

## GEOGRAPHIC RANGES, POPULATION STRUCTURE, AND AGES OF SEXUAL AND PARTHENOGENETIC SNAIL LINEAGES

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**Abstract.**—Asexual reproduction is thought to doom organisms to extinction due to mutation accumulation and parasite exploitation. Theoretical models suggest that parthenogens may escape the negative effects of conspecifics and biological enemies through escape in space. Through intensive sequencing of a mitochondrial DNA (mtDNA) and a nuclear intron locus in sexual and parthenogenetic freshwater snails (*Campeloma*), I examine three questions: (1) Are sexual mtDNA lineages more restricted geographically than parthenogenetic mtDNA lineages? (2) Are independent parthenogenetic lineages shorter lived than sexual lineages? and (3) Do parthenogens have higher intraindividual nuclear sequence diversity and form well-differentiated monophyletic groups as expected under the Meselson effect? Geographic ranges of parthenogenetic lineages are significantly larger than geographic ranges of sexual lineages. Based on coalescence times under different demographic assumptions, asexual lineages are short lived, but there is variation in clonal ages. Although alternative explanations exist, these results suggest that asexual lineages may persist in the short term through dispersal, and that various constraints may cause geographic restriction of sexual lineages. Both allotriploid and diploid *Campeloma* parthenogens have significantly higher allelic divergence within individuals, but show limited nuclear sequence divergence from sexual ancestors. In contrast to previous allozyme evidence for nonhybrid origins of diploid *Campeloma* parthenogens, cryptic hybridization may account for elevated heterozygosity.

**Key words.**—*Campeloma*, cryptic hybridization, geographic range, mtDNA, nuclear intron, parthenogenesis, sexual reproduction.

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Aside from a few scandalous examples (Mark Welch and Meselson 2000), obligately asexual organisms are evolutionarily short lived (Bell 1982; Judson and Normark 1996). This short-term limitation of asexual lineages represents a long-standing paradox because of the putative reproductive advantages of clonal reproduction (Maynard Smith 1978; Bell 1982). Theoretical arguments and empirical evidence suggest that asexual organisms accumulate deleterious mutations (Muller 1964; Kondrashov 1988) and may be exploited by rapidly evolving enemies (Hamilton et al. 1990). How asexual lineages may temporarily escape these forces may be due to the geographic distribution of closely related sexual and asexual lineages.

The spatial isolation, broader geographic distribution, and relegation to marginal habitats of asexual lineages relative to their sexual ancestors may provide insight into potential mechanisms by which asexual lineages might persist (Glesener and Tillman 1978; Peck et al. 1998; Law and Crespi 2002a,b). In contrast to sexual ancestors, asexuals may have larger geographic ranges because, in the colonization of marginal environments, they do not suffer from mate limitation or outbreeding/inbreeding depression (Peck et al. 1998; Haag and Ebert 2004). Host-parasite interactions may also favor widespread dispersal of asexuals because coevolutionary interactions between hosts and their virulent parasites may be lessened if asexual hosts have higher rates of dispersal (Ladle et al. 1993). These disparate models share a common element: asexuals may persist if they can escape negative interactions with either conspecific sexuals and/or biological enemies. Sexual lineages should be more restricted geographically, and sequence variation in sexuals should exhibit finer-scale differentiation. Studies indicate that asexuals are either more broadly distributed or geographically isolated than their sex-

ual ancestors (Law and Crespi 2002a,b; Vorburger et al. 2003; Wilson et al. 2003; Neiman et al. 2005). Using the southeastern United States freshwater snail *Campeloma*, I examine the geographic ranges of sexual and parthenogenetic lineages, and test whether sexual lineages show finer-scale differentiation, relative to asexual lineages, using mitochondrial DNA (mtDNA) and nuclear sequence variation.

In the southeastern United States, there have been multiple, independent origins of nonhybrid and hybrid parthenogens from sexual ancestors in the freshwater prosobranch snail *Campeloma* (Johnson and Bragg 1999; Johnson and Leefe 1999). Based on allozyme variation, *Campeloma* diploid parthenogens from the Atlantic coastal plain and the Florida peninsula arose spontaneously without interspecific hybridization. Another group of sexual and parthenogenetic *Campeloma* (Gastropoda: Viviparidae) inhabits Florida Gulf Coast freshwater streams and rivers. *Campeloma geniculum* are obligate sexuals and *C. parthenum* are highly heterozygous triploids formed by hybridization between *C. geniculum* and Atlantic Coast *Campeloma*. There are problems with inferences of spontaneous origins of parthenogens from allozyme data. Studies of aphid parthenogens indicate that allozymes underestimate hybrid origins of parthenogens because they show heterozygote deficit whereas microsatellites show heterozygote excess, presumably due to strong selection for homozygosity in allozymes (Hales et al. 1997; Delmotte et al. 2002). I examine evidence for hybrid origins of diploid *Campeloma* parthenogens using nuclear sequence variation.

Another component of the current study is to assess the ages of sexual and parthenogenetic *Campeloma* lineages and to qualitatively address causes of variation in clonal longevity. A recent study of the parthenogenetic New Zealand snail, *Potamopyrgus antipodarum*, revealed considerable variation

in ages of asexual lineages, and longer-lived asexual lineages were found in habitats with low frequencies of potential sexual competitors (Neiman et al. 2005). An advantage of examining variation in the longevity of sexual and parthenogenetic *Campeloma* lineages is that considerable data exist on costs and benefits of sexual versus parthenogenetic reproduction. Experimental and field studies indicate that triploid hybrid parthenogens from the Florida Gulf Coast exhibit considerable heterotic advantages in terms of survivorship and growth rates as well as a two-fold reproductive advantage (Johnson 2005). Additionally, spontaneous diploid parthenogens from the Atlantic Coast, relative to sympatric diploid sexuals, have significantly higher fecundity and similar laboratory survivorship and growth rates when controlling for maternal effects. However, inferences about the ages of asexual lineages require considerable caution.

Inferences about clonal age are derived primarily from patterns of mtDNA sequence divergence from sexual ancestors (Avice et al. 1992; Johnson and Bragg 1999; Law and Crespi 2002a) and allelic divergence of nuclear genes within asexual individuals (Normark 1999; Mark Welch and Meselson 2000). In addition to large confidence limits around molecular clock estimates of asexual ages (Hillis et al. 1996), invalid inferences of long-lived asexuals from mtDNA divergence may stem from extinction of sexual ancestors or inadequate sampling of sexual populations, if sexual populations exhibit considerable sequence divergence among populations, (Johnson and Bragg 1999; Law and Crespi 2002a; Delmotte et al. 2003). In the aphid *Rhopalosiphum radi*, an unknown sibling species was discovered that discounts previous claims of moderate age (1.4 million years) of a parthenogenetic lineage (Delmotte et al. 2003). Therefore, intensive sampling of sexuals and asexuals is paramount. Another major assumption in estimating times to most recent common ancestor is that population sizes have remained constant. This assumption may be violated in parthenogenetic lineages due to extreme bottlenecks upon origin and the potential for exponential growth. In the current study, I assess clonal ages under different demographic assumptions.

For nuclear sequence polymorphism, long-lived asexuals should exhibit considerable allelic divergence within individuals and asexual haplotypes should form well-differentiated monophyletic groups, the so-called Meselson effect (Mark Welch and Meselson 2000). Determining whether asexuals are ancient has met with mixed success because various processes such as gene conversion and meiotic parthenogenesis can destroy heterozygosity even in long-lived asexuals (Normark 1999). Another factor that may obscure attempts to infer clonal longevity is hybridization. If elevated allelic divergence within individuals is present in the absence of divergent asexual haplotypes, hybridization is probably the main contributor to within-individual allelic divergence.

Here, I address three questions: (1) Are sexual mtDNA lineages more restricted geographically than parthenogenetic mtDNA lineages, and do sexuals exhibit finer-scale differentiation for mtDNA and nuclear sequences? (2) Under different demographic conditions, are independent parthenogenetic lineages shorter lived than sexual lineages? (3) Do parthenogens exhibit greater allelic divergence within individuals, and do asexual nuclear haplotypes form highly di-

vergent monophyletic groups? This study reports that parthenogenetic lineages have significantly larger geographic ranges, and sequence variation in sexuals exhibits finer-scale differentiation relative to parthenogens. Based on mtDNA and intron sequences, parthenogens are relatively short lived, but there is variation in clonal ages. Additionally, in hybrid and nonhybrid parthenogens, within-individual sequence diversity is significantly greater compared to sexuals, but there is no or limited divergence from common sexual haplotypes.

## MATERIALS AND METHODS

### *Geographic Sampling and Molecular Methods*

I sampled 58 sites containing *Campeloma* sp. from Atlantic and Florida Gulf coast populations (Fig. 1; See Appendix available online only at <http://dx.doi.org/10.1554/06-062.1.s1>). Parthenogenetic localities contained only females whereas most sexual populations contained at least 40% males. Two populations with biased female sex ratios (20–25% males) may contain both parthenogenetic and sexual females (see discussion below). I focused sampling within river drainages that were known to contain both sexual and parthenogenetic *Campeloma* populations from previous studies (Johnson and Bragg 1999; Johnson 2000). Despite intensive searching, I was unable to find *Campeloma* from river drainages, such as the tributaries of the Apalachicola, Ochlockonee, and Suwannee Rivers in central and southern Georgia (see Fig. 1), probably due to severe pollution and habitat degradation (S. G. Johnson, pers. obs.). All specimens were frozen in liquid nitrogen and then stored at  $-80^{\circ}\text{C}$ . For each population, five to 15 individuals were sequenced for a mtDNA gene (a 331 bp portion of cytochrome *b*) and four to nine individuals for a nuclear intron (calmodulin). For each population, the same individuals were sequenced for both markers, although only a subset of individuals was sequenced for intron variation. Total DNA was extracted from foot tissue using the Qiagen (Valencia, CA) DNA prep kit and the mtDNA gene was amplified with primers previously described (Johnson and Bragg 1999). Mitochondrial cytochrome *b* amplifications were performed in 50- $\mu\text{l}$  solutions containing 10 mM Tris (pH 8.3), 50 mM KCl, 5.5 mM  $\text{MgCl}_2$ , each dNTP at 200 mM, 30 pmol of each primer, 1–2  $\mu\text{l}$  of undiluted template DNA, and 2 units of *Taq* polymerase. The polymerase chain reaction (PCR) cycling parameters were 45 sec at  $92^{\circ}\text{C}$ , 50 sec at  $47^{\circ}\text{C}$ , and 75 sec at  $72^{\circ}\text{C}$  for 30 cycles followed by  $72^{\circ}\text{C}$  for five min. Amplification products were purified with Wizard PCR Preps (Promega, Madison, WI) or GeneClean (QBIOScience, Irvine, CA), and sequenced with Big-Dye Ready Reaction mix 1.1 on either an ABI 377 or 3100 (Applied Biosystems, Foster City, CA) using the upstream primer. Sequences were aligned using Clustal W (Thompson et al. 1994).

A calmodulin nuclear intron situated between exons III and IV (Swanson et al. 1990) was amplified using an exon-primed, intron-crossing PCR, with specific primers for *Campeloma*: Cam-1 (5'GATTCTGATGGTATGTTTCTG3') and Cam-2 (5'ATTTTGATTGGTTGAAGAG3'). These specific primers produced a single, distinct PCR band. Thirty pmol of each of these primers was used in amplification reactions of 50  $\mu\text{l}$ , with a final  $\text{Mg}^{2+}$  concentration of 1.5 mM, 5  $\mu\text{l}$

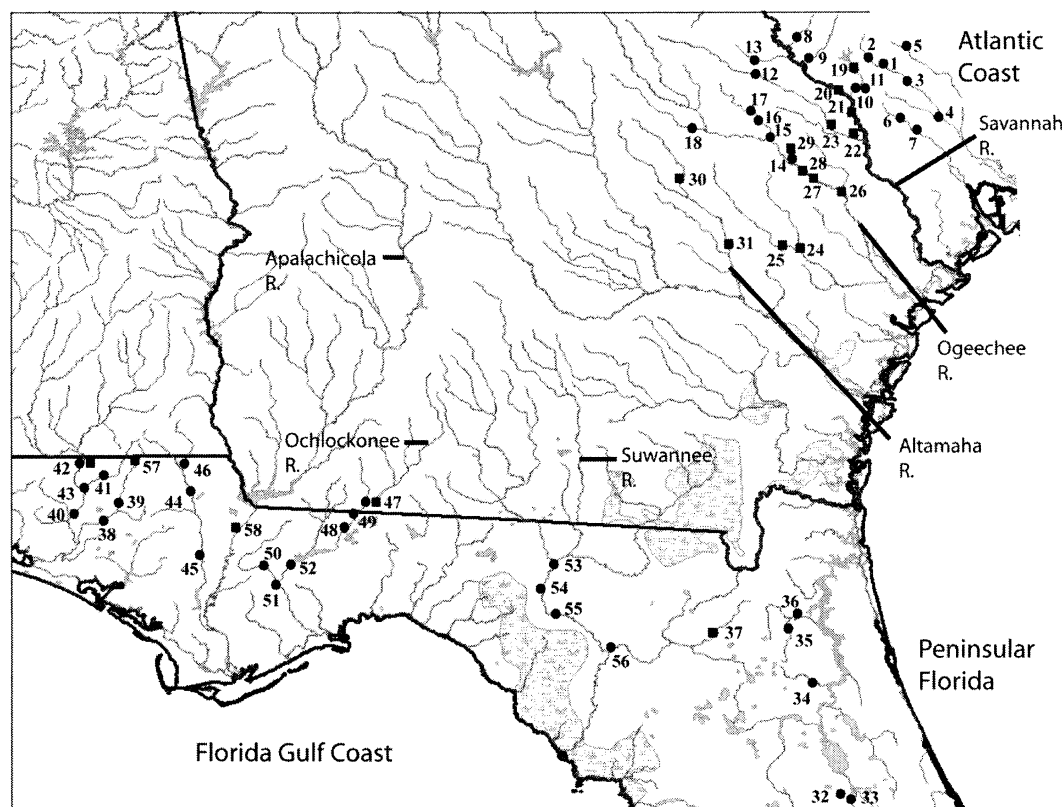


FIG. 1. Map of 58 sampling locations. Circles and squares represent sexual and parthenogenetic populations, respectively. See Appendix (available online only) for sample sizes for mtDNA and intron sequences, and more detailed locality information.

of undiluted genomic DNA, and otherwise standard conditions. The PCR program consisted of a denaturation step of 2 min at 92°C, followed by 30 cycles of 45 sec at 92°C, 50 sec at 56°C, 75 sec at 72°C, and ended with a final extension step of 5 min at 72°C. Amplification products were purified with Wizard PCR Preps or GeneClean, and sequenced with Big-Dye Ready Reaction mix 1.1 on either an ABI 377 or 3100.

To detect heterozygous sites, I used Factura (ABI Sequencing analysis) to infer IUB heterozygous codes if the ratio of the second highest peak to the highest peak at a particular sites was greater than 50%. To determine allelic phases of individuals that were heterozygous at two or more nucleotide sites, I used a combination of statistical methods and cloning. After I obtained a large sample of direct sequences for individuals that were homozygous or contained a single heterozygous site, I inferred allelic phases of heterozygous individuals using PHASE (Stephens et al. 2001). This Bayesian method uses the population samples of direct sequences to inform haplotype reconstruction and also estimates the uncertainty associated with the phase call. If the phase call for a particular site was less than 90%, I cloned the PCR product for these individuals, and directly sequenced four to six clones per individual using the Cam-1 primer to determine allelic phases. Multiple sequences were aligned using Clustal W (Thompson et al. 1994). Indels were collapsed to a single base substitution.

#### *Clade Ages, Geographic Ranges, and Population Structure from mtDNA*

Phylogenetic relationships among cytochrome *b* haplotypes were reconstructed using maximum likelihood (Swofford 2001). Selection of the best-fitting, simplest model of sequence evolution was based on hierarchical likelihood-ratio tests performed in MrModeltest 2.2 (Nylander 2004). This method identified the GTR + I + G as the simplest model. I used this model of sequence evolution and its parameter estimates to perform a heuristic maximum-likelihood search with 10 replications of stepwise addition and tree bisection-reconnection branch swapping in PAUP\* (Swofford 2001). MrBayes (Huelsenbeck 2000) was used to estimate the clade support by running four Markov chains (one cold and three heated chains) for 5 million generations and using a burn-in of 500,000. Trees were sampled every 5000 generations. Maximum likelihood and Bayesian reconstructions were identical. The remaining trees were imported into PAUP\*, and the numbers at interior branches of a majority-rule consensus tree were used as the posterior probabilities of particular clades existing.

For estimates of time to most recent common ancestor (tMRCA) for sexuals, I included all sexual haplotypes in clades for which there were >0.95 posterior probabilities (12 clades). I estimated asexual clade ages based on coalescence to a common ancestor within each asexual clade (solid cir-

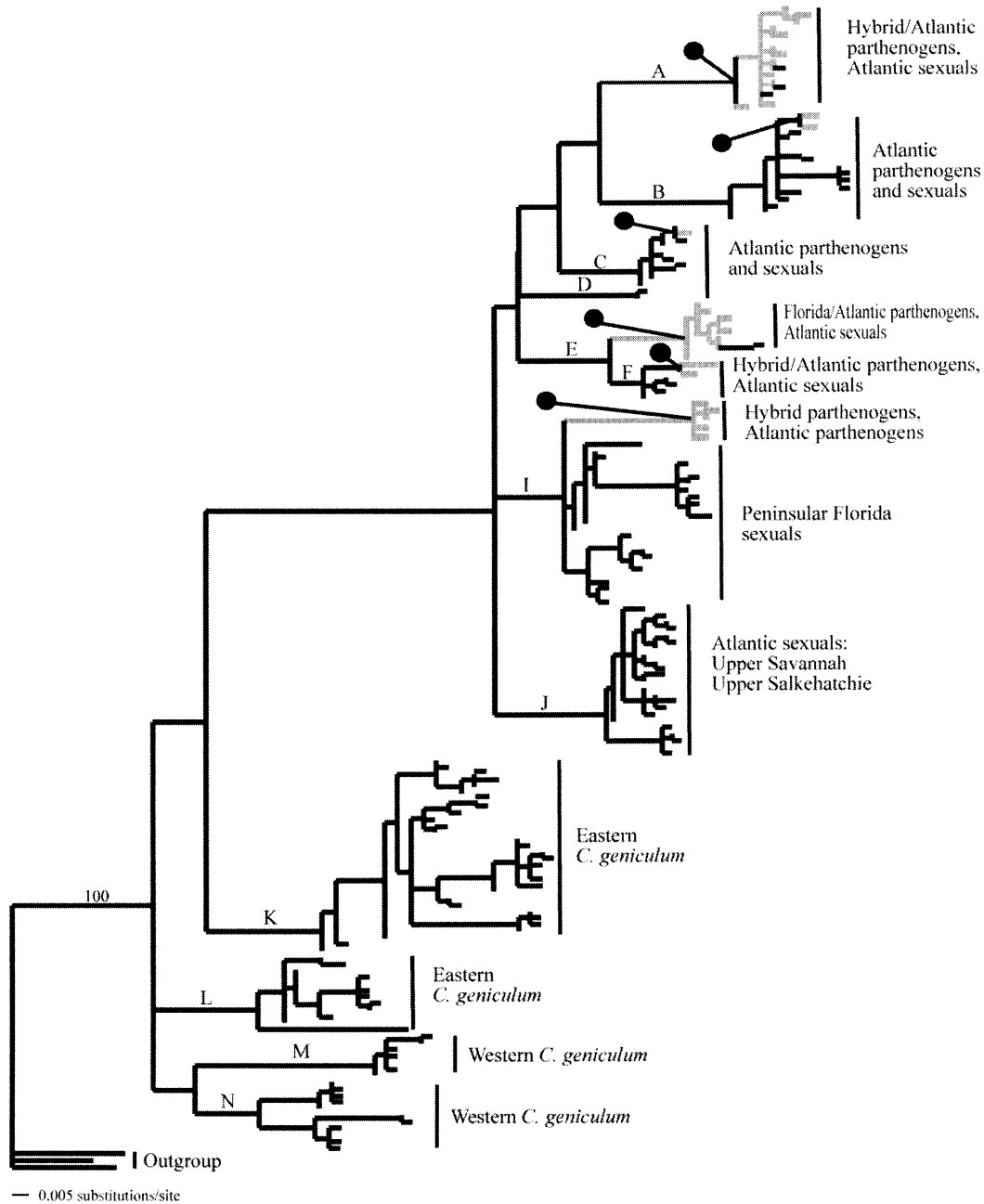


FIG. 2. Maximum likelihood phylogram for 174 unique cytochrome *b* haplotypes with outgroup rooting (*Campeloma decisum*). The model of sequence evolution was GTR + I + G. Letters on branches represent nodes for which Bayesian posterior probabilities exceeded 0.95. Closed circles represent nodes for which time to most recent ancestor (tMRCA) was estimated for six parthenogenetic clades. Letters represent nodes (to the right of letter) at which tMRCA was estimated for sexual clades. Terminal dark branches represent sexual haplotypes, and gray branches represent parthenogenetic haplotypes. Inferred sexual and parthenogenetic branches are presented in black and gray, respectively. The scale bar at the bottom left is proportional to branch length, measured as the number of DNA substitutions per site.

cles; Fig. 2). This is a more conservative estimate of clonal age than time to most recent sexual ancestor. Because parthenogenetic clades could also undergo exponential population growth due to high reproductive rates during colonization, I also determined clonal age under a demographic model of exponential growth. Divergence dates were estimated using BEAST 1.3, which employs a Bayesian Markov chain Monte Carlo algorithm (Drummond and Rambaut

2003). A GTR + I + G model of sequence evolution was employed, with a substitution rate based on the standard 2.0% sequence divergence per million years (Collins et al. 1996). Five million chains were run with a burn-in of 500,000. Two replicates were run, and the average tMRCA was calculated from these replicates.

To test whether there were significant differences in the geographic ranges of parthenogenetic and sexual clades, I

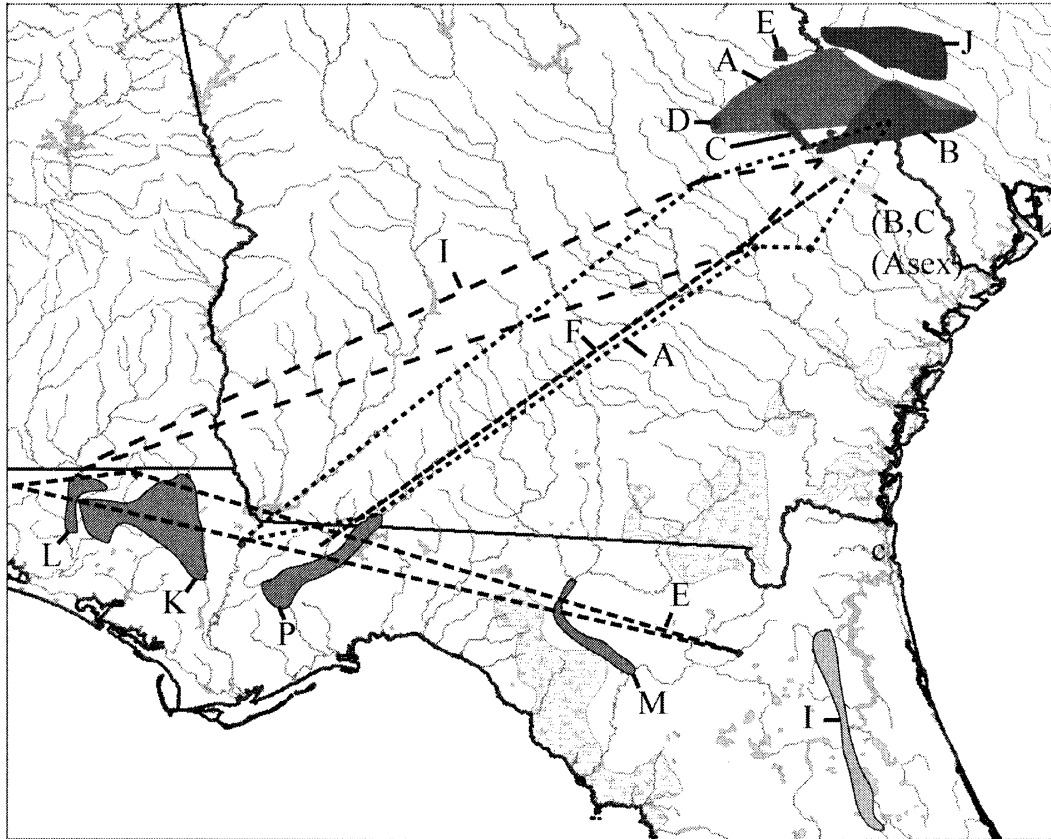


FIG. 3. Geographic distribution of sexual and parthenogenetic clades from cytochrome *b* tree. Refer to Figure 2 for letter designation of particular clades associated with reproductive modes and geographic region. Shaded regions represent geographic ranges of 12 sexual clades. Dashed lines represent geographic ranges of the four widespread parthenogenetic clades and the clades B and C (light shading) represent the two narrowly distributed parthenogenetic clades.

used GeoDis (Posada et al. 2000) to estimate clade distances for parthenogenetic and sexual clades. Clade distance measures the average distance of an individual from the geographic center of all individuals within that clade and is thus a measure of the geographical spread of a particular clade. A Kolmogorov-Smirnov Z-test was used to test whether the geographic ranges of sexual and parthenogenetic clades differed significantly. All statistical analyses employed SPSS (SPSS 2003). Because drainage isolation should contribute to genetic divergence in freshwater organisms, I employed analysis of molecular variance (AMOVA) to examine patterns of spatial differentiation in Atlantic Coast sexuals, Atlantic Coast parthenogens, and Florida Gulf Coast sexuals. Florida Gulf Coast parthenogens were not included because there were not multiple populations within drainages. The distance matrix was computed from pairwise differences, so nucleotide substitutions and indels were included. For each of these three groups, I partitioned the total sequence variation into differences among drainages ( $\Phi_{CT}$ ), among localities within drainages ( $\Phi_{SC}$ ), and within populations ( $\Phi_{ST}$ ). Significance of each estimator was based on 1000 permutations and all analyses were run in ARLEQUIN 2.0 (Schneider et al. 2000).

#### *Tests of the Meselson Effect and Hierarchical Analysis of Intron Sequence Diversity*

To test the Meselson effect, I assessed the allelic divergence within individuals. For each individual, I determined the number of sites containing either two nucleotides or indels. This measure is analogous to the number of within-individual pairwise differences (WIPD). To examine whether the distribution of WIPD differed between sexuals and parthenogens from the Florida Gulf Coast and Atlantic Coast, I conducted a nonparametric Kolmogorov-Smirnov Z-test. To assess the divergence of parthenogenetic and sexual haplotypes, a haplotype network was constructed using TCS 1.13 (Clement et al. 2000). Closed loops were present in this Cam network, but most loops were resolved based on coalescent theory (Crandall et al. 1994). Some closed loops could not be resolved.

To test the hypothesis that drainage isolation plays the primary role in generating genetic subdivision, I employed AMOVA to examine genetic structuring among river drainages for Atlantic Coast sexuals, Atlantic Coast parthenogens, and Florida Gulf Coast sexuals. The total sequence variation was partitioned in the same way as the cytochrome *b* data. I also determined the multilocus genotypes for each individ-

TABLE 1. Hierarchical analysis of mtDNA (cytochrome *b*) and nuclear intron (calmodulin) sequence variation for Atlantic Coast sexuals, Atlantic Coast parthenogens, and Florida Gulf Coast sexuals. For each of these three groups, total sequence variation is partitioned into differences among drainages ( $\Phi_{CT}$ ), among localities within drainages ( $\Phi_{SC}$ ), and within populations ( $\Phi_{ST}$ ). \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ .

Gene/region/reproductive mode	$\Phi_{CT}$	$\Phi_{SC}$	$\Phi_{ST}$
<b>Cytochrome <i>b</i></b>			
Atlantic Coast sexuals	20.0***	53.1***	26.9***
Atlantic Coast parthenogens	18.6	36.2***	45.2***
Florida Gulf Coast sexuals	56.0***	31.9***	12.1***
<b>Calmodulin</b>			
Atlantic Coast sexuals	15.3**	20.5***	64.2***
Atlantic Coast parthenogens	3.5	14.5***	82.0***
Florida Gulf Coast sexuals	38.4***	30.1***	31.5***

ual that was sequenced for cytochrome *b* and calmodulin. To qualitatively examine whether multilocus genotypes from Atlantic Coast parthenogens showed evidence of greater spatial ranges relative to sexuals, I determined the number and spatial distribution of unique multilocus haplotypes for Atlantic Coast sexuals and parthenogens.

## RESULTS

Mitochondrial cytochrome *b* sequences were obtained from 527 individuals from 58 *Campeloma* populations throughout the southeastern United States (see Fig. 1 and Appendix available online for localities and sample sizes). Using the mtDNA genetic code of echinoderms in DnaSP (Rozas et al. 2003), there were no stop codons, indicating that these are mtDNA sequences instead of nuclear translocations. There were 123 variable sites from the 331 bp fragment, of which 106 were parsimony informative. Synonymous changes were more frequent than replacement changes (86 vs. 24). There is considerable sequence mtDNA divergence between groups, ranging from 6.4% divergence of Atlantic Coast sexuals and parthenogens to more than 11% between Florida Gulf Coast sexuals and Atlantic/peninsular Florida sexuals and parthenogens. Phylogenetic relationships among the 174 unique mtDNA haplotypes (GenBank accession nos. DQ131229–DQ131402) are presented in Figure 2.

There were six independent origins of parthenogens (clades A, B, C, E, F, and I) from Atlantic Coast and Peninsular Florida sexuals, and there were 12 sexual clades (clades A–P) with strong Bayesian support ( $>0.95$  posterior probabilities; Fig. 2). Sexual clades show restricted geographic ranges, whereas most parthenogenetic clades have large geographic ranges (Fig. 3). The average clade distances of sexual and parthenogenetic clades were 19.8 and 99.1 km, respectively. Clade distances were significantly larger for parthenogenetic clades ( $Z = 1.33$ ,  $P = 0.05$ ). The hierarchical analysis of mtDNA sequence variation indicates that there is a significant effect of drainage isolation in sexuals from the Florida and Atlantic Gulf Coast, whereas nearly half of the total variation in Atlantic Coast parthenogens occurs within populations (Table 1). Additionally, Atlantic Coast sexuals show considerable differentiation among populations within river drainages. These patterns are consistent with finer-scale

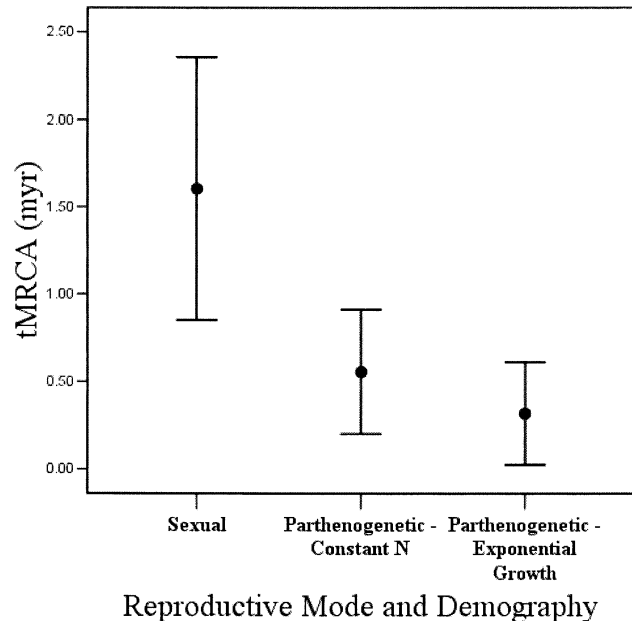


FIG. 4. Estimates of average ( $\pm 95$  confidence intervals) time to most recent common ancestor (tMRCA) for sexual clades, parthenogenetic clades with constant population size, and parthenogenetic clades undergoing exponential growth.

differentiation of Atlantic and Florida Gulf coast sexuals relative to Atlantic Coast parthenogens.

The tMRCA for sexual clades and parthenogenetic clades under constant population size or exponential growth indicates that parthenogenetic clades are shorter lived than sexual clades (Fig. 4). The average tMRCA for sexual clades (1.6 million years; my) is significantly greater than parthenogenetic clades under constant population size (0.56 my;  $U = 14$ ;  $P < 0.05$ ) and exponential growth (0.32 my;  $U = 7$ ;  $P < 0.01$ ). Under the assumption of exponential growth, the tMRCA is reduced by nearly half, although the difference is not significant ( $P > 0.05$ ).

Calmodulin nuclear intron sequences were obtained from 340 individuals from 58 populations (see Appendix available online for localities and sample sizes). Seventy-five unique calmodulin nuclear intron haplotypes have been deposited in GenBank (accession nos. DQ131403–DQ131477). There were 29 variable sites from an approximately 443–457 bp fragment, of which 21 were parsimony informative. There were also four indels, ranging in size from 1–8 bp. In striking contrast to mtDNA sequence divergence, there is limited sequence divergence, ranging from 0.75% divergence of Atlantic Coast sexuals and parthenogens to 1% between Atlantic Coast parthenogens and Florida Gulf Coast sexuals. To address the hypothesis that WIPD is greater in parthenogens relative to sexuals, I compared the distribution of WIPD for sexuals and parthenogens from the Atlantic and Florida Gulf coast. For both Atlantic and Florida Gulf coast parthenogens and sexuals, WIPD was significantly greater in parthenogens compared to sexuals ( $P < 0.05$ ; Fig. 5A,B). Florida Gulf coast allotriploid parthenogens also have significantly higher WIPD relative to diploid Atlantic Coast parthenogens ( $Z = 2.89$ ;  $P < 0.001$ ). To test whether alleles from parthenogens

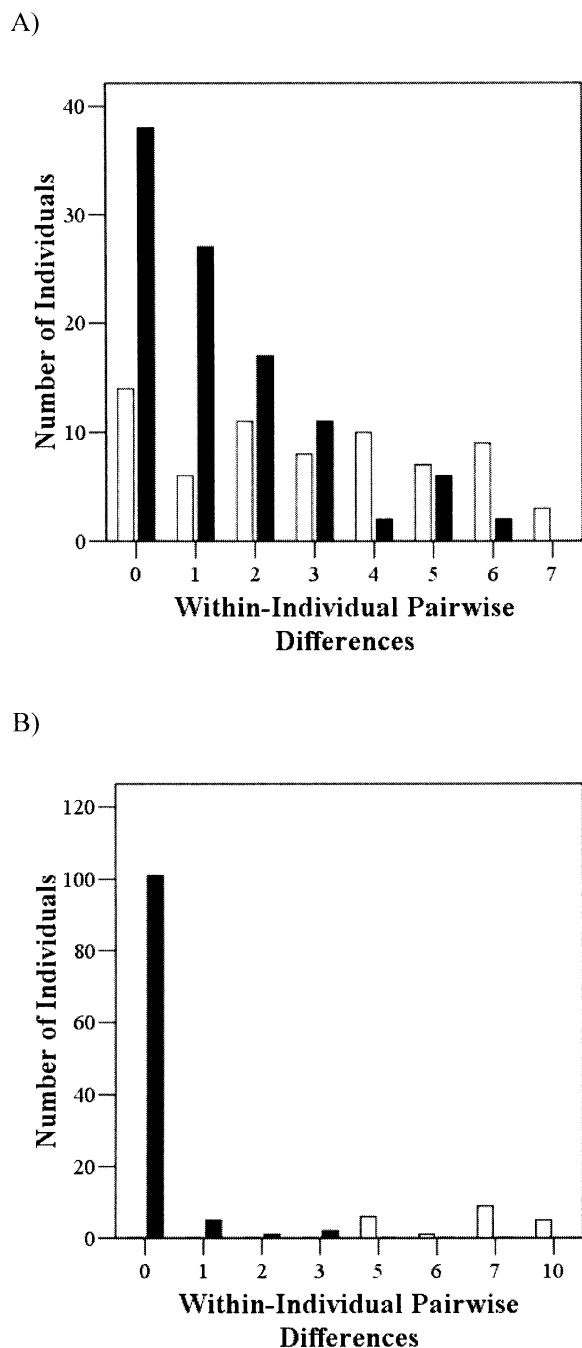


FIG. 5. Frequency distributions of within-individual pairwise differences for (A) Atlantic sexuals (closed bars) and parthenogens (open bars;  $Z = 2.2$ ) and (B) Florida panhandle sexuals (closed bars) and hybrid parthenogens (open bars;  $Z = 4.2$ ).

are divergent from ancestral sexual haplotypes (i.e., the Meselson effect) and form monophyletic clades, a TCS network was constructed for the 75 unique intron haplotypes (Fig. 6). There is little evidence for highly divergent haplotypes in either Atlantic or Florida Gulf coast parthenogens. For example, high-frequency haplotypes ( $>20$  haplotypes; uppercase letters in Fig. 6) are present in Atlantic Coast sexuals and parthenogens, and rare haplotypes ( $<8$  haplotypes) show only limited divergence ( $\leq 2$  mutational steps) from these

common haplotypes. Similarly, the divergent alleles in hybrid parthenogens group with the Atlantic Coast sexuals and Florida Gulf coast sexuals, with only minimal divergence from alleles present in the Florida sexuals ( $\leq 2$  mutational steps).

The hierarchical analysis of calmodulin sequence variation indicates that there is a significant effect of drainage isolation in Florida and Atlantic Gulf Coast sexuals, whereas 82% of the total variation in Atlantic Coast parthenogens occurs within populations (Table 1). Consistent with the results from mtDNA sequence variation, these patterns are consistent with finer-scale differentiation of sexuals from the Atlantic and Florida Gulf coast relative to Atlantic Coast parthenogens. In contrast to the widespread spatial distribution of mtDNA haplotypes, there were 70 multilocus genotypes in Atlantic Coast parthenogens of which 75% were unique, of which nearly all genotypes were restricted to a single locality. Similarly, there were 122 Atlantic Coast sexual multilocus genotypes, of which 86% were unique and nearly all were restricted to a single locality.

#### DISCUSSION

Through intensive sequencing of a mitochondrial gene and a nuclear intron from multiple individuals and populations of parthenogenetic and sexual *Campeloma*, there were three major findings. First, parthenogenetic lineages have significantly larger geographic ranges than sexual lineages, and sexual *Campeloma* exhibit finer-scale population differentiation for both genetic markers. Second, although Atlantic and Florida Gulf Coast parthenogens exhibit greater within-individual allelic divergence compared to sexuals, parthenogenetic mtDNA clades have significantly shorter times to most recent common ancestor under different demographic assumptions, and parthenogenetic intron haplotypes do not form divergent monophyletic groups. These data suggest that parthenogenetic lineages are not long lived, although there is variation in the ages of parthenogenetic lineages. Lastly, both mtDNA and nuclear sequences indicate numerous origins of parthenogenesis, and Atlantic Coast diploid *Campeloma* parthenogens may have arisen through cryptic hybridization rather than spontaneous origins.

#### *Geographic Ranges, Population Structure and Ages of Sexual and Parthenogenetic Campeloma*

The current study indicates that parthenogenetic mtDNA clades have greater geographic ranges than sexual clades, and mtDNA and nuclear intron genetic differentiation occurs at much smaller spatial scales in Atlantic and Gulf Coast sexuals compared to Atlantic Coast parthenogens. Although many parthenogens have larger geographic ranges than their sexual ancestors or are geographically isolated from their sexual ancestors (Glesener and Tilman 1978; Law and Crespi 2002a,b; Stenberg et al. 2003), I am unaware of any studies examining hierarchical spatial patterns of both mitochondrial and nuclear sequence differentiation in sexuals and parthenogens. The larger geographic ranges and low population structure among drainages of parthenogenetic *Campeloma* may stem from relaxed selection on dispersal in parthenogens, because they do not suffer from mate limitation or outbreeding and inbreeding depression when colonizing new

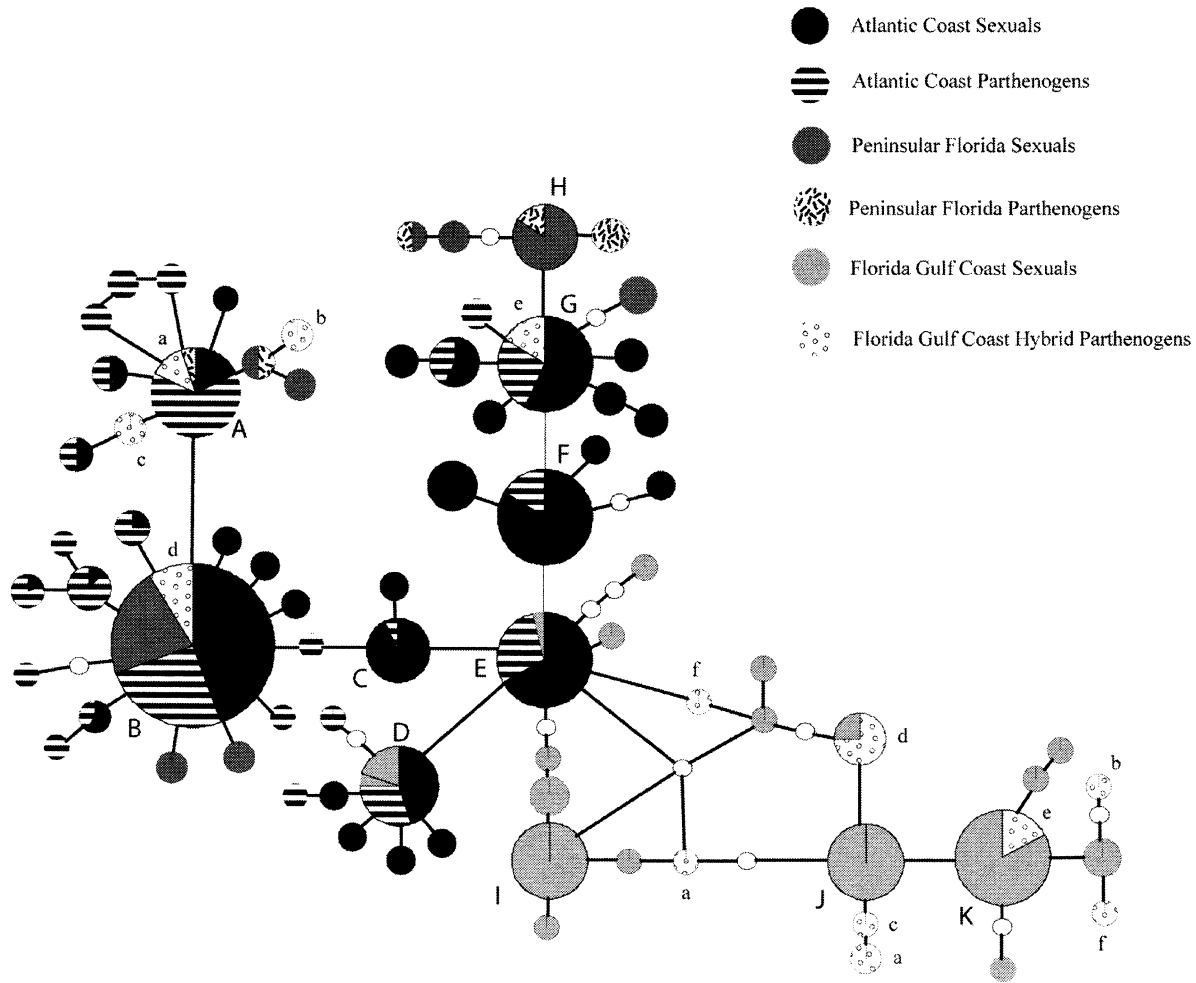


FIG. 6. Haplotype network estimated from the calmodulin intron sequences. Haplotype frequencies are proportional to circle area. Small open circles are inferred missing haplotypes that were not observed in the data. Lowercase letters represent alleles present in individual hybrid parthenogens and uppercase letters represent common haplotypes.

environments (Peck et al. 1998; Haag and Ebert 2004). Alternatively, greater geographic ranges of *Campeloma* parthenogens may also lessen local adaptation of parasites due to higher host dispersal rates of parthenogens. Distinguishing between these two explanations will require experimental studies of local adaptation of parasites, and fitness comparisons of sexual and parthenogenetic females under sperm limitation.

A recent study of the parthenogenetic New Zealand snail, *Potamopyrgus antipodarum*, revealed variation in ages of asexual lineages, and longer-lived asexual lineages were found in habitats with low frequencies of potential sexual competitors (Neiman et al. 2005). Also, a long-lived asexual walkingstick is geographically isolated from its sexual ancestor and occurs at higher latitudes (Law and Crespi 2002a). Although the ages of mtDNA parthenogenetic clades and limited intron divergence of parthenogenetic haplotypes from sexual haplotypes indicate that parthenogens only persist over short periods of time, there is variation in the ages of these lineages. Certain parthenogenetic lineages may have higher fitness and, therefore, be capable of expanding their ranges especially into marginal habitats. Additional evidence

suggests that parthenogenetic lineages differ in their persistence times, and some clones may have higher fitness. Based on intraclade sequence divergence, clade A parthenogens are longer lived than clade I parthenogens. I corroborated this finding by examining the frequency of rare calmodulin haplotypes in individuals from these two clades. Clade A asexuals have a significantly higher frequency of rare haplotypes than clade I asexuals (78.2% versus 18.2%, respectively;  $G = 14.4$ ,  $df = 1$ ,  $P < 0.001$ ). Clade A asexuals are also the most frequent (62% of all asexuals), consistent with higher fitness of these parthenogens. Recent work also indicates that certain aphid clones are widespread and common (Haack et al. 2000; Vorburger et al. 2003), although the exact mechanisms of their success remain unclear.

Assumptions about constant population sizes and no selection will lead to overestimates of clonal ages. Recent evidence indicates that the ratio of amino acid to silent substitutions ( $K_a/K_s$ ) in parthenogenetic *Campeloma* clades is six times higher than ratios in sexual clades (S. G. Johnson and R. Howard, unpubl. ms.), suggesting that parthenogenetic lineages accumulate slightly deleterious mutations. If purifying selection is weaker in parthenogenetic lineages and

most molecular clocks are based on sexually reproducing lineages, then clonal ages are probably overestimated using these molecular clocks. In the current study, ages of sexual lineages may be underestimated because sexual individuals from two clades (A and E) appear to represent reversions to sexuality, which is highly unlikely for various genetic and developmental reasons. The likeliest explanation is that these represent asexual females coexisting with sexual females within two localities. This hypothesis is further supported by high  $K_a/K_s$  ratios for these two clades compared to all other sexual clades (S. G. Johnson and R. Howard, unpubl. ms.). Future work will concentrate on distinguishing sexual and parthenogenetic females through segregation patterns of microsatellites.

*Enhanced Within-Individual Pairwise Differences of Triploid and Diploid Parthenogens and Limited Nuclear Divergence of Parthenogens from Sexual Ancestors*

In the present study, both parthenogens exhibit higher WIPD relative to their sexual counterparts. Elevated WIPD in Florida parthenogens was not unexpected given that they are derived from interspecific hybridization. As suggested by Normark (1999), polyploid hybrid asexuals may be the ideal group to examine whether asexual lineages are long lived because heterozygosity-destroying processes such as meiotic parthenogenesis or gene conversion may be minimized. The current study indicates that elevated WIPD has been maintained in *Campeloma* hybrid parthenogens, but many hybrid nuclear alleles are identical to nuclear haplotypes in the hybridizing sexual taxa. For example, triploid hybrids from the Choctawhatchee River ("e" in Cam network) contain divergent alleles present in Atlantic Coast parthenogens/sexuals and nearby sexual *C. geniculum* populations in the western Florida panhandle. However, some hybrid parthenogens show slight allelic divergence from both sexual ancestors. For example, hybrid parthenogens from the Ochlockonee River ("b") are two mutational steps from common ancestors in the Atlantic Coast and Florida panhandle.

In contrast to the expectation of elevated WIPD in interspecific hybrid parthenogens, there was no expectation that Atlantic Coast parthenogens would have elevated WIPD, because there was no evidence of elevated heterozygosity from allozyme variation (Johnson and Leefe 1999). However, Atlantic Coast parthenogens have significantly higher WIPD compared to Atlantic Coast sexuals. Studies of aphid parthenogens indicate that allozyme variation provide no evidence for hybrid origins, although other markers indicate hybrid origins of parthenogens (Hales et al. 1997; Delmotte et al. 2002, 2003). By integrating information from mtDNA and intron sequences, we can provide greater insight into hybrid origins of these diploid *Campeloma*. For example, diploid parthenogens throughout the Savannah River drainage occur exclusively in mtDNA clade A (see Appendix available online). These parthenogens have divergent alleles found primarily in two groups of common calmodulin haplotypes (A/B and G/F). Sexual A/B haplotypes are most frequent in the Ogeechee River, whereas sexual F/G haplotypes are most frequent in the Salkehatchie and Coosawatchie Rivers, suggesting that hybridization between divergent sex-

uals in these two river drainages may have led to the origin of these Savannah River parthenogens. Although there is strong evidence for hybrid origins of diploid parthenogens, these parthenogens show considerable variation in intron heterozygosity (0–7 sites). Given evidence for heterosis in *Campeloma* (Johnson 2005), comparison of fitness components of homozygous versus highly heterozygous clones may shed light on whether heterosis acts on this locus or associated loci.

*Diverse Parthenogenetic Origins from Atlantic Coast Campeloma*

There have been numerous independent origins of parthenogenesis, and Atlantic Coast sexuals are the maternal ancestor of these parthenogens. Based on calmodulin sequences, there have been numerous origins of Atlantic Coast parthenogens from common sexual haplotypes. Also, the high frequency of unique multilocus genotypes in these parthenogens suggests diverse origins from sexual ancestors. Another striking pattern is that both Atlantic Coast sexuals and parthenogens show elevated heterozygosity compared to Florida Panhandle sexuals, where more than 95% of individuals are homozygous. An unresolved issue is why there is a higher rate of origin of parthenogens from Atlantic Coast *Campeloma*, and no spontaneous or cryptic hybridization origins of parthenogens in the Florida Gulf Coast. As mentioned above, one possibility is that hybridization between individuals from genetically divergent clades occurs more readily in the Atlantic Coast. Given that well-differentiated clades are in close proximity in the Atlantic Coast, stream capture or river coalescence may allow exchange between drainages at small and large spatial scales. For example, highly divergent mtDNA clades C and J (7% average sequence divergence) occur primarily in the currently isolated Salkehatchie and Ogeechee Rivers, but coalescence of river drainages on the broad coastal plain during Pleistocene glacial periods may allow exchange (Webb 1990). Given that the area from northern Florida to the Savannah River is one of Remington's (1968) classic suture zones where hybridizing taxa meet, the elevated heterozygosity in this region may also derive from cryptic hybridization between peninsular Florida and Atlantic *Campeloma*. For example, clade I parthenogens appear to be derived from a Florida peninsula maternal ancestor. These parthenogens include an allotriploid parthenogen in the Florida Gulf Coast and diploid parthenogens in the Altamaha and Ogeechee Rivers on the Atlantic Coast, suggesting dispersal westward and northward after the initial hybridization. Why cryptic hybridization has not led to parthenogens in the Florida Gulf coast may be due to very little spatial overlap of major sexual clades in this region.

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