

# Clonal diversity and polyphyletic origins of hybrid and spontaneous parthenogenetic *Campeloma* (Gastropoda: Viviparidae) from the south-eastern United States

S. G. JOHNSON & W. R. LEEFE

Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA

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## Abstract

Some theories for the maintenance of sexual reproduction indicate that parthenogens may persist if there is high clonal diversity and high dispersal rates. Using allozymic variation, we report on the origin, clonal diversity and population structure of hybrid and spontaneous parthenogens from south-eastern United States populations of the freshwater snail *Campeloma*. Independent origins of triploid hybrid parthenogens in the Florida panhandle occurred by hybridization between an Atlantic coastal species (*C. limum* or *C. floridense*) and the Florida sexual species (*C. geniculum*). Allozyme genotypic diversity is similar between these hybrid parthenogens and sexuals. Diploid spontaneous parthenogens originated multiple times from nonlocal *C. limum* sexual populations in Atlantic coastal rivers, and levels of genotypic diversity are significantly higher in sexual *C. limum*. How parthenogens originate, the degree of clonal diversity, and their subsequent dispersal influence whether basic assumptions of evolution-of-sex models are met.

## Introduction

In lineages capable of parthenogenetic and sexual reproduction, a fundamental paradox is why parthenogenetic females do not rapidly replace sexual females (Maynard Smith, 1971; Williams, 1975). Because parthenogenetic females only produce the fecund sex while sexual females produce 50% males, parthenogenetic females have a two-fold advantage every generation and should quickly replace sexual females. Given the cost of sexual reproduction, plausible theories for the maintenance of sexual reproduction must outline mechanisms by which sexual females can recoup a two-fold benefit. Under the Red Queen model, sexual progeny may temporarily escape infection by common parasite genotypes whereas clonal females cannot generate rare resistance genotypes (Hamilton, 1980; Bell, 1982; Hamilton *et al.*, 1990; Lively, 1996). If parasites are highly virulent and have high transmission rates, sexual females can replace asexual females (Howard & Lively,

1994). However, the Red Queen hypothesis and other evolution-of-sex models rest on two critical assumptions: (1) the all-else-equal assumption; and (2) the population structure and genetic diversity of sexual and parthenogenetic populations.

Whether the 'all-else-equal' assumption is met depends on how parthenogens originate (Vrijenhoek, 1989). The two-fold cost to sex is more likely to be correct if parthenogenetic females arise from local sexual populations and have similar fecundity to sexual females. Parthenogens either arise from hybridization between genetically divergent sexual species or arise spontaneously from within a single sexual population (Suomalainen *et al.*, 1987; Johnson, 1992). Most hybrid parthenogens violate the all-else-equal assumptions: they are usually highly heterozygous polyploids whereas the sexual ancestors are diploid and have lower levels of heterozygosity. If there is heterosis for fitness in hybrid parthenogens then the cost of sex is more than two-fold. Spontaneous parthenogens may be more similar to ancestral sexuals, although the mechanism of parthenogenesis may influence fitness. Forms of automictic parthenogenesis that are analogous to selfing could result initially in considerable fitness reduction (Templeton, 1982).

Correspondence: Dr Steven G. Johnson, Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA.  
Tel: +1 504 280 7040; fax: +1 504 280 6121;  
e-mail: sgjohnso@uno.edu

The population structure of parthenogens can influence whether parthenogens can escape elimination by virulent parasites (Ladle *et al.*, 1993; Judson, 1995). Under metapopulation structure combined with dispersal, parthenogens may be able to persist indefinitely if the asymmetry between host and parasite dispersal is large (Ladle *et al.*, 1993; Judson, 1995). If parthenogens have high dispersal rates compared with parasites, they can escape parasites and persist indefinitely within the metapopulation. Higher dispersal rates of parthenogens into marginal habitats may also reduce the strength of selection against deleterious mutations (Peck, 1996; Lively *et al.*, 1998). In the case of competition in stable habitats, synergistic effects of mutations may be enhanced, whereas selection against deleterious mutations is relaxed in asexuals inhabiting marginal habitats where competition is reduced.

Another assumption of ecological models of sex such as the Tangled Bank and the Red Queen is that sexual populations harbour higher levels of genetic diversity. However, drift erodes genetic variation in small sexual populations, and numerous mechanisms can generate high levels of clonal diversity. In recent simulation studies, parasites can select for clonal diversity and the advantage to sex diminishes as clonal diversity increases (Lively & Howard, 1994). Mutational divergence within monophyletic clonal lineages or multiple (polyphyletic) origins generate clonal diversity in hybrid and spontaneous parthenogens. Polyphyletic origins of parthenogenesis are the primary means in clonal invertebrate taxa (Harshman & Futuyma, 1985; Crease *et al.*, 1989; Dufresne & Hebert, 1994, 1995, 1997; Dybdahl & Lively, 1995). A common pathway for the origin of allotriploid parthenogens is the 'genome addition' hypothesis (Schultz, 1969; Quattro *et al.*, 1992). Under this hypothesis, matings between diploid hybrids and males from one or both species produce allotriploid parthenogens. Repeated hybridization and backcrossing between sexual populations with high levels of genetic diversity will elevate clonal diversity. Spontaneous parthenogens capture a portion of the genetic diversity present in the sexual populations. These parthenogens do not have elevated levels of heterozygosity and, except for mutational divergence, contain alleles that are present in sexual populations. Clonal diversity will depend upon the rate at which spontaneous parthenogens spin off from sexual populations, the dispersal rate of parthenogenetic females and the strength of selection on parthenogens.

In the present study, we describe the origin and clonal diversity of hybrid and spontaneous parthenogens in Southeastern United States populations of the freshwater snail, *Campeloma*. Using allozymic variation, we specifically address four major aspects of the origin and maintenance of these parthenogens: (1) test for deviations in sex ratio and H-W equilibrium, and assess ploidy levels of parthenogens; (2) determine whether parthe-

nogenesis arose via hybrid or spontaneous origins in *Campeloma*, and determine the ancestral sexual species of hybrid parthenogens; (3) compare indices of genotypic diversity between sympatric sexuals and parthenogens; and (4) determine the number of independent origins of parthenogenesis, and assess population structure and dispersal of *C. limum* parthenogens. First, we outline previous studies of parthenogenesis in *Campeloma*.

*Campeloma* is an ovoviviparous, dioecious snail found in lakes and rivers throughout eastern North America (Burch, 1989). Johnson (1992) demonstrated previously that highly heterozygous clones in glaciated regions of the northern US arose by hybridization. These hybrid parthenogens dominated northern environments and there was low clonal diversity within or among populations. Spontaneous parthenogens were rare in northern environments, but they were the only parthenogens found during limited sampling in North Carolina. No allozymic variation was present within or among parthenogenetic and sexual populations. The low levels of allozymic variability in parthenogens and sexuals violate the assumption of greater genetic variation in sexuals. However, because of the large geographical separation of sexuals and both hybrid and spontaneous parthenogens, tests of the Red Queen hypothesis by correlating parasite loads (castrating digenetic trematodes) and levels of recombination were not possible. In the present study, we focused on *Campeloma* populations in the southeastern United States where greater levels of allozymic variation are present, and sexuals and parthenogens coexist on a smaller geographical scale. Another advantage of studying patterns of genetic variation in southeastern US *Campeloma* is that the historical factors involved in isolation and potential dispersal of freshwater organisms in this region are well understood (Bermingham & Avise, 1986; Avise, 1992, 1996).

## Materials and methods

### *Campeloma* species and populations

We sampled 55 populations of five *Campeloma* species throughout the south-eastern United States (Table 1; Fig. 1). We sampled *Campeloma decisum* from rivers draining into Lake Pontchartrain to the Pascagoula River in Mississippi. We collected *Campeloma geniculum* from the Choctawhatchee river drainage in the Florida panhandle to the Suwannee river in northern peninsular Florida. Found in isolated river drainages of the Florida panhandle, *Campeloma parthenum* may be a triploid hybrid parthenogen, although the putative sexual ancestors are unknown. Earlier work indicated that *C. parthenum* is a triploid, fixed-heterozygote (Karlín *et al.*, 1980; Dougherty, 1982). *Campeloma floridense* is an insular endemic species restricted to the St. John's river drainage in eastern Florida. We collected *Campeloma limum* from the upper Suwannee river drainage in

**Table 1** Population characteristics. For each population the following are given: geographical coordinates, sex ratio (% male), reproductive mode (RM: Sex = Sexual, HPar = Hybrid Parthenogen, SPar = Spontaneous Parthenogen), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), inbreeding coefficient ( $f$ ), genotypic diversity ( $G_o$ ), evenness (Ev), and number of multilocus genotypes and sample size ( $\#/n$ ). The number before each population refers to that given in Fig. 1.

| Species and population           | Coordinates     | Sex ratio | RM       | $H_o$ | $H_e$ | $f$      | $G_o$ | Ev   | $\#/n$ |
|----------------------------------|-----------------|-----------|----------|-------|-------|----------|-------|------|--------|
| <i>C. decisum</i>                |                 |           |          |       |       |          |       |      |        |
| 1. Twelve-mile Cr.               | 30.46°N 90.41°W | 43.8      | Sex      | 0.042 | 0.066 | 0.37     | 1.87  | 0.63 | 4/20   |
| 2. Colyell Bay                   | 30.20°N 90.50°W | 48.0      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 3. Natalbany R.                  | 30.26°N 90.33°W | 63.7      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 4. Big Creek                     | 30.48°N 90.27°W | 60.0      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 5. Tchefuncta R.                 | 30.49°N 90.18°W | 35.5      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 6. Pearl R.                      | 30.47°N 89.48°W | 55.1      | Sex      | 0.022 | 0.040 | 0.45     | 1.51  | 0.57 | 3/15   |
| 7. White Sandy Cr.               | 30.49°N 89.38°W | 43.0      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 8. Pascagoula R.                 | 30.38°N 88.36°W | 40.5      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| <i>C. parthenum/C. geniculum</i> |                 |           |          |       |       |          |       |      |        |
| 9. Yellow R.                     | 30.45°N 86.38°W | 0.1       | HPar     | 0.667 | 0.343 | -1.000*  | 1.26  | 0.52 | 2/17   |
| 10. Choctawhatchee R.            | 30.57°N 85.51°W | 41.9      | Sex/HPar | 0.012 | 0.036 | 0.66*    | 2.06  | 0.75 | 2/20   |
| 11. Parrot Cr.                   | 30.58°N 85.51°W | 38.1      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 12. Ten Mile Cr.                 | 30.53°N 85.43°W | 49.0      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 13. Wright's Cr.                 | 30.52°N 85.42°W | 45.2      | Sex      | 0.042 | 0.049 | 0.16     | 1.80  | 0.68 | 3/20   |
| 14. Holmes Cr.                   | 30.44°N 85.37°W | 42.7      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 15. Holmes Cr.                   | 30.55°N 85.33°W | 52.9      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 16. Holmes Cr.                   | 30.47°N 85.37°W | 45.6      | Sex      | 0.017 | 0.016 | -0.03    | 1.22  | 0.29 | 2/20   |
| 17. Holmes Cr.                   | 30.58°N 85.32°W | 0.4       | HPar     | 0.633 | 0.341 | -0.90*   | 1.10  | 0.29 | 2/20   |
| 18. Holmes Cr.                   | 30.59°N 85.31°W | 4.8       | HPar     | 0.600 | 0.354 | -0.73*   | 1.22  | 0.47 | 2/20   |
| 19. Wright's Cr.                 | 30.58°N 85.37°W | 10.8      | HPar     | 0.633 | 0.349 | -0.85*   | 1.10  | 0.29 | 2/20   |
| 20. Wright's Cr.                 | 30.59°N 85.36°W | 0.0       | HPar     | 0.667 | 0.342 | -1.00*   | 1.00  | 0.00 | 1/20   |
| 21. Cowart's Cr.                 | 31.01°N 85.14°W | 46.5      | Sex      | 0.033 | 0.047 | 0.30     | 1.65  | 0.63 | 3/20   |
| 22. Chipola R.                   | 30.48°N 85.13°W | 54.0      | Sex      | 0.071 | 0.110 | 0.36     | 2.97  | 0.86 | 4/14   |
| 23. Dry Cr.                      | 30.41°N 85.14°W | 47.5      | Sex      | 0.052 | 0.059 | 0.12     | 2.03  | 0.76 | 3/16   |
| 24. Ochlockonee R.               | 30.35°N 84.22°W | 42.6      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 25. Ochlockonee R.               | 30.40°N 84.18°W | 47.4      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 26. Ochlockonee R.               | 30.44°N 84.14°W | 50.6      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/19   |
| 27. Little R.                    | 30.31°N 84.32°W | 0.0       | HPar     | 0.667 | 0.344 | -1.00*   | 1.00  | 0.00 | 1/16   |
| 28. Suwanee R.                   | 30.23°N 83.11°W | 58.0      | Sex      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| <i>C. limum/C. floridense</i>    |                 |           |          |       |       |          |       |      |        |
| 29. Santa Fe R.                  | 29.55°N 82.26°W | 0.0       | Par      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 30. Little R.                    | 31.23°N 83.32°W | 0.0       | Par      | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 31. Alapaha R.                   | 31.32°N 83.24°W | 0.0       | Par      | 0.010 | 0.010 |          | 1.12  | 0.32 | 2/17   |
| 32. Ocmulgee R                   | 31.47°N 82.59°W | 0.0       | SPar     | 0.075 | 0.060 | -0.27    | 1.98  | 0.99 | 2/20   |
| 33. Sturgeon Cr.                 | 31.46°N 83.04°W | 0.0       | SPar     | 0.096 | 0.084 | -0.15    | 2.80  | 0.96 | 3/19   |
| 34. Spar Lake                    | 31.47°N 82.56°W | 0.0       | SPar     | 0.158 | 0.111 | -0.44    | 1.90  | 0.66 | 4/20   |
| 35. Little Ocmulgee R            | 31.56°N 82.40°W | 0.0       | SPar     | 0.019 | 0.114 | 0.84***  | 2.49  | 0.78 | 4/18   |
| 36. Ohoopsee R.                  | 32.23°N 82.19°W | 0.0       | SPar     | 0.042 | 0.037 | -0.12    | 1.60  | 0.81 | 2/20   |
| 37. Ohoopsee R.                  | 32.26°N 82.23°W | 0.0       | SPar     | 0.017 | 0.082 | 0.80***  | 2.30  | 0.84 | 3/20   |
| 38. Ohoopsee R.                  | 32.30°N 82.30°W | 0.0       | SPar     | 0.025 | 0.152 | 0.84***  | 2.94  | 0.79 | 5/20   |
| 39. Canoochee R.                 | 32.09°N 81.47°W | 0.0       | SPar     | 0.167 | 0.085 | -1.00*** | 1.00  | 0.00 | 1/20   |
| 40. Ogeechee R                   | 32.52°N 82.19°W | 0.0       | SPar     | 0.017 | 0.016 | -0.03    | 1.22  | 0.47 | 2/20   |
| 41. Ogeechee R                   | 32.49°N 82.08°W | 27.1      | Sexual   | 0.070 | 0.059 | -0.21    | 2.11  | 0.77 | 3/19   |
| 42. Horse Creek                  | 32.41°N 81.50°W | 35.7      | Sexual   | 0.033 | 0.031 | -0.09    | 1.47  | 0.72 | 2/20   |
| 43. Buckhead Creek               | 32.48°N 81.58°W | 47.1      | Sexual   | 0.058 | 0.054 | -0.09    | 1.68  | 0.67 | 3/20   |
| 44. Ogeechee R                   | 32.36°N 81.46°W | 0.0       | SPar     | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 45. Brier Cr.                    | 32.52°N 81.37°W | 0.0       | SPar     | 0.133 | 0.097 | -0.39*** | 1.72  | 0.56 | 5/20   |
| 46. Savannah R.                  | 32.56°N 81.30°W | 0.0       | SPar     | 0.000 | 0.000 |          | 1.00  | 0.00 | 1/20   |
| 47. Little Hell Landing          | 33.04°N 81.34°W | 0.0       | SPar     | 0.008 | 0.008 | 0.00     | 1.11  | 0.29 | 2/20   |
| 48. Lower Three-Runs Cr.         | 33.04°N 81.29°W | 50.0      | Sexual   | 0.050 | 0.085 | 0.42     | 2.94  | 0.99 | 3/20   |
| 49. Little Salkehatchie R.       | 33.04°N 80.55°W | 47.1      | Sexual   | 0.108 | 0.104 | -0.04    | 3.64  | 0.88 | 5/20   |
| 50. Salkehatchie R.              | 33.03°N 81.06°W | 50.0      | Sexual   | 0.026 | 0.025 | -0.06    | 1.34  | 0.63 | 2/19   |

Table 1 (Continued)

| Species and population | Coordinates     | Sex ratio | RM     | $H_o$ | $H_e$ | $f$   | $G_o$ | Ev   | #/n   |
|------------------------|-----------------|-----------|--------|-------|-------|-------|-------|------|-------|
| 51. Whippy Swamp       | 32.57°N 81.03°W | 41.4      | Sexual | 0.114 | 0.106 | -0.07 | 4.25  | 0.94 | 5/19  |
| 52. Coosawhatchie      | 32.48°N 81.03°W | 44.3      | Sexual | 0.075 | 0.085 | 0.12  | 2.82  | 0.97 | 3/20  |
| 53. Lowndes Lake       | 32.46°N 81.01°W | 38.0      | Sexual | 0.100 | 0.082 | -0.23 | 2.17  | 0.82 | 3/20  |
| 54. S. Fork Edisto     | 33.29°N 81.19°W | 47.1      | Sexual | 0.044 | 0.071 | 0.39* | 1.69  | 0.64 | 3/19  |
| 55. Alexander Springs  | 29.05°N 81.34°W | 40.0      | Sexual | 0.183 | 0.242 | 0.25  | 10.53 | 0.96 | 13/20 |

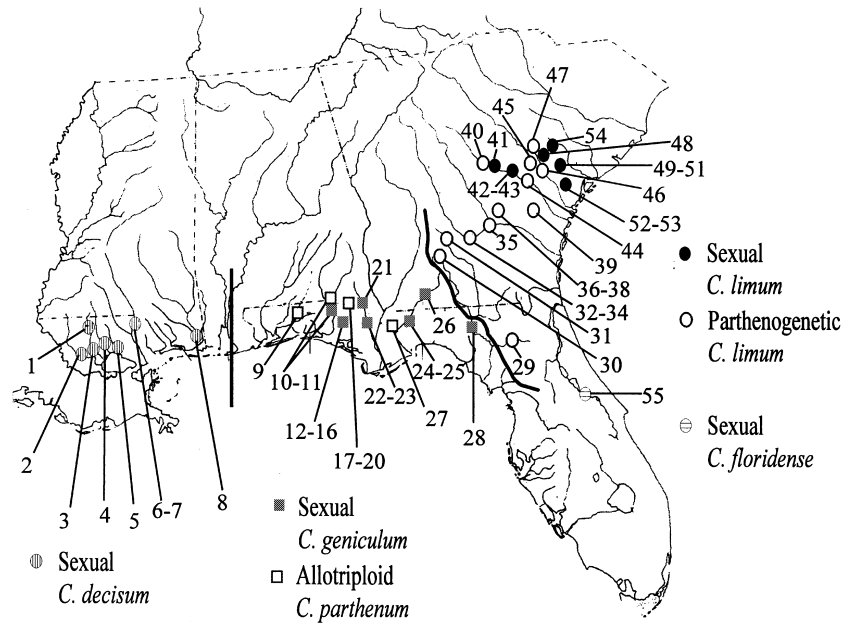


Fig. 1 Map of collection sites. Solid heavy lines represent major genetic discontinuities among Southeastern US *Campeloma*. The name of each population is given in Table 1.

northern peninsular Florida to the Edisto river drainage in South Carolina.

Except where noted (Table 1), we collected approximately 75 individuals within each population along a 15-m transect running parallel to the riverbank. We transported snails to the laboratory where we determined the sex of each individual and stored snails at  $-80^{\circ}\text{C}$ .

### Allozyme analysis

We used allozyme variation to examine origins and variation of sexuals and parthenogens. Before electrophoresis, small portions of gill, foot and digestive gland were placed in 80–100  $\mu\text{L}$  of distilled water, homogenized for 10 s and centrifuged at 10 000 r.p.m. for 15 min at  $4^{\circ}\text{C}$ . We performed cellulose acetate electrophoresis. Six enzyme loci showed excellent resolution and were polymorphic among all populations (*Gpi*, *Pgm-1*, *Pgm-2*, *Idh*, *Pgdh* and *Acoh*). Stain and buffer recipes followed Richardson *et al.* (1986). We used the following buffers: Tris-Maleate (*Acoh* and *Idh*), Phosphate (*Gpi* and *Pgm*) and Tris-EDTA-Borate (*Pgdh*). We conducted elec-

trophoresis at  $4^{\circ}\text{C}$  and run times varied between 20 and 40 min.

### Statistical analyses

We calculated observed and expected heterozygosity for sexual and spontaneous parthenogenetic populations using Genepop v.3.1 (Raymond & Rousset, 1995). For hybrid parthenogens, we choose an allele from each ancestral sexual species. We calculated the inbreeding coefficient,  $f$ , and exact probability tests of significant deviations from H-W (excess homozygotes or excess heterozygotes) for single loci and for each population. We used sequential Bonferroni techniques within loci and species to minimize Type I error in multiple comparisons (Rice, 1989).

Elevated levels of heterozygosity and significant deviations from H-W characterize hybrid parthenogens. To determine the putative sexual ancestors, we determined which four sexual species could possibly have contributed alleles at all six loci in the hybrid parthenogens. We inferred triploidy if individuals have three alleles or if

dosage patterns of the alleles differ. In dimeric enzymes, triploid heterozygotes may express an unbalanced staining intensity of 2:2:1 instead of the typical pattern of 1:2:1 in diploids.

We calculated the observed genotypic diversity of each population as  $G_o = 1/\sum p_i^2$ , where  $p_i$  is the frequency of the  $i$ th multilocus genotype for  $k$  multilocus genotypes (Stoddart, 1983).  $G_o$  varies from 1 (monoclonal populations) to a maximum that equals the total number of multilocus genotypes if there is one individual of each genotype. We calculated the evenness of genotypic diversity as  $\text{Evenness} = H'/\ln S$ , where  $S$  = total number of multilocus genotypes in sample and  $H' = -\sum p_i \ln p_i$  ( $p_i$  is the proportion of individuals of  $i$ th multilocus genotype for  $k$  multilocus genotypes in the sample). Evenness is constrained between 0 (extreme unevenness) and 1 (complete evenness).

For observed heterozygosity and both measures of diversity, we used Mann–Whitney to test the null hypothesis that observed heterozygosity or diversity does not differ between sympatric sexuals and parthenogens.

### Phylogeny reconstruction and genetic differentiation among sympatric sexuals and parthenogens

We computed the distance measure (mean character differences) from allele frequencies using Paup 4\* (Swofford, 1998). We constructed trees using minimum evolution as the optimality criterion and tree-bisection-reconnection (TBR) as the branch-swapping algorithm. We obtained starting trees for branch-swapping by neighbour-joining. We estimated node support by bootstrapping (100 replicates) the minimum evolution tree. We rooted the phylogram using allele frequencies from *Lioplax pilbyri*, which is a member of the same subfamily as *Campeloma* (Viviparinae). We mapped changes in reproductive mode along the minimum evolution tree, assuming transitions from sex to parthenogenesis are irreversible (MacClade; Maddison & Maddison, 1992).

We tested genetic differentiation between sexual *C. limum* and spontaneous *C. limum* populations inhabiting the same river drainage (Savannah and Ogeechee rivers) using exact tests of allele frequency differences in GENEPOP v.3.1 (Raymond & Rousset, 1995). Within river drainages, parthenogenetic populations were tested for significant genic differentiation at polymorphic loci. If there was no differentiation, we pooled individuals and then tested for genic differentiation between sexuals and parthenogens at individual loci.

## Results

### Sex ratio, H–W equilibrium and ploidy levels

Collecting localities of the 55 populations are given in Table 1 and Fig. 1. Sex ratios for each population are also

in Table 1. We distinguished sexual populations from parthenogenetic populations by sex ratio. Average ( $\pm 1$  SE) sex ratio (percentage males) in *C. decisum*, *C. geniculum* and sexual *C. limum* was nearly even ( $48.7 \pm 3.5$ ,  $45.9 \pm 1.4$  and  $42.5 \pm 2.1$ , respectively). The average sex ratio in *C. parthenum* was  $2.7 \pm 1.8$ . Based on allozymic banding patterns presented below, there is a low frequency of sexuals in some of these *C. parthenum* parthenogenetic populations. Parthenogenetic *C. limum* populations have all-female populations (Table 1).

Allele frequencies at the six polymorphic loci are shown in Table 2. Based on  $f$ -values, sexual populations of *C. decisum*, *C. geniculum* and *C. limum* were in H–W equilibrium (Table 1). *Campeloma parthenum* populations had a significant excess of heterozygotes, with most  $f$ -values approaching  $-1$ , suggesting hybrid origins. Of the 12 parthenogenetic *C. limum* populations that were polymorphic, five had significant  $f$ -values. Three populations in the Altamaha drainage (35, 37 and 38) had excess homozygotes and two populations in the Savannah (45) and Canoochee (39) rivers had excess heterozygotes.

In *C. parthenum* from Holmes and Wrights Creek (17–20), the presence of three alleles (C, D and E) at *Acoh* (a monomeric enzyme) in each individual and symmetric band intensities are consistent with triploidy. Also, individuals with two alleles had asymmetric band intensities in the expected proportion in dimeric (*Gpi*, *Idh* and *Pgdh*) and monomeric (*Acoh*) enzymes. In heterozygous *C. limum* from all-female populations, only two alleles are present and band intensity was symmetrical at *Acoh* and *Pgdh*, which is consistent with diploidy.

**Table 2** Allele frequencies in sexual *C. decisum*.

| Locus        | Drainages & populations           |      |      |      |      |       |      |            |
|--------------|-----------------------------------|------|------|------|------|-------|------|------------|
|              | Lake Ponchartrain & Lake Maurepas |      |      |      |      | Pearl |      | Pascagoula |
|              | 1                                 | 2    | 3    | 4    | 5    | 6     | 7    | 8          |
| <i>Gpi</i>   |                                   |      |      |      |      |       |      |            |
| A            | 0.17                              | –    | –    | –    | –    | –     | –    | –          |
| C            | 0.83                              | 1.00 | 1.00 | 1.00 | 1.00 | 0.87  | 1.00 | 1.00       |
| D            | –                                 | –    | –    | –    | –    | 0.13  | –    | –          |
| <i>Pgm-1</i> |                                   |      |      |      |      |       |      |            |
| C            | 1.00                              | 1.00 | 1.00 | 1.00 | 1.00 | 1.00  | 1.00 | 1.00       |
| <i>Pgm-2</i> |                                   |      |      |      |      |       |      |            |
| A            | 0.05                              | –    | –    | –    | 1.00 | 1.00  | 1.00 | 1.00       |
| B            | 0.95                              | 1.00 | 1.00 | 1.00 | –    | –     | –    | –          |
| <i>Idh</i>   |                                   |      |      |      |      |       |      |            |
| B            | 1.00                              | 1.00 | 1.00 | 1.00 | 1.00 | 1.00  | 1.00 | 1.00       |
| <i>Pgdh</i>  |                                   |      |      |      |      |       |      |            |
| C            | 1.00                              | 1.00 | 1.00 | 1.00 | 1.00 | 1.00  | 1.00 | 1.00       |
| <i>Acoh</i>  |                                   |      |      |      |      |       |      |            |
| D            | 1.00                              | 1.00 | 1.00 | 1.00 | 1.00 | 1.00  | 1.00 | 1.00       |



**Table 2** (Continued) Allele frequencies in sexual *C. limum* (bold type), spontaneous parthenogenetic *C. limum* and *C. floridense*.

|              |   | Drainages & populations |      |      |      |      |          |      |      |      |      |          |      |      |      |      |          |      |      |      |      |                            |      |      |      |      |                                 |      |  |
|--------------|---|-------------------------|------|------|------|------|----------|------|------|------|------|----------|------|------|------|------|----------|------|------|------|------|----------------------------|------|------|------|------|---------------------------------|------|--|
|              |   | Suwannee                |      |      |      |      | Altamaha |      |      |      |      | Ogeechee |      |      |      |      | Savannah |      |      |      |      | Coosawhatchie/Salkehatchie |      |      |      |      | Canochee, Edisto, and St. Johns |      |  |
| Locus        |   | 29                      | 30   | 31   | 32   | 33   | 34       | 35   | 36   | 37   | 38   | 40       | 41   | 42   | 43   | 44   | 45       | 46   | 47   | 48   | 49   | 50                         | 51   | 52   | 53   | 39   | 54                              | 55   |  |
| <i>Gpi</i>   | C | 1.00                    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                       | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                            | 1.00 |  |
|              | D | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | 0.17                       | -    | -    | -    | -    | -                               | 0.03 |  |
|              |   |                         |      |      |      |      |          |      |      |      |      |          |      |      |      |      |          |      |      |      |      |                            |      |      |      |      |                                 |      |  |
| <i>Pgm-1</i> | A | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | 0.05 | -        | -    | -    | -    | -    | -                          | -    | -    | -    | -    | -                               | -    |  |
|              | B | 1.00                    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 0.95 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                       | 1.00 | 1.00 | 1.00 | 1.00 | 0.50                            | 1.00 |  |
|              | C | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -                          | -    | -    | -    | -    | 0.50                            | -    |  |
| <i>Pgm-2</i> | B | 1.00                    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                       | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                            | 0.84 |  |
|              | C | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -                          | 0.08 | -    | -    | -    | -                               | 0.16 |  |
|              |   |                         |      |      |      |      |          |      |      |      |      |          |      |      |      |      |          |      |      |      |      |                            |      |      |      |      |                                 |      |  |
| <i>Idh</i>   | A | 1.00                    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                       | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                            | 1.00 |  |
|              |   |                         |      |      |      |      |          |      |      |      |      |          |      |      |      |      |          |      |      |      |      |                            |      |      |      |      |                                 |      |  |
| <i>Pgdh</i>  | A | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | -    | -    | -    | -    | -                          | -    | -    | -    | -    | -                               | 0.65 |  |
|              | B | -                       | -    | -    | -    | -    | -        | -    | -    | -    | -    | -        | 0.03 | -    | -    | -    | -        | -    | -    | -    | -    | -                          | -    | -    | -    | -    | -                               | -    |  |
|              | C | 1.00                    | 1.00 | 0.97 | 0.23 | 0.11 | 0.38     | 0.86 | 0.88 | 0.40 | 0.30 | 0.05     | 0.79 | 1.00 | 0.88 | 1.00 | 1.00     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                       | 1.00 | 1.00 | 1.00 | 1.00 | 1.00                            | 0.35 |  |
|              | D | -                       | -    | 0.03 | 0.77 | 0.89 | 0.62     | 0.14 | 0.12 | 0.60 | 0.70 | 0.95     | 0.18 | -    | 0.12 | -    | -        | -    | -    | -    | -    | -                          | -    | -    | -    | -    | -                               | -    |  |
| <i>Acch</i>  | A | -                       | -    | -    | -    | -    | -        | -    | -    | 0.18 | -    | -        | -    | -    | -    | -    | 0.08     | -    | 0.03 | 0.45 | -    | -                          | -    | -    | -    | -    | -                               | -    |  |
|              | B | -                       | -    | 1.00 | 0.00 | 0.38 | -        | 0.31 | -    | -    | 0.10 | -        | 1.00 | 0.10 | -    | -    | 0.45     | -    | -    | -    | 0.20 | -                          | 0.50 | 0.48 | 0.40 | -    | 0.95                            | 0.60 |  |
|              | C | 1.00                    | 1.00 | 0.00 | 1.00 | 0.62 | 1.00     | 0.69 | 1.00 | 0.82 | 0.90 | 1.00     | -    | 0.90 | 1.00 | 1.00 | 0.47     | 1.00 | 0.97 | 0.55 | 0.80 | 1.00                       | 0.21 | 0.52 | 0.48 | 1.00 | 0.05                            | 0.40 |  |

**Table 3** Comparison of alleles present at six polymorphic enzyme loci in sexual *C. decisum*, sexual *C. geniculum*, hybrid parthenogenetic *C. parthenum*, sexual *C. limum* and spontaneous parthenogenetic *C. limum*. Single locus genotypes are given for triploid hybrid *C. parthenum* and numbers represent populations in Table 1 and Fig. 1.

| Species                         | Locus                  |                               |                          |                          |                          |                               |                          |
|---------------------------------|------------------------|-------------------------------|--------------------------|--------------------------|--------------------------|-------------------------------|--------------------------|
|                                 | <i>Gpi</i>             | <i>Pgm-1</i>                  | <i>Pgm-2</i>             | <i>ldh</i>               | <i>Pgdh</i>              | <i>Acoh</i>                   |                          |
| <i>C. decisum</i>               | A, C, D                | C                             | A, B                     | B                        | C                        | D                             |                          |
| <i>C. geniculum</i>             | A, C                   | B                             | B, C                     | B                        | C, D, E                  | D, E                          |                          |
| <i>C. parthenum</i>             | 9<br>10<br>17–20<br>27 | AAC, ACC<br>CCD<br>AAC<br>ACC | BBB<br>BBB<br>BBB<br>BBB | BBB<br>BBB<br>BBB<br>BBB | ABB<br>ABB<br>ABB<br>AAB | CCE<br>CCC<br>CCE, CEE<br>CCE | CDD<br>CEE<br>CDE<br>BBD |
| Sexual <i>C. limum</i>          | C, D                   | B                             | B                        | A                        | A, B, C, D               | A, B, C                       |                          |
| Parthenogenetic <i>C. limum</i> | C                      | B, C                          | B                        | A                        | C, D                     | A, B, C                       |                          |
| <i>C. floridense</i>            | C                      | B                             | B, C                     | A                        | A, C                     | B, C                          |                          |

### Hybrid or spontaneous origins of parthenogenesis?

Average ( $\pm$ SE) observed heterozygosity was significantly higher in *C. parthenum* compared with *C. geniculum* ( $0.645 \pm 0.01$  and  $0.017 \pm 0.026$ , respectively; Mann–Whitney  $U = 0.000$ ,  $P < 0.0001$ ). All hybrid parthenogens were fixed heterozygotes at four of the six enzyme loci. An examination of the combination of alleles in *C. parthenum* indicated that *C. geniculum* and either *C. limum* or *C. floridense* were the ancestral species. *Campeloma parthenum* had one of the alleles present in *C. limum* or *C. floridense* at all six loci (Table 3). This finding is also consistent with the mtDNA evidence that *C. limum* or *C. floridense* is the maternal ancestor of these hybrid clones (Johnson & Bragg, submitted). Likewise, the allozyme signature of sexual *Campeloma geniculum* is found at all loci in the hybrid clones, suggesting that *C. geniculum* was the paternal ancestor of these hybrid clones. This is not surprising given that all hybrid clones are sympatric with *C. geniculum*. At *Acoh*, the presence of allele E in the hybrid clones also implicates *C. geniculum* as the paternal ancestor because this allele is only present in *C. geniculum*. We reject *Campeloma decisum* as the paternal ancestor of these hybrid clones because hybrids should be fixed heterozygotes at PGM-1 if the hybridization event involved *C. decisum* and any other species. Additionally, given the mitochondrial evidence, we can exclude *Campeloma decisum* because allele E at *Pgdh* and allele E at *Acoh* in the hybrid clones were not found in *C. decisum*.

Average ( $\pm$ SE) observed heterozygosity was significantly lower in spontaneous parthenogenetic *C. limum* relative to sexual *C. limum* ( $0.048 \pm 0.015$  and  $0.078 \pm 0.014$ , respectively;  $U = 45.4$ ,  $P = 0.036$ ), suggesting that these clones did not originate from interspecific hybridization. Except for the C allele at *Pgm-1* in the Canoochee river population (39), all alleles in parthenogenetic *C. limum* were a subset of alleles found in sexual *C. limum* (Table 3). Lower heterozygosity of parthenogens and the ability to account for nearly all

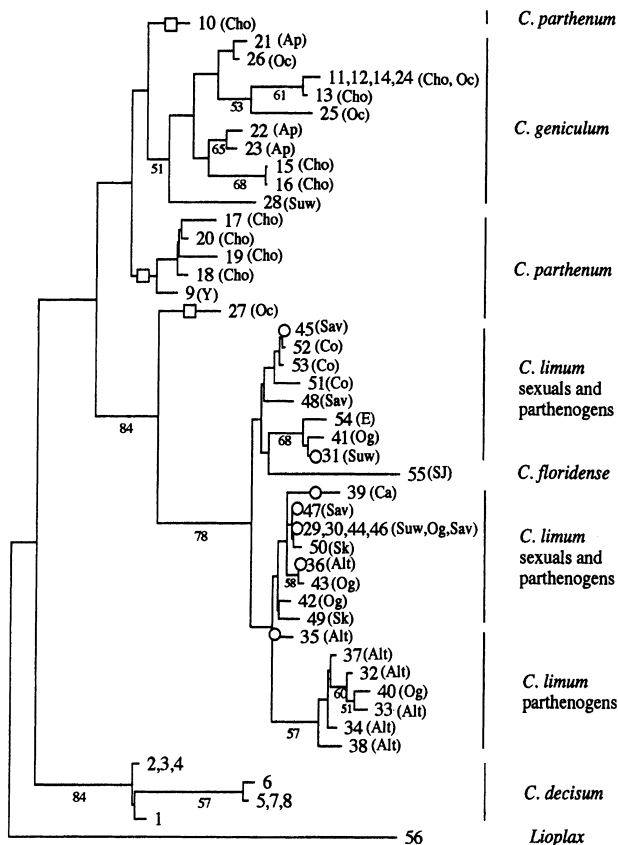
alleles in parthenogens from sexuals suggest that these parthenogens arose spontaneously from sexual populations. Two plausible explanations can account for fixed heterozygotes (BC) at PGM-1 in the Canoochee river: mutational divergence after parthenogenesis or we did not sample the C allele in sexual populations due to its rarity.

### Genotypic diversity and evenness in sympatric sexuals and parthenogens

Mean genotypic diversity ( $\pm$ SE) did not differ significantly between *C. geniculum* and *C. parthenum* ( $1.37 \pm 0.18$  and  $1.11 \pm 0.04$ , respectively;  $U = 32$ ,  $P = 0.75$ ). There were no significant differences in evenness between *C. geniculum* and *C. parthenum* (*C. geniculum* =  $0.25 \pm 0.10$ , *C. parthenum* =  $0.07 \pm 0.02$ ;  $U = 33$ ,  $P = 0.35$ ). Sexual *C. limum* had significantly higher genotypic diversity compared with spontaneous parthenogenetic *C. limum* (sexuals =  $3.15 \pm 0.79$ , parthenogens =  $1.64 \pm 0.17$ ;  $U = 39.5$ ,  $P = 0.016$ ). Evenness was also significantly higher in sexual *C. limum* relative to parthenogenetic *C. limum* ( $0.82 \pm 0.04$  and  $0.47 \pm 0.10$ , respectively;  $U = 43$ ,  $P = 0.026$ ).

### Polyphyletic origins of hybrid and spontaneous parthenogens

Figure 2 presents the minimum evolution phylogram of the relationships among all sexual and parthenogenetic populations. Other reconstruction techniques such as neighbour-joining and UPGMA showed similar relationships, although relationships among populations within species showed slight variation (trees available from author upon request). There was bootstrap support for *C. decisum* and Atlantic coastal populations (*C. limum* and *C. floridense*) forming distinct monophyletic lineages. Although quite distinct as evidenced by a long branch length, the insular species, *C. floridense*, groups with some



**Fig. 2** Rooted phylogenetic tree of *Campeloma* populations using minimum evolution of Manhattan distances. Open squares represent hybrid parthenogens and open circles represent spontaneous parthenogens. Numbers above branches represent percentage of bootstrapped trees supporting each node. Nodes with no number have <50% bootstrap support. The following acronyms were used for river drainages: *C. geniculum* & *C. parthenum* – Cho: Choctawhatchee, Ap: Apalachicola, Oc: Ocklockonee, Suw: Suwannee; Y: Yellow; *C. limum* – Suw: Suwannee, Alt: Altamaha, Ca: Canoochee, Og: Ogeechee, Sav: Savannah, Co: Coosawhatchie, Sk: Salkehatchie; *C. floridense* – SJ: St. John's.

*C. limum* populations. Changes in reproductive mode along this minimum evolution phylogram are consistent with polyphyletic origins of hybrid and spontaneous parthenogenesis. Because there is no bootstrap support (<75%) for many branches, we present this tree as a working hypothesis of the relationships among populations within species.

There are three distinct groups of hybrid parthenogens: Choctawhatchee river hybrids (10), Yellow river and Holmes and Wrights creek hybrids (9, 17–20) and Little river hybrids (27). Asymmetric band intensities in triploid, fixed heterozygotes allowed us to assign multilocus genotypes to each clone (Table 3). All hybrids west of the Apalachicola (9–10, 17–20) have one copy of the *limum* genome and two copies of the *geniculum* genome. At *Idh*, for example, hybrid clones from the Yellow river and

Holmes and Wrights creek have two A alleles from *C. geniculum* and one copy of the B allele from *C. limum*. At *Acoh*, *C. limum* contributed a C allele and *C. geniculum* contributed alleles D and E. The pattern of allele contribution by the sexual ancestors at *Acoh* supports the genome addition hypothesis. Because *C. limum* is the maternal ancestor of these hybrid clones and only one copy of the *limum* genome is present, then a sexual *C. limum* female with allele C probably hybridized with a male *C. geniculum* with either a D or an E allele. A backcross with a male *C. geniculum* probably added the additional allele at this locus (either D or E). Choctawhatchee hybrids differed from other hybrids west of the Apalachicola river at *Gpi*, *Pgdh* and *Acoh*. At *Gpi*, these hybrids had two copies of the C allele from *geniculum* and one copy of a rare D allele from *limum*. At *Pgdh*, these clones were homozygous for the C allele, which occurred in *C. geniculum* and *C. limum*. Only two alleles were present at *Acoh*: two copies of E from *geniculum* and a C allele from *limum*. These hybrids cluster with *C. geniculum* because they have two copies of the *geniculum* genome. However, the Little river parthenogens group with *C. limum*/*C. floridense*, and asymmetric staining intensities at *Idh* and *Acoh* indicate that they have two copies of the *C. limum* or *C. floridense* genome and one copy of the *C. geniculum* genome. Because *C. limum* and *C. floridense* are fixed for *Idh*-A, an AAB triploid has two copies of the *C. limum* or *C. floridense* genome. Likewise, the B allele at *Acoh* was found only in *C. limum* and a BBD triploid has two copies of the *C. limum* or *C. floridense* genome.

The phylogenetic relationships among sexual and spontaneous parthenogenetic *C. limum* are consistent with polyphyletic origins of spontaneous parthenogens from sexuals (Fig. 2). One difficulty with inferring the number of independent origins of spontaneous parthenogens from this tree is that most branches do not have >75% bootstrap support, probably because of limited allozyme variation. Therefore, we are cautious about identifying the exact number of independent origins from the tree. There are two other ways to address polyphyletic origins: genic differentiation between parthenogenetic populations and the number of multilocus clonal genotypes derived from sexual multilocus genotypes. Except for the Savannah river parthenogens (47) and the widespread clone (29, 30, 44 and 46), parthenogenetic populations with open circles show significant genic divergence from each other ( $P < 0.001$ ). With regard to multilocus clonal genotypes found in sexual populations, nine of the 14 multilocus clonal genotypes are present in sexual populations. Five unique multilocus clones occur in Canoochee and Altamaha river parthenogens. These patterns suggest multiple, independent origins of spontaneous parthenogenesis from sexual *C. limum*, rather than mutational divergence generating the majority of multilocus clones.

### Dispersal of spontaneous clones

We inferred dispersal of clones from two pieces of evidence: the geographical distribution of single multi-locus clones and genic differentiation of clones and sexuals within the same river drainage. As mentioned previously, one clone occurs in widely separated rivers in the Suwannee, Ogeechee and Savannah rivers (29, 30, 44, 46). Other clones (31 in the Suwannee and 36 in the Altamaha) form well-supported groups with sexuals from other river drainages. These patterns suggest dispersal of clones away from their site of origin. Significant genic differentiation occurs between most clones and sexuals in the same river drainage. In the Ogeechee river, sexuals and spontaneous parthenogens coexist. Two spontaneous parthenogenetic populations in the Ogeechee (40 and 44) showed nearly fixed differences at *Pgdh*, and strong differentiation ( $P < 0.00001$ ). Therefore, we did not pool these two populations when testing for genic differentiation between sexuals and parthenogens. As expected from the phylogram, the spontaneous parthenogenetic population (40) that groups with Altamaha parthenogens was significantly differentiated from all three sexual populations ( $P < 0.00001$  for each comparison). There was no significant genic differentiation between spontaneous parthenogen 44 and two of the sexual populations (42 and 43;  $P > 0.10$  for each comparison), but there was strong genic differentiation between 44 and sexual population 41 ( $P < 0.00001$ ). In the Savannah river system, three spontaneous parthenogenetic populations and one sexual population coexist. Significant differentiation existed between Brier Creek (45) and Savannah river parthenogens (46 and 47) at *Acoh* ( $P < 0.0001$ ). There was no significant differentiation between the two Savannah river parthenogens ( $P = 1$ ), so we pooled these two populations. Significant genic differentiation exists between Lower Three Runs creek sexuals (48) and Brier Creek parthenogens ( $P < 0.0001$ ) and the Savannah river parthenogens ( $P < 0.0001$ ).

### Discussion

This study represents the first detailed investigation into the origin and genetic diversity of hybrid and spontaneous parthenogenetic *Campeloma* from the south-eastern United States. The key findings from this study are: (1) evidence for hybrid and spontaneous origins of allotriploid *C. parthenum* and multiple, spontaneous origins of diploid *C. limum* parthenogens; (2) geographical differences in patterns of genotypic diversity in sympatric sexuals and parthenogens; and (3) dispersal of spontaneous parthenogens away from their site of origin. We discuss these issues within the context of evolution-of-sex models, and the origin and diversity of other clonal organisms.

In the Florida panhandle and the Atlantic coastal plain, sex ratios show discrete distributions. Except for mixtures

of hybrid parthenogenetic females and a few sexual *C. geniculum* females, most populations are all-female or nearly equal mixtures of males and females. This pattern contrasts with the occurrence of sexual and parthenogenetic females within the same lake in the New Zealand snail, *Potamopyrgus antipodarum* (Dybdahl & Lively, 1995; Fox *et al.*, 1996). In *Campeloma*, populations evolve towards either extreme (all-female or 1 : 1 sex ratio). Even in river drainages where both parthenogens and sexuals are present, they show very little spatial overlap. Hybrid parthenogens occur in the upper headwaters of Holmes and Wrights creek or disturbed areas in the Yellow and Little rivers. Spontaneous parthenogens occur throughout the Altamaha river drainage, the upper Suwannee river, and main river channels of the Ogeechee and Savannah rivers. We need to determine if these habitats have a lower risk of parasitism by digenetic trematodes (Lively, 1996) or undergo periodic extinction and recolonization where parthenogens may have an advantage (Peck *et al.*, 1998). Sexuals may have an advantage in more stable habitats with a higher risk of parasitism (Lively, 1996) or stronger selection against deleterious mutations due to higher levels of intraspecific competition (Peck, 1996; Lively *et al.*, 1998).

*Campeloma parthenum* and sexual *C. geniculum* differ in heterozygosity and ploidy. *Campeloma parthenum* is a fixed heterozygote at four loci and we recently confirmed triploidy by flow cytometry (Johnson *et al.*, submitted). In a comparison of *C. parthenum* and *C. geniculum* there is a violation of the all-else-equal assumption. However, there is no consensus about whether highly heterozygous triploids differ significantly in life history characteristics from sexual ancestors (Cullum, 1997; Vrijenhoek & Pfeiler, 1997). Recent experimental results indicated that *C. parthenum* has similar fecundity, higher growth rate and five-fold higher survivorship than sexual *C. geniculum* under stressful conditions (Johnson & Cargille, submitted). In a New Zealand snail, allotriploid parthenogens have similar fitness to diploid sexuals and a two-fold higher population growth rate (Jokela *et al.*, 1997).

Another important result of this study is the dual mechanisms by which highly heterozygous triploids originate. Because of the limited number of individuals and loci screened, we were unable to distinguish whether *C. limum* or *C. floridense* was involved in the hybridization event. However, the allozyme signature of *C. geniculum* and either *C. limum* or *C. floridense* was evident at all six loci. Fortunately, the combination of allozymic and mtDNA sequence variation provides a complete answer. Triploid hybrids west of the Apalachicola have two copies of the *geniculum* genome and one copy of the *limum* genome, whereas triploid hybrids in the Little river have two copies of the *limum* genome and one copy of the *geniculum* genome. There is strong evidence from mitochondrial sequence data that *C. limum* is the maternal ancestor of all hybrid clones, except that *C. floridense* is the maternal ancestor of Choctawhatchee hybrids (Johnson

& Bragg, in revision). Given the mitochondrial evidence and allele contribution at *Acoh*, Holmes and Wrights creek parthenogens arose through an initial hybridization event between a male *C. geniculum* and a female *C. limum* and a male *C. geniculum* contributed another allele after the initial hybridization event. The two multilocus clonal genotypes in the Yellow river and Holmes Creek hybrids arose by different male genotypes involved in the initial hybridization and the subsequent backcross. This result is consistent with the genome-addition hypothesis (Schultz, 1969; Quattro *et al.*, 1992). In the Little river parthenogens, however, it is more likely that parthenogenesis arose spontaneously in *C. limum* before hybridization. If *C. limum* is the maternal ancestor of the Little river parthenogens, two alternative hypotheses can account for the ploidy level and multilocus genotypes. First, a *C. geniculum* male fertilized a diploid parthenogenetic *C. limum*. The second possibility is that a sexual *C. limum* female hybridized with a male *C. geniculum* and was subsequently fertilized by a male *C. limum*. The first scenario seems more likely because spontaneous parthenogens are widespread in *C. limum* and the Little river parthenogens are more likely to encounter sympatric *C. geniculum* males. Because the allozyme data are not variable enough to resolve these issues, phylogenetic reconstruction of mtDNA haplotypes of triploid clones and *C. limum* sexuals and parthenogens might provide insight into the maternal ancestor of triploid hybrid parthenogens.

Evidence for spontaneous origins of parthenogenesis in *C. limum* comes from several quarters: (1) nearly all alleles in all-female populations are present in sexual *C. limum* populations, (2) heterozygosity is significantly lower in parthenogens and (3) five populations show deviations from H-W equilibrium. Two populations showed an excess of heterozygotes at two loci (*Acoh* and *Pgm-1*) whereas three populations showed an excess of homozygotes at two loci (*Acoh* and *Pgdh*). Even in three populations with excess homozygosity, heterozygotes occur at these two loci, suggesting that modes of automictic parthenogenesis that are analogous to selfing do not occur in parthenogenetic *C. limum*. Possible mechanisms of parthenogenesis are apomixis or premeiotic doubling. Considering the lack of asymmetric band intensities in heterozygous parthenogens, these parthenogens are probably diploid. We recently confirmed diploidy by flow cytometry (Johnson *et al.*, submitted). These features suggest that there may be no violation of the all-else-equal assumption in a comparison of *C. limum* sexuals and parthenogens.

Sympatric sexuals and parthenogens differ dramatically in a comparison of genetic diversity between Atlantic coast populations vs. Florida Gulf Coast populations. *Campeloma limum* sexuals have significantly higher genotypic diversity compared with *C. limum* parthenogens, whereas *C. geniculum* and *C. parthenum* have similar, low levels of genotypic diversity. Drift or founder effect

during colonization may account for the low genetic diversity in *C. geniculum*. Except for the Apalachicola river drainage where the highest level of genotypic diversity occurs, Pleistocene high sea stands probably inundated many of these rivers. Most ecological models of sex assume that sexuals have greater levels of genetic variation and can therefore respond more effectively to spatial or temporal heterogeneity. For example, under the Tangled Bank model, the advantage to sex is eliminated if clonal diversity is high (Case & Taper, 1986). Correlative tests of the Red Queen hypothesis usually relate some index of recombination such as sex ratio with risk of parasitism among populations, under the assumption that sex ratio is a good approximation of levels of genetic diversity (Lively, 1987, 1992; Schrag *et al.*, 1994). This assumption may be invalid if sexual populations have experienced drift or if multiple clones of polyphyletic origin exist.

Recent simulations of the Red Queen hypothesis indicate that the presence of 2–4 multilocus clones within a population can prevent elimination of clones by sexuals (Lively & Howard, 1994). In the New Zealand snail, *Potamopyrgus antipodarum*, genotypic diversity is lower in spontaneous triploid clones compared with nearby sexuals, but clonal diversity is extremely high (Fox *et al.*, 1996). Many *Daphnia* parthenogens also have considerable clonal diversity (Hebert *et al.*, 1989; Dufresne & Hebert, 1994, 1995, 1997). In these cases, parasites may select for rare clonal genotypes, and diverse mixtures of clones may replace sexuals. Although many populations of parthenogenetic *C. limum* are uniclonal, populations in the Altamaha drainage are considerably more diverse with up to five multilocus genotypes within a population. Possible mechanisms for clonal diversity are higher mutation rates to spontaneous parthenogenesis from diverse sexual lineages. We recognize that the phylogenetic reconstruction does not provide strong evidence concerning independent origins of parthenogens. However, the combined evidence of genic differentiation of parthenogenetic populations and the presence of most multilocus clonal genotypes in ancestral sexuals suggests that multiple origins account for most of the clonal diversity rather than mutational diversification within clonal lineages. We plan to examine more variable nuclear markers, such as microsatellites, to further address this issue.

Another important mechanism by which parthenogens can persist under the Red Queen hypothesis is dispersal away from locally adapted parasites (Ladle *et al.*, 1993; Judson, 1995). Some *Campeloma limum* parthenogens were geographically widespread and grouped with nonlocal sexual populations on the minimum evolution tree, and most sexuals and parthenogens coexisting in the Ogeechee and Savannah showed significant genic differentiation. Dispersal of parthenogens away from their source of origin could explain this pattern. Parthenogens with limited clonal

diversity may only persist by dispersal away from locally adapted parasites. A recent study of mtDNA sequence variation corroborates higher dispersal of parthenogens relative to sexuals (Johnson, submitted). During Pleistocene glacial periods, river drainages probably coalesced on a broad coastal plain (Swift *et al.*, 1986; Webb, 1990), thus allowing spontaneous clones to disperse away from their site of origin. We are currently testing the prediction from the Red Queen hypothesis that dispersal by parthenogens provides an escape from castrating digenetic trematodes. An alternative hypothesis is that higher dispersal of parthenogens relaxes selection against deleterious mutations (Peck, 1996; Lively *et al.*, 1998). Clearly, we need experimental tests to distinguish among these alternative hypotheses.

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