

Mode of origin differentially influences the fitness of parthenogenetic freshwater snails

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How parthenogenetic lineages arise from sexual ancestors may strongly influence their persistence over evolutionary time. Hybrid parthenogens often have elevated heterozygosity and ploidy, thus making it difficult to disentangle the influence of reproductive mode, hybridity and ploidy on their relative fitness. By comparing the relative fitness of both hybrid and non-hybrid parthenogens to their sexual ancestors, further insight may be gained into how these three factors influence the maintenance of sexual and parthenogenetic reproduction. In the present study, hybrid and non-hybrid parthenogenetic and sexual snails (*Campeloma* sp.) were compared for the following characteristics: female size-fecundity curves, offspring size, survivorship, and growth. Compared to nearby sexual populations, triploid hybrid parthenogens from the Florida Gulf coast have similar fecundity and offspring size, five-times higher survivorship, and 60% higher growth. Relative to nearby sexual populations, non-hybrid parthenogenetic *C. limum* from the Atlantic coast have significantly higher fecundity, smaller offspring size, similar survivorship and slightly lower growth. Given the considerable fitness advantages of parthenogens, especially hybrid parthenogens, it is enigmatic as to why these parthenogens occupy marginal natural habitats.

Keywords: sexual reproduction; parthenogenesis; heterosis; *Campeloma*; fitness

1. INTRODUCTION

The rarity of parthenogenetic reproduction in most eukaryotes is paradoxical because parthenogens may have a two-fold reproductive advantage over sexuals, and should rapidly eliminate sexuals (Maynard Smith 1971). The magnitude of this reproductive advantage may depend on how parthenogens originate from their sexual ancestors (Vrijenhoek 1998; Kearney & Shine 2004). In many plants and animals, a common mechanism that may lead to the origin of apomictic parthenogenesis is interspecific hybridization, which is often associated with increased ploidy (Stebbins 1950, 1971; Avise *et al.* 1992; Johnson & Bragg 1999; Adams *et al.* 2003; Delmotte *et al.* 2003). Given the ubiquity of polyploid parthenogens derived from interspecific hybridization (Simon *et al.* 2003), heterosis may play an important role in the establishment of these lineages (Wetherington *et al.* 1987; Corley *et al.* 2001; Anderson *et al.* 2002). However, tight linkage among asexuality, hybridity and polyploidy makes it difficult to assess their relative influence on fitness components. One way to overcome the confounding influences of these three factors is to examine fitness components in non-hybrid, diploid parthenogens and their sexual ancestors. Depending upon their mode of origin, parthenogenetic lineages may have reduced or higher fitness relative to their sexual ancestors.

In the southeastern United States, there have been multiple, independent origins of non-hybrid and hybrid parthenogens from sexual ancestors in the freshwater prosobranch snail *Campeloma* (Johnson & Bragg 1999; Johnson & Leefe 1999). *Campeloma limum* diploid parthenogens from the Atlantic coastal plain arose

spontaneously without interspecific hybridization, and parthenogenetic juveniles had lower survivorship in a laboratory experiment (Johnson & Bragg 1999; Johnson & Leefe 1999; Johnson 2000). However, a shortcoming of this experiment was that parthenogenetic offspring were significantly smaller than sexual offspring, so that maternal effects could have played an important role in these survivorship differences. The current study addresses this shortcoming. Another group of sexual and parthenogenetic *Campeloma* (Gastropoda: Viviparidae) occurs in Florida Panhandle freshwater streams and rivers. Using allozyme and mitochondrial DNA sequence variation, *Campeloma parthenum* are highly heterozygous triploids formed by hybridization between two species, *C. geniculum* and *C. limum* (Johnson & Bragg 1999; Johnson & Leefe 1999; Johnson *et al.* 1999). *Campeloma geniculum* is an obligate sexual found in the same freshwater streams and rivers.

The present study examines how reproductive mode influences various fitness components and whether the magnitude of fitness differences between parthenogens and sexuals depends on how parthenogens originate. Two questions are examined for parthenogenetic and sexual *Campeloma* from these two geographic regions: (1) do sexuals and parthenogens show similar relationships between female size and fecundity, and is mean offspring size similar for sexuals and parthenogens?; and (2) under common laboratory conditions, do coexisting sexuals and parthenogens show similar juvenile survivorship and growth rates? The current study indicates that, relative to nearby sexuals, hybrid parthenogens have similar fecundity and offspring size, five-times higher survivorship, and 60% higher growth rate, whereas non-hybrid parthenogenetic *C. limum* have significantly higher

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Table 1. Collecting localities.

(For each population the following are given: geographic coordinates, reproductive mode (RM: Hpar=Hybrid parthenogens; Par=Parthenogens; Sex=Sexuals), and data collected from each population (F=fecundity, S=survivorship/growth rates experiment).)

drainage and creek/river	species	location	RM, data
<i>Choctawhatchee</i>			
Wright's Cr.	<i>C. geniculum</i>	30°52N 85°42W	Sexual (F, S)
Holmes Cr.	<i>C. geniculum</i>	30°55N 85°33W	Sexual (F, S)
Holmes Cr.	<i>C. parthenum</i>	30°58N 85°32W	HPar (F,S)
Wright's Cr.	<i>C. parthenum</i>	30°58N 85°37W	HPar (F,S)
<i>Salkehatchie/Coosawhatchie</i>			
Coosawhatchie R.	<i>C. limum</i>	32°54.31N 81°13.65W	Sexual (F, S)
<i>Savannah</i>			
Lower three runs Cr.	<i>C. limum</i>	33°04.39N 81°28.61W	Sexual (F, S)
Brier creek	<i>C. limum</i>	32°51.64N 81°36.73W	Par (F, S)
<i>Ogeechee</i>			
Horse creek	<i>C. limum</i>	32°41.11N 81°50.41W	Sexual (F)
Ogeechee river	<i>C. limum</i>	32°29.69N 81°33.34W	Par (F, S)
<i>Altamaha</i>			
Ocmulgee R.	<i>C. limum</i>	31°49.25N 83°07.01W	Par (F)

fecundity, smaller offspring size, similar survivorship, and slightly lower growth. These findings suggest that both parthenogens have more than a two-fold advantage.

2. MATERIAL AND METHODS

For Florida Gulf Coast sexuals and hybrid parthenogens, reproductive individuals (>10 mm aperture width) were collected from sexual and hybrid parthenogenetic populations in Holmes Creek and Wright's Creek (table 1). In each creek, the hybrid parthenogenetic and sexual populations occur within 5 km of one another. These triploid hybrid parthenogens have two copies of the *C. geniculum* genome and one copy of the Atlantic coast *Campeloma* genome, and triploidy was derived from fertilization of a diploid hybrid by a male *C. geniculum* (Johnson & Bragg 1999). For Atlantic Coast sexuals and parthenogens (table 1), reproductive females were collected from three sexual populations and three parthenogenetic populations. Snails were transported alive to the laboratory where they were sexed and aperture length was measured to the nearest 0.1 mm. Because *Campeloma* is ovoviviparous, the number of offspring from the brood chamber of reproductive females from each population was counted and the size of the five largest offspring was measured. Only the five largest offspring from the distal end of the brood chamber were used because juvenile snails develop asynchronously. Analysis of covariance was used to determine whether the number of offspring differed by reproductive mode and/or mother size. Within each geographic region, the interaction between reproductive mode and size was tested to examine whether fecundity increases more rapidly as a function of maternal size in parthenogens versus sexuals. If homogeneity of slopes was not rejected, size-adjusted fecundity of parthenogens and sexuals was examined. For each geographic region, mean offspring size of sexuals and parthenogens was examined by one-way ANOVA.

To examine survivorship and growth, parthenogenetic and sexual juveniles were raised together under identical conditions. Washed and sifted sand from *Campeloma* habitat in southeastern Louisiana was used as substrate. Juvenile snails

were raised in 10 l aquaria at approximately 20 °C under natural photoperiods. For the Florida Gulf coast experiment, twenty females were randomly chosen from each of the four populations (two sexual and two parthenogenetic). The five largest offspring from each female were individually marked with colored, numbered bee tags and randomly assigned across five different aquaria. Each aquarium contained 80 juvenile snails, with all populations represented within each aquaria and each female had one offspring in each aquarium. Mixed colonies of sexuals and parthenogens were used to mimic conditions of competition between sexuals and parthenogens. In a separate experiment using Atlantic Coast sexuals and parthenogens, the design was similar except that only four offspring were collected from 20 reproductive females per population. Offspring from two sexual and two parthenogenetic populations were used (table 1). Each aquarium contained 80 juvenile snails, with all populations represented within each of the four aquaria. For each experiment, snails were fed 0.004 g of trout chow pellets per live individual every 12 days. Both experiments terminated after 8 months, and shell length was measured.

For each geographic region, a logistic regression model was used employing a forward-stepwise likelihood ratio test to determine which variables (initial size or reproductive mode) explained significant variation in the dependent variable, survival. Aquarium was also included in the logistic regression model to assess aquarium effects. A two-way analysis of variance (randomized complete block design) was used with aquarium (block) and reproductive mode as sources of variation. The primary purpose was to test the null hypothesis that offspring growth (shell length at end of experiment – initial shell length) does not differ between sexuals and parthenogens. All analyses were conducted using SPSS (Rel. 11.5.2.1, SPSS Inc. 2003).

3. RESULTS

The slope of the relationship between fecundity and mother size did not differ between hybrid parthenogens and sexuals ($F_{\text{reproductivemode} \times \text{mothersize}} = 0.245$, d.f. = 1,142, $p > 0.5$; figure 1a). The standardized

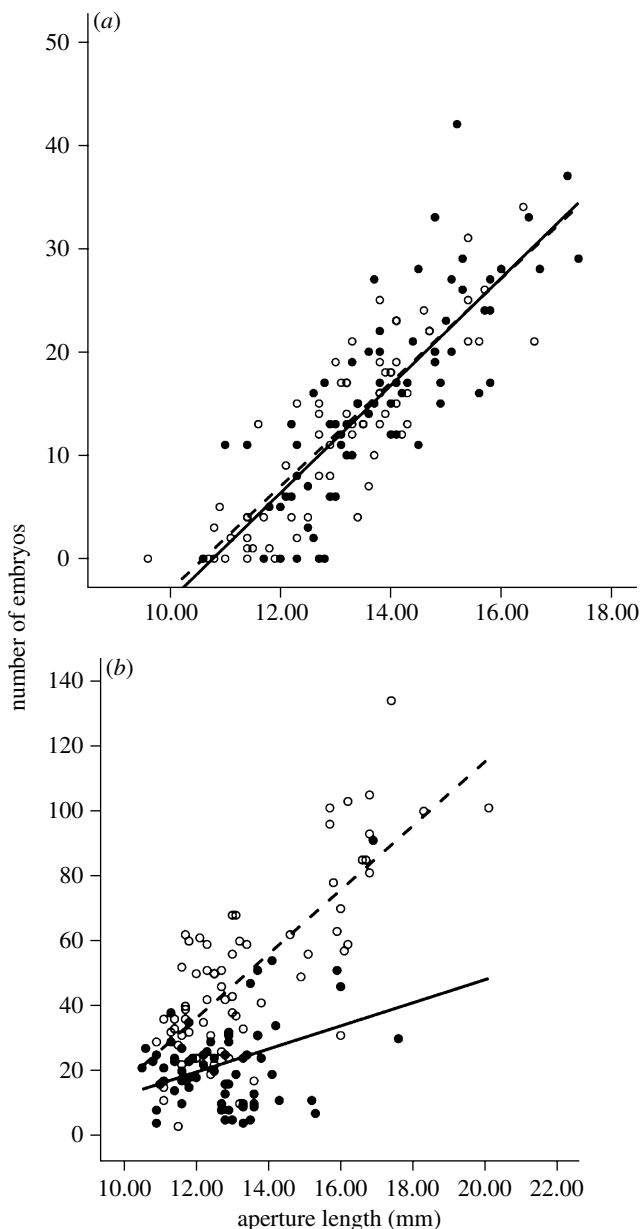


Figure 1. Relationship between number of embryos and female size in parthenogenetic (open circles and dashed regression line) and sexual (filled circles and solid regression line) *Campeloma* from the (a) Florida and (b) Atlantic coastal plains.

regression coefficient (\pm standard error of regression coefficient) was $5.02 (\pm 0.36)$ and $5.30 (\pm 0.44)$ for hybrid parthenogens and sexuals, respectively. The estimated marginal means (\pm s.e.) for fecundity evaluated at a size of 13.53 mm, under the assumption of homogenous slopes, was $14.61 (\pm 0.63)$ and $14.25 (\pm 0.59)$ for hybrid parthenogens and sexuals, respectively. Initial offspring size did not differ significantly between Florida hybrid parthenogens and sexuals ($F_{1,388} = 0.328$, $p > 0.5$). Mean (\pm s.e.) offspring size was 3.80 ± 0.02 and 3.78 ± 0.03 for Florida hybrid parthenogens and sexuals, respectively. In contrast, Atlantic coast parthenogens had a significantly steeper relationship between fecundity and female size ($F_{\text{reproductivemode} \times \text{mothersize}} = 15.46$, d.f. = 1, 142; $p < 0.001$; figure 1b). The standardized regression coefficient was $9.95 (\pm 0.986)$ and $3.56 (\pm 1.14)$ for parthenogens and sexuals, respectively. Offspring size was

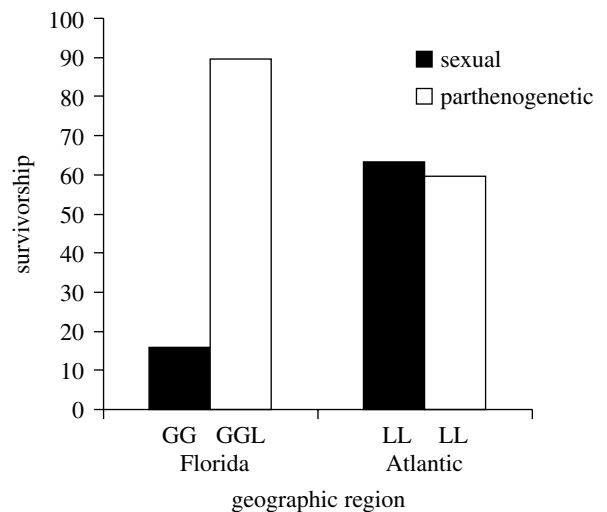


Figure 2. Survivorship under laboratory conditions of parthenogenetic (open bars) and sexual (filled bars) *Campeloma* from Florida and Atlantic coastal plains. Survivorship of hybrid parthenogens and sexual *C. geniculatum* was 89.5 and 16%, respectively. Survivorship of parthenogenetic and sexual *C. limum* was 59.7 and 63.2%, respectively. Genome dosages are presented for diploid sexuals (GG, LL) and parthenogens (LL) and triploid hybrid parthenogens (GGL).

significantly smaller in parthenogenetic *C. limum* (mean size \pm s.e. = 3.26 ± 0.02) relative to sexual *C. limum* (3.43 ± 0.02 ; $F_{1,636} = 54.7$, $p < 0.001$). However, for the laboratory survival experiment, initial offspring size did not differ significantly between Atlantic coast parthenogens and sexuals ($F_{1,318} = 0.023$, $p > 0.8$): mean offspring size (\pm s.e.) was 3.33 ± 0.02 and 3.34 ± 0.02 for Atlantic coast parthenogens and sexuals, respectively.

For Florida hybrid parthenogens and sexuals, the logistic regression indicated that reproductive mode had a significant influence on survivorship (Wald statistic = 154.8, d.f. = 1, $p < 0.001$) whereas there was no effect of initial size on survivorship (Wald statistic = 0.276, d.f. = 1, $p > 0.5$) or aquarium (Wald statistic = 0.987, d.f. = 4, $p > 0.1$). Survivorship of hybrid parthenogens (89.5%) was nearly five times higher than the survivorship of sexuals (16%; figure 2). Hybrid parthenogens had significantly higher growth than sexual *C. geniculatum* ($F_{1,196} = 44.7$, $p < 0.001$). The mean (\pm s.e.) growth of hybrid parthenogens and sexuals was 2.02 ± 0.03 mm and 1.32 ± 0.09 mm, respectively. There was significant variation in growth rates among aquaria ($F_{4,196} = 3.54$, $p < 0.01$).

For Atlantic coast *C. limum* parthenogens and sexuals, the logistic regression indicated that only initial size had a significant influence on survivorship (Wald statistic = 9.171, d.f. = 1, $p < 0.01$) whereas there was no effect of reproductive mode (Wald statistic = 0.155, d.f. = 1, $p > 0.6$; figure 2) or aquarium (Wald statistic = 0.358, d.f. = 3, $p > 0.5$) on survivorship. Survivorship of parthenogenetic and sexual *C. limum* was 59.7 and 63.2%, respectively. There was a significant differences in mean growth of parthenogenetic and sexual *C. limum* ($F_{1,133} = 4.61$, $p < 0.05$). The mean (\pm s.e.) growth of parthenogenetic and sexual *C. limum* was 1.02 ± 0.08 mm and 1.20 ± 0.07 mm, respectively. There was a significant effect of aquaria on growth rates ($F_{3,133} = 7.31$, $p < 0.01$).

4. DISCUSSION

The most important findings from the present study are that, in the two geographic areas with different origins of parthenogenesis from sexual ancestors, there are considerable differences in fitness components between sympatric sexuals and parthenogens. Florida sexuals and hybrid parthenogens show similar patterns of female size–fecundity and offspring size, but hybrid parthenogens have five-fold higher survivorship and 60% higher growth. Under additive effects of combined genomes in these hybrids (Kearney & Shine 2004), survivorship and growth rates would be more similar to *C. geniculum* than to *C. limum* given two doses of the *C. geniculum* genome. Instead, there are strong non-additive effects on these two fitness components. The extreme survivorship and growth rates of allotriploids compared to both parental species is also consistent with transgressive hybridization (Lexer *et al.* 2003; Rieseberg *et al.* 2003). Given that these fitness comparisons were conducted in novel laboratory situations, future studies will examine whether allotriploids also exhibit extreme fitness phenotypes in their native habitats and in habitats where sexuals dominate.

In contrast, diploid, non-hybrid *C. limum* parthenogens have much higher relative fecundity but relatively smaller offspring size compared to *C. limum* sexuals, and similar survivorship and slightly lower growth rates when controlling for maternal effects. These patterns also suggest a fitness advantage to these autodiploid parthenogens, although there is no evidence for transgressive phenotypes. *C. limum* parthenogens dominate in main river channels and exhibit a trade-off for fecundity and offspring size. Preliminary data analysis indicates that the organic content of substrates in main river channels is much lower than creeks, probably attributable to lower allochthonous input in main river habitats (Johnson, unpublished data). A shortcoming of the present study is that fecundity and offspring size were estimated from field-collected samples, which are subject to both environmental and genetically based differences between populations. Therefore, reciprocal transplant experiments will be necessary to assess the contribution of environmental and genetic factors to these life history differences.

Given the considerable fitness advantage of allotriploid and autodiploid parthenogens, why are parthenogens restricted to particular habitats (headwaters and main rivers) and why have they not replaced sexuals? The most likely explanation is that the fitness advantage of parthenogens is habitat specific. Numerous mechanisms could restrict the fitness advantage of parthenogens to particular habitats. First, transgressive hybrids may experience fitness advantages only under novel environmental conditions (Rieseberg *et al.* 2003). Given their rapid growth and survivorship advantage, hybrid parthenogens may out compete sexuals during colonization of these environments. Sexual *Campeloma* may colonize marginal environments, but may not persist because they suffer from inbreeding depression (Vrijenhoek 1998; Haag & Ebert 2004). The relatively low survivorship of sexual *C. geniculum* is consistent with inbreeding depression. Second, rapidly evolving parasites may be absent from marginal environments, leading to relaxed selection for genetic diversity in snail hosts only in these environments. If habitats where sexuals dominate have higher parasite densities, parthenogens may suffer higher parasitism in

these habitats if parasites overexploit common clonal genotypes (Johnson 2000). Lastly, parthenogens may not successfully invade sexual populations because of destabilizing hybridization. Lynch (1984) argued that parthenogenetic lineages could only be established and maintained if they avoid backcrosses with sexual ancestors. There is no indication of elevated ploidy in either parthenogen (Johnson *et al.* 1999), suggesting that backcrossing events are rare or there is strong selection against elevated ploidy early in development. These three mechanisms are not mutually exclusive and, in combination, may explain the restricted spatial distribution of both parthenogens.

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