Phylogeography of an Island Endemic, the Puerto Rican Freshwater Crab (*Epilobocera sinuatifrons*)

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Abstract

The endemic Puerto Rican crab, *Epilobocera sinuatifrons* (Pseudothelphusidae), has a freshwater-dependant life-history strategy, although the species has some capabilities for terrestrial movement as adults. In contrast to all other freshwater decapods on the island (e.g., caridean shrimp), *E. sinuatifrons* does not undertake amphidromous migration, and is restricted to purely freshwater habitats and adjacent riparian zones. As Puerto Rico has a dynamic geologic history, we predicted that both the life history of *E. sinuatifrons* and the geological history of the island would be important determinants of phylogeographic structuring in the species. Using a fragment of the cytochrome *c* oxidase subunit 1 mtDNA (mitochondrial DNA) gene, we tested for deviations from panmixia among and within rivers draining Puerto Rico and used statistical phylogeography to explore processes that may explain extant patterns of genetic variation in the species. While populations of *E. sinuatifrons* were significantly differentiated among rivers, they were likely to be recently derived because nested clade analysis (NCA) indicated evolutionarily recent restricted gene flow with isolation by distance (IBD) and contiguous range expansion at various spatial scales. Ongoing drainage rearrangements associated with faulting and land slippage were invoked as processes involved in sporadic gene flow among rivers throughout the Pleistocene. Patterns of genetic differentiation conformed to IBD and population demographic statistics were nonsignificant, indicating that although recently derived, populations from different rivers were in drift-mutation equilibrium. A shallow (0.6 million years ago), paraphyletic split was observed in the haplotype network, which NCA indicated arose via allopatric fragmentation. This split coincides with an area of high relief in central Puerto Rico that may have experienced relatively little drainage rearrangements. Shallow but significant genetic isolation of populations of *E. sinuatifrons* among Puerto Rican rivers suggests phylogeographic patterns that are intermediate to terrestrial habitat specialists (highly divergent populations) and other freshwater biota, such as amphidromous species and insects with aerial adult dispersal (highly connected populations).

Oceanic islands typically harbor a high proportion of endemic species on account of their isolation, among other factors (MacArthur and Wilson 1967). However, the freshwater biota of islands (excluding insects) is dominated by amphidromous species (i.e., freshwater species that undertake migration as larvae to marine habitats, followed by postlarval upstream migration) that are typically distributed throughout archipelagos (McDowall 2004). For example, while individual islands of the Hawaiian archipelago often harbor endemic terrestrial species (Carlquist 1970; Carson and Clague 1995), several Hawaiian freshwater fishes, shrimps, and gastropods are distributed throughout the archipelago (Chubb et al. 1998; McDowall 2003; Bebler and Foltz 2004). Amphidromy in these species prohibits the evolution of island-specific clades (Bebler and Foltz 2004),

indicating that populations of these species may be genetically continuous among rivers (Cook et al., forthcoming) and among islands (Chubb et al. 1998). Similarly, for insects with aquatic nymph stages (e.g., Canary Island caddisflies), aerial dispersal as adults facilitates genetic continuity among catchments and among islands (Kelly et al. 2001). However, many terrestrial habitat specialists show strong population subdivision among isolated habitats within an island (Brown and Pestano 1998; Holland and Hadfield 2002; Vandergast et al. 2004), with geological history, discontinuous habitat, and disturbance being dominant forces that influence their phylogeographic patterns (Emerson 2002). For freshwater species that are not amphidromous or do not have abilities for flight as adults, discontinuous riverine habitats and disturbance may
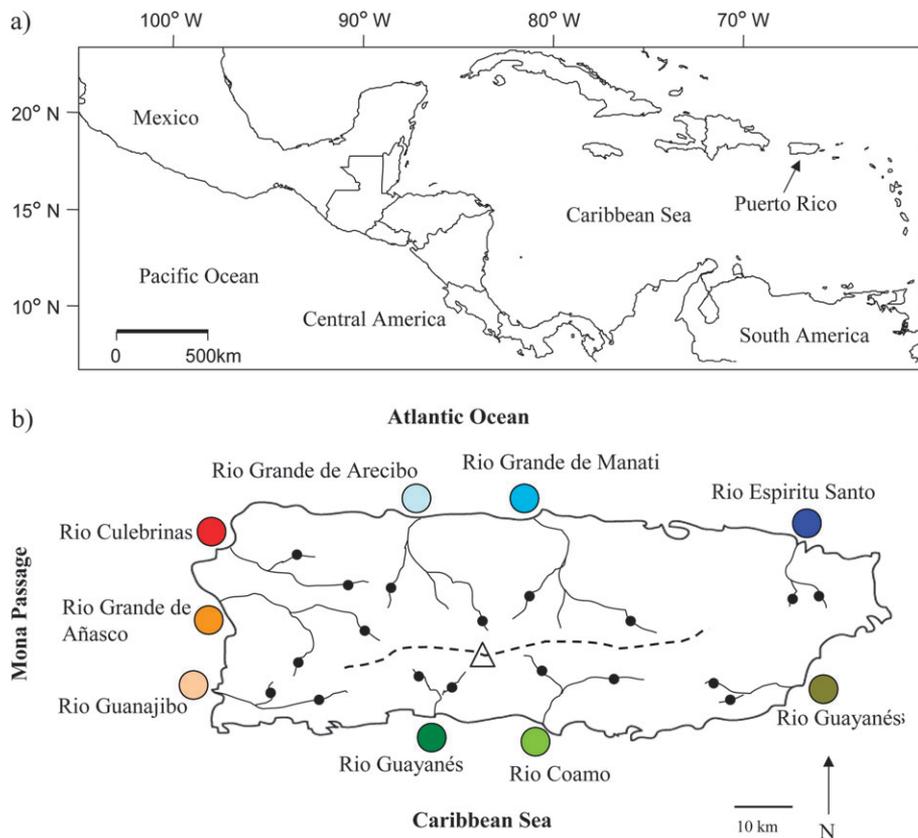
Figure 1. (**a**) Map of Central America and Caribbean showing regional location of Puerto Rico. (**b**) Map of Puerto Rico showing the sites (black dots) and rivers sampled. Each river is color coded, and these colors correspond to colors used in the haplotype network (Figure 2). The triangle represents the mountain Cerro de Punta; the dashed line represents the approximate location of the Cordillera Central mountain range. Only sampled rivers are shown.

promote the evolution of phylogeographic patterns that resemble patterns commonly observed in terrestrial species.

The freshwater biota of the Caribbean archipelago is dominated by 2 main groups of decapod crustaceans: 1) amphidromous, widespread caridean shrimp (e.g., genera *Atya, Xiphocaris, Macrobrachium*), and 2) freshwater-associated (semiterrestrial, not amphidromous), island endemic crabs (e.g., genera *Epilobocera, Guinotia*) (Rodríguez and Williams 1995; Fièvet 1998). Islands of the Greater Antilles (i.e., Cuba, Jamaica, Hispaniola, Puerto Rico) each have one to several endemic species of *Epilobocera* (Pseudothelphusidae), with 1 species (*Epilobocera sinuatifrons*) endemic to Puerto Rico and Saint Croix (Chace and Hobbs 1969; Rodríguez 1986). Endemism and the life-history characteristics of these crabs have made them interesting systems for regional biogeographic studies (Banarescu 1990; Ng and Rodríguez 1995; Rodríguez and López 2003) and also make them interesting systems for studying within-island phylogeographic patterns. All species of *Epilobocera* have direct development, an absence of a free-swimming larval stage (Ng and Rodríguez 1995) and the ability for air breathing (Rodríguez 1986). Juveniles of *E. sinuatifrons* are commonly found in pool habitats in Puerto Rico (Zimmerman and

Covich 2003), and adults are commonly found under rocks in saturated sections of stream banks (Covich and McDowell 1996; Zimmerman and Covich 2003) and are reported to consume leaf-based detritus, forest fruits, and terrestrial invertebrates on the rainforest floor adjacent to streams (Covich and McDowell 1996; March and Pringle 2003). Thus, the species has a strong dependency on freshwater habitats as juveniles and adults, although adults have the ability to traverse terrestrial habitats to some extent. The competing influence of strong freshwater association and ability for terrestrial movement may be important in population genetic and phylogeographic structuring in *E. sinuatiforns* on Puerto Rico.

Puerto Rico has a dynamic geological history characterized by ancient (Eocene) volcanic and plutonic activity and ongoing (Oligocene–recent) deformation of high-relief regions by tectonic stress, erosion, and land slippage processes (Monroe 1980; van Gestel et al. 1999; Renken et al. 2002). The Central Range (Cordillera Central) is thus a highly folded, faulted, and eroded mountain range, with the highest peaks occurring in the vicinity of Cerro de Punta (1338 m; Figure 1). Furthermore, most Puerto Rican rivers have their headwaters in the Cordillera Central, with

Table 1. Sample size, molecular diversity, and population demographic statistics for *Epilobocera sinuatifrons* for populations from each river

River	Sample size		π	h	Tajima's D	Fu's Fs
	SC 1	SC 2				
ESP	11	11	0.004 ± 0.002	0.697 ± 0.102	−1.231 (0.105)	−2.058 (0.176)
MAN	11	0	0.004 ± 0.003	0.764 ± 0.107	−0.722 (0.270)	−0.117 (0.481)
ARI	9	4	0.002 ± 0.001	0.692 ± 0.075	1.214 (0.840)	0.610 (0.634)
CUL	9	5	0.005 ± 0.003	0.791 ± 0.067	−0.395 (0.389)	0.679 (0.681)
ANA	5	10	0.004 ± 0.003	0.810 ± 0.078	0.474 (0.717)	−0.697 (0.375)
JIB	8	10	0.004 ± 0.002	0.824 ± 0.071	−0.080 (0.528)	−1.454 (0.234)
YAN	7	7	0.003 ± 0.002	0.813 ± 0.094	−0.065 (0.518)	−1.438 (0.228)
COA	9	6	0.003 ± 0.002	0.562 ± 0.143	−1.350 (0.086)	−0.105 (0.504)
GUA	4	0	0.004 ± 0.004	0.833 ± 0.222	−0.065 (0.705)	0.251 (0.426)

SC, subcatchments; ESP, Rio Espiritu Santo; MAN, Rio Grande de Manati; ARI, Rio Grande de Arecibo; CUL, Rio Culebrinas; ANA, Rio Grande de Añasco; JIB, Rio Guanajibo; YAN, Rio Guayanés; COA, Rio Coamo; GUA, Rio Guayanés. *P* values for demographic parameters are shown in parentheses.

present-day mass movement of hill slopes delivering large quantities of sediment to rivers leading to highly dynamic river morphology (Ahmad et al. 1993). The geologically dynamic history of the island thus raises the prospect for geological history to be an important determinant of phylogeographic patterning in *E. sinuatifrons*. Thus, if discontinuous rivers demarcate discrete populations in *E. sinuatifrons*, we expected to reveal divergent populations associated with river habitats. In contrast, we expected to find no genetic subdivision among rivers in the species if terrestrial dispersal is an effective mechanism of among-river dispersal. Finally, we explored the role of geological history, notably ancient volcanism and ongoing deformation processes, on population patterning in the species using statistical phylogeographic techniques.

Materials and Methods

Field and Laboratory Methods

Epilobocera sinuatifrons were sampled from 2 subcatchments in each of 9 rivers in Puerto Rico (Figure 1). We aimed to sample 10 individuals at each site, although this was not always possible (Table 1). A leg was taken from large individuals and preserved in 70% ethanol, and these individuals were released otherwise unharmed, although whole juveniles were taken.

Genomic DNA was extracted from each individual using a standard phenol–chloroform procedure, and a fragment of the cytochrome *c* oxidase subunit 1 (COI) mtDNA (mitochondrial DNA) gene was amplified via polymerase chain reaction (PCR) using primers COI-CR-F and COI-CR-R (Cook et al., forthcoming). The forward and reverse primers align with base pair positions 20–41 and 898–917, respectively, of the complete COI mtDNA gene of the Hawaiian volcano shrimp (*Halocaridina rubra*) (GenBank accession number: NC 008413; Ivey and Santos 2007). PCR reactions contained approximately 40 ng of template DNA, 0.4 μM of each primer, 0.2 mM dNTP (Astral Scientific, Caringbah, New South Wales, Australia), 2 mM MgCl$_2$, 1.25 μl of 10× polymerase reaction buffer and 0.25 unit of *Taq*

polymerase (Fisher Biotech, Subiaco, Australia), adjusted to a final volume of 12.5 μl with ddH$_2$O. The thermal cycling profile followed: 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C; an additional extension phase of 5 min at 72 °C; and a final hold at 4 °C. PCR product was purified with the exonuclease I–shrimp alkaline phosphatase method, using 2.5-μl PCR product, 2.0 μl shrimp alkaline phosphatase (Promega), and 0.5 μl exo-nuclease I (Fermenta), and a 2-step thermal cycling profile: 35 min at 37 °C and 20 min at 80 °C. Sequencing reactions contained 0.5-μl purified product, 0.32 μl forward primer, 2 μl BigDye v1.1 (Applied Biosystems, Foster City, CA), and 2 μl BigDye 5× sequencing buffer (Applied Biosystems), and the following thermal cycling conditions were used: 1 min at 96 °C; 30 cycles of 10 s at 96 °C, 5 s at 50 °C, and 4 min at 60 °C; and a hold period of 4 °C. Sequencing was conducted on a 3130xl Capillary Electrophoresis Genetic Analyzer (Applied Biosystems), and sequences were aligned and edited using SEQUENCHER version 4.1.2 (Gene Codes). Two exemplars of each haplotype were sequenced from the 3′ end to verify bases at polymorphic sites.

Data Analysis

Population subdivision within and among rivers was assessed using 10 000 randomizations of the observed genotypes in the AMOVA framework (Excoffier et al. 1992), as implemented in ARLEQUIN (Schneider et al. 2000). Φ-indices (Excoffier et al. 1992), which incorporate haplotype frequency and divergence, were used in the AMOVA rather than *F*-statistics, which incorporate only haplotype frequency. Pairwise analyses of Φ_{ST} among sites and among rivers (whereby subcatchments were pooled for the analysis) were also performed in ARLEQUIN. The relationship between mtDNA divergence and geographic distance among sites [i.e., isolation by distance (IBD)] was tested using Mantel tests (Mantel 1967) in PRIMER version 5.2.8 (Clarke and Gorley 2001). Both straight-line distance among sites and hydrological distance (incorporating stream and coastline distance) were used in tests for IBD. For each population AMOVA indicated, we calculated haplotype (*h*)

Table 2. Pairwise Φ_{ST} among rivers

	ESP	MAN	ARI	CUL	ANA	JIB	YAN	COA
MAN	**0.493**							
ARI	**0.565**	**0.297**						
CUL	**0.406**	**0.146**	**0.142**					
ANA	**0.428**	**0.133**	**0.265**	0.041				
JIB	**0.429**	**0.235**	**0.218**	0.080	0.009			
YAN	**0.433**	**0.089**	**0.189**	0.054	−0.014	0.020		
COA	**0.500**	−0.042	**0.306**	**0.165**	**0.168**	**0.270**	**0.094**	
GUA	−0.007	**0.625**	**0.792**	**0.576**	**0.576**	**0.577**	**0.659**	**0.557**

Abbreviated river names are shown in Table 1; significant values are indicated in bold.

and nucleotide (π) diversity, and the population demographic parameters D (Tajima 1989) and F_s (Fu 1997), using DNASP (Rozas et al. 2003). The significance of the demographic parameters was assessed using 10 000 coalescent simulations given the observed number of segregating sites. A haplotype network was constructed using TCS version 1.18 (Clement et al. 2000), and cladistic analysis of the nested haplotypes [nested clade analysis (NCA); Templeton et al. 1995] was implemented using GeoDis version 2.4 (Posada et al. 2000). The November 2005 NCA inference key (available at Darwin.uvigo.es/download/geodisKey_11Nov05.pdf) was used to discriminate between putative evolutionary events that account for present-day patterns of genetic diversity. For clades that NCA indicated arose via allopatric fragmentation, we used the coalescent program MDIV (Nielsen and Wakeley 2001) to estimate the timing of the population split. MDIV was implemented using a finite sites (HKY) model, 5 000 000 Markov chain iterations and a 25% burn-in. M_{max} and T_{max} were set to 10 and 5, respectively. The highest likelihood values for these parameters were then converted into years (Nielsen and Wakeley 2001) using a COI mtDNA substitution rate of 1.4% per million years, as reported for other tropical decapod crustaceans (Knowlton and Weigt 1998).

Results

Sequencing of 126 individuals yielded 548 bp of unambiguous sequence of which 25 (4.56%) were polymorphic. These polymorphisms resulted in 26 haplotypes being detected in the sample (GenBank accession numbers: EU004914–EU004939). The transition:transversion ratio was 5.5:1, and percent nucleotide composition was C: 15.4, T: 38.7, A: 29.4, and G: 16.5. AMOVA indicated population subdivision in *E. sinuatifrons* among rivers (Φ_{CT} = 0.288, $P < 0.001$), but no substructure was evident among subcatchments within rivers (Φ_{SC} = 0.005, P = 369). These results were largely confirmed by pairwise analyses of Φ_{ST} as most pairwise comparisons among rivers were significant (Table 2) and no pairwise comparisons within rivers were significant (data not shown). Notably, pairwise analyses of Φ_{ST} among rivers revealed genetic homogeneity among rivers at the western end of the island (i.e., Rio

Culebrinas, Rio Grande de Añasco, Rio Guanajibo, Rio Guayanés; Table 2). Tests for IBD were highly significant (straight-line distance: ρ = 0.724, $P < 0.001$; hydrological distance: ρ = 0.509, $P < 0.001$). As there was no population subdivision in *E. sinuatifrons* within rivers, subcatchments within rivers were pooled for calculations of molecular diversity and population demographic parameters. Nucleotide diversity (π) and haplotype diversity (h) were both low and did not differ substantially among the rivers, and all population demographic parameters were not significantly different from zero (Table 1). NCA revealed a succession of fragmentation, IBD, and dispersal events over various geographical scales (Figure 2; Table 3). While clade 3-2 is restricted to the eastern end of the island, clade 3-1 is distributed throughout the island, indicating a paraphyletic break at the centre of the island. Clade 3-2 was inferred to have arisen via allopatric fragmentation, and the timing of the population split between clades 3-1 and 3-2, as inferred using MDIV, was 0.577 million years ago (MYA), indicating a Pleistocene fragmentation event.

Discussion

Among-River Population Genetics

Interestingly, results indicated 2 contrasting patterns of among-river gene flow in *E. sinuatifrons*. The first result is the absence of genetic population subdivision among the western rivers, in which NCA revealed contiguous range expansion (clade 2-1), indicating evolutionarily recent dispersal. Such dispersal could have been mediated by either intrinsic (i.e., walking ability) or extrinsic (i.e., drainage rearrangements) factors. If walking was the principle mechanism of among-river dispersal in *E. sinuatifrons*, we would have expected other pairwise analyses of Φ_{ST} among rivers to also be significant, as walking would facilitate gene flow among other geographically proximate rivers. However, the second result concerning among-river patterns of gene flow contradicted this expectation and showed genetic discontinuity among populations of *E. sinuatifrons* from all other rivers. This indicates that walking ability alone is unlikely to facilitate among-river dispersal in this species and that extrinsic factors had a role in genetic continuity among the western rivers. Abilities for terrestrial movement in
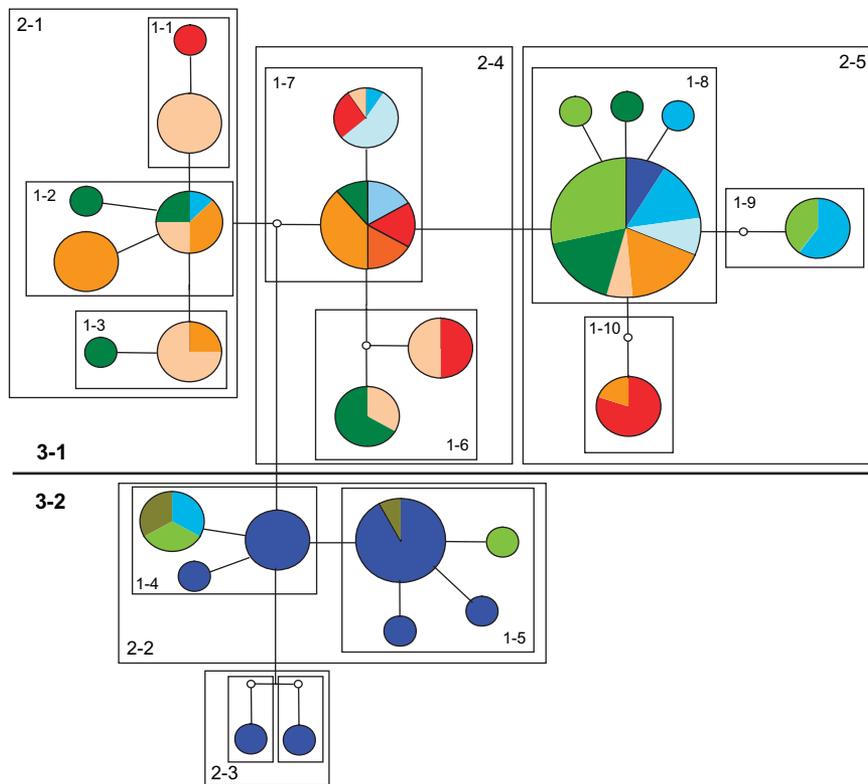
Figure 2. Haplotype network for *Epilobocera sinuatifrons*, showing genealogical relationships among mtDNA haplotypes and nesting levels used in NCA.

spiny crayfish from eastern Australia (*Euastacus* spp.) are insufficient for facilitating gene flow among populations from discontinuous streams (Baker et al. 2004; Ponniah and Hughes 2006), and idiosyncratic patterns of genetic continuity among isolated rivers have often been interpreted as evidence for historical geological processes, such as drainage rearrangement (Hurwood and Hughes 1998; McGlashan and Hughes 2000). It is therefore likely that recent gene flow between rivers at the western end of the island was facilitated by recent (Holocene) drainage rearrangements associated with faulting and erosion processes (Monroe 1980; Ahmad et al. 1993; Renken et al. 2002) and that these populations are not in genetic drift–gene flow equilibrium (Wade and McCauley 1988).

Population demographic analyses indicated that riverine populations in *E. sinuatifrons* are in drift–mutation equilibrium and have not experienced recent demographic fluctuations (population expansions or bottlenecks).

Table 3. Results of NCA for *Epilobocera sinuatifrons*

Clade level	Significant constituent clades	Distance type	Inference chain	Inferred evolutionary process
Clade 1-8	Clade v (interior)	Dc (large)	1-2-3-4: no	Restricted gene flow with IBD
		Dn (large)		
	I-T	Dc (large)		
		Dn (large)		
Clade 2-1	Clade 1-1 (tip)	Dn (large)	1-2-11-12: no	Contiguous range expansion
	Clade 1-2 (interior)	Dc (small)		
		Dn (small)		
	I-T	Dc (small)		
		Dn (small)		
Clade 2-5	Clade 1-10 (tip)	Dc (small)	1-2-3-5-15: no	Past fragmentation or long distance colonization
	I-T	Dn (large)		
		Dc (large)		
Clade 3-1	Clade 2-5 (tip)	Dc (small)	1-2-3-4: no	Restricted gene flow with IBD
		Dn (small)		
Total cladogram	Clade 3-2 (tip)	Dc (small)	1-2-3-4-9: no	Allopatric fragmentation

Similarly, regionally stable population pattern typically exhibit signatures of IBD in genetic data (Hutchison and Templeton 1999), as we found for *E. sinuatifrons*. Despite stable patterns of population subdivision among most rivers in *E. sinuatifrons*, among-river genetic exchange appears to have occurred sporadically throughout the Pleistocene as some tip haplotypes are shared among rivers and the oldest split in the genealogy is only about 0.6 MYA. A similar result was revealed for the South African burrowing freshwater crab (*Potamonautes calcaratus*), whereby recently derived populations were significantly differentiated among isolated waterholes (Daniels et al. 2002). In contrast, populations of the anchialine shrimp (*H. rubra*) in distinct subterranean watersheds throughout the Hawaiian islands are more ancient and are characterized by extremely large genetic divergence (Santos 2006; Craft et al., forthcoming). Similarly, for terrestrial habitat specialists on islands, discontinuous habitat promotes substantial cladogenesis among populations (Brown and Pestano 1998; Holland and Hadfield 2002; Vandergast et al. 2004). The geologically dynamic history of Puerto Rico appears to have sporadically and repeatedly facilitated continuity in riverine habitat over evolutionary time, thereby prohibiting strong divergence among populations of *E. sinuatifrons* in different rivers.

The terrestrial walking ability of *E. sinuatifrons* means that "river capture" (i.e., when a stream become disconnected from the original river and connected to a new river via erosion and fault processes) need not have been necessary to promote population continuity across catchment divides. In some instances, rearranged drainage lines in close proximity may have been sufficient for gene flow among rivers. Thus, although drainage rearrangement via faulting and erosion processes was likely the dominant process in historical gene flow among rivers, walking ability in *E. sinuatifrons* may mean that altered drainage patterns had a more substantial influence on genetic exchange than what would be expected for species that cannot leave in-stream habitat, such as nonmigratory fish (Hurwood and Hughes 1998; Burridge et al. 2006).

Within-River Population Genetics

Although geological activity, such as drainage rearrangements, is likely needed for gene flow among rivers, walking appears to facilitate high rates of gene flow within rivers. Although various Indo-Pacific atyid shrimp (e.g., *Paratya* spp., Hughes et al. 1995; Cook et al. 2007; *Caridina lanceolata*, Roy et al. 2006) are isolated by steep stream gradients (i.e., waterfalls, chutes, cascades), these have no apparent influence on patterns of genetic connectivity in *E. sinuatifrons* within rivers as walking likely allows the species to negotiate such sections of stream. An African freshwater crab (*Potamonautes unispinus*) is reported to undertake potamodromous migration (i.e., migration within purely freshwater sections of rivers) in ephemeral streams in Zimbabwe (Gratwicke 2004), indicating that other freshwater crabs also appear to have well-developed abilities to move within rivers. Patterns of habitat use by *E. sinuatifrons* differ significantly between forested and agricultural areas (Zimmerman and Covich 2003) and source-sink dynamics in population structure between forested and agricultural habitats has been reported for the crab (*Potamonautes odhneri*) in East Africa (Dobson et al. 2007). It would be interesting and important for conservation to determine if *E. sinuatifrons* also displays source-sink dynamics in movement within rivers, as also reported for caridean shrimp in Puerto Rican rivers (Greathouse et al. 2005). Cold-adapted species of spiny crayfish (*Euastacus* spp.) are restricted to montane streams in eastern Australia; thus, hydrologically connected streams that coalesce in lowland tropical or subtropical environments harbor discrete populations of spiny crayfish (Baker et al. 2004; Ponniah and Hughes 2006). In contrast, *E. sinuatifrons* occurs in both upland and lowland environments in Puerto Rico, indicating that temperature variation associated with elevation does not influence their patterns of distribution or gene flow within rivers.

The Role of Cerro de Punta in Phylogeographic Patterning

NCA indicated that a succession of allopatric fragmentation, contiguous range expansion, and restricted gene flow with IBD have been important processes in the biogeographic history of the species. The most notable phylogeographic pattern revealed is the paraphyletic association of haplotypes from clades 3-1 and 3-2. Interestingly, the geographical location of this east–west split coincides with the location of the tallest mountain in Puerto Rico (Cerro de Punta, 1338 m; Figure 1). At least 5 other volcanic peaks in excess of 1000-m elevation also occur in the area. Although Eocene volcanism in Puerto Rico predates the late Pleistocene split between clades 3-1 and 3-2, the legacy of volcanism (i.e., a large area of very high relief) may have functioned as a barrier to historical sporadic population connectivity among rivers. It is possible that drainage rearrangements associated with faulting and erosion processes were uncommon in this area of high relief surrounding Cerro de Punta, resulting in allopatric fragmentation of eastern and western clades. NCA also indicated that dispersal from the west to the east (as clade 3-2 is restricted to the eastern half of the island) has occurred more recently than the fragmentation event. Although drainage rearrangements were likely to be uncommon in the central area of high relief, the rare times they occurred likely facilitated idiosyncratic dispersal in *E. sinuatifrons*, such as the unidirectional pattern of gene flow we have found in the species.

Conclusion

Rivers demarcate stable population units for *E. sinuatiforns*, although riverine populations are recently derived as sporadic and repeated past gene flow among rivers characterizes the biogeographic history of the species. As rivers and their catchment areas have a long legacy of anthropogenic disturbance on Puerto Rico (Greathouse et al. 2006), rivers would represent appropriate management

units for conservation of this endemic species. Recent phylogeographic and population genetic studies of sympatric congeners of freshwater decapod have revealed striking differences in population patterning in common landscapes (Cook et al. 2007; Page and Hughes 2007). Cuba, Jamaica, and Hispaniola each have several codistributed species of *Epilobocera* (Banarescu 1990; Rodríguez 1995; Rodríguez and López 2003), indicating that this genus would be an interesting study system for phylogeographic studies on these islands. Shallow (but significant) genetic population subdivision in *E. sinuatifrons* among rivers suggests phylogeographic patterns that are intermediate to phylogeographic patterns on islands reported for terrestrial habitat specialists (highly divergent populations, Holland and Hadfield 2002; Vandergast et al. 2004) and freshwater biota with different life histories, such as amphidromous species and insects with aerial adult dispersal (highly connected populations, Kelly et al. 2001; Cook et al., forthcoming.).

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