations. Conversely, if phenotypic divergence is less than genetic divergence, the trait is under stabilizing selection. Finally, if the two measures of population differentiation do not differ significantly, then trait differences between populations may be due to genetic drift. Thus, the degree of differentiation in quantitative traits between populations can be compared to an expectation derived from a null model of neutral genetic drift.

Using this model, the importance of drift and selection in generating divergence in several phenotypic traits was tested in a flightless ground cricket, *Amphiacusta sanctaecrucis*, which is distributed throughout the Virgin Islands (the U.S. and British Virgin Islands) (Figure 1). Its island distribution renders this species a good candidate for identifying the processes mediating phenotypic divergence because island populations may be small and particularly susceptible to the kinds of stochastic events (e.g., bottlenecks, genetic drift) that may lead to non-selective phenotypic divergence. Furthermore, isolation on islands reduces or eliminates the homogenizing effects of gene flow, allowing populations to evolve along separate trajectories.

The Virgin Islands have experienced a dynamic geological history of recent changes in size and connection with other islands. Volcanic in origin, they emerged together as part of the Puerto Rican Bank in the middle Miocene (~12 mya), and remained contiguous with one another (except Saint Croix) until they eventually separated with changing sea levels during the Pleistocene, approximately 8,000 years ago (Donn *et al.,* 1962; Farrand, 1962). These changes in sea level also caused the modern Virgin Islands to be considerably smaller than in the past (Heatwole & MacKenzie, 1967).

Some of the six Virgin Islands included in this study differ in the habitats occupied by *A. sanctaecrucis*, with some habitats characterized by moist, high-elevation tropical forest, and others by low elevation dry tropical forest. These differences in habitat may reflect ecological differences between populations of *A. sanctaecrucis*, and may be one factor mediating population divergence. In addition, species within the genus *Amphiacusta* are characterized by significant interspecific differences in male genitalia shape, a trait thought to be under sexual selection via female choice and male fertilization success (Eberhard 1985; Arnqvist & Danielsson 1999; Rivera *et al.* 2004). If populations are diverging following their recent separation, then differences may be reflected in differences in ecological and sexual traits. For this reason, population differentiation in two phenotypic traits of potentially ecological importance (body size and shape and mandible shape) and one potentially sexually selected trait (genitalia shape) was quantified. The degree of differences in these traits was compared to the neutral divergence between populations, as estimated with microsatellite markers.

Because some islands have similar habitats, while others differ, it is possible that some populations are under stabilizing selection while others are experiencing divergent selection. To investigate whether ecological differences or similarities among islands was reflected in the kind of selection acting on potentially ecological traits, pairwise population comparisons between islands with similar environmental characteristics were performed, as well as pairwise comparisons between populations in close proximity but inhabiting different ecological regimes.

MATERIALS AND METHODS

Genetic Analysis

A total of 172 individuals collected on six Virgin Islands (Beef Island, Saint Croix, Saint John, Saint Thomas, Tortola, and Virgin Gorda) were used to estimate population genetic differentiation. These specimens are now registered at the University of Michigan Museum of Zoology. Genomic DNA was extracted from the femur of each individual using a Qiagen DNeasy kit. Ten dinucleotide microsatellites were isolated using the

protocol outlined in Glenn and Schable (2005). Clone sequences are deposited in Genbank under accession numbers (XXXXX). An average of 28 individuals per population were genotyped for 8 loci (AS15, AS18, AS19, AS29, AS44, AS54, AS69, AS70; primers are described in Appendix 1). Microsatellites were amplified in a 10-μL reaction containing 5.7 μL H_2O, 1.0 μL 10x buffer minus $MgCl_2$, 0.3 μL $MgCl_2$, 0.5 μL of each 10 μM primer, 0.4 μL bovine serum albumin, 0.6 μL of 2.5mM dNTPs, and 0.04 μL Taq polymerase (Invitrogen) under the following conditions: 2 min of initial denaturation at 94 °C and then 35 cycles of 94°C for 15 s, 30 s from 50°C to 56°C, 30 s at 72 °C and a final extension of 4 min at 72 °C. PCR products were genotyped on an ABI Model 3730 sequencer with a standard of ROX 500 (ABI). Alleles were scored using GeneMarker v1.70 (Soft Genetics).

The program Micro-Checker (van Oosterhaut et al 2004) was used to detect the presence of null-alleles. Each locus was tested for Hardy-Weinberg equilibrium using a Markov-chain approximation with 100,000 permutations in ARLEQUIN 2.00 (Schneider et al., 2000), with *p*-values Bonferroni corrected for multiple tests (Rice, 1989). Weir and Cockerham's (1984) F_{ST} values for pairwise population comparisons were calculated in FSTAT v2.9.3.2 (Goudet, 1995) with 95% confidence intervals determined from 1000 bootstrap replicates. The level of significance of population differentiation was determined using ARLEQUIN 2.00.

Morphological Analysis

Collection and Measurement of Traits

Patterns of phenotypic divergence were characterized across populations: Beef Island (n=14), Saint Croix (n=23), Saint John (n=25), Saint Thomas (n=29), Tortola (n=21), and Virgin Gorda Peak (n=27), and Virgin Gorda Baths (n=14) (Table 1). Virgin Gorda Peak and Baths populations occur on the same island but in different habitats, with Virgin Gorda Peak characterized by high elevation, wet tropical forest, and Baths by a low elevation, scrubby habitat near the shore. Only males were used since a test for selective divergence arising from sexual selection relies on male genitalic differences. Digital photographs were taken with a Leica DFC320 dissecting scope of the femur, tibia, front

femur, thorax and head. Linear measurements of the femur length, tibia length, thorax width and breadth, labrum width, and the distance between the eyes were taken with Image-Pro Plus software (Media Cybernetics). Lateral views of the C-sclerite (Fig. 2) were obtained from dissected genitalia placed in sand or glass beads in a watchglass submersed in ethanol. Extended depth-of-field composite images were taken with a Leica DC300 dissecting scope and compiled from a standardized set of 10-20 focal planes using Discovery-Pro and Scope-Pro Plus imaging software. Digital images of the mandibles (Fig. 3) were taken using the same protocol. Multiple images (n = 5) were taken of each structure for each individual to take into account potential errors with the digitizing procedure.

MANOVA was performed on the femur length, tibia length, front femur length, thorax length and width, labrum width and eye span to determine whether significant differences in the characters among populations exist. A principal components analysis was performed to summarize the variation in these structures.

Population differences in C-sclerite and mandible shape were quantified using geometric morphometrics. Nine landmarks were digitized using tpsDig1.44 software (Rohlf, 2004) and used to summarize the shape of the mandibles. Five landmarks and 74 sliding semilandmarks were used to measure C-sclerite shape. The semilandmarks approach enables the researcher to identify fixed homologous points (landmarks) (Rohlf & Marcus, 1993; Bookstein, 1996) and then allows other points to slide along curves connecting those points (semilandmarks), and is useful for quantifying the shape of objects that have a few landmarks connected by homologous curves (Bookstein, 1997; Adams *et al*. 2004). A generalized Procrustes analysis (GPA) was performed using tpsRelw1.44 (Rohlf, 2004), which rescales, translates, and rotates the images to remove the effects of size and orientation, while minimizing the distances between the shape coordinates of each individual. A relative warps analysis, which is equivalent to a principal components analysis, was performed in tpsRelw1.44 to summarize the variation. The relative warps were averaged across the 5 replicate images for each specimen. A MANOVA was used to assess the significance of differences in the mandible and C-sclerite shape between populations using the linear measurements and relative warps.

Quantifying phenotypic divergence

As a comparable measure to the expected neutral population divergence among populations (i.e., the degree of genetic divergence measured by F_{ST}), patterns of phenotypic divergence were characterized using P_{ST}. Unlike Q_{ST}, P_{ST} includes the genetic as well as the environmental component of variation. The measure Q_{ST} assumes that the phenotypic trait of interest has an additive genetic basis, and furthermore, that only the phenotypic differentiation resulting from additive genetic variation (and not environmental variation) is included in the measure of population differentiation. The challenges of controlling for maternal and environmental effects in the field make it difficult to use this measure in studies of natural populations.

P_{ST} was estimated as

$$P_{ST} = \frac{\sigma^2_{PB}}{\sigma^2_{PB} + 2h^2\sigma^2_{PW}}$$

where σ^2_{PB} is the between group variance, and σ^2_{PW} the within group variance in a phenotypic trait, and h^2 is the heritability of the trait. P_{ST} is an acceptable measure of phenotypic divergence when Q_{ST} cannot be estimated (e.g., in field studies that cannot estimate heritability) (Storz, 2002; Saint-Laurent *et al*., 2003). Morphological traits typically exhibit high additive genetic variance (Houle, 1992; Schluter, 1996; Lynch & Walsh, 1998; Santos 2002), and a heritability of 0.5 was used for each trait, which is within the range of heritability values commonly reported in the literature for morphological traits (Houle, 1992; Lynch & Walsh, 1998). This includes both morphological traits thought to be under sexual selection (Arnqvist & Thornhill, 1998; Morrow & Gage, 2001; Blanckenhorn, 2002; Mühlhäuser & Blanckenhorn, 2004), and traits measured in natural populations (Grant & Grant, 1995; Manier *et al*., 2007). A sensitivity analysis was performed for each comparison to assess the impact of the assumed heritability of characters on the conclusions drawn.

P_{ST} was calculated from the relative warp (RW) scores of the mandibles and C-sclerite, and directly from the multivariate data of linear measurements. To estimate the

standard error, 95% confidence intervals were calculated for P_{ST} by bootstrapping with 1000 replicates.

Testing for selection

F_{ST} and P_{ST} values were compared among islands for each trait, with Virgin Gorda represented by the Peak population. I did this because the Peak and the Baths populations are significantly different from one another, both morphologically as well as genetically (Oneal, unpublished data), and the purpose of this study was to determine the factors affecting the divergence of populations on different islands from one another. Substituting the Baths for the Peak population or combining the two populations did not influence the direction or the significance of the results. To further test for a link between neutral genetic divergence and phenotypic divergence, I compared matrices of pairwise genetic and phenotypic distances using a Mantel tests with 10,000 permutations to generate null expectations.

In addition, pairwise comparisons of F_{ST} and P_{ST} of mandible and body shape were performed between Saint Croix, Saint John and Saint Thomas, because crickets on these islands occur in very similar habitats (wet tropical forest), and most were collected at similar elevations; therefore, comparing these islands may yield evidence of stabilizing selection. To test for diversifying selection, comparisons in these ecological traits were made between Tortola and Beef Island, and between the Virgin Gorda Peak and Baths populations. In both cases this consisted of a comparison between a wet, high-elevation forest habitat (Tortola and Virgin Gorda Peak) and a dry, low-elevation scrubby habitat (Beef Island and Virgin Gorda Baths).

RESULTS

Population genetic differentiation

All loci conformed to Hardy-Weinberg expectations (Table 2), except that AS18 showed low levels of heterozygosity in 3 of 6 populations, and AS19 and AS54 each exhibited

low levels of heterozygosity in 1 population each ($p < 0.001$). Genetic differentiation among the six islands was low ($F_{ST} = 0.014 \pm 0.006$), and not significantly different ($p > 0.10$). Pairwise genetic differentiation was as low as 0.003 ± 0.010 (between Saint John and Tortola) and as high as 0.026 ± 0.010 (between Saint Croix and Tortola).

Morphological Divergence

A MANOVA of femur length, tibia length, front femur length, thorax width and breadth, labrum width, and eye span finds that populations differ significantly in these traits (Pillai's trace = 0.976, $p < 0.001$). The first principal component (PC1) explains 95.1% of the variance in these variables (Table 3). The loadings for the PC1 are all in the same direction, with the highest loadings on femur, front femur, and tibia length, indicating that it likely summarizes variation in size, as especially limb size. An ANOVA of PC1 is significant ($F = 8.789$, $p < 0.001$). The high levels of differentiation in PC1, as well as the low amount of variation summarized by the other principal components, indicates that populations differ principally in size but not in body shape, or the relative proportions of the traits measured. Because all crickets were collected within the months of May and June 2005 and 2007, and all were adult males, size differences are unlikely to reflect the effect of seasonality on growth rates or size at adulthood.

The first 8 relative warps (RWs) summarized 93.75% of the variation in mandible shape and the first 13 RWs summarized 94.12% of the variation in C-sclerite shape. Islands differed significantly in the shape of both structures (mandibles MANOVA: Pillai's trace 0.831, $p < 0.001$; C-sclerite MANOVA: Pillai's trace = 1.39, $p < 0.001$).

In general, P_{ST} for all three quantitative traits was significantly greater than F_{ST} when examined over all populations, with no overlap in confidence intervals between the two measures (Table 4). An assumption of heritability of 0.5 did not affect these conclusions, as the findings remained the same when heritability ranged from 0.1 to 0.9 (Fig. 4), well within values reported in the literature for morphological traits (Grant & Grant, 1995; Schluter, 1996; Roff, 1998; Monteiro *et al*., 2002; Funk *et al*., 2005), including biological shape (Santos, 2002)

Pairwise Population Comparisons

For all traits measured, Beef Island *A. sanctaecrucis* were smaller than Tortola *A. sanctaecrucis*, and these differences were significant (MANOVA: Pillai's trace = 0.6955, $p < 0.001$). In contrast, crickets from the Virgin Gorda Baths population were larger than those of the Virgin Gorda Peak population for 6 out of 7 traits (MANOVA: Pillai's trace = 0.3799, $p = 0.036$). There were no consistent pattern of size differences between Saint Croix, Saint John and Saint Thomas, but these populations did differ from each other (MANOVA: Pillai's trace = 0.6083, $p < 0.001$).

All pairwise population comparisons showed that P_{ST} was significantly greater than F_{ST}, for a range of heritability values from 0.1 to 0.9, except for Saint Croix vs. Saint John (body size), and Saint Croix vs Saint Thomas (body size and mandible shape) (Table 4). For all population pairwise comparisons, there was no correlation between differentiation in body size and genetic differentiation ($r = -0.1447$, $p > 0.10$). The same was true for mandible shape ($r = 0.0494$, $p > 0.10$), and for C-sclerite shape ($r = 0.1677$, $p > 0.1$).

DISCUSSION

In a test for selective divergence across all six populations, as well as in most of the population comparisons, phenotypic divergence was significantly greater than differentiation at neutral genetic markers. This overall pattern of selective divergence was consistent for most pairwise population comparisons, including those between islands with similar habitats (Saint John, Saint Croix and Saint Thomas), in which stabilizing selection on ecological traits might be expected to result in a level of phenotypic differentiation less than the genetic differentiation. The results implicate divergent natural and sexual selection in driving the phenotypic evolution on these islands and suggest that *A. sanctaecrucis* on Saint John, Saint Croix, and Saint Thomas experience differing ecological environments that may not be captured by the rough estimate of the habitat variability made here.

An assumption of this work is that phenotypic and genetic variances are correlated within populations, and that environmental variance within populations is

similar to that among populations. This assumption may not be met, and may cloud the issue of whether selection can be conclusively inferred, especially in pairwise comparisons that involve strikingly different environments, such as Tortola vs. Beef Island and Virgin Gorda Baths vs. Virgin Gorda Peaks. This issue is unlikely to have affected an overall conclusion of selective divergence for Virgin Island populations of *A. sanctaecrucis*. Divergence was considerably greater than neutral divergence for a range of assumed heritabilities ranging from 0.1 to 0.9, as revealed in the sensitivity analysis for C-sclerite shape, body size, and mandible shape. In addition, most studies have concluded that phenotypic variance and genetic variance are proportional for morphological traits (Cheverud, 1988; 1996; Roff, 1995, 1997; Koots & Gibson, 1996), and an analysis by Leinonen *et al*. (2008) found that studies based on wild phenotypes did not tend to yield higher estimates of phenotypic divergence than studies based on full- or half-sib designs that isolated the additive genetic variance component of phenotypic divergence. Finally, the potential for environmental variance to affect conclusions is probably greatest for size differentiation among populations; this should be less of a concern for conclusions about mandible shape and genitalia shape, in which the effect of size was removed, and for which environmental variance should be low (ref).

Levels of genetic differentiation were low, a finding that is not surprising given the recent timing of the flooding of the Puerto Rico/Virgin Island Bank and the separation between these islands. Such low levels of neutral divergence underscore the high levels of phenotypic divergence seen overall and in multiple pairwise comparisons of island populations. Divergent selection has been strong over the course of the last several thousand years, and has produced significant differentiation in a suite of characters that may be both ecologically and sexually important for male *A. sanctaecrucis*. In addition to the recentness of their separation, one possible explanation for the low levels of genetic differentiation between populations is ongoing gene flow. This raises the possibility that selection has maintained and contributed to phenotypic divergence in the face of gene flow that would tend to erase differences among populations. Despite the existence of significant phenotypic differences, gene flow between islands may still have shaped the extent of differentiation, a question that can be further examined by calculating migration rates and relating that to character divergence.

Most studies that tested explicitly for selective divergence by employing a null model of neutral evolution found phenotypic divergence to be greater than neutral divergence (e.g., Merilä & Crnokrak, 2001; Leinonen *et al*., 2008; but see Waldman & Andersson, 1998; Weaver *et al*., 2007). A review found that the measures are positively correlated, suggesting that, to a certain extent, genetic divergence is predictive of phenotypic divergence (Leinonen *et al*., 2008), and that demographic processes affecting divergence at neutral markers may also be acting on phenotypic traits. Thus, by examining correlations between these measures of divergence, it may be possible to detect a role for drift even when natural or sexual selection is the primary mechanism behind quantitative trait divergence between populations. The lack of a correlation between phenotypic and neutral divergence in this study indicates that selection may be the sole process by which these phenotypic traits have diverged, despite reductions in island size that may have led to reductions in population size, a demographic factor that should affect both genetic and phenotypic evolution within a population (Whitlock *et al*., 2002).

While it is possible that selection is the only force driving population differentiation in these potentially important ecological and sexual traits, it is also possible that populations have experienced alternating periods of drift-dominated or selection-dominated divergence. Disentangling the relative contributions of these mechanisms remains a challenge; for example, when comparing trait values between populations or across species, it is difficult to distinguish between a pattern of change produced by weak selection over a period of time and that of a brief period of strong selection followed by stabilizing selection or drift (Lande, 1990; Roff, 2000). One possibility for dissecting the roles of drift and selection in producing phenotypic change is comparing the variation in the additive genetic variance covariance matrix (the **G** matrix) between two (or more) groups of interest. The variance associated with drift or selection can then be partitioned (Roff, 2000), allowing more explicit conclusions to be drawn about the importance of those mechanisms. Further elucidating the roles of both selection and drift in driving phenotypic divergence in *A. sanctaecrucis* will require experimentally determining the structure of the **G** matrix.

Natural and Sexual Selection in *A. sanctaecrucis*

As body size and mandible shape, two ecologically relevant traits, are selectively divergent among populations of *A. sanctaecrucis*, it is worthwhile to investigate further the selective pressures that have contributed to this divergence. Populations occur in a range of habitats on the Virgin Islands, from the low-elevation wet tropical forest on Saint Thomas, Saint John and Saint Croix, to the relatively high-elevation moist habitat on Tortola and Virgin Gorda, as well as low-elevation dry, scrubby habitat, such as that found on Beef Island. Indeed, the pairwise population comparison between Beef Island and Tortola, two islands separated by 50 m of water (and now connected by a man-made bridge) showed the greatest divergence in all three of the traits measured, despite exhibiting a relatively low level of genetic divergence. Beef Island *A. sanctaecrucis* were smaller than Tortola *A. sanctaecrucis* for all seven of the body traits measured, suggesting that high temperatures and aridity might select for smaller body size. In addition to body size differentiation, populations are divergent in mandible shape. Mandible shape is known to be reflective of trophic ecology (Liem, 1973), and divergence in mandible shape may represent divergence in the resources exploited by *A. sanctaecrucis* on different islands. Further investigation into the climatic, habitat, and trophic factors mediating ecological divergence could shed light on the specific selective pressures promoting differentiation in these crickets.

Male genitalia are some of the most diverse characters in animal taxa with internal fertilization (Eberhard, 1985; Hosken & Stockley, 2004), particularly in insects, where even closely related species may show striking differences in male genitalia shape (Knowles & Otte 2000; Rivera *et al*. 2004). Empirical evidence increasingly supports the view that variation in male genitalia is driven by postmating sexual selection on male fertilization success (Arnqvist & Danielsson 1999; Rivera *et al.* 2004). Furthermore, female genitalia sometimes also show evidence of diversification and in some cases male and female genitalia may interact, either through sperm competition (Arnqvist & Danielsson, 1999) or through external conflict over male mating opportunity (Arnqvist & Rowe, 2002). The C-sclerite is inserted directly into the female genital tract during mating in *A. sanctaecrucis*, and its close relative, *A. pronauta* (Oneal, personal observation). Moreover, females, as well as males, show evidence of species-specific

variation (Desutter-Grandcolas & Otte 1997). These facts, coupled with evidence of divergent selection suggests the potential for coevolution between male and female genitalia in *A. sanctaecrucis*, in which males in different populations are continually selected to achieve an optimum driven either by female choice (Eberhard, 2004), male-male competition or sexual conflict (Alexander *et al.*, 1997).

Patterns of *Amphiacusta* diversity

The genus *Amphiacusta* represents a radiation of more than 75 species of flightless crickets distributed throughout the West Indies, most of which are found on the islands of the Greater Antilles (Cuba, Jamaica, Hispaniola, and Puerto Rico), and exhibit habitat differentiation similar to that of *A. sanctaecrucis*. Additionally, *Amphiacusta* also exhibit significant variation in genitalia shape. The findings presented here shed light on the factors that may be driving diversification in genitalia shape and ecological traits like body size and mandible shape. Further work on the ecological and sexual context in which divergent selection is occurring in *A. sanctaecrucis* could illuminate the mechanisms driving the radiation of this genus throughout the Caribbean.

FIGURE LEGENDS

Figure 1. Map of the Virgin Islands.

Figure 2. Drawing of the male genitalia of *A. sanctaecrucis* showing the C-sclerite. Drawing by Dan Otte.

Figure 3. Drawing of the mandible of *A. sanctaecrucis* showing placement of landmarks

Figure 4. Plot of the effect of heritability (h^2) values ranging from 0.1 to 0.9 on overall measures of P_{ST} for (A) body size, (B) mandible shape, and (C) C-sclerite shape. Horizontal line $F_{ST} = 0.014$ indicates level of neutral divergence. For a range of heritability values, for comparisons of all six islands, neutral divergence is never greater than phenotypic divergence.

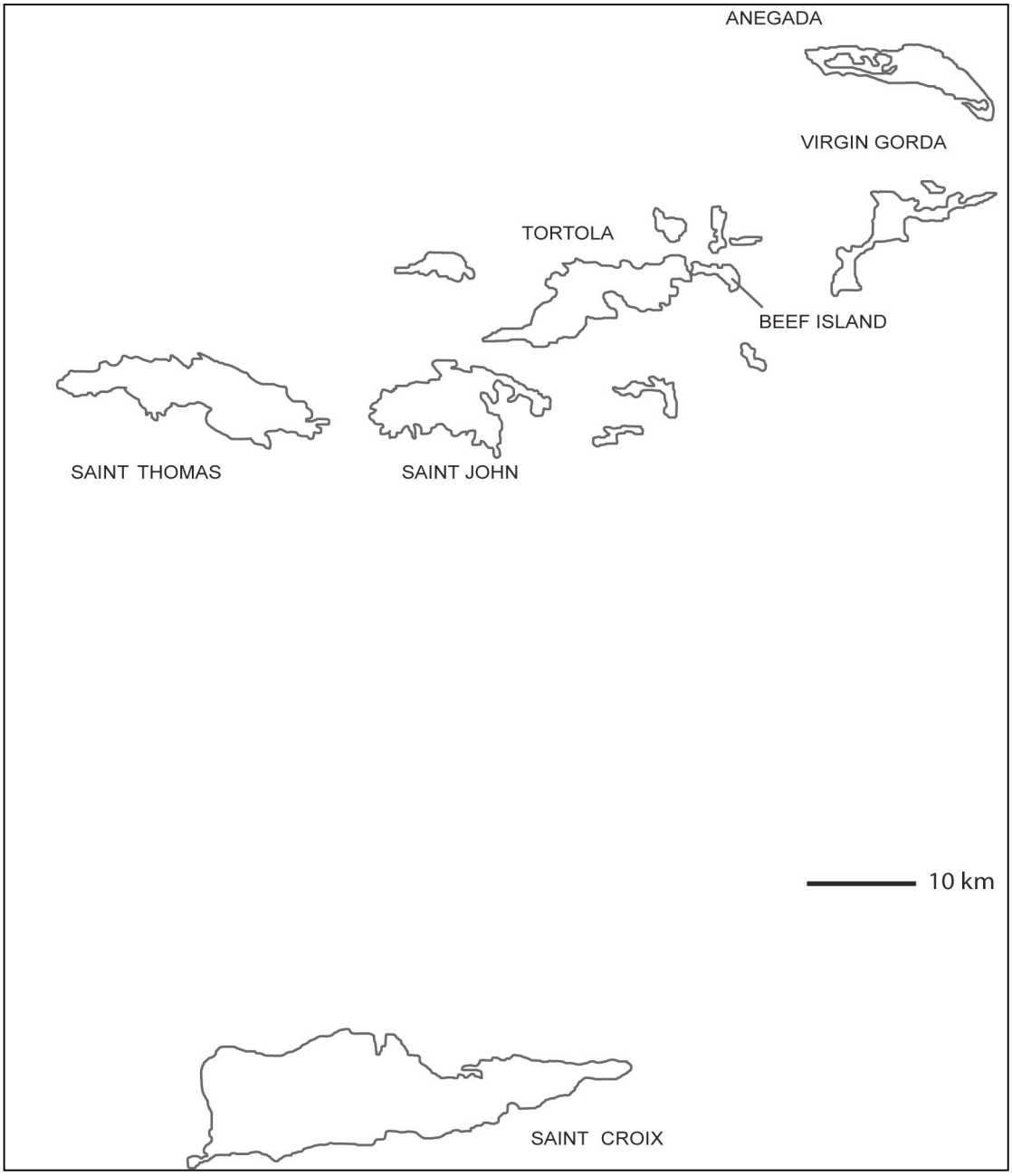
ANEGADA
VIRGIN GORDA
TORTOLA
BEEF ISLAND
SAINT THOMAS
SAINT JOHN
10 km
SAINT CROIX

Figure 1.

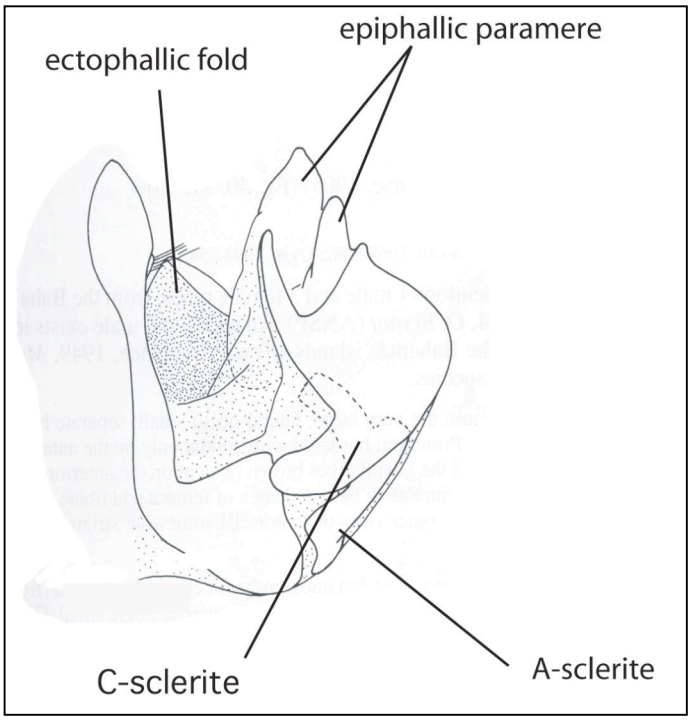
epiphallic paramere
ectophallic fold
C-sclerite
A-sclerite

Figure 2.

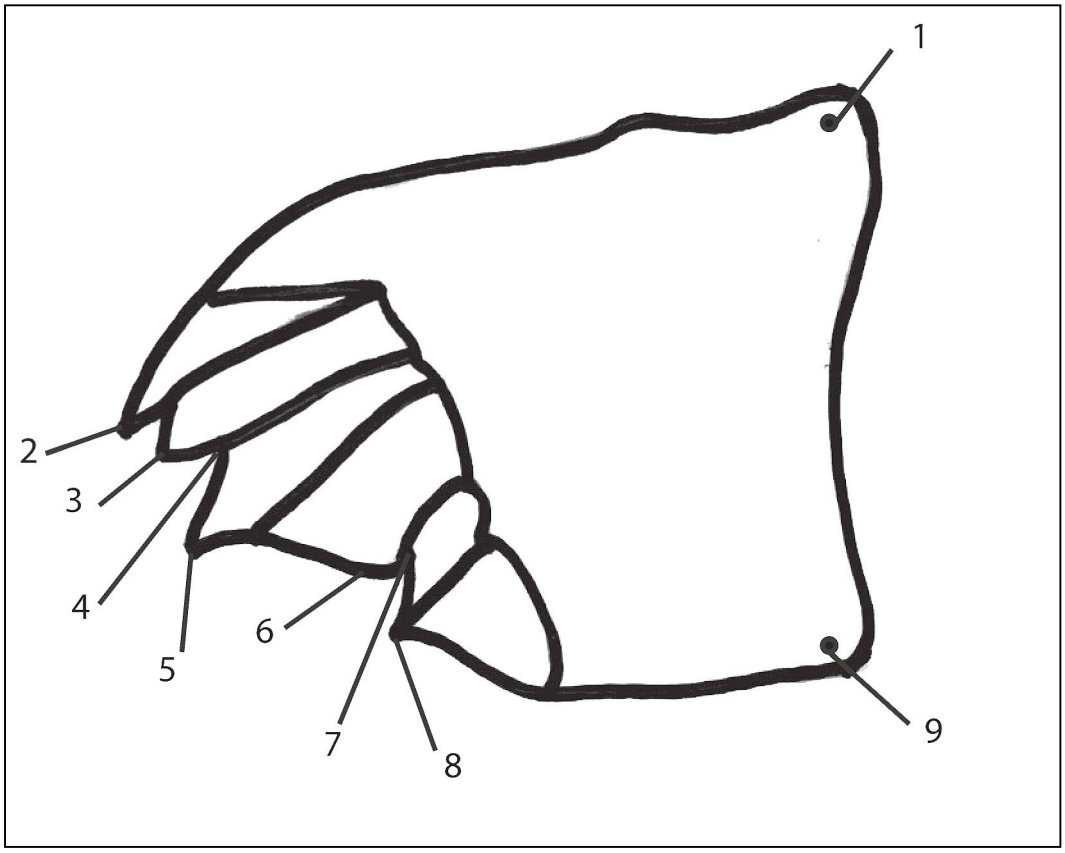

Figure 3

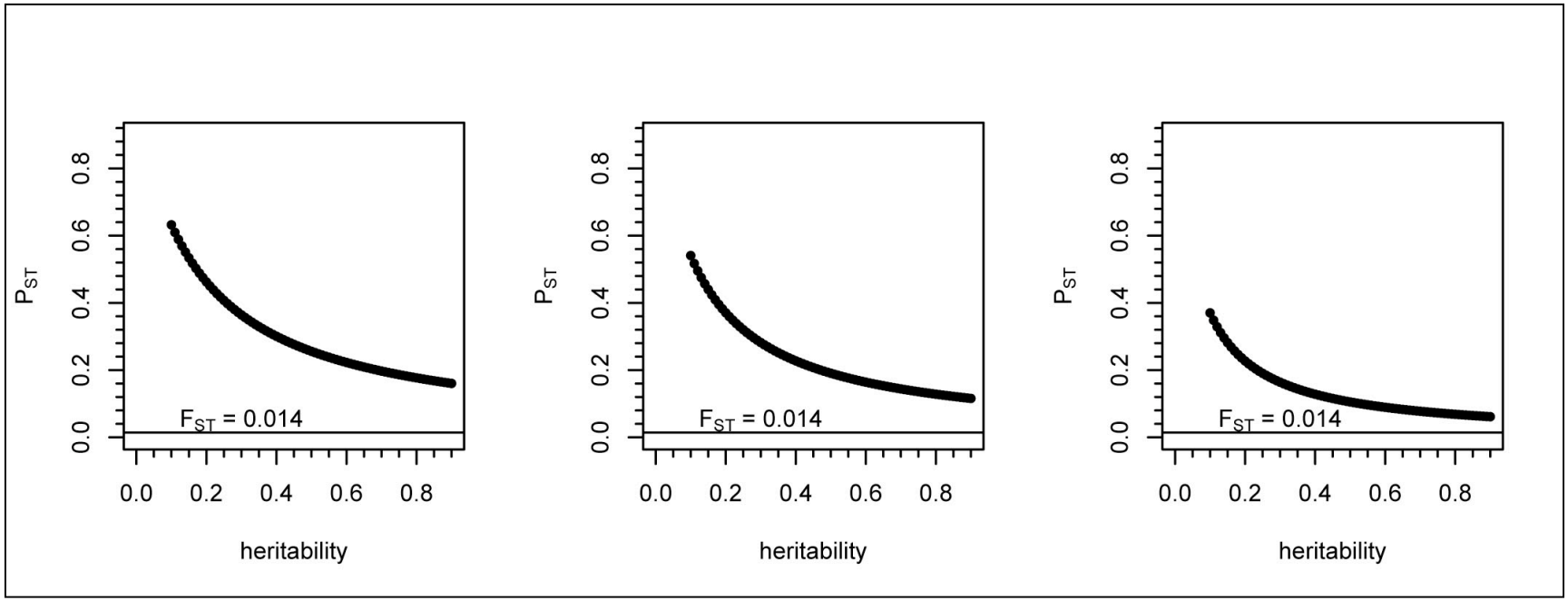

Figure 4.

Table 1. Sampling locations, dates, and elevation for each of six Virgin Islands.

Island	Location	Geoposition	Elev	Date
Beef Island	0.3 km south of Trellis Bay	18.221667 N, 64.532778 W	15 m	7 May 2005
Saint Croix	Mahogany Rd	17.737500 N, 64.841667 W	165 m	10 May 2005
				31 May 2007
Saint John	Cinnamon Bay	18.352200 N, 64.760556 W	15 m	23 May 2005
	Centerline Road	18.343056 N, 64.765000 W	190 m	24 May 2007
Saint Thomas	Route 33	18.363333 N, 64.974167 W	255 m	3 May 2007
	Crown Mountain Rd	18.355833 N, 64.974167 W	415 m	22 May 2007
Tortola	Sage Mountain	18.400833 N, 64.661111 W	460 m	6 May 2005
				24 May 2007
Virgin Gorda	Virgin Gorda Peak	18.477778 N, 64.403333 W	320 m	8 May 2005
				27 May 2007
	Virgin Gorda Baths	18.426994 N, 64.443889 W	10 m	8 May 2005
				27 May 2007

Table 2. Characterization of genetic diversity for each island population, where n = number of individuals sampled, k = average numbers of alleles per locus, A_R = allelic richness, H_E = expected heterozygosity, H_O = observed heterozygosity, and F_{IS} = and inbreeding coefficient, averaged across 8 microsatellite loci.

Sample Population	n	k	A_R	H_E	H_O	F_{IS}
Beef Island	16	13.88	13.65	0.849	0.899	0.054
Saint Croix	26	14.38	11.92	0.889	0.823	0.074
Saint John	31	17.38	13.71	0.805	0.924	0.126
Saint Thomas	37	19.12	13.88	0.924	0.827	0.102
Tortola	32	17.00	13.44	0.860	0.923	0.065
Virgin Gorda	30	19.25	14.77	0.847	0.926	0.082

Table 3. Summary of a principal components analysis of body measurements including the percent variation explained by each component and the factor loadings on each structure.

	PC1	PC2
% variation	95.1	2.8
femur	0.603	-0.508
tibia	0.649	-0.539
front femur	0.412	0.794
upper pronotum	0.101	0.108
lower pronotum	0.171	0.250
snout	0.018	-0.001
eyes	0.059	0.041

Table 4. F_{ST} and P_{ST} with confidence intervals determined by bootstrapping. Reported P_{ST} values assume heritability (h^2) is 0.5. Comparisons for which P_{ST} is significantly greater than F_{ST} are identified by *. BI = Beef Island, STC = Saint Croix, STJ = Saint John, STT = Saint Thomas, TOR = Tortola, VGP = Virgin Gorda, VGB = Virgin Gorda Baths. Comparisons among STC, STJ and STT are reported because *A. sanctaecrucis* occurs in similar habitats (low-elevation wet tropical forest) on these islands, raising the possibility that stabilizing selection may be responsible for population differentiation on these islands. Conversely, comparisons between TOR and BI, and VGP and VGB represent comparisons between high-elevation forest (Tortola and VG Peak) and dry scrubby habitat (BI and VGB), two very different habitats, demonstrating potential for selective divergence among populations.

Population Comparison	F_{ST}	Body P_{ST}	Mandibles P_{ST}	C-sclerite P_{ST}
Overall	0.014 ± 0.006	0.221 ± 0.001*	0.191 ± 0.001*	0.105 ± 0.001*
BI vs. TOR	0.010 ± 0.016	0.407 ± 0.009*	0.301 ± 0.003*	0.105 ± 0.002*
STC vs. STJ	0.016 ± 0.008	0.006 ± 0.002	0.055 ± 0.002*	0.051 ± 0.001*
STC vs. STT	0.020 ± 0.005	0.014 ± 0.002	0.025 ± 0.024*	0.066 ± 0.002*
STJ vs. STT	0.008 ± 0.008	0.016 ± 0.003*	0.021 ± 0.001*	0.067 ± 0.002*
VGB vs. VGP	0.004 ± 0.007	0.044 ± 0.004*	0.032 ± 0.003*	0.083 ± 0.003*

Appendix. Primer sequences and annealing temperatures for microsatellites used in this study.

Primer pair	Primer sequence (5' - 3')	Annealing temperature (ºC)
AS15	CGTGACGGAATGGTTTTCA GGGGAAGGAGGAAAAGTGAG	56
AS18	CAATGAGTCCTAGCGGTGGT GTCGTCGGTCATCATTTCCT	52
AS19	TGCACATCTCATCACGCTAA ACCGGCTGTAACATGTGAAT	52
AS29	GCCACCTCTTTAATCATACTG CGGGACGCTTTAGATAGACC	50
AS44	CCTTGCGGGCTTTGTTTAC TACCTCGTCCGCTCATCCTA	56
AS54	CCACCGAAACTCAATAGATGG CCCTTGACATGTTACGAATCC	50
AS69	GGGGGATGTTCAAATGTTTC GACCGTGGGTATTGGAGGAT	50
AS70	GACAGCGTGAACACATTACGA ACGCGATAGGTTTTCGACAG	50

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Chapter 3

Morphological divergence is not influenced by gene flow in *Amphiacusta sanctaecrucis*

INTRODUCTION

Oceanic islands represent unique opportunities to study the process of phenotypic differentiation among populations, and specifically, the balance between the effects of local selection pressures and gene flow between populations. Investigations into the patterns of diversification among island populations have contributed greatly to our understanding of the mechanisms affecting ecological and sexual divergence (Robichaux et al. 1990, Kambysellis & Craddock 1997). Such studies rely upon the assumption that isolation on islands results in the cessation of gene flow, allowing local adaptive differentiation to occur. Yet movement between island populations does occur (Heaney, 2007) and such events can have important consequences for population dynamics and adaptive divergence. Furthermore, immigration may not be random, but may reflect a number of influences, most notably the distance between islands (Illera *et al.* 2007), a history of connecting land bridges (Liggins *et al.* 2008), or the prevailing direction of ocean currents between islands (Calsbeek & Smith 2003). Current levels of connectivity between populations via migration, as well as their history and timing of isolation, have the potential to influence the phenotypic divergence between island populations. Each island represents an independent replicate in a natural experiment on the effects of these factors on phenotypic differentiation.

Species in the diverse genus *Amphiacusta* are distributed throughout the Caribbean and exhibit both ecological and sexual differentiation. These flightless ground crickets are found to occur in a variety of habitats, including caves and wet and dry

tropical forests at a range of elevations (Desutter-Grandcolas & Otte, 1997, Otte & Perez, unpublished manuscript). In addition, like many insects, species exhibit significant differentiation in male genitalia (Desutter-Grandcolas & Otte, 1997), structures that are thought to be under sexual selection and which may contribute to the pace and pattern of diversification (Eberhard 1985, Arnqvist 1998). Interestingly, patterns of ecological and sexual divergence at the species level are also reflected in differentiation among populations of *Amphiacusta sanctaecrucis*, a species occurring on several of the Virgin Islands (Figure 1). *A. sanctaecrucis* populations inhabit both wet and dry tropical forests across the islands. These differences in habitat, coupled with isolation among island populations, are associated with differentiation in two ecological traits (body size and mandible shape (Figure 2)) as well as male genitalia (in particular, the shape of the C-sclerite, Figure 3). Moreover, these phenotypic differences appear to be the result of selectively driven divergence. Comparison of the degree of phenotypic differentiation to that of neutral molecular markers showed that the observed morphological divergence was greater than expected from genetic drift (Oneal, in preparation).

Despite this pattern of selective ecological and sexual divergence, levels of neutral genetic differentiation between island populations, as determined by microsatellite loci, are low (Oneal, in preparation). Such low levels of genetic divergence could be the result of recent isolation, in which insufficient time has passed for populations to accumulate substantial neutral genetic divergence, and phenotypic divergence solely reflects the effects of selection operating independently in isolated populations. Alternatively, populations may continue to experience gene flow, and selection is promoting phenotypic divergence in spite of its potentially homogenizing effects. In this case, in addition to selection, other factors such as the connectivity between populations, could influence the process of phenotypic divergence.

In this study, microsatellite loci are used to quantify the amount of gene flow between islands. This framework is then used to compare levels of morphological population differentiation to the estimated levels of gene flow to investigate the potential for gene flow to influence the pattern of inter-population morphological divergence. The comparison is used to address two questions. First, to what extent do extrinsic factors that govern levels of gene flow influence patterns of phenotypic differentiation? For example,

if patterns of gene flow are not only concordant with the direction of ocean currents, but also correlated with patterns of phenotypic divergence, it would suggest that the geographic configuration of islands, in addition to the selective pressures within an island, has played an important role in population divergence. Second, are selective pressures sufficiently strong to promote divergence, irrespective of the level of gene flow occurring among islands? In this case, the patterns of inter-population morphological divergence are not expected to correlate with the amount of genetic exchange experienced by the populations.

MATERIALS AND METHODS

Characterizing microsatellite variation

A total of 172 male and female *Amphiacusta sanctaecrucis* were sampled on six Virgin Islands (Beef Island, Saint Croix, Saint John, Saint Thomas, Tortola, and Virgin Gorda) (Table 1). Genomic DNA was extracted from the femur of each individual using a Qiagen DNeasy kit. Eight dinucleotide microsatellites were amplified using fluorescently labeled primers in a polymerase chain reaction (PCR). An average of 29 individuals per population were genotyped for 9 loci (AS15, AS18, AS19, AS29, AS44, AS54, AS69, AS70; primers are described in Appendix 1). Microsatellites were amplified in a 10-μL reaction containing 5.7 μL H_2O, 1.0 μL 10x buffer minus $MgCl_2$, 0.3 μL $MgCl_2$, 0.5 μL of each 10 μM primer, 0.4 μL bovine serum albumin, 0.6 μL of 2.5mM dNTPs, and 0.04 μL Taq polymerase (Invitrogen) under the following conditions: 2 min of initial denaturation at 94 °C and then 35 cycles of 94°C for 15 s, 30 s from 50°C to 56°C, 30 s at 72 °C and a final extension of 4 min at 72 °C. PCR products were genotyped on an ABI Model 3730 sequencer with a standard of ROX 500 (ABI). Alleles were scored using GeneMarker v1.70 (Soft Genetics).

Calculating Gene Flow

The program Micro-Checker (van Oosterhaut *et al*. 2004) was used to detect the presence of null-alleles at each locus. Each locus was tested for Hardy-Weinberg equilibrium using a Markov-chain approximation with 100,000 permutations in ARLEQUIN 2.00

(Schneider *et al*. 2000), with *p*-values Bonferroni corrected for multiple tests (Rice, 1989).

A global F_{ST} (θ) and F_{ST} for pairwise population comparisons were calculated in FSTAT v2.9.3.2 (Goudet, 1995) with 95% confidence intervals determined from 1000 bootstrap replicates. The level of significance of population differentiation was determined using ARLEQUIN 2.00.

Levels of gene flow between island populations were calculated with the program MIGRATE 3.0 (Beerli & Felsenstein 1999; Beerli 2003) using a Bayesian framework. Bayesian analysis is more accurate than maximum-likelihood (ML), and performs better at recovering "true" migration rates from simulated data sets, especially when the data show low or high levels of heterozygosity (Beerli 2006). MIGRATE 3.0 calculates θ, or $4N_e\mu$, for each population, and pairwise migration rates between populations (M), which is reported as m/μ, where m is the migration rate and μ is the mutation rate, here assumed to be constant over all loci. A preliminary analysis was conducted assuming uniform prior distributions of these parameters, with a search of 3 long chains of a total of 300,000 steps, sampled every 20 steps, and a burn-in of 10,000 trees. The resulting values for θ and M from these preliminary runs were then averaged and used as boundaries for the exponential prior distributions of a second, longer run, which was run for 10.5 million steps, sampled every 50 steps and with a burn-in of 105,000 trees. The search was conducted with adaptive heating (temperatures: 1.00, 2.33, 3.67, 5.00) and repeated with three independent runs to test for convergence. Estimates of N_em, or the effective migration rate, were obtained as $0.25 \times \theta \times M$; the mode, the median, and 95% confidence intervals are reported (Table 2).

Rayleigh's test of directionality (Batschelet 1981), which determines whether a sample of directions shows evidence of one-sidedness, was used to test whether the direction of gene flow between islands was nonrandom with respect to the direction of regional ocean currents. Because Beef Island and Tortola are separated by only 50 m of water and are now connected by a man-made bridge, comparisons between these two islands were not included in an analysis of directionality of gene flow. A model of isolation-by-distance for population genetic differentiation was tested with a Mantel test in R, with 10,000 permutations, using straight-line distances between collecting sites.

Quantifying morphological divergence

Patterns of phenotypic divergence were characterized across populations: Beef Island (n=14), Saint Croix (n=23), Saint John (n=25), Saint Thomas (n=29), Tortola (n=21), and Virgin Gorda Baths (n=14). Only males were used since examining the effects of gene flow on sexual traits relies on quantifying male genitalic differences. Digital photographs were taken with a Leica DFC320 dissecting scope of the femur, tibia, front femur, thorax and head. Linear measurements of the femur length, tibia length, thorax width and breadth, labrum width, and the distance between the eyes were taken with Image-Pro Plus software (Media Cybernetics). Lateral views of the C-sclerite (Fig. 1) were obtained from dissected genitalia using an extended depth-of-field composite images taken with a Leica DC300 dissecting scope and compiled from a standardized set of 10-20 focal planes using Discovery-Pro and Scope-Pro Plus imaging software. Digital images of the mandibles were taken using the same protocol. Multiple images (n = 5) were taken of each structure for each individual to take into account potential errors with the digitizing procedure.

Population differences in C-sclerite and mandible shape were quantified using geometric morphometrics. Nine landmarks were digitized using tpsDig1.44 software (Rohlf 2004) and used to summarize the shape of the mandibles, while five landmarks and 74 sliding semilandmarks were used to measure C-sclerite shape. The semilandmarks approach enables the researcher to identify fixed homologous points (landmarks) (Rohlf & Marcus 1993; Bookstein 1996) and then allows other points to slide along curves connecting those points (semilandmarks), and is useful for quantifying the shape of objects that have a few landmarks connected by homologous curves (Bookstein 1997; Adams *et al*. 2004). A generalized Procrustes analysis (GPA) was performed using tpsRelw1.44 (Rohlf 2004), which rescales, translates, and rotates the images to remove the effects of the size of the structure and its orientation, while minimizing the distances between the shape coordinates of each individual. GPA yields principal warps (PWs), which are coordinates along partial warp axes that describe the shape of the structure (Zelditch *et al*. 2004).

A relative warps analysis, which is a weighted principal components analysis of partial warp scores (Bookstein 1991), was performed in tpsRelw1.44 to summarize the variation. Relative warps were averaged across the 5 replicate images for each specimen. MANOVA was used to assess whether populations differed significantly in mandible and C-sclerite shape and body size and shape. For mandibles and body variables, the original partial warps were used; because of the limitations imposed by sample size on the number of variables permitted by MANOVA, the relative warps were used to test for differences in C-sclerite shape between populations. Finally, a principal components analysis of the meristic traits (femur length, tibia length, etc.) was performed to illustrate the contributions of each measure to the total variation among populations in these traits.

The partial warp scores for mandibles and C-sclerite were averaged within populations and a matrix of distances between the populations was calculated. A distance matrix was similarly computed directly from the population averages of linear measurement. These matrices were compared to a matrix of gene flow estimates using a partial Mantel test of the significance of a correlation between morphological pairwise distances and pairwise levels of gene flow. A Mantel test was also performed to test for a correlation between morphological divergence and straight-line distance between populations.

RESULTS

Genetic Differentiation and Gene Flow

All loci conformed to Hardy-Weinberg expectations except AS18, which showed low heterozygosity in 3 populations (St. John, St. Thomas and Virgin Gorda), and AS54, which exhibited low heterozygosity in 1 population (St. John) ($p < 0.001$). There was no evidence of null alleles. Genetic differentiation among the six islands was low (F_{ST} = 0.014 ± 0.006), and not significant ($p > 0.1$). Pairwise F_{ST}-values ranged from 0.003 ± 0.010 (between Saint John and Tortola) to 0.026 ± 0.010 (between Saint Croix and Tortola); no pairwise comparison was significant. Levels of gene flow between islands

were generally low with narrow 95% confidence intervals (Table 2), averaging 0.552 effective immigrants per generation.

The dominant current in the Lesser Antilles of the Caribbean is the Antilles current, which flows through the Virgin Islands in a northwesterly direction (CIMAS: Institute for Marine and Atmospheric Studies: http://oceancurrents.rsmas.miami.edu/atlantic/antilles.html; Defense Mapping Agency, US Government). There is no significant increase in gene flow in concordance with the direction of ocean currents in the Virgin Islands (Rayleigh's test: r = 0.153, $p > 0.9$). There was a significant isolation-by-distance effect on the pattern of genetic differentiation among islands (r = 0.849, $p = 0.008$).

Morphological divergence

Populations differ significantly in a suite of nonsexual morphological traits (femur length, tibia length, front femur length, thorax width and breadth, labrum width, and eye span) (MANOVA: Pillai's trace = 0.976, $p < 0.001$). The first principal component (PC1) explains 95.1% of the variance in these variables, with PC2 accounting for 2.8% (Table 3). The loadings for the PC1 are all in the same direction, indicating that it likely summarizes variation in size. In contrast, loadings for PC2 are in different directions, so this component probably summarizes some factors of body shape, such as disproportional changes in body variables, such as limb length, between islands. Populations also differ significantly in mandible shape (MANOVA of principal warps, Pillai's trace: 1.09, $p < 0.001$), with Beef Island showing the most divergence in shape (Fig. 5). The first 13 RWs summarized 94.12% of the variation in C-sclerite shape, and a MANOVA of the RWs found that populations differ significantly in male genitalia shape (Pillai's trace = 1.39, $p < 0.001$).

There was no association between total levels of gene flow between pairs of island populations and pairwise distances in body size and shape (one-tailed test, r = -0.094, $p > 0.1$), mandible shape (one-tailed test, r = 0.120, $p > 0.1$), and C-sclerite shape (one-tailed test, r = 0.175, $p > 0.1$). In addition, there was no association between morphological divergence and distance between populations for any of the traits (body

size and shape: r = - 0.1848, $p > 0.1$; mandible shape: r = -0.1572, $p > 0.1$; C-sclerite shape: r = -0.1452, $p > 0.1$, all tests one-tailed).

DISCUSSION

Local adaptation arises from a balance between divergent selection and gene flow, and gene flow may influence the extent of divergence between populations. While spatially variable ecological and sexual selection may drive population differentiation in morphological and behavioral traits (Schluter 2000), this process may be hindered by the homogenizing effect of gene flow (Riechert 1993, Storfer *et al.* 1998, Moore *et al.* 2007). In addition, gene flow may not be uniform, but instead may be geographically structured, with populations characterized by varying levels of connectivity. Geographic barriers or facilitators to migration may thus serve as extrinsic factors that may influence the dynamic process of adaptive population divergence.

The Virgin Islands are volcanic in origin, emerging during the late Eocene and experiencing periods of orogeny alternating with submergence until the late Miocene (~ 8 mya), when they became fully emergent. They are part of a shelf that includes Puerto Rico, with which they formed a single landmass during the Pleistocene (with the exception of Saint Croix, which remained separate). These connections existed until at least 14,000 years ago and were severed only about 8,000 years ago with the rising of sea levels to their present level (Heatwole & MacKenzie 1967).

In contrast with other Caribbean taxa (Calsbeek & Smith 2003), gene flow is not spatially structured with ocean currents, and cannot explain the low pairwise population Fst values reported here, these values are very low—in all cases less than one migrant per generation, the amount required to erase the effects of genetic drift on population genetic variation (Wright 1931). The lack of concordance between ocean currents and what gene flow does exist implies that, like other flightless insects, crickets may be dispersed rarely and primarily by wind during storm events (Gressit 1966; Peck 1994). Rather, it is the recency of isolation among the Virgin Islands that accounts for the low levels of differentiation at microsatellite loci between populations of *A. sanctaecrucis*.

Furthermore, a strong pattern of isolation by distance suggests that the spatially structured genetic variation uncovered here may have arisen before rising Pleistocene sea levels severed land connections between island populations.

The evidence of divergence among populations in three morphological traits mirrors previous findings. In the case of body size and shape and mandible shape, phenotypic differences between populations are suggestive of ecologically and sexual selectively driven adaptive divergence. For example, while population differences in the meristic traits measured appear to be of overall size, the trait loadings on PC2 imply some changes in the relative sizes of the femur, front femur, and tibia. *A. sanctaecrucis* occupy both wet and dry tropical forests at low and high elevations, and like other island taxa differences in limb length may be the result of ecological divergence in habitat use (Losos *et al.* 1998). Differences in mandible shape among *A. sanctaecrucis* populations, particularly in the case of Beef Island, which is drier than the other islands and is characterized by a low, scrubby habitat, implicate local adaptation to divergent selection pressures (Liem 1973), the nature of which should be explored through further study on the trophic ecology of these crickets. Population variation in the shape of the C-sclerite, a component of male genitalia, reflects a general pattern of diversity in this structure in the genus *Amphiacusta* and suggests that selection on genitalia shape may play an important role in the process of population and species divergence in *A. sanctaecrucis* and in the genus as a whole.

Unlike neutral genetic variation, phenotypic differentiation among populations is not spatially structured—there is no correspondence between geographic distance and pairwise phenotypic divergence, and in addition, no evidence that gene flow has influenced morphological divergence between populations. It should be noted that levels of gene flow are so low, and the confidence intervals of estimates so narrow, that there may be a lack of power in the estimation of these parameters which renders conclusions about the spatial structure and influence of gene flow problematic. Nevertheless, in conjunction with other findings that selection is driving morphological divergence in these populations, a lack of concordance between morphology and gene flow implies a primary role for selection operating within recently isolated populations in driving divergence in *A. sanctaecrucis*. Furthermore, population variation in the shape of male

genitalia reflects a general pattern of diversity in this structure in the genus *Amphiacusta* and suggests that selection on genitalia shape may play an important role in the process of population and species divergence in *A. sanctaecrucis* and in the genus as a whole. Further work on the ecological and sexual context in which these traits are diverging will shed light on the selective pressures promoting local adaptation.

CONCLUSION

A pattern of spatially structured genetic variation in Virgin Island populations of *A. sanctaecrucis* is likely the result of spatially correlated migration when lower sea levels during the Pleistocene exposed land connections between the Virgin Islands. In contrast to findings for other ocean taxa, levels of gene flow between populations are very low and show no concordance with the prevailing direction of ocean currents between islands. Rather, the evidence suggests that *A. sanctaecrucis* are rarely dispersed to other islands during storm events, possibly by wind. Despite such low levels of genetic differentiation, island populations are significantly divergent in two ecological and one sexual trait. This morphological population differentiation is not influenced by extrinsic factors such as geographic distance between populations or low levels of pairwise gene flow; rather, adaptive divergence is occurring in response to local selection pressures. Further work on the ecology and reproductive behavior of *A. sanctaecrucis* is needed to elucidate on the functional significance of morphological divergence in body size, mandible shape, and C-sclerite shape, and the context in which adaptive divergence is occurring in these crickets.

FIGURE LEGENDS

Figure 1. Map of the Virgin Islands showing the region's currents, which flow in a northwesterly direction.

Figure 2. Drawing of the mandible of *A. sanctaecrucis* showing placement of landmarks.

Figure 3. Drawing of the male genitalia of *A. sanctaecrucis* showing the C-sclerite. Drawing by Dan Otte.

Figure 4. Pairwise $F_{ST}/(1-F_{ST})$ values plotted against geographical distance (km). A mantel test indicates a significant isolation by distance effect ($r = 0.849$, $p = 0.008$).

Figure 5. Thin plate spline representations of the average mandible shape in six Virgin Island populations, which differ significantly in shape (Pillai's trace = 1.09, $p < 0.001$). A plot of the first two principal components (relative warps), which together summarize 58.03% of the variation, suggests that most of the differences can be attributed to Beef Island, which exhibits a longer mandible than the other populations.

Figure 6. Thin plate spline representations of the average C-sclerite shape in six Virgin Island populations, which differ significantly in shape (Pillai's trace = 1.39, $p < 0.001$). A plot of the first two principal components (relative warps), which together summarize 48.26% of the variation, shows a great deal overlap among populations, with most of the differences attributable to Beef Island.

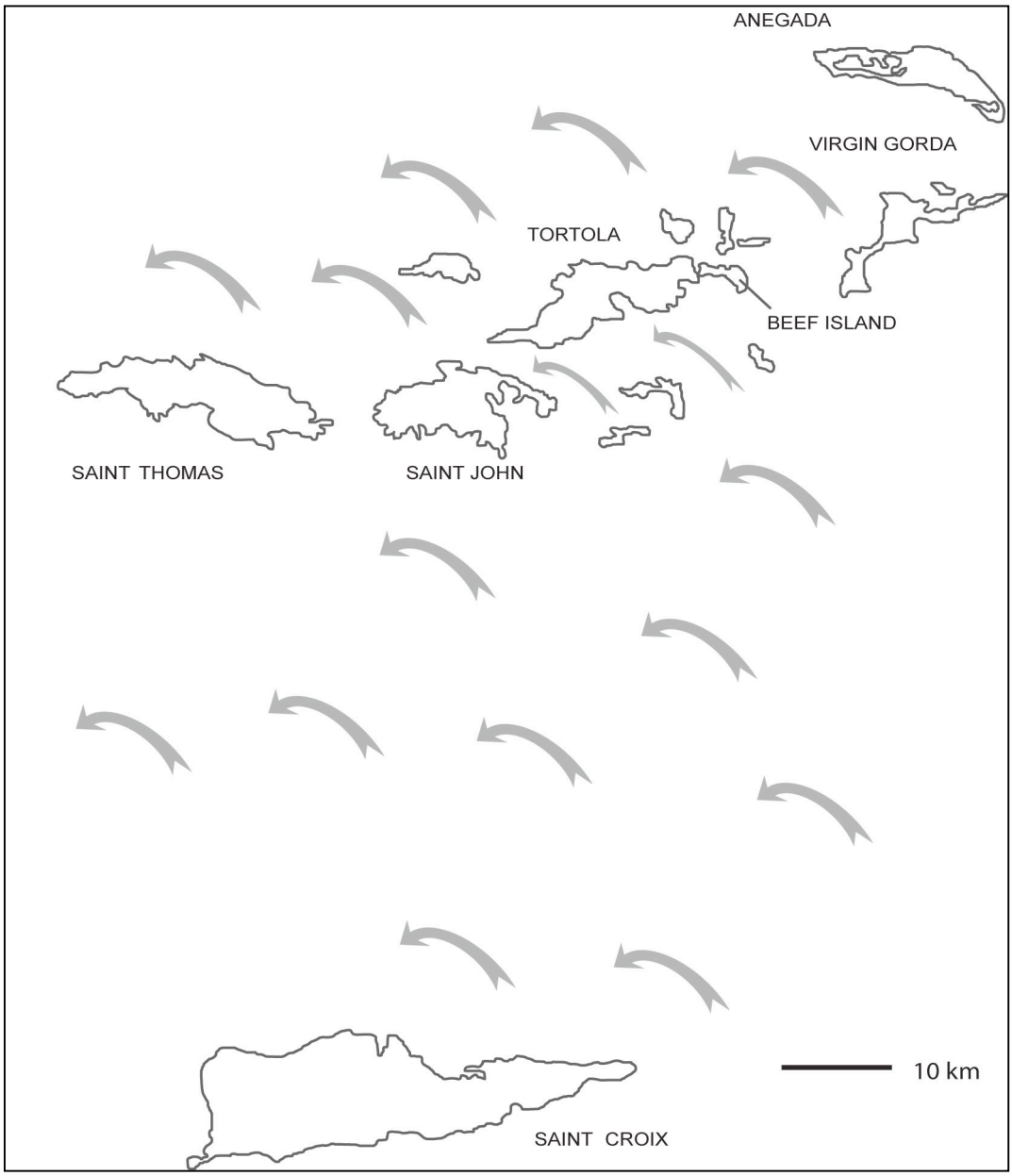
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Fig. 1

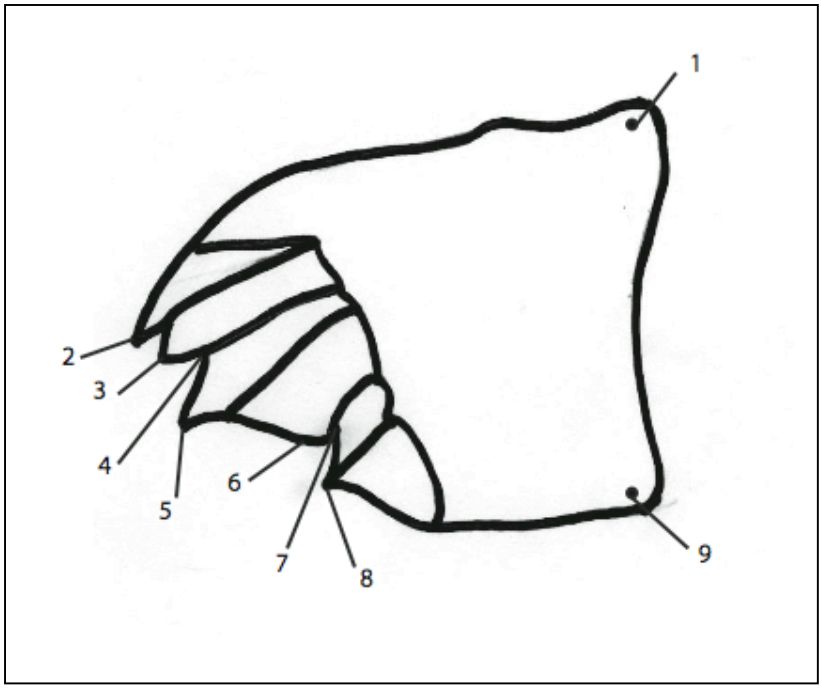
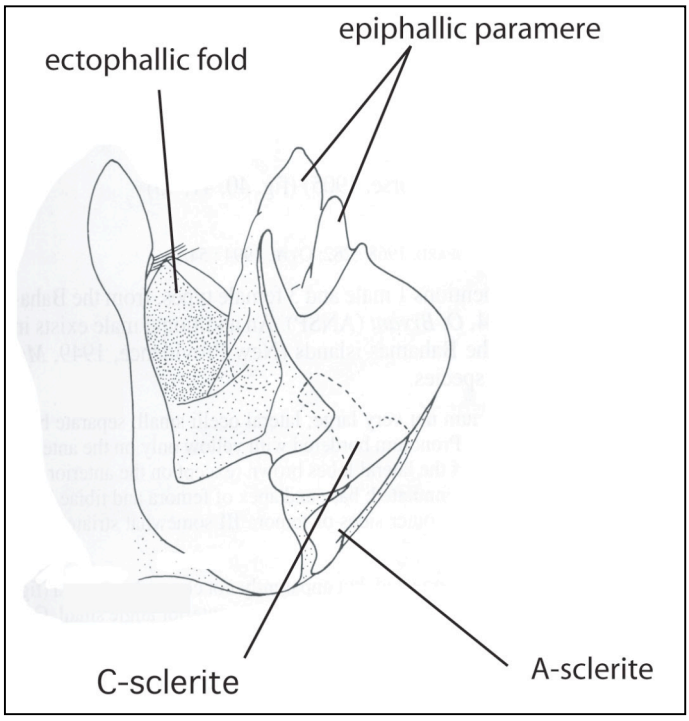


Fig 2.


epiphallic paramere
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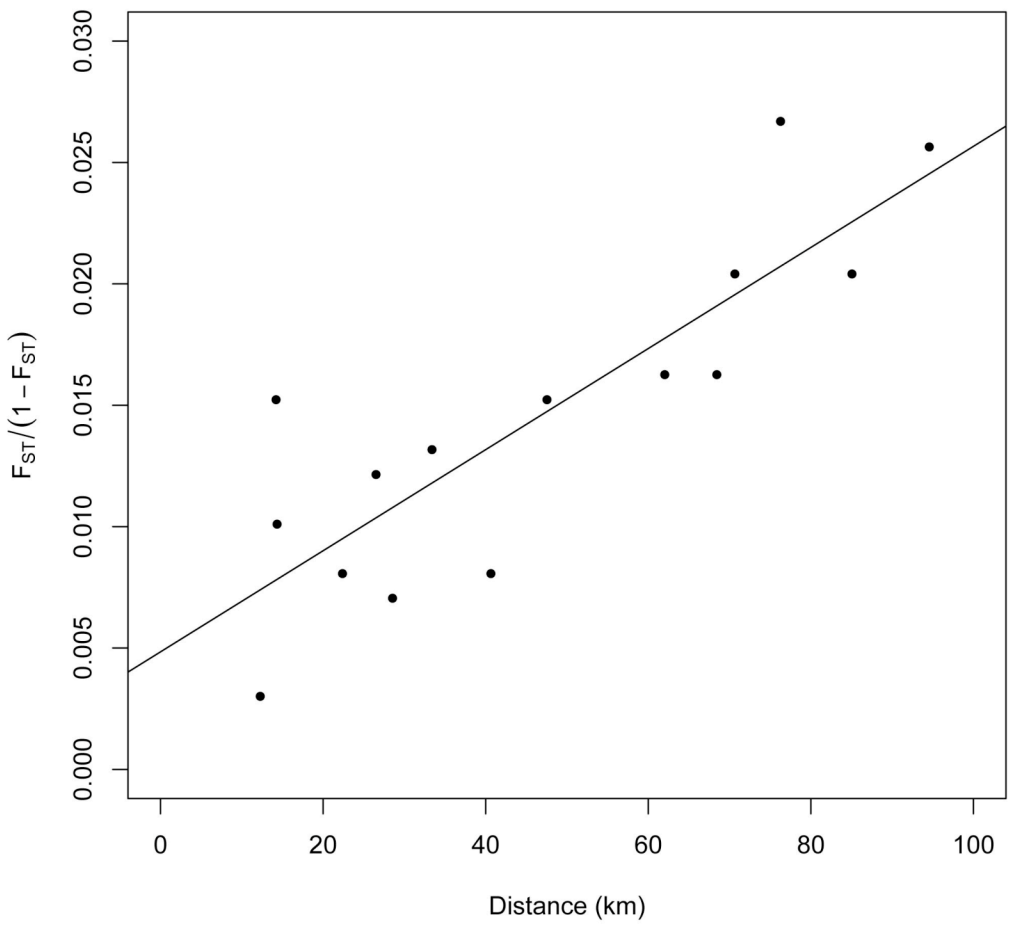
C-sclerite
A-sclerite

Fig 3.


$F_{ST}/(1-F_{ST})$
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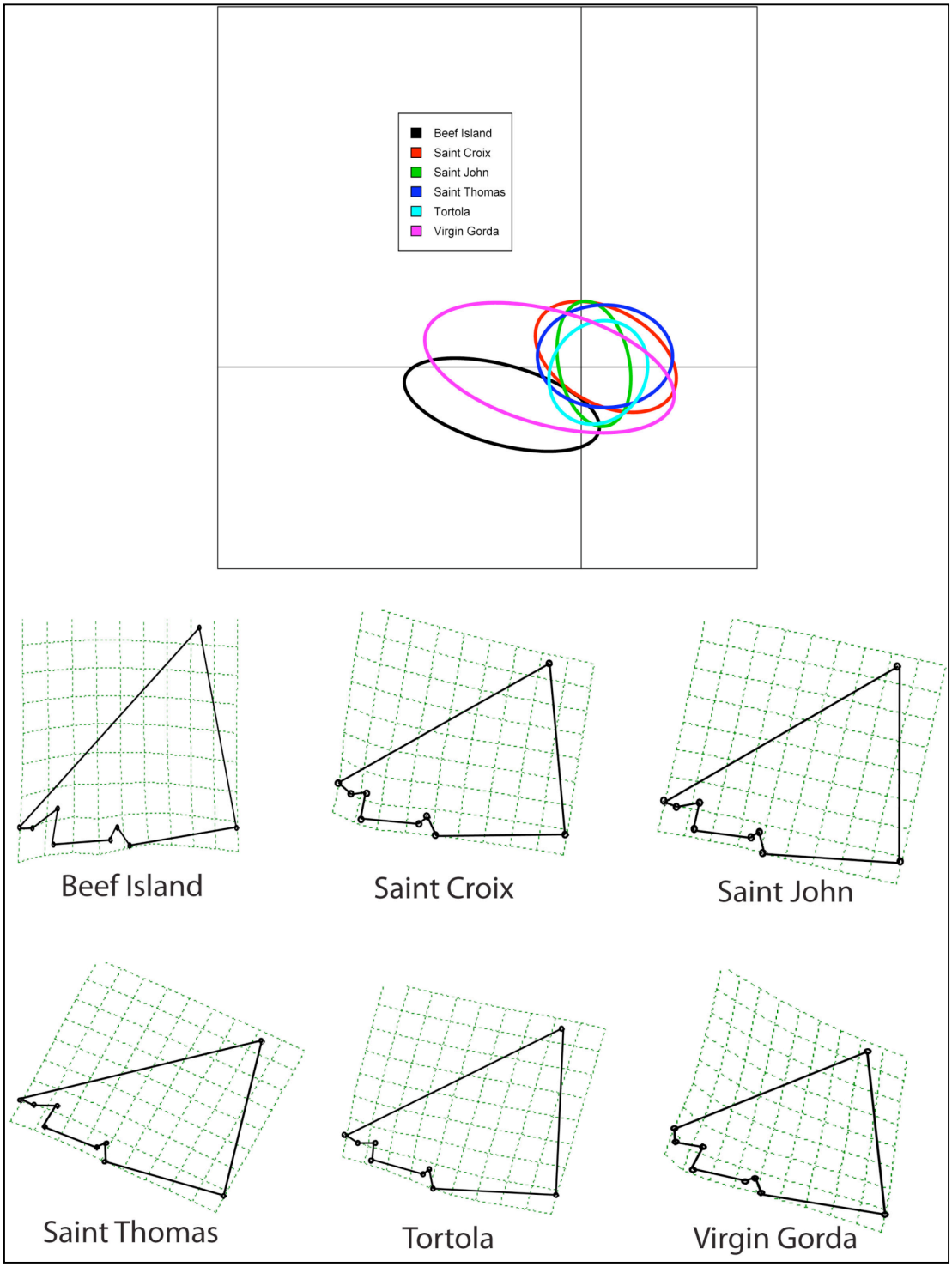
20
40
60
80
100
Distance (km)

Fig. 4


Beef Island
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Beef Island
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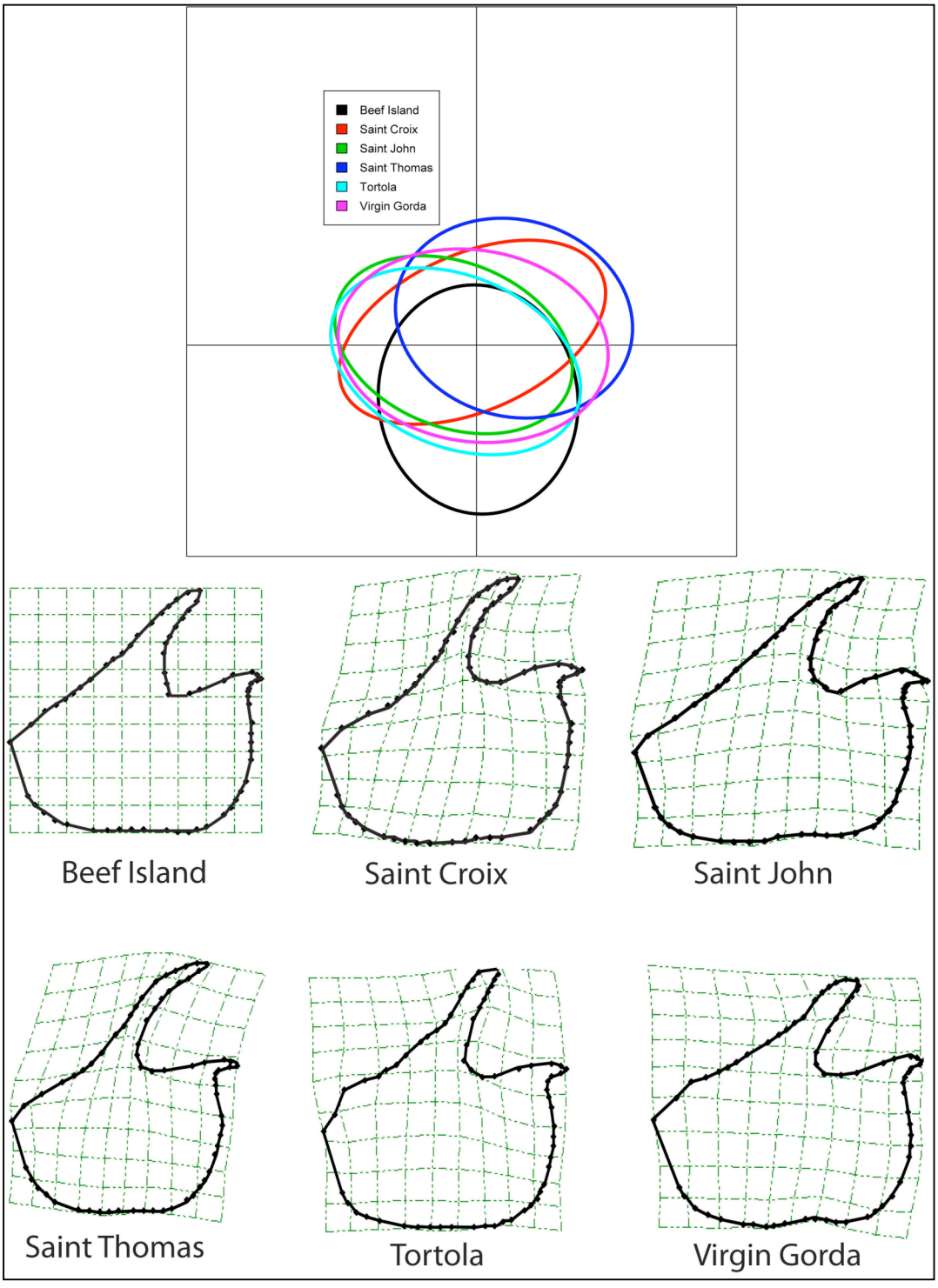
Virgin Gorda

Fig. 5


Beef Island
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Beef Island
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Virgin Gorda

Figure 6

Table 1. Sampling locations, dates and elevation for each collecting site on six Virgin Islands. Latitude and longitude were calculated using BioGeomancer.

Island	Location	Geoposition	Date	N
Beef Island	0.3 km south of Trellis Bay	18.221667 N, 64.532778 W	7 May 2005	16
Saint Croix	Mahogany Rd	17.737500 N, 64.841667 W	10 May 2005	18
			31 May 2007	8
Saint John	Cinnamon Bay	18.352200 N, 64.760556 W	23 May 2005	18
	Centerline Road	18.343056 N, 64.765000 W	24 May 2007	13
Saint Thomas	Route 33	18.363333 N, 64.974167 W	3 May 2007	16
	Crown Mountain Rd	18.355833 N, 64.974167 W	22 May 2007	21
Tortola	Sage Mountain	18.400833 N, 64.661111 W	6 May 2005	19
			24 May 2007	12
Virgin Gorda	Virgin Gorda Peak	18.477778 N, 64.403333 W	8 May 2005	11
			27 May 2007	19

Table 2. Effective migration rates ($N_e m = \theta M/4$) between 6 island populations. BI = Beef Island, STC = Saint Croix, STJ = Saint John, STT = Saint Thomas, TOR = Tortola, VG = Virgin Gorda.

Parameter	mode	median	95% CI
$N_e m_{STC \text{ to } STT}$	0.511	0.542	0.412 – 0.692
$N_e m_{STT \text{ to } STC}$	0.511	0.524	0.408 – 0.651
$N_e m_{STC \text{ to } STJ}$	0.634	0.624	0.412 – 0.738
$N_e m_{STJ \text{ to } STC}$	0.489	0.492	0.394 – 0.564
$N_e m_{STC \text{ to } TOR}$	0.630	0.619	0.479 – 0.733
$N_e m_{TOR \text{ to } STC}$	0.342	0.364	0.272 – 0.414
$N_e m_{STC \text{ to } VG}$	0.580	0.597	0.470 – 0.779
$N_e m_{VG \text{ to } STC}$	0.379	0.387	0.295 – 0.478
$N_e m_{STC \text{ to } BI}$	0.662	0.606	0.322 – 0.738
$N_e m_{BI \text{ to } STC}$	0.612	0.568	0.329 – 0.715
$N_e m_{STT \text{ to } STJ}$	0.561	0.561	0.444 – 0.656
$N_e m_{STJ \text{ to } STT}$	0.447	0.460	0.299 – 0.564
$N_e m_{STT \text{ to } TOR}$	0.598	0.615	0.485 – 0.752
$N_e m_{TOR \text{ to } STT}$	0.493	0.505	0.394 – 0.624
$N_e m_{STT \text{ to } VG}$	0.712	0.720	0.575 – 0.843
$N_e m_{VG \text{ to } STT}$	0.799	0.765	0.597 – 0.902
$N_e m_{STT \text{ to } BI}$	0.771	0.761	0.598 – 0.925
$N_e m_{BI \text{ to } STT}$	0.411	0.423	0.311 – 0.528
$N_e m_{STJ \text{ to } TOR}$	0.607	0.629	0.439 – 0.802
$N_e m_{TOR \text{ to } STJ}$	0.493	0.496	0.300 – 0.615
$N_e m_{STJ \text{ to } VG}$	0.479	0.496	0.380 – 0.637
$N_e m_{VG \text{ to } STJ}$	0.502	0.515	0.399 – 0.647
$N_e m_{STJ \text{ to } BI}$	0.498	0.496	0.371 – 0.596
$N_e m_{BI \text{ to } STJ}$	0.616	0.619	0.512 – 0.711
$N_e m_{TOR \text{ to } VG}$	0.525	0.524	0.371 – 0.711
$N_e m_{VG \text{ to } TOR}$	0.475	0.487	0.349 – 0.624
$N_e m_{TOR \text{ to } BI}$	0.749	0.747	0.624 – 0.852
$N_e m_{BI \text{ to } TOR}$	0.292	0.300	0.204 – 0.391
$N_e m_{VG \text{ to } BI}$	0.712	0.715	0.583 – 0.829
$N_e m_{BI \text{ to } VG}$	0.457	0.455	0.343 – 0.555

Table 3. Summary of a principal components analysis of body measurements (i.e., femur length, tibia length, front femur length, upper and lower pronotum length, snout width and eye span), including the percent variation explained by each component and the factor loadings on each structure.

	PC1	PC2
% variation	95.1	2.8
femur	0.603	-0.508
tibia	0.649	-0.539
front femur	0.412	0.794
upper pronotum	0.101	0.108
lower pronotum	0.171	0.250
snout	0.018	-0.001
eyes	0.059	0.041

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