Evolution, 59(10), 2005, pp. 2125–2138

POPULATION STRUCTURE AT TWO GEOGRAPHIC SCALES IN THE BURROWING CRUSTACEAN *CALLICHIRUS ISLAGRANDE* (DECAPODA, THALASSINIDEA): HISTORICAL AND CONTEMPORARY BARRIERS TO PLANKTONIC DISPERSAL

A. Lelania Bilodeau,[1,2,3] Darryl L. Felder,[3] and Joseph E. Neigel[3,4]
[1]*U.S. Department of Agriculture-Agricultural Research Service, Catfish Genetics Research Unit, 141 Experiment Station Road, Box 38, Stoneville, Mississippi 38776*
[2]*E-mail: abilodeau@ars.usda.gov*
[3]*Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana 70504*
[4]*E-mail: jneigel@louisiana.edu*

Abstract.—There has been much recent interest in the extent to which marine planktonic larvae connect local populations demographically and genetically. Uncertainties about the true extent of larval dispersal have impeded our understanding of the ecology and evolution of marine species as well as our attempts to effectively manage marine populations. Because direct measurements of larval movements are difficult, genetic markers have often been used for indirect measurements of gene flow among marine populations. Here we examine data from allozymes, mitochondrial DNA sequences, and microsatellite length polymorphisms to assess the extent of gene flow among populations of the burrowing crustacean *Callichirus islagrande*. All three types of markers revealed a genetic break between populations separated by the Louisiana Chenier Plain. The extent of mitochondrial sequence divergence across this break indicates that the nominal species, *C. islagrande*, consists of at least two lineages that have been reproductively isolated for about a million years. Within the eastern lineage microsatellite allele frequencies were significantly heterogeneous among populations as little as 10 km apart. Maximum likelihood estimates of gene flow and effective population size based on a coalescent model for the microsatellite data indicated that local populations are nearly closed. A model-based clustering method identified four or five groups from the microsatellite data, although individuals sampled from each location generally consisted of mixtures of these groups. This suggests a mechanism that would lead to genetic differentiation of open populations: gene flow from different source populations that are themselves genetically distinct.

Key words.—*Callichirus islagrande*, gene flow, larval dispersal, open versus closed populations, phylogeography, population structure.

Received November 4, 2004. Accepted July 25, 2005.

It often has been assumed that populations of marine species with planktonic larvae are demographically ''open,'' with recruitment mostly from external sources (Gooch and Schopf 1972; Berger 1973; Crisp 1978; Caley et al. 1996). This view implies that local adaptation is unlikely in marine populations (Brown et al. 2001) and that speciation requires isolation by strong physical barriers (Mayr 1963; Vermeij 1978). It also leads to the practical suggestion that most marine species can be managed as single stocks. However, this simplistic view of open marine populations has recently been challenged. Larval tagging studies have shown that the degree to which populations of reef fish are open varies greatly and depends on hydrographic conditions (Swearer et al. 1999, 2002). Molecular systematic studies have suggested that marine species can form without strong physical barriers to gene flow (Miya and Nishida 1997; Hellberg 1998). Population genetic studies have provided evidence of restricted gene flow among conspecific marine populations without any obvious barriers to dispersal (Karl and Avise 1992; Burton and Lee 1994; Pogson et al. 1995; Knutsen et al. 2003; Taylor and Hellberg 2003). These and other observations have led to a reevaluation of the proposition that planktonic dispersal implies open populations (Warner and Cowen 2002).

Genetic differentiation among populations reflects a dynamic balance between the forces of genetic drift, gene flow, selection, and mutation. Interpretation of the distributions of genetic markers often requires that some of these forces be assumed negligible. For example, it has often been assumed that selection and mutation can be ignored when F_{ST} is used to estimate gene flow (Slatkin and Barton 1989). Inferences about the strength of gene flow based on F_{ST} also require assumptions about the strength of genetic drift, because F_{ST} is used to estimate the magnitude of gene flow (m) relative to genetic drift ($1/N_e$). If the forces involved are relatively weak, equilibria will be approached very slowly and historical effects can be important as well (Crow and Aoki 1984; Neigel 1997). This confounding of gene flow and genetic drift is especially problematic for populations of marine species that conceivably could have either very large effective sizes because they have large numbers of individuals or very small effective sizes because reproductive success is limited to a small number of individuals (Hedgecock et al. 1992; Hedgecock 1994a; Neigel 1994, 2002).

Different types of genetic markers are expected to vary in how their distributions respond to forces other than gene flow, and in the statistical power they provide for the detection of genetic differentiation among populations (Neigel 1997). Allozymes have strongly influenced our views of marine larva dispersal (e.g., Burton 1983). If sufficient numbers of polymorphic allozyme loci are used, they can provide considerable statistical power for detection of genetic differentiation (Slatkin 1985; Slatkin and Barton 1989), although it is likely that some early claims of significant allozyme differentiation among marine populations can be attributed to biases in contemporaneous methods for estimation of F_{ST} (Cockerham and Weir 1993). However any interpretation of allozyme data should acknowledge the possibility that allozymes are at least occasionally subject to strong selection (e.g., Koehn et al. 1980; Hilbish 1985; Karl and Avise 1992).

© 2005 The Society for the Study of Evolution. All rights reserved.

Mitochondrial DNA (mtDNA) variation has been especially useful for revealing the phylogeographic structure of species (Avise 2000). Because we do not expect to see deep phylogeographic divisions among populations interconnected by gene flow their discovery in marine species has been interpreted as evidence for strong barriers to gene flow (Barber et al. 2000; Barber 2002; Taylor and Hellberg 2003). However such cases beg the question of whether we are seeing isolation that developed in situ under contemporary oceanographic conditions or secondary boundaries between cryptic species that had achieved reproductive isolation under different conditions. This distinction is more than semantic because very different types of mechanisms could be at work, such as dispersal-limiting versus postdispersal barriers to gene flow.

Microsatellite loci are often highly polymorphic because of high rates of length mutation (Hughes and Queller 1993). Remarkably precise estimates of migration rates and effective population sizes can be obtained with microsatellite data and maximum likelihood estimators based on coalescent models (Beerli and Felsenstein 1999, 2001; Knowles and Maddison 2002). Microsatellite loci are expected to be less useful for resolving phylogenetic relationships among isolated populations because high mutation rates can quickly saturate measures of population differentiation (Neigel 1997; Hedrick 1999) and only a weak correlation is expected between number of mutation steps and allelic differences in length (Slatkin 1995).

Estimates of gene flow from genetic data have traditionally relied on population genetic models that relate gene flow and other population genetic processes to observed distributions of genetic markers. Initially, these models were very simplistic, with all populations assumed to be identical in size and to have a uniform rate of gene flow among them (Neigel 1997). Recently, considerable progress has been made in developing more complex coalescent models that can estimate population genetic parameters by likelihood methods (Beerli and Felsenstein 1999, 2001; Knowles and Maddison 2002), although there are still practical computational limits to the use of these models. An alternative to such ''indirect'' estimates of gene flow is the use of genetic markers to first characterize distinct populations and then to identify migrants by matching their genotypes to probable source populations (Paetkau et al. 1995; Rannala and Mountain 1997; Waser and Strobeck 1998; Cornuet et al. 1999; Pritchard et al. 2000). These so-called direct methods do not assume explicit population genetic models, although they do make other assumptions, such as that all potential source populations have been sampled.

Here we analyze new data on mtDNA and microsatellite variation among populations of the thalassinidean crustacean, *Callichirus islagrande* and reexamine previous data on allozyme variation. This species is restricted to relatively coarse siliceous sediments characteristic of open coast and barrier island habitats of the northern and western Gulf of Mexico (GOM) (Felder 2001). Dispersal is via a planktonic larval phase that consists of five zoeal stages and can persist for up to two weeks under laboratory conditions. After metamorphosis, individuals settle and burrow into intertidal or shallow subtidal sediments where they develop into adults (Strasser and Felder 2000). Adult burrows can extend >2 m below the surface and typically occur at densities of 5–100 m^{-2}, which implies that continuous populations on single beaches can number in the millions or more (Felder and Griffis 1994; Felder 2001).

Allozyme variation across the range of *C. islagrande* was previously surveyed by Staton and Felder (1995). Four of six polymorphic loci exhibited large differences in allele frequencies between eastern and western populations (Fig. 1). The location of this genetic break corresponds to a gap in the distribution of *C. islagrande* across the Chenier Plain (CP) of Louisiana where predominantly muddy coastline sediments exclude establishment of dense or persistent populations of *C. islagrande* (Strasser and Felder 1998; Felder 2001). Three of the loci were nearly fixed for alternative alleles on either side of this break, which led Staton and Felder (1995) to suggest that the nominal species *C. islagrande* is actually a complex of at least two biological species. The type locality for *C. islagrande* is east of the break (Grand Isle, LA), and so we here refer to the populations east of the CP as *C. islagrande* sensu stricto (s.s.) and the entire complex as *C. islagrande* sensu lato (s.l.). Although the allozyme data provide strong evidence of a barrier to gene flow across the CP, it is more difficult to interpret differences in allele frequencies among the populations of *C. islagrande* s.s. In the four populations that were surveyed only three allozyme loci were polymorphic. At two of these loci (*ACP-1* and *GPI*), the same allele was fixed or nearly fixed in all populations, so these loci are minimally informative. At the third polymorphic locus (*LDH*), two common alleles varied considerably in frequency among populations (Fig. 1), which suggests strong barriers to gene flow. However because this pattern is shown by only one locus it is also consistent with the effects of differential selection.

We examined DNA sequence variation within a portion of the mitochondrial large subunit ribosomal RNA gene (16S) to confirm the genetic break in *C. islagrande*, gauge its phylogenetic depth and estimate the time of separation. We also conducted a survey of microsatellite variation within the range of *C. islagrande* s.s. to investigate gene flow among populations that do not appear to have had a long history of isolation. In combination with the previous allozyme data, this study provides two complementary views of genetic population structure: a phylogeographic view of an ancient division maintained by a contemporary barrier to gene flow and a population genetic view of the pattern of gene flow among benthic populations connected by planktonic dispersal of larvae.

Materials and Methods

Study Site and Collection

Adult specimens of *C. islagrande* were collected from intertidal burrows at 11 sites along the northeastern coast of the GOM between January 1998 and June 2000 (Table 1, Fig. 1). The sites provided sampling on two geographic scales, as most sites were >75 km apart, but seven were clustered along a 75-km stretch of barrier islands along the coast of Louisiana. Juveniles, defined as individuals up to 11 mm in carapace length (Felder and Griffis 1994), were col-

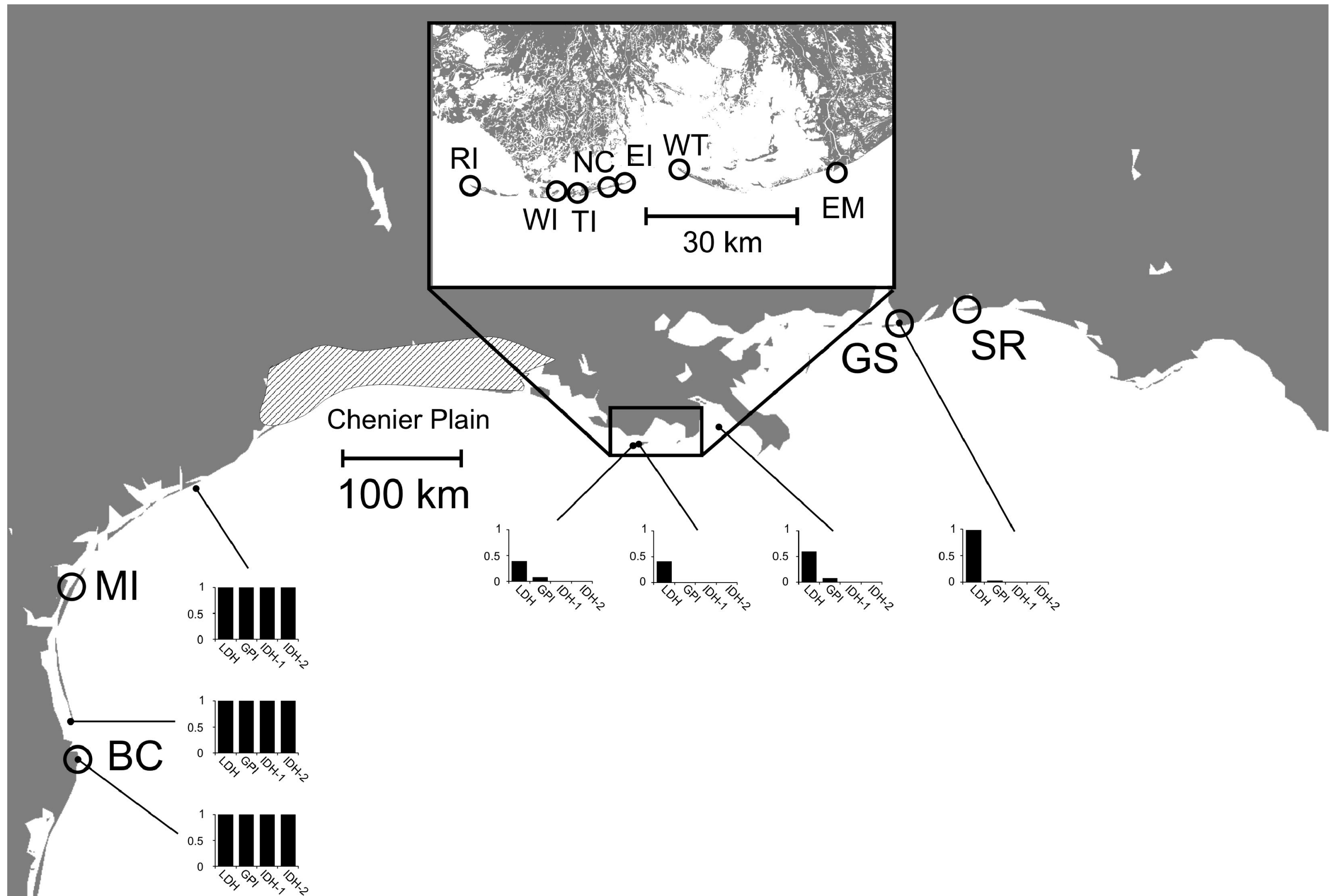


FIG. 1. Surveys of genetic variation in *Callichirus islagrande*, northwestern Gulf of Mexico. Open circles with adjacent two-letter abbreviations indicate collections used for the surveys reported here. Full place names and geographic coordinates are given in Table 1. The inset shows collections from Louisiana barrier islands at a finer scale. For the allozyme survey of Staton and Felder (1995), lines ending in solid circles indicate collection sites, and small histograms show the frequencies of the most common allele at each of four loci that exhibited a geographic break.

lected from two of these sites (EI and WT). Nine of the sites were within the range of *C. islagrande* s.s. Of these, four sites (WI, EM, GS, and SR) were either on beaches that faced the open GOM or on beaches near the GOM-facing ends of passes between islands. The other sites for *C. islagrande* s.s. (RI, TI, NC, EI, and WT) were either on beaches that faced estuaries or near estuary-facing ends of passes between islands. All animals were collected with a hand-operated suction corer known as a yabby pump (Manning 1975). Within hours of collection, the minor cheliped of each individual was removed, placed by itself in a sealed cryotube and frozen in liquid nitrogen. The remainder of each animal was then preserved as a voucher specimen in 95% ethanol.

Molecular Methods

Genomic DNA was extracted and purified following PureGene (Gentra Systems, Minneapolis, MN) and Prep-A-Gene (Bio-Rad, Hercules, CA) protocols for animal tissue. Extracts were prepared from fresh muscle tissue of the minor chela and quantified with a Hoefer (San Francisco, CA) TKO100 Fluorometer. Mitochondrial DNA sequences were determined for a total of 40 individuals sampled from all 11 locations. A 540-bp region of the mitochondrial large subunit ribosomal DNA gene (16S) was amplified by polymerase chain reaction (PCR) from samples of genomic DNA with the crustacean-specific primers (1471: CCTGTTTANCA AAAACAT; 1472: AGATAGAAACCAACCTGG) (Crandall and Fitzpatrick 1996). Amplification conditions were optimized with a Stratagene (La Jolla, CA) Robo-Cycler and used for all subsequent reactions. The amplification profile was: 10 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 48°C, and 1 min at 72°C with a final 10 min at 72°C. Each 25-μl reaction included 10 pmol of each primer; 31 μmol of each dNTP; 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin; PE Applied Biosystems, Foster City, CA); 1.25 U Taq polymerase (PE Applied Biosystems); and template DNA (10–25 ng). Amplification of single products was verified by electrophoresis through 2% agarose gels stained with ethidium bromide. Amplicons were sequenced with Big Dye (Applied Biosystems) terminator cycle sequencing, purified with Microcon 100 centrifugation columns (Millipore Corporation, Billerice, MA) and analyzed with an ABI 310 Genetic Analyzer with Sequencing Analysis software (PE Applied Biosystems).

TABLE 1. Location names and abbreviations, GIS coordinates, and the distribution of haplotype variation in sequences of the mitochondrial large subunit ribosomal RNA gene (16S).

Location	Coordinates	Haplotype									
		1	2	3	4	5	6	7	8	9	10
Boca Chica, TX (BC)	25°58.92′N 97°08.99′W	2	1	0	0	2	0	0	0	0	0
Mustang Island, TX (MI)	27°38.49′N 97°11.26′W	0	1	3	1	2	0	0	0	0	0
Raccoon Island, LA (RI)	29°3.50′N 90°57.60′W	0	0	0	0	0	0	0	0	1	2
Whiskey Island, LA (WI)	29°3.01′N 90°47.32′W	0	0	0	0	0	0	0	0	0	3
Trinity Island, LA (TI)	29°2.72′N 90°45.8′W	0	0	0	0	0	0	0	0	0	3
New Cut, LA (NC)	29°3.46′N 90°41.12′W	0	0	0	0	0	0	0	0	0	2
East Island, LA (EI)	29°3.81′N 90°39.51′W	0	0	0	0	0	0	0	0	0	3
West Timbalier, LA (WT)	29°5.42′N 90°32.31′W	0	0	0	0	0	0	0	0	0	3
Elmer's Island, LA (EM)	29°4.50′N 90°12.28′W	0	0	0	0	0	1	0	0	0	4
Gulf Shores, AL (GS)	30°17.04′N 87°48.06′W	0	0	0	0	0	0	0	1	0	2
Santa Rosa Island, FL (SR)	30°20.74′N 87°03.93′W	0	0	0	0	0	0	1	0	0	2

Microsatellite loci were isolated from a genomic library of *C. islagrande* s.s. The library was made in the plasmid vector pZERO-2.1 (Invitrogen, Carlsbad, CA). Miniprep DNA samples from 428 recombinant clones were UV-cross-linked to Magnagraph nylon membranes (Micron Separations, Westboro, MA) and sequentially probed with: $(GA)_n$, $(CA)_n$, and $(AAT)_n$) digoxigenin-labeled oligonucleotides (synthesized by Boehringer Mannheim, Indianapolis, IN). Positive clones were sequenced with Big Dye Terminator Sequencing chemistry on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems). PCR primers were designed for microsatellite sequences that had a minimum of 12 repeats and suitable flanking sequences. Amplification conditions were optimized with a Stratagene Robo-Cycler, and were used for all subsequent reactions. The amplification profile was: 10 min at 95°C, followed by 39 cycles of 1 min at 95°C, 1 min at the optimized annealing temperature and 1 min at 72°C (Table 2). Each 25-μl reaction included 10 pmol of each primer; 2 mM of each dNTP; 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, PE Applied Biosystems); 1.25 U Taq polymerase (PE Applied Biosystems); and template DNA (10–25 ng). The name, repeat unit, primer sequences, annealing temperatures, and number of alleles for each microsatellite locus are provided in Table 2. We attempted to determine microsatellite genotypes at all seven loci for 449 adult individuals sampled from nine collection sites and 107 juvenile individuals from two sites. However, not all amplifications produced scorable products. Among the 3143 combinations of individual × locus for adults, 483 were unsuccessful (15.4%), so missing data for those combinations of locus and individual decreased sample sizes. The percentages of unsuccessful amplifications varied by locus from 4.7% to 33% (Table 2).

Data Analysis

We aligned 16S sequences with LaserGene Seqman II (DNAStar, Madison, WI). Our sequence comparisons were based on 469 bp of the 540-bp amplicon. We used the Perl script MrAIC 1.3.pl (Nylander 2004) to run PHYML (Guindon and Gascuel 2003) for selection of the most appropriate sequence evolution model for phylogenetic analysis by the Akaike information criterion (AIC; Akaike 1974). MEGA version 2.1 (Kumar et al. 1993) was used to calculate measures of divergence between individual haplotypes and average divergence between groups of haplotypes as well as to construct neighbor-joining (NJ) and maximum parsimony (MP) trees. PHYML was used to construct maximum likelihood trees. Our estimate of sequence divergence accumulated after the separation of *C. islagrande* s.s. employed the correction of Nei and Li (1979) for ancestral divergence. This

TABLE 2. Name, repeat unit, primer sequences, annealing temperature, number of alleles, and percentage of unsuccessful amplifications for seven microsatellite loci of *Callichirus islagrande*.

Locus	Repeat unit	Primer	Ta	A	% unsuccessful
1–3	CR	For: TGAAGACAACCAGAAGTGAAGA Rev: CGACGACAATACACATACCTCG	51	51	4.7
1–10	GA	For: ATGATAAAAAGGAAAGATGACA Rev: GTAAGACTAACGACGCCGAAC	59	48	9.6
1–46	AAT	For: TAGGGGAAACTGGTCGCATACT Rev: CAGCCTTAGTTATGGTGTCTTG	56	32	6.7
2–83	CT	For: TCGCAACACAGTCAGCCTCATC Rev: GCCTTCCTCCCAACCTCCCGGA	60	14	23.6
2–90	CT	For: TATGCCCTACAAGAGAACTAAA Rev: TGAGGATGGCAGCGAGGAAT	54	54	7.8
3–13	AAT	For: TACCAGTTGTGTCGGATAAT Rev: CATTACAGCCAAACAGGTCG	51	30	33.0
4–55	AAT	For: CTTCTCTAGGCTGAAACTGAGG Rev: TCAGACTCAGCGTTTTCACT	51	29	22.3

estimate was then converted to the approximate time of separation with molecular clock calibrations for 16S sequences from other decapod crustaceans. Three published calibrations were considered: 0.9% per million years (my) for ocypodid crabs (Strumbauer et al. 1996), 0.65–0.88% per my for grapsid crabs (Schubart et al. 1998), and 0.38% per my for paguroid crabs (Cunningham et al. 1992). We tested for global molecular clocks for each set of sequences used for these calibrations in combination with the 16S haplotype 1 sequence of *C. islagrande*. A partial 16S sequence from *Fenneropenaeus chinensis* (GenBank accession AF245113) was selected as an outgroup because it aligned over a greater span of the haplotype 1 sequence than any other sequence in GenBank from the suborder Dendrobranchiata. We aligned each set of sequences with ClustalW 1.83 (Thompson et al. 1994) using a gap opening penalty of 10, a gap extension penalty of 0.20, divergent sequences delayed 30%, a transition weight of 0.5, and the International Union of Biochemistry DNA weight matrix. These alignments were inspected by eye and appeared reasonable. We used MrAIC 1.3 pl and PHYML to select a model of sequence evolution by the AICc criterion (AIC corrected for small sample size) and to infer a phylogenetic tree. The trees were rooted with the sequence of *F. chinensis* and used with the aligned sequences to test for global molecular clocks with the molecular clock script in the HyPhy Package (Pond et al. 2005), the model of evolution selected by the AICc criterion, global parameters and lengths estimated independently for each branch.

For analysis of microsatellite data, we used GENEPOP (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium and to calculate pairwise values of F_{ST} (following Weir and Cockerham 1984). Likelihood ratio tests for differences in allele frequencies were conducted with a program written by J. E. Neigel. Following Hernandez and Weir (1989), the probability of each observed log-likelihood ratio under the null hypothesis was evaluated by comparison with the distribution generated from 10,000 randomizations of the data. Levels of significance for all pairwise tests were adjusted with the sequential Bonferroni method (Rice 1989). We used the Mantel test (Mantel 1967) to determine whether matrices of F_{ST} and geographic distances between local populations were correlated, as implemented by the ISOLDE program of GENEPOP (Raymond and Rousset 1995) with 10,000 bootstraps. Chord distances (Cavalli-Sforza and Edwards 1967) were calculated with the program GENETIX (Belkhir et al. 2002). A NJ phenogram based on the chord distance was generated by MEGA version 2.1 (Kumar et al. 1993). Sample gene diversity for each microsatellite locus was calculated as shown by Nei (1987). Tests for differences in average gene diversity between samples were based on the method for closely related populations (Nei 1987).

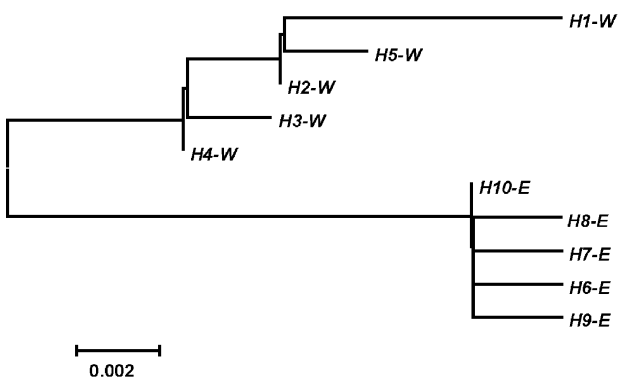
Evolutionary or historical effective population size, a long-term measure of the net effects of genetic drift on the accumulation of genetic variation within a species (Avise et al. 1988), was estimated from our 16S sequence data with the program FLUCTUATE (Kuhner et al. 1998) and a mutation rate of 10^{-8}, which is an order-of-magnitude estimate based on the crustacean molecular clock calibrations cited above. Maximum likelihood estimates of the product of effective population size and migration rate ($N_e m$) and the parameter θ, which is four times the product of effective population and mutation rate ($4N_\mu$), were obtained by analyzing microsatellite data with the program MIGRATE (Beerli 2002). The MIGRATE program also provides 95% confidence limits (CL) for these parameters by comparison of profile likelihood ratios of each parameter with the quantiles of a χ^2 distribution with one degree of freedom (Beerli and Felsenstein 2001). For all runs of MIGRATE, we used the stepwise mutation model, and unless otherwise specified, 10 short chains with 500 genealogies sampled at increments of 20, three long chains with 5000 genealogies sampled at increments of 20, and the FST option for initializing the migration matrix.

Population structure and admixture analysis was conducted with the program STRUCTURE 2.0 (Pritchard et al. 2000; Falush et al. 2003). The following options were used for each run: 10^6 replicates after a burn-in of 10^5; admixture model; α inferred with an initial value of 1, a maximum value of 10, a uniform prior, and the same value for all populations; different values of F_{ST} for different subpopulations; prior mean F_{ST} of 0.01; a prior SD of 0.0; and constant λ with a value of 1.

Results

Ten haplotypes of the 16S mtDNA sequence were represented among 40 individuals, with 17 variable positions, a 2 bp indel, and a 1 bp indel. These haplotypes, numbered 1 through 10, have been deposited in GenBank with accession numbers DQ069703 through DQ069712. Five (half) of the haplotypes were found only at locations east of the Chenier Plain (CP), within the range of *C. islagrande* s.s., whereas the other five were found only at locations west of the CP in the broader range of *C. islagrande* s.l. (Table 1). The Tamura-Nei substitution model (Tamura and Nei 1993) without a gamma correction or invariant sites was selected by the AIC criterion. The NJ and maximum likelihood trees based on this model and the MP tree all separated the haplotypes into two groups that correspond to the geographic division in the distribution of haplotypes (Fig. 2). For all three methods, bootstrap support for this partition was high (95–100%). The two groups were also distinguished by both indels: all western haplotypes had a 2-bp deletion and a 1-bp insertion relative to eastern haplotypes.

Likelihood ratio tests of a global molecular clock were conducted to determine which, if any, published clock calibrations for other decapod groups could be applied to 16S sequences from *C. islagrande*. Three groups of sequences were tested (see Materials and Methods); each consisted of sequences that had been previously used for clock calibrations of decapods within the suborder Pleocyemata, haplotype 1 of *C. islagrande* (also in the Pleocyemata) and, for an outgroup, a sequence from *Fenneropenaeus chinensis* (within the sister suborder Dendrobranchiata). A global molecular clock was rejected at the $\alpha = 0.05$ level when the haplotype 1 sequence was included with paguroid sequences ($P = 0.014$), but not with either grapsid ($P = 0.095$) or ocypodid sequences ($P = 0.288$). We therefore considered our clock calibration for 16S sequences from *C. islagrande* as the range that encompasses both the grapsid and ocypodid calibrations: 0.65–



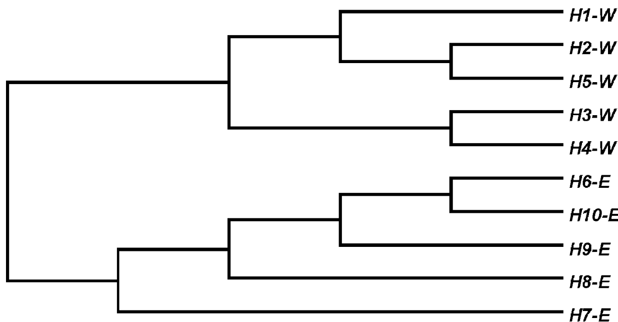


FIG. 2. Neighbor-joining and maximum parsimony trees of mitochondrial large subunit ribosomal RNA gene (16S) haplotypes from *Callichirus islagrande*. The maximum likelihood tree (not shown) was topologically identical to the neighbor-joining tree and had similar branch lengths. An ''E'' is appended to the names of haplotypes found east of the Chenier Plain, a ''W'' for those west of the Chenier Plain.

0.9% per my per lineage. Average estimated sequence divergence between the western and eastern groups of haplotypes of *C. islagrande* was 0.016 after correction for within-species polymorphism. Because this estimate is for divergence between two lineages, it corresponds to an estimated divergence time of between 0.89 and 1.2 million years ago.

All seven microsatellite loci were highly polymorphic. The number of alleles at each locus ranged from 30 to 54; expected heterozygosity ranged from 0.71 to 0.97. Of the 63 combinations of locus and population, genotype proportions for 21 were significantly different ($\alpha = 0.05$) from Hardy-Weinberg (HW) expectations after sequential Bonferroni correction (Rice 1989). All of the deviations were heterozygote deficiencies, which could have been caused by either null alleles or the Wahlund effect. Although null alleles are common at microsatellite loci, the distribution of heterozygote deficiencies was more variable than might be expected: there were no loci with significant HW deviations in all samples (collection sites), nor were there any samples with significant deviations at all loci.

Histograms of microsatellite allele frequency distributions for all combinations of locus and collection site are shown in Figure 3. The most pronounced difference in allele frequency distributions is at locus 4–55 between the BC sample (west of the CP) and the others (east of the CP); the distributions differ to the extent that the BC sample has few alleles in common with the others. Pronounced differences at other loci were in the shapes of the distribution rather than allelic composition; for example, at locus 2–83 the allele frequency distribution for the BC sample shares one peak but not a second with the distributions from the other samples.

There were no pronounced differences in microsatellite allele frequency distributions among samples collected from within the range of *C. islagrande* s.s. (Fig. 3), although for all loci combined the differences are statistically significant. A null hypothesis of homogeneity of allele frequencies across all loci and collection sites was strongly rejected by a likelihood ratio test ($P < 0.0001$). Microsatellite allele frequencies were also significantly different in all pairwise comparisons between collection sites ($\alpha = 0.05$ with sequential Bonferroni correction) when data from all loci were combined in likelihood ratio tests.

F_{ST} estimates were low, as expected for highly polymorphic microsatellites. The estimate of F_{ST} across all collection sites and loci was 0.015. Estimates of F_{ST} between pairs of collection sites ranged from 0.0003 to 0.043 (Table 3). Estimates of pairwise F_{ST} for comparisons between the sample from BC and those from other sites (mean 0.038) were substantially higher than for comparisons among sites excluding BC (0.0054).

Although microsatellite allele frequencies were significantly different among locations, there was little evidence of hierarchical geographic structuring within the range of *C. islagrande* s.s. A Mantel test of isolation by distance (sensu Slatkin 1993) based on the expected correlation between geographic distance and F_{ST} was not significant for the full set of locations (including the BC sample). There was a weakly significant correlation ($P = 0.04$) for the Isles Dernieres group of islands (RI, WI, TI, NC, and EI), but this would not be significant if included with any additional comparisons and subjected to a Bonferroni correction. There was only a slight tendency for samples to be grouped by geographic region in a neighbor-joining phenogram of chord distances (Cavalli-Sforza and Edwards 1967) calculated from microsatellite allele frequencies (Fig. 4). For example, samples from the three most closely spaced locations (WI, NC, and EI) were grouped together, but not with a sample from a nearby location (WT).

Because genetic divergence between populations is the product of both restricted gene flow and genetic drift, we examined the possibility that genetic drift was an extremely strong force in populations of *C. islagrande*. For *C. islagrande* s.s., long-term evolutionary effective population size was estimated by FLUCTUATE to be 699,000. Although this number must be many orders of magnitude smaller than the census size of the species, it is not small enough to indicate that genetic drift has been as strong in *C. islagrande* as has been suggested for species with ''sweepstakes reproduction''

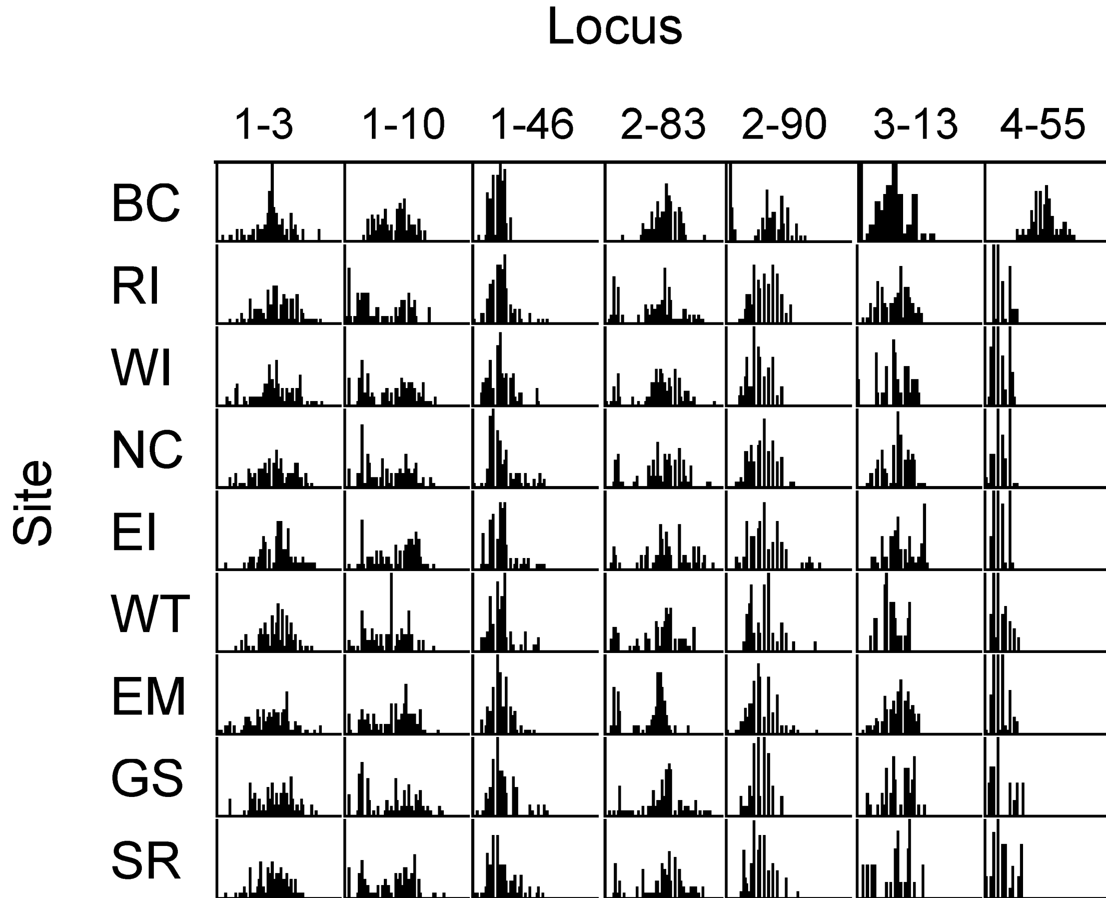


FIG. 3. Allele frequencies at seven microsatellite loci for samples of *Callichirus islagrande* from nine locations. Site abbreviations are in Table 1. The scale of the vertical axis (allele frequency) is 0.16, and the horizontal axis (allele) spans 60 allele size classes.

(Hedgecock et al. 1992; Hedgecock 1994b). Another potential indicator of strong genetic drift is reduced gene diversity in recruits relative to adults (Li and Hedgecock 1998; Flowers et al. 2002). For two sites, it was possible to collect numbers of juveniles sufficient for statistically meaningful comparisons of microsatellite gene diversity with adults from the same sites. At both sites, average gene diversity of microsatellite loci was actually slighter higher for juveniles, although these differences were not significant (Table 4). We also included these samples of juveniles in the neighbor-joining phenogram shown in Figure 4. The sample of juveniles from EM clustered with adults from EM, whereas the sample from WT did not cluster with WT adults.

Initial parameter values used by MIGRATE were based on F_{ST} for the first run and on the estimates obtained from the first run for the second run. These two runs produced maximum likelihood estimates (MLEs) of $\theta(4N_e\mu)$ and migration rates (N_em) with similar mean values, but only moderate consistency for individual parameter estimates as judged by either the correlation coefficient ($r^2 = 0.54$) or the proportion of parameter estimates with overlapping 95% confidence limits (0.28). Because this lack of consistency could indicate

TABLE 3. Measures of genetic differentiation of adult populations of *Callichirus islagrande*, based on data generated from seven microsatellite loci. *P*-values for log-likelihood tests are given above the diagonal. All were significant at the 0.05 level after Bonferroni correction; an asterisk represents significance at the $\alpha = 0.01$ level. F_{ST} estimates are given below the diagonal. Place names and geographic coordinates for site abbreviations are in Table 1; locations are mapped in Figure 1.

	BC	RI	WI	NC	EI	WT	EM	GS	SR
BC	—	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
RI	0.0433	—	0.0101	<0.0001*	<0.0001*	0.0005*	0.0036	0.0021	0.0014
WI	0.0345	0.0053	—	0.0058	<0.0001*	<0.0001*	0.0238	0.0086	0.0160
NC	0.0419	0.0091	0.0021	—	0.0019	<0.0001*	<0.0001*	0.0001*	0.0001*
EI	0.0395	0.0060	0.0024	0.0016	—	0.0011	<0.0001*	<0.0001*	0.0031
WT	0.0336	0.0059	0.0036	0.0129	0.0064	—	0.0002*	<0.0001*	0.0002*
EM	0.0395	0.0040	0.0003	0.0109	0.0078	0.0048	—	<0.0001*	0.0002*
GS	0.0389	0.0050	0.0019	0.0106	0.0088	0.0028	0.0080	—	0.0067
SR	0.0320	0.0040	0.0030	0.0097	0.0055	0.0033	0.0047	0.0017	—

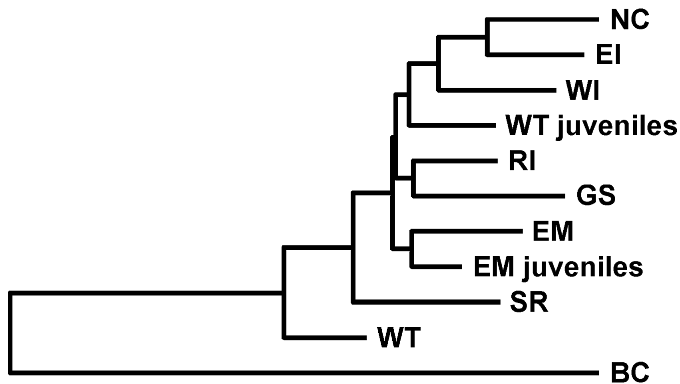


FIG. 4. Neighbor-joining tree of chord distances between samples of *Callichirus islagrande* from nine locations (abbreviations are in Table 1) based on allele frequencies at seven microsatellite loci.

TABLE 4. Sample sizes (number of individuals, n) and estimates of gene diversity (H) for microsatellite markers in *Callichirus islagrande*. Place names and geographic coordinates for site abbreviations are in Table 1; locations are mapped in Figure 1.

Site	n	H
BC	48	0.912
RI	54	0.903
WI	56	0.921
NC	53	0.911
EI	41	0.913
WT	43	0.904
WT juveniles	45	0.915
EM	61	0.916
EM juveniles	59	0.921
GS	49	0.901
SR	44	0.919

insufficient sampling of genealogies (Beerli and Felsenstein 2001) we increased the sampling increments for both short and long chains by a factor of 10, ran three full replicates, and combined data from the last chains of each replicate for the calculation of MLEs. This ''long run'' required three weeks on a 2.8 GHz Pentium 4 PC. The mean values of the MLEs from the long run were similar in magnitude to those from the previous runs. The correlation between the first run (initial values based on F_{ST}) and the long run was $r^2 = 0.7$, whereas the correlation between the second run (initial values based on parameter estimates from the first run) and the long run was $r^2 = 0.47$. The results from all three runs were thus similar overall but differed in the precise values of individual parameter estimates. The results shown in Tables 5, 6, and 7 are from the long run, which is expected to be the most accurate.

MLEs of θ and migration rates indicated that *C. islagrande* populations are effectively large and exchange substantial numbers of migrants, but are not open populations as this term is generally applied (Caley et al. 1996). Maximum likelihood estimates of the parameter θ for each population ranged from 0.54 to 1.0 (Table 5). For these values to be interpreted as estimates of N_e they must be scaled to mutation rates, which have not been measured for microsatellite loci in *C. islagrande*. Microsatellite mutation rates in mammals typically range between 10^{-3} and 10^{-5} (Dallas 1992; Weber and Wong 1993; Ellegren 1995), whereas in *Drosophila melanogaster* they are lower, averaging 5×10^{-6} (Schug et al. 1997, 1998; Schlotterer et al. 1998; Vazquez et al. 2000). If the mutation rates of the microsatellite loci we surveyed are near the middle of this range (i.e., 10^{-5} to 10^{-4}) the values of θ we estimated would correspond to N_e values between 1350 and 13,500.

MLEs for migration rates (N_em) ranged from 1.7 to 11.4 among the nine sites for which microsatellite data were available. If effective population sizes are on the order of 10^4 to 10^5, this implies migration rates are on the order of 0.01 or lower. Among barrier islands along the coast of Louisiana (Table 6) estimates for eastward migration rates (mean 5.8) were not substantially different from westward migration rates (mean 6.2), despite the predominantly westward flow of surface currents over coastal shelf during nonsummer months (Ohlmann and Niiler 2005). More surprisingly, MLEs of migration rates from the BC site to other sites (mean 4.7) and from those to the BC site (mean 5.5) were not substantially lower than the estimates for migration rates between closely spaced barrier islands (Tables 6 and 7), despite the pronounced phylogeographic division that separates the BC site from the others. Because this last result appeared questionable, we ran a second analysis using only those microsatellite loci that were in Hardy-Weinberg proportions in most samples and thus possibly more reliable (loci 1–3, 2–90, 1–46, and 4–55). Parameter estimates based on this reduced dataset were similar in magnitude to estimates based on the full dataset and also failed to show any reduction in migration between the BC site and others (results not shown).

STRUCTURE was used to calculate a posteriori probabilities for the number of populations represented in our microsatellite data. Among replicate runs, the highest probabilities were for four or five populations, and probabilities were very low for all other numbers (Table 8). The documentation for STRUCTURE suggests that the smallest number of populations with a significant probability (in this case four populations) be considered the preferred estimate. For runs that assumed four populations, one of the inferred populations corresponded closely to the BC sample of individuals; 90% of the BC sample was assigned to this ''BC population'' versus a range from 5.4% to 16.2% for the samples that represented *C. islagrande* s.s. Furthermore, the inferred

TABLE 5. Maximum likelihood estimates and approximate 95% confidence intervals (CI) for θ ($4N_e\mu$) computed with MIGRATE from microsatellite data for samples from nine local populations of *Callichirus islagrande*. Place names and geographic coordinates for site abbreviations are in Table 1; locations are mapped in Figure 1.

Site	θ	CI
BC	1.00	0.93–1.08
RI	0.62	0.58–0.67
WI	0.84	0.78–0.92
NC	0.82	0.76–0.88
EI	0.55	0.50–0.60
WT	0.57	0.52–0.62
EM	0.79	0.74–0.84
GS	0.61	0.56–0.67
SR	0.54	0.50–0.60

TABLE 6. Maximum likelihood estimates and approximate 95% confidence intervals (CI) for directional migration rates (N_em) computed with MIGRATE from microsatellite data for five barrier island samples of *Callichirus islagrande*. On the left are migration rates in a west to east direction; on the right, east to west. Place names and geographic coordinates for site abbreviations are in Table 1; locations are mapped in Figure 1.

Locations	N_em	CI	Locations	N_em	CI
RI to WI	11.42	9.83–13.18	WI to RI	4.14	3.40–4.98
RI to NC	7.92	6.81–9.15	NC to RI	4.8	4.00–5.69
RI to EI	5.66	4.70–6.75	EI to RI	3.37	2.71–4.12
RI to WT	3.74	2.95–4.65	WT to RI	6.04	5.14–7.03
WI to NC	7.11	6.06–8.28	NC to WI	7.35	6.09–8.77
WI to EI	3.63	2.87–4.52	EI to WI	8.45	7.09–9.98
WI to WT	3.93	3.13–4.87	WT to WI	10.68	9.14–12.39
NC to EI	6.92	5.85–8.12	EI to NC	1.71	1.22–2.31
NC to WT	4.46	3.60–5.45	WT to NC	8.5	7.35–9.77
EI to WT	3.34	2.60–4.20	WT to EI	6.73	5.68–7.92
Mean	5.8			6.2	

ancestry of every individual in the BC sample was always highest for the BC population. For 10.2% of the individuals from the other samples, inferred ancestry was highest for BC population, although only one non-BC individual had an ancestry of over 90% for the BC population.

DISCUSSION

A major genetic break divides the nominal species *C. islagrande* across the Louisiana CP. This break was first reported as fixed or nearly fixed differences for several allozyme loci (Staton and Felder 1995). Here we have shown that this break also corresponds to a deep phylogeographic division in mtDNA haplotypes and pronounced differences in microsatellite allele frequencies. The consistency of these genetic differences supports the previous suggestion (Staton and Felder 1995) that the nominal species *C. islagrande* probably represents two or more phylogenetic species. The divergence in 16S sequences across this division is within the range reported for congeneric decapod species. For example, estimates of 16S divergence ranged from 0.007 to 0.139 among four species in the anomuran genus *Pagurus* (Cunningham et al. 1992) and from 0.0 to 0.094 among 36 pairs of congeners from two genera in the brachyuran family Panopeidae (Schubart et al. 2000). These estimates were corrected for multiple hits but not for divergence prior to speciation, and so are directly comparable to the Tamura-Nei distance of 0.021 for the division in *C. islagrande*. Although this value appears at the low end of the ranges cited above for congeneric decapod species, it should be noted that the upper ends of these ranges include comparisons between species that were likely assigned to the same genera incorrectly (Cunningham et al. 1992; Schubart et al. 2000).

Our estimate for the time at which the division in *C. islagrande* occurred is on the order of one million years ago, when oceanographic conditions and the distribution of suitable habitats were likely to have been profoundly different than they are today. The muddy shoreline habitat of the CP could be responsible for the current position or maintenance of the genetic break, but is not likely to have been the cause. The formation of the CP began when sediments were deposited by coastal streams during the last glacial melt (Saucier 1974). The mudflats were formed later by the deposition of sediments from the Mississippi River as it ran to the western edge of its deltaic plain (Kemp 1986). The CP was thus formed within the last 10,000 years, or two orders of magnitude more recently than our estimate for the time at which the phylogeographic break in *C. islagrande* occurred. Of course it is possible that the division of *C. islagrande* occurred near its present location during an earlier Pleistocene glacial melt. Alternatively, these lineages could have separated elsewhere with subsequent range extensions to their present locations. For example, cooling during the Pleistocene could have eliminated *C. islagrande* from the northern GOM and led to the formation of allopatric populations in

TABLE 7. Maximum likelihood estimates with approximate 95% confidence intervals (CI) of directional migration rates (N_em) between the BC sample of *Callichirus islagrande* from west of the Chenier Plain and eight samples from east of the Chenier Plain computed with MIGRATE from microsatellite data. Place names and geographic coordinates for site abbreviations are in Table 1; locations are mapped in Figure 1.

Locations	N_em	CI	Locations	N_em	CI
BC to RI	4.03	3.24–4.94	RI to BC	4.64	3.86–5.52
BC to WI	4.48	3.64–5.43	WI to BC	7.85	6.54–9.32
BC to NC	4.08	3.29–4.99	NC to BC	5.53	4.61–6.57
BC to EI	4.36	3.53–5.30	EI to BC	6.97	5.89–8.17
BC to WT	4.73	3.87–5.70	WT to BC	5.86	4.86–6.98
BC to EM	6.86	5.81–8.02	EM to BC	4.09	3.36–4.93
BC to GS	4.72	3.86–5.70	GS to BC	3.44	2.68–4.33
BC to SR	4.64	3.86–5.52	SR to BC	5.69	4.61–6.92
Means	4.7			5.5	

TABLE 8. Probabilities of the data for different number of populations, *K*, of *Callichirus islagrande* estimated by STRUCTURE from microsatellite data.

K	Probability
1	7.0×10^{-137}
2	1.2×10^{-77}
3	6.6×10^{-39}
4	0.99
5	1.0×10^{-2}
6	3.2×10^{-24}
7	1.5×10^{-72}
8	4.6×10^{-91}
9	6.2×10^{-129}

Mexico and Florida. These and similar scenarios have been proposed to explain why there appears to be a vicariant zone in the northern GOM for many fish and invertebrate taxa (Briggs 1974; Wiley and Mayden 1985). However, none of these scenarios explains why the division is at its present location at the CP, considerably to the west of other species boundaries and hybrid zones in the northern GOM (e.g., Bert 1986; McClure and McEachran 1992; Harrison 2004).

We consider two plausible explanations for how the phylogeographic division in *C. islagrande* persists: larvae do not cross the CP, or larvae that cross the CP have reduced fitness and fail to effect gene flow. The muddy sediments of the CP are a poor environment for the benthic life stage of *C. islagrande* (Strasser and Felder 1998; Felder 2001) and represent a large distance for larvae to traverse in the course of a two-week planktonic phase. Surface currents over the Louisiana shelf are wind driven and follow an annual cycle. From September to May they flow westward, whereas from June to August they flow eastward. Velocities are typically about 0.2 m/sec (Ohlmann and Niiler 2005), which would advect passive larvae across the CP (about 200 km) in just under two weeks (12 days). This estimate is consistent with data from other marine species on the relationship between larval period and dispersal distance (Shanks and Grantham 2001). However, there are also many examples of decapod larvae that are retained within embayments or local oceanographic features and that settle near where they were released (McConaugha 1992). The shallow water habitat of *C. islagrande* could facilitate retention and delay or reduce entry of larvae into the coastal current. Nevertheless, population genetics theory predicts that even a trickle of gene flow, on the order of a few individuals per generation, or occasional episodes of higher gene flow should prevent the fixation of different neutral alleles in separate populations (Wright 1951). It would seem likely that even if most larvae of *C. islagrande* are locally retained, some would at least occasionally cross the CP.

A reduction in fitness for larvae that cross the CP could arise in several ways. The trip itself could be harmful, subjecting larvae to unfavorable conditions or detrimental prolongation of larval life. There could be local adaptation to differences in habitat that influence settlement, survival, or reproductive successes. Even without actual differences in habitat, local populations could differ in mate preferences so that immigrants suffer a reduction in mating success. If there are differences in habitat, they are likely to be subtle. There are no obvious differences in the physical environments on either side of the CP and to our knowledge no biogeographic breaks like those that have been associated with some phylogeographic divisions in other marine species (Avise 1994; Burton 1998).

The use of genetic markers for indirect estimates of gene flow in marine species with planktonic dispersal has been problematic. Estimates of F_{ST} for marine populations are often too low to be used as the basis for parameter estimation (Hedgecock 1994b; Waples 1998; Hellberg et al. 2002). In this study, we explored several recent developments that could extend our ability to estimate gene flow in marine populations. Our results are encouraging, but they also indicate several important caveats. The first is that our microsatellite data could contain artifacts. We detected heterozygote deficiencies for 21 of 63 combinations of locus and sample, which could indicate that some alleles failed to amplify and were thus incorrectly scored. Other caveats concern the fit of the coalescent model implemented in MIGRATE to our data. Although this model is much more realistic than the models used to estimate gene flow from F_{ST}, it is still necessarily a simplified representation of a more complex reality. The population model used in MIGRATE assumes populations are discrete (rather than continuous with isolation by distance), generations are nonoverlapping, every source of gene flow is represented in the data, and parameters (N_e, m, and μ) are constant over time (Beerli and Felsenstein 2001; Beerli 2002). It is realistic to assume that each of these assumptions has been violated. Furthermore, microsatellite mutation processes are known to be more complex than the model used in MIGRATE (Amos and Rubinsztein 1996; Primmer et al. 1996; Di Rienzo et al. 1998). Because of these caveats, we do not suggest that the parameter estimates we have obtained from MIGRATE should be interpreted literally.

Maximum likelihood estimates of migration rate ($N_e m$) computed by MIGRATE from microsatellite data suggest that there is no restriction of gene flow across the CP, although fixed differences in both mtDNA and allozyme markers and relatively large F_{ST} estimates for microsatellite loci constitute strong evidence to the contrary. Analysis of the same microsatellite data with STRUCTURE also indicated that gene flow across the CP is very restricted. A plausible explanation for this discrepancy is that the microsatellite mutation model in MIGRATE does not accurately represent the accumulated effects of a large number of mutations, such as would occur between species. For example, there could be constraints on the number of repeats at a microsatellite locus that canalize allele lengths and result in less divergence than would be expected from a simple model of random length changes. By this reasoning, we would expect microsatellite markers to be best suited for the analysis of population structure whereas markers such as mtDNA sequences that progressively accumulate point mutations would be better for the detection of deep phylogenetic divisions.

We found significant differences in microsatellite allele frequencies among local populations of *C. islagrande*, even between populations that were separated by as little as 10 km (Fig. 1 and Table 3). This result by itself indicates that these populations are not panmictic; even at this fine geographic scale gene flow has not eliminated the heterogeneity generated by genetic drift (or possibly selection). One possibility is that gene flow is not weak in absolute terms, but only in relation to genetic drift that is exceptionally strong because all recruitment is derived from a very small number of individuals. This model of sweepstakes reproduction has received some empirical support from observations of temporal variance in allele frequencies in bivalve mollusks (Hedgecock et al. 1992; Hedgecock 1994a,b; Li and Hedgecock 1998). However, this scenario requires that some individuals have extremely high fecundity (e.g., 10^6 or higher), whereas fecundity in *C. islagrande* in our observations is typically on the order of 10^3 eggs per clutch. There is also no evidence for either sweepstakes reproduction or small effective pop-

ulation sizes in our microsatellite data. In our limited sampling of juveniles, gene diversity was as at least as high as in adults, and all microsatellite loci were highly polymorphic.

Although microsatellite allele frequencies were significantly heterogeneous among populations, the magnitude of divergence, as measured by F_{ST}, appears to be extremely low. Estimates of F_{ST} below 0.01, which indicate that only a small fraction of the detectable variation is partitioned among populations, are sometimes interpreted as evidence of panmixia. However, very low values of F_{ST} will be found for highly polymorphic markers regardless of population structure (Slatkin 1995). The high mutation rate of microsatellite loci prevents individual alleles from approaching fixation and places a numerical upper limit on the value of F_{ST} when it is calculated as a statistic rather than estimated as a demographic parameter (Hedrick 1999; Neigel 2002).

Analyses of microsatellite data for *C. islagrande* by F_{ST} and MIGRATE indicated that dispersal is limited, even among neighboring sites. These populations would be considered demographically closed if, as indicated by MIGRATE, migration rates between neighboring populations were generally below 0.01. These analyses indicate that lack of pronounced genetic differentiation among population is due to large effective population sizes (reduced genetic drift) rather than high rates of gene flow. This example demonstrates that low values of F_{ST} cannot be simply equated with high rates of gene flow. Such limited dispersal is compatible with contemporary ideas about larval transport mechanisms (e.g., Largier 2003) if most of the larvae of *C. islagrande* remain in the coastal boundary layer. These larvae would remain in the immediate vicinity of their source populations, while a smaller proportion would enter surrounding waters, mix with larvae from other sources and be transported by diffusive processes to nearby populations. Strong directional transport, such as advection in coastal currents, would seldom occur. The CP would act as a barrier to gene flow for larvae that seldom enter offshore waters where advection would be strong enough to carry them across the CP. Populations could be adapted to local conditions, so that larvae that do occasionally cross the CP could suffer a selective disadvantage that further reduces gene flow.

The analysis of the microsatellite data for *C. islagrande* by STRUCTURE also indicated significant population structure, but suggested a more complex scenario. When samples were partitioned into populations without regard to their locations of origin, the samples of *C. islagrande* s.s. represented a mixture of three or four distinct populations. This could be an artifactual result caused by errors in the data (such as null alleles) or by departures from the underlying model used by STRUCTURE. However, it is also compatible with a scenario of source and sink populations. If each sink population receives a distinct mixture of larvae from genetically differentiated source populations, the sink populations would themselves become genetically differentiated. This is an important possibility to consider, because it has been argued that any degree of genetic differentiation between marine populations can be interpreted as evidence for restricted gene flow (Palumbi 2003), an interpretation that could suggest the erroneous conclusion that sink populations are nearly closed or ''self-seeding.''

Selection has also been proposed as a cause of heterogeneity in allozyme allele frequencies over small spatial and temporal scales (Tracey et al. 1975; Johnson and Black 1984). For the case of the intertidal limpet *Siphonaria jeanae*, recruitment from different populations could not explain the observed temporal fluctuations because these fluctuations exceeded differences among potential source populations (Johnson and Black 1984). However, there was no evidence of large temporal changes in microsatellite allele frequencies for our comparisons between juveniles and adults from the same populations of *C. islagrande*, so we do not invoke selection to explain the patterns that we have observed.

The interpretation of fine-scale genetic population structure in *C. islagrande* depends on the underlying model used to analyze the data. In the framework of a simple island model of population structure with bidirectional migration, the effective rate of migration among populations appears to be so low that populations can be considered essentially closed. An alternative interpretation is that most populations are open but are genetically differentiated because they receive migrants from different source populations. Analysis of microsatellite data with MIGRATE suggests the first interpretation, although simulation studies have shown that MIGRATE is not always effective at distinguishing unidirectional migration (i.e., source-sink relationships) from bidirectional migration (Beerli and Felsenstein 2001). Analysis with STRUCTURE suggests the second interpretation; every sample of *C. islagrande* s.s. that we analyzed appeared to be a mixture of individuals from several genetically differentiated populations. However, we have not identified any of these hypothetical source populations, and there is also the possibility that our analyses were affected by artifacts such as null alleles. Both MIGRATE and STRUCTURE are expected to be sensitive to artifacts or errors in the data.

New methods for the analysis of genetic marker data such as those implemented in MIGRATE and STRUCTURE offer fresh opportunities to revisit old questions about gene flow and dispersal in marine populations. However, these methods are not without assumptions and can yield ambiguous results. Neither MIGRATE nor STRUCTURE is based on a model in which patterns of gene flow change over time. If a marine species with a meroplanktonic life history is genetically structured over relatively small scales (as appears to be the case for *C. islagrande*) then it is likely that dispersal is influenced by complex oceanographic dynamics in the nearshore environment. For example, coastal currents in the northern GOM and along many other coasts periodically reverse in direction. Simple models that fail to represent these dynamics may be inappropriate. New methods are being developed to consider both contemporary and historical connections between populations (Knowles 2004), but more complex models generally require much more data (Nielsen and Slatkin 2000). An additional assumption of methods that estimate migration rates between specific pairs of populations is that all sources of migration are represented in the data (Slatkin 2005). It is unlikely that our data for *C. islagrande* meet this assumption, because every site we sampled was genetically distinct. If sites as little as 10 km apart represent different populations, then it would be impractical to sample every population. In analyses of simulated data with MIGRATE, gene flow be-

tween pairs of populations was overestimated when both populations received gene flow from an unknown third population (Beerli 2004). This situation seems likely in marine populations.

The application of genetic markers to analyze planktonic dispersal could be made much more powerful by the incorporation of prior knowledge of a species' history, ecology, and demography. Rather than attempting to reconstruct gene flow scenarios from whole cloth, we could use genetic markers to test specific dispersal hypotheses developed from a combination of physical oceanographic and larval dispersal data. This approach has seldom been used because direct measurements of dispersal are notoriously difficult. However, there has been significant progress in the development of physical oceanographic models for larval dispersal (Largier 2003) and in methods for direct measurement of larval dispersal (Thorrold et al. 2002). We expect that an integrated approach will be needed to resolve the complexities of planktonic dispersal.

Acknowledgments

We extend thanks to R. Bourgeois for help with specimen collection and to M. Hellberg for useful comments on the manuscript. This study was funded by grants from the U.S. Department of Energy (DE-FG02–97ER12220) and the National Science Foundation (0326383 and 0315995). This is contribution number 106 from the University of Louisiana Laboratory for Crustacean Research.

Literature Cited

Akaike, H. 1974. A new look at statistical model identification. IEEE Trans. Automat. Contr. AC 19:716–723.

Amos, W., and D. C. Rubinsztein. 1996. Microsatellites are subject to directional evolution. Nat. Genet. 12:13–14.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

———. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, MA.

Avise, J. C., R. M. Ball, and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. 5: 331–344.

Barber, P. H. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes and consequences. Mol. Ecol. 11: 659–674.

Barber, P. H., S. R. Palumbi, M. V. Erdmann, and M. K. Moosa. 2000. Biogeography: a marine Wallace's line? Nature 406: 692–693.

Beerli, P. 2002. MIGRATE: documentation and program, part of LAMARC. Available via http://popgen.csit.fsu.edu/migrate.download.html.

———. 2004. Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. Mol. Ecol. 13:827–836.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763–773.

———. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98: 4563–4568.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2002. GENETIX 4.04, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Berger, E. 1973. Gene-enzyme variation in three sympatric species of *Littorina*. Biol. Bull. 145:83–90.

Bert, T. M. 1986. Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. Mar. Biol. 93: 157–170.

Briggs, J. C. 1974. Marine zoogeography. McGraw Hill, New York.

Brown, A. F., L. M. Kann, and D. M. Rand. 2001. Gene flow versus local adaptation in the northern acorn barnacle, *Semibalanus balanoides*: insights from mitochondrial DNA variation. Evolution 55:1972–1979.

Burton, R. S. 1983. Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar. Biol. Lett. 4: 193–206.

———. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745.

Burton, R. S., and B. N. Lee. 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. Proc. Natl. Acad. Sci. USA 91:5197–5201.

Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. Annu. Rev. Ecol. Syst. 27:477–500.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19:233–257.

Cockerham, C. C., and B. S. Weir. 1993. Estimation of gene flow from *F*-statistics. Evolution 47:855–863.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153: 1989–2000.

Crandall, K. A., and J. F. Fitzpatrick. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst. Biol. 45:1–26.

Crisp, J. 1978. Genetic consequences of different reproductive strategies in marine invertebrates. Pp. 257–273 *in* B. Battaglia and J. Beardmore, eds. Marine organisms: genetics, ecology and evolution. Plenum Press, New York.

Crow, J. F., and K. Aoki. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci. USA 81:6073–6077.

Cunningham, C. W., N. W. Blackstone, and L. W. Buss. 1992. Evolution of king crabs from hermit crab ancestors. Nature 355: 539–542.

Dallas, J. F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mammal. Genome 5: 32–38.

Di Rienzo, A., P. Donnelly, C. Toomajian, B. Sisk, A. Hill, M. L. Petzl-Erler, G. K. Haines, and D. H. Barch. 1998. Heterogeneity of microsatellite mutations within and between loci, and implications for human demographic histories. Genetics 148: 1269–1284.

Ellegren, H. 1995. Mutation rates at porcine microsatellite loci. Mammal. Genome 6:376–377.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Felder, D. L. 2001. Diversity and ecological significance of deep-burrowing macrocrustaceans in coastal tropical waters of the Americas (Decapods: Thalassinidea). Interciencia 26:2–12.

Felder, D. L., and R. B. Griffis. 1994. Dominant infaunal communities at risk in shoreline habitats: burrowing thalassinid Crustacea. OCS Study/MMS 94-0007. U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Regional Office, New Orleans, LA. No. 86.

Flowers, J. M., S. C. Schroeter, and R. S. Burton. 2002. The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. Evolution 56: 1445–1453.

Gooch, J. L., and T. J. M. Schopf. 1972. Genetic variability in the deep-sea in relation to environmental variability. Evolution 26: 545–552.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52:696–704.

Harrison, J. S. 2004. Evolution, biogeography, and the utility of mitochondrial 16s and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapods: Pinnnotheridae). Mol. Phylogenet. Evol. 30:743–754.

Hedgecock, D. 1994a. Does variance in reproductive success limit effective population sizes of marine organisms? Pp. 199–207 *in* T. Kawasaki, S. Tanaka, Y. Toba, and A. Taniguchi, eds. Long-term variability of pelagic fish populations and their environment. Pergamon Press, Oxford, U.K.

———. 1994b. Temporal and spatial genetic structure of marine animal populations in the California Current. CalCOFI Rep. 35: 73–81.

Hedgecock, D., V. Chow, and R. S. Waples. 1992. Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture 108:215–232.

Hedrick, P. W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313–318.

Hellberg, M. E. 1998. Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. Evolution 52:1311–1324.

Hellberg, M. E., R. S. Burton, J. E. Neigel, and S. R. Palumbi. 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70(Suppl.):273–290.

Hernandez, J. L., and B. S. Weir. 1989. A disequilibrium coefficient approach to Hardy-Weinberg testing. Biometrics 45:53–70.

Hilbish, T. J. 1985. Demographic and temporal structure of an allele frequency cline in the mussel *Mytilus edulis*. Mar. Biol. 86: 163–171.

Hughes, C. R., and D. C. Queller. 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Mol. Ecol. 2:131–137.

Johnson, M. S., and R. Black. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38:1371–1383.

Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256: 100–102.

Kemp, G. P. 1986. Mud deposition at the shoreface: wave and sediment dynamics on the Chenier Plain of Louisiana. Ph.D. diss. Louisiana State University, Baton Rouge, LA.

Knowles, L. L. 2004. The burgeoning field of statistical phylogeography. J. Evol. Biol. 17:1–10.

Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. Mol. Ecol. 11:2623–2635.

Knutsen, H., P. E. Jorde, C. Andre, and N. C. Stenseth. 2003. Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol. Ecol. 12:385–394.

Koehn, R. K., R. I. Newell, and F. Immerman. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Natl. Acad. Sci. USA 77:5385–5389.

Kuhner, M. K., J. Yamato, and J. Felsenstein. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434.

Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular evolutionary genetics analysis Ver. 1.0. Pennsylvania State University, University Park, PA.

Largier, J. L. 2003. Considerations in estimating larval dispersal distances from oceanographic data. Ecol. Appl. 13:S71–S89.

Li, G., and D. Hedgecock. 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. Can. J. Fish. Aquat. Sci. 55:1025–1033.

Manning, R. B. 1975. Two methods for collecting crustaceans in shallow water. Crustaceana 29:317–319.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, MA.

McClure, M. R., and J. D. McEachran. 1992. Hybridization between *Prionotus alatus* and *P. paralatus* in the northern Gulf of Mexico (Pisces Triglidae). Copeia 1992:1039–1046.

McConaugha, J. R. 1992. Decapod larvae: dispersal, mortality and ecology, a working hypothesis. Am. Zool. 32:512–523.

Miya, M., and M. Nishida. 1997. Speciation in the open sea. Nature 389:803–804.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

Neigel, J. E. 1994. Alternative approaches to the detection of genetic structure in marine populations. CalCOFI Rep. 35:82–89.

———. 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. Annu. Rev. Ecol. Syst. 28: 105–128.

———. 2002. Is F_{ST} obsolete? Conserv. Genet. 3:167–173.

Nielsen, R., and M. Slatkin. 2000. Likelihood analysis of ongoing gene flow and historical association. Evolution 54:44–50.

Nylander, J. A. A. 2004. MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Ohlmann, J. C., and P. P. Niiler. 2005. Circulation over the continental shelf in the northern Gulf of Mexico. Progr. Oceanogr. 64:45–81.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4:347–354.

Palumbi, S. 2003. Population genetics, demographic connectivity and the design of marine reserves. Ecol. Appl. 13:S146–S158.

Pogson, G. H., K. A. Mesa, and R. G. Boutilier. 1995. Genetic population structure and gene flow in the Atlantic Cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139:375–385.

Pond, S. L. K., S. D. W. Frost, and S. V. Muse. 2005. HyPhy: hypothesis testing using phylogenies. Bioinformatics 21: 676–679.

Primmer, C. R., H. Ellegren, N. Saino, and A. P. Møller. 1996. Directional evolution in germline microsatellite mutations. Nat. Genet. 13:391–393.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. USA 94: 9197–9201.

Raymond, M., and F. Rousset. 1995. Genepops (Ver. 1.2). Population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Saucier, R. T. 1974. Quaternary geology of the Lower Mississippi valley. Ark. Arch. Surv. Res. 6:1–26.

Schlotterer, C., R. Ritter, B. Harr, and G. Brem. 1998. High mutation rate of a long microsatellite allele in *Drosophila melanogaster* provides evidence for allele-specific mutation rates. Mol. Biol. Evol. 15:1269–1274.

Schubart, C. D., R. Diesel, and S. B. Hedges. 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature 393:363–365.

Schubart, C. D., J. E. Neigel, and D. L. Felder. 2000. Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. Mar. Biol. 137:11–18.

Schug, M. D., T. F. C. Mackay, and C. F. Aquadro. 1997. Low mutation rates of microsatellite loci in *Drosophila melanogaster*. Nat. Genet. 15:99–102.

Schug, M. D., C. M. Hutter, K. A. Wetterstrand, M. S. Gaudette, T. F. C. Mackay, and C. F. Aquadro. 1998. The mutation rates of di-, tri- and tetranucleotide repeats in *Drosophila melanogaster*. Mol. Biol. Evol. 15:1751–1760.

Shanks, A., and B. Grantham. 2001. Propagule dispersal distance and the size and spacing of marine reserves. Ecol. Appl. 13: S159–S169.

Slatkin, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393–430.

———. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

———. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

———. 2005. Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. Mol. Ecol. 14:67–73.

Slatkin, M., and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368.

Staton, J. L., and D. L. Felder. 1995. Genetic variation in populations of the ghost shrimp genus *Callichirus* (Crustacea, Decapoda, Thalassinidea) in the western Atlantic and Gulf of Mexico. Bull. Mar. Sci. 56:523–536.

Strasser, K. M., and D. L. Felder. 1998. Settlement cues in successive developmental stages of the ghost shrimps *Callichirus major* and *C. islagrande* (Crustacea: Decapoda: Thalassinidea). Mar. Biol. 132:599–610.

———. 2000. Larval development of the ghost shrimp *Callichirus islagrande* (Decapoda: Thalassinidea: Callianassidae) under laboratory conditions. J. Crustacean Biol. 20:100–117.

Strumbauer, C., J. S. Levinton, and J. Christy. 1996. Molecular phylogeny analysis of fiddler crabs: test of the hypothesis of increasing behavioral complexity in evolution. Proc. Natl. Acad. Sci. USA 93:10855–10857.

Swearer, S., E. J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402:799–802.

Swearer, S., S. Thorrold, J. Shima, M. Hellberg, G. Jones, D. Robertson, K. Selkoe, G. Ruiz, S. Morgan, and R. Warner. 2002. Evidence for self-recruitment in benthic marine populations. Bull. Mar. Sci. 70:251–272.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

Taylor, M. S., and M. E. Hellberg. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107–109.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Thorrold, S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer, J. E. Neigel, S. G. Morgan, and R. R. Warner. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. Bull. Mar. Sci. 70: 291–308.

Tracey, M. L., N. F. Bellet, and C. D. Gravem. 1975. Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus*. Mar. Biol. 32:303–311.

Vazquez, J. F., T. Perez, J. Albornoz, and A. Dominguez. 2000. Estimation of microsatellite mutation rates in *Drosophila melanogaster*. Genet. Res. 76:323–326.

Vermeij, G. J. 1978. Biogeography and adaptation: patterns of marine life. Harvard Univ. Press, Cambridge, MA.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. 89:438–450.

Warner, R. R., and R. K. Cowen. 2002. Local retention of production in marine populations: evidence, mechanisms, and consequences. Bull. Mar. Sci. 70:245–249.

Waser, P. M., and C. Strobeck. 1998. Genetic signatures of interpopulation dispersal. Trends Ecol. Evol. 13:43–44.

Weber, J. L., and C. Wong. 1993. Mutation of human short tandem repeats. Hum. Mol. Genet. 2:1123–1128.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wiley, E. O., and R. L. Mayden. 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Mo. Bot. Gard. 72:596–635.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323–353.

Corresponding Editor: D. McHugh