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Spontaneous and hybrid origins of parthenogenesis in *Campeloma decisum* (freshwater prosobranch snail)

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A previous study hypothesized that parthenogenesis arose spontaneously in the freshwater prosobranch snail, *Campeloma decisum*, and was subsequently maintained by strong sterility selection against males caused by a digenetic trematode, *Leucochloridiomorpha constantiae*. The present study examines whether parthenogenesis arose spontaneously or by hybridization between genetically distinct sexual ancestors. Genetic variation was examined at 19 enzyme loci in 11 parthenogenetic and seven sexual populations. Parthenogens from North Carolina and Wisconsin are homozygous at all loci, which suggests that parthenogenesis arose spontaneously in the absence of hybridization. Parthenogens from other northern populations are heterozygous at numerous loci and these individuals have gene combinations found in distinct sexual lineages. These findings suggest that parthenogenesis arose both spontaneously, followed by strong selection of these families from parasitic castration of males, and by hybridization. Low levels of clonal diversity occurred within and among parthenogenetic populations and clonal diversity is derived from patterns of genetic diversity present in sexual populations. Lastly, fixed allelic differences between southeastern and southern sexual individuals suggest that these areas are inhabited by cryptic species.

Keywords: *Campeloma decisum*, clonal diversity, hybridization, parthenogenesis, spontaneous origins.

Introduction

Study of the origin of parthenogenetic reproduction from sexual ancestors is fraught with problems inherent in the unravelling of historical events. Various mechanisms have been invoked to explain the presence of parthenogenetic taxa within predominately sexual clades. Adaptive hypotheses state that the predominance of parthenogens at high altitudes and latitudes may reflect selection for females capable of parthenogenesis in response to the absence or low density of males during colonization (Stebbins, 1950; Lloyd, 1980) or in response to lessened competition or parasitism (Levin, 1975; Glesener & Tillman, 1978; Lively, 1987). Parthenogenesis is assumed to arise rarely through spontaneous mutation, and the subsequent spread and fixation of parthenogenesis is derived from the selective advantages accrued from reproductive assurance and/or escape from competitors or parasites. Although some sexual females seem capable of parthenogenetic reproduction when males are unavailable (Carson, 1967; Templeton *et al.*, 1976; Templeton, 1982), modification of gametogenesis without depression of fertility and stable development may be difficult obstacles to overcome during initial stages of parthenogenesis (Moritz *et al.*, 1989a). These genetic constraints may account for the rarity of parthenogenesis. Nonetheless, the study of invertebrate parthenogens has shown consistent patterns of parthenogens being found in disturbed, glaciated regions where male density may be low or where levels of competition and parasitism are lower (Suomalainen & Saura, 1973; Cuellar, 1977; Suomalainen *et al.*, 1987).

The most common explanation for the origin of parthenogenesis in vertebrates and many invertebrates, however, is hybridization between genetically distant sexual taxa (Schultz, 1969; White, 1973; Hewitt, 1975; Parker & Selander, 1976; Vrijenhoek, *et al.*, 1977; Moritz, 1983; Dawley & Bogart, 1989; Honeycutt & Wilkinson, 1989; Moritz *et al.*, 1989b, 1989c). Numerous parthenogenetic taxa are heterozygous at enzyme loci for which the putative sexual ancestors

have the opposite allele fixed. This pattern of parthenogens sharing alleles derived from the sexual ancestors is often considered evidence of a direct relationship between hybridization and the origin of parthenogenesis. The mode of origin of parthenogenesis, whether by spontaneous mutation or hybridization, may dramatically affect genotypic diversity and the concomitant evolutionary consequences of parthenogenesis.

This study examines the hypothesis that hybridization between sexual ancestors of *Campeloma decisum*, a freshwater snail, is a factor in the origin of parthenogenesis by describing patterns of electrophoretic variation of proteins within and among parthenogenetic and sexual populations throughout eastern North America. The primary purpose is to examine whether parthenogens have enzyme profiles consistent with a spontaneous origin of parthenogenesis or with a hybrid origin between genetically distinct sexual ancestors. If parthenogenesis arose spontaneously, parthenogens should have similar or lower levels of polymorphism and heterozygosity relative to their sexual ancestors. The hybrid hypothesis predicts elevated levels of heterozygosity in the parthenogens compared to their respective sexual ancestor. Because little information exists on the genetic structure of parthenogenetic *C. decisum*, two questions with important evolutionary consequences are addressed. First, is parthenogenesis apomictic or automictic? Apomixis (ameiotic reproduction) should result in the retention of heterozygosity whereas automixis (various forms of meiotic reproduction and subsequent restoration of diploidy) should result in fixed homozygosity, although two forms of automixis, central fusion and premeiotic doubling, may allow retention of heterozygosity (Uzzel, 1970; Suomalainen *et al.*, 1987). Apomictic parthenogenesis occurs in other populations of *Campeloma decisum* (Selander *et al.*, 1977) and other *Campeloma* species (Mattox, 1937; Karlin *et al.*, 1980. Secondly, the degree of clonal diversity is assessed within and among parthenogenetic populations, in an attempt to determine whether patterns of clonal diversity result from mutation, repeated hybridization events, or founding of clones by genetically divergent sexual ancestors (Moritz *et al.*, 1989a).

Tests of hypotheses for the maintenance of parthenogenesis in *C. decisum*

Campeloma decisum is a freshwater, prosobranch snail endemic to lakes and rivers of eastern North America. The distribution of parthenogenetic and sexual populations was believed previously to follow a classical pattern of northern, parthenogenetic populations in glaciated regions and more southernly, sexual populations in unglaciated areas (van der Schalie, 1965; Bell, 1982). However, recent work has revealed that obligate parthenogenesis has arisen in southern populations of both *C. decisum* (Johnson, 1991; Fig. 1) and *C. parthenum* (Vail, 1979). The presence of parthenogens in unglaciated regions suggests that colonization of glaciated regions is unnecessary for the spread of parthenogenesis in this snail. Elsewhere, obligate parthenogenesis in this snail was postulated to have been maintained as a direct consequence of sterility selection against males by the metacercariae of *Leucochloridiomorpha constantiae* (Trematoda: Brachylaemidae), a digenetic trematode capable of sperm ingestion or sperm blockage in females (Johnson, 1991).

Parthenogenesis is assumed to have arisen spontaneously; therefore, heterozygosity should be similar to the sexual ancestral lineage. All parthenogenetic populations sampled throughout eastern North America were infected with unencysted metacercariae found in the brood chamber of female snails, whereas no individuals from sexual populations were infected with the metacercariae. The prevalence of infection (percentage of individuals infected) was often 70–80 per cent within host populations and the intensity of infection (mean number of metacercariae per host within host populations) usually ranged from 25–80 metacercariae per host. The prevalence of infection increased throughout the lifespan of individuals and snails were unable to reduce infections by immune response. Clearly, the high prevalence and intensity of infection could result in strong selection against males based on the following argument. It was reasoned that the historical introduction of this parasite into sexual snail populations resulted in strong selection for females capable of parthenogenetic reproduction because of the severe sperm limitation caused by this parasite. Metacercariae would have direct access to stored sperm in the seminal receptacle in female snails because the brood chamber and seminal receptacle are contiguous (S. G. Johnson personal observation; Vail, 1977).

Materials and methods

Allozyme variation was assessed for 358 snails from seven sexual populations and 11 parthenogenetic populations throughout the range of *C. decisum* (Appendix, Table A1; Fig. 1). For each population, 50–60 individuals were sexed, and parthenogenetic populations were defined by the absence of males, whereas sexual populations contained males (ranging from 25–55 per cent males). Populations with female-

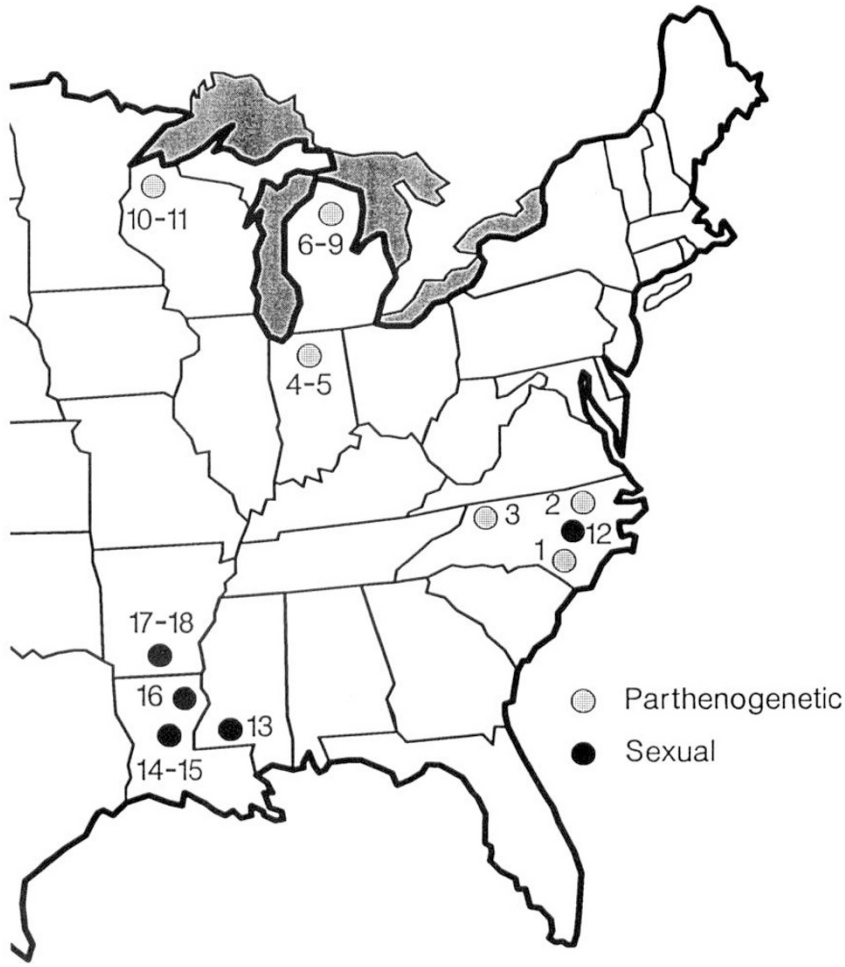

Fig. 1 Collection sites of parthenogenetic and sexual populations of *C. decisum*. See Appendix, Table A1 for more exact location.

biased sex ratios may represent cases of mixtures of sexual and parthenogenetic females (Johnson, 1991). Parasitized tissues were removed from infected individuals and whole snails were stored in liquid nitrogen. They were placed at −80°C upon return to the laboratory.

Homogenates of foot muscle, digestive gland, and gill were centrifuged the day prior to staining and the supernatant was stored at −80°C. Individual tissues consisting of the three tissue types were screened for tissue-specificity and, as a result of this screening, mixed-tissue homogenates were used.

Twenty individuals were scored for every population except Diamond Lake, WI and Chippewa River, WI ($n = 19$). Genetic variation at 19 presumptive genetic loci encoding 12 enzymes was assayed by horizontal starch gel (12 per cent) electrophoresis using standard staining techniques (Harris & Hopkinson, 1976). Buffer systems and the presumptive gene loci are presented in the Appendix, Table A2.

Alleles at a locus were designated alphabetically in order of decreasing mobility; the most anodal locus in a system was numbered '1' with more cathodal loci receiving higher numbers. Calculations of individual heterozygosity (H) and the percentage of loci polymorphic per population were estimated from individual genotypes of all loci for each population. Nei's genetic distance was calculated and phenetic relationships among all sexual and parthenogenetic populations were determined by UPGMA clustering (Sneath & Sokal, 1973). All analyses were performed on the BIOSYS = 1 package (Swofford & Selander, 1989).

Results

Genetic variation of sexual and parthenogenetic populations

Parthenogenetic and sexual populations from southeastern regions (populations 1–3, 12) are genetically identical at the 19 enzyme loci examined. They were monomorphic for the following alleles at the eight variable loci: *G3phd*^{A}, *6-Pgdh*^{B}, *Acon-1*^{B}, *Ck-1*^{A}, *Pgm-1*^{B}, *Lap-1*^{A}, *Est-1*^{A}, *Icdh-1*^{B} (Table 1). Parthen-

ogens from Diamond Lake, WI (population 10) are entirely homozygous and differ from southeastern parthenogens only by the presence of *Lap-1*^{B}. These fixed-homozygote parthenogens probably arose spontaneously and automictic parthenogenesis preserves homozygosity at these 19 loci.

Sexual populations from Mississippi, Louisiana, and Arkansas (populations 13–18) are nearly fixed for an alternative allele at these loci (Table 1). Parthenogenetic populations from Indiana and Michigan (populations 4–9) consist of individuals heterozygous at six or seven loci for the fixed alleles occurring in the southeastern and southern sexual populations. The pattern of elevated heterozygosity compared to sexual ancesters may indicate hybrid origins of these parthenogens and maintenance of elevated heterozygosity by apomictic parthenogenesis. Parthenogens from Chippewa River (population 11) are polyclonal, containing both homozygous individuals most similar to southeastern *C. decisum* and heterozygous individuals.

Sources of genetic variation in parthenogenetic populations

Only five multilocus genotypes were present among the 218 parthenogenetic individuals sampled (Table 2), a relatively low number of clones. There are two general classes of multilocus genotypes: homozygous genotypes and heterozygous genotypes. The single gene difference at the *Lap-1* between the two homozygous clones from North Carolina (*ABBABAAB*) and Wisconsin (*ABBABBAB*) populations suggests a very close genetic affinity. This slight difference may be attributable to mutation or founding by sexuals with different genotypes. Likewise the three heterozygous clones from Michigan and Indiana differ only at two enzyme loci (*6-Pgdh* and *Icdh-1*). The multilocus genotype *CBCCCCCB* may represent silencing of one allele at *6-Pgdh* from the multilocus genotype *CCCCCCCB*. The Wolf Creek clone (*CCCCCCCA*) represents a slight divergence at the *Icdh-1* locus.

Genetic relatedness among sexual and parthenogenetic populations

Three major clusters are evident in the phenogram (Fig. 2) based on Nei's genetic distances (Table 3): sexual populations and homozygous clones from North Carolina and Wisconsin, heterozygous clones from Michigan and Indiana, and sexual populations from the southern United States (compare Fig. 1). The separation of the Wisconsin clones from North Carolina populations is due to their slight, genetic differences at the *Lap-1* locus. Within the heterozygous northern clones, the uniclonal populations (Potato Creek, Black Mallard, and Burt Lake) cluster and the polyclonal populations (Thunder Bay and Carp Lake River) cluster together. The southern, sexual popula-

Table 1 Allele frequencies at eight polymorphic loci in parthenogenetic and sexual populations of *Campeloma decisum*. P and S refer to parthenogenetic and sexual populations, respectively. See Appendix 1 for number designations and exact localities of populations

		Population																
Locus	Allele	1P	2P	3P	4P	5P	6P	7P	8P	9P	10P	11P	12S	13S	14S	15S	16S	17S	18S
G3pdh	A	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.76	1.00	—	—	0.03	0.03	0.05	0.05
	B	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	—	0.24	—	1.00	1.00	0.97	0.97	0.95	0.95
6-Pgdh	A	—	—	—	—	—	0.40	—	—	0.43	—	—	—	—	—	0.00	0.03	0.13	0.13
	B	1.00	1.00	1.00	1.00	1.00	0.60	1.00	1.00	0.57	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.87	0.87
Acon-1	A	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	—	0.27	—	1.00	1.00	1.00	1.00	1.00	1.00
	B	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.63	1.00	—	—	—	—	—	—
Ck-1	A	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.76	1.00	—	—	—	—	—	—
	B	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	—	0.24	—	1.00	0.97	0.85	1.00	1.00	0.90
	C	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	0.15	—	—	0.10
Pgm-1	A	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	—	0.21	—	1.00	1.00	0.97	1.00	1.00	0.97
	B	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.79	1.00	—	—	0.03	—	—	0.03
Lap-1	A	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	—	0.21	1.00	—	—	—	—	—	—
	B	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.79	—	1.00	1.00	1.00	1.00	1.00	1.00
Est-1	A	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.79	1.00	—	—	—	—	—	—
	B	—	—	—	0.50	0.50	0.50	0.50	0.50	0.50	—	0.21	—	1.00	1.00	1.00	1.00	1.00	1.00
Icdh-1	A	—	—	—	—	1.00	—	—	—	—	—	—	—	1.00	1.00	1.00	1.00	1.00	1.00
	B	1.00	1.00	1.00	1.00	—	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—	—	—	—	—

Table 2 Allozymic heterozygosity, percentage polymorphic loci, non-random genotype frequencies, and clonal diversity in parthenogenetic and sexual populations of *Campeloma decisum*. Heterozygosity in parthenogenetic populations is based on direct counts

Population	Percentage polymorphic	Heterozygosity	Clone
Parthenogenetic			
Waccamaw Lake, NC	0.00	0.00	*ABBABAAB*
Flat River, NC	0.00	0.00	*ABBABAAB*
S. Fork New River, NC	0.00	0.00	*ABBABAAB*
Diamond Lake, WI	0.00	0.00	*ABBABBAB*
Chippewa River, WI	31.58	0.14	*ABBABBAB*(11) *CBCCCCCB*(8)
Potato Creek, IN	31.58	0.32	*CBCCCCCB**
Black Mallard River, MI	31.58	0.32	*CBCCCCCB**
Burt Lake, MI	*31.58*	*0.32*	*CBCCCCCB**
Thunder Bay River, MI	*36.84*	*0.36*	*CBCCCCCB**(4) *CCCCCCCB*(16)
Carp Lake River, MI	36.84	0.36	*CBCCCCCB**(3) *CCCCCCCB*(17)
Wolf Creek, IN	31.58	0.32	*CBCCCCCA**
Sexual			
Black River, NC	0.00	0.00	
Tangipohoa River, MS	0.00	0.00	
Old River, LA	5.26	0.003	
Iatt River, LA	15.79	0.019	
Bayou Bartholomew, LA	10.53	0.005	
Ouachita River, AR	10.53	0.017	
Saline River, AR	21.05	0.029	

*Indicates significant departure from Hardy–Weinberg equilibrium. Letters for multilocus clonal genotype refer to the 8 polymorphic loci in order of presentation in Table 1. A = fast allele present, B = slow allele present, C = fixed heterozygote. See Appendix 1 for sample sizes.

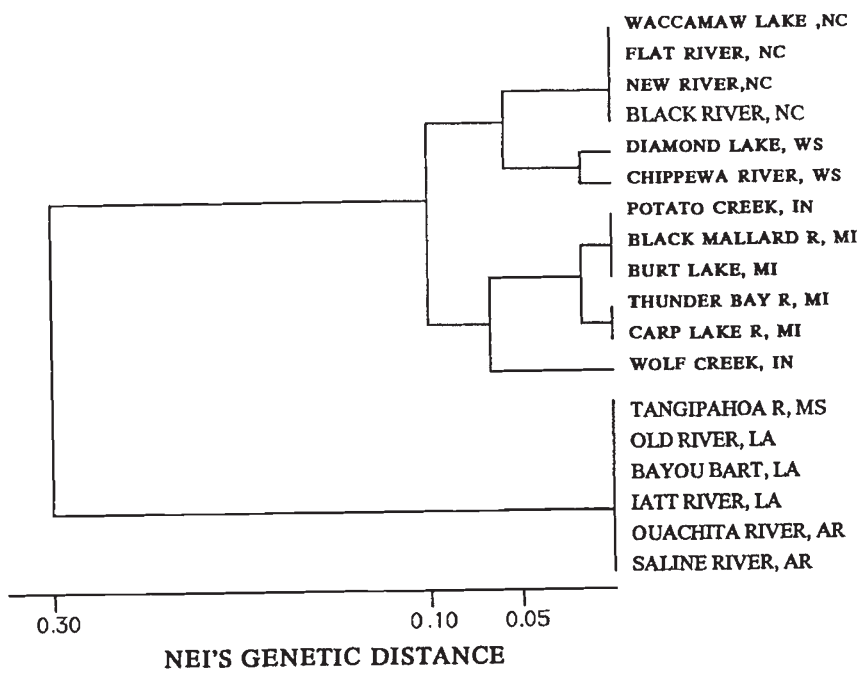

Fig. 2 UPGMA cluster analysis of sexual and parthenogenetic populations of *C. decisum* based on Nei's genetic distances. Sexual populations are indicated by larger capital letters.

tions are quite genetically divergent from the southeastern sexual populations, suggesting that these groups of populations may represent cryptic species. There is, however, very little genetic differentiation among these sexual populations.

Mode of parthenogenesis

The presence of fixed frequencies (0.5) for alternate alleles in the heterozygous parthenogens indicates apomictic parthenogenesis in which an unreduced egg undergoes mitotic divisions resulting in clonal replication of maternal genotypes. The fixed homozygosity at these 19 loci in various other parthenogens (1–3, 10–11) suggests that automixis may be the mode of reproduction, because we would expect some heterozygosity through accumulation of mutations in apomicts or central fusion automicts.

Discussion

This study provides critical evidence on the origins of parthenogenesis and the sources of genetic diversity within and among sexual and parthenogenetic populations of *Campeloma decisum*. Three salient results emerged from this study: first, the presence of two classes of parthenogens, fixed homozygotes and fixed heterozygotes, suggests that both the spontaneous origin of parthenogenesis and hybridization between sexual ancestors may have occurred; second, clonal diversity is minimal within and among parthenogenetic populations, with most of the clonal diversity stemming from variation present in ancestral sexual populations; lastly, cryptic allozyme variation exists between southeastern and southern sexual populations of *C. decisum*.

Hybridization and the origin of parthenogenesis

Numerous parthenogenetic taxa have dramatically higher levels of heterozygosity compared to their putative sexual ancestors and, furthermore, the gene combinations in parthenogenetic taxa are often derived from fixed alleles present in the ancestral sexual taxa (Schultz, 1969; White, 1973; Parker & Selander, 1976; Vrijenhoek *et al.*, 1977; Honeycutt & Wilkinson, 1989; Moritz *et al.*, 1989c). This pattern, derived mostly from unisexual vertebrates, focused attention on hybridization as a key factor in the origin of parthenogenesis. These results, however, suggest that, although hybridization between genetically divergent sexual ancestors may have been responsible for the origin of some parthenogens, hybridization is not necessary for the origin of parthenogenesis in *C. decisum*. The presence of fixed homozygotes at these 19 loci in parthenogens from North Carolina and Wisconsin, provides the strongest evidence that parthenogenesis can arise in the absence of hybridization. There is no indication that the North Carolina or Wisconsin clones are derived from hybridization, unless these parthenogens are derived from two sexual ancestors that are genetically identical at these 19 loci, but differ at other loci. Assuming these loci represent a somewhat random

Table 3 Nei's coefficient of genetic distance based on 19 presumptive genetic loci in parthenogenetic and sexual populations of *C. decisum*. Sample numbers correspond to those in Appendix 1

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	S12	S13	S14	S15	S16	S17	S18
P1	—	0.00	0.00	0.08	0.15	0.09	0.08	0.08	0.09	0.05	0.04	0.00	0.46	0.46	0.45	0.46	0.46	0.45
P2		—	0.00	0.08	0.15	0.09	0.08	0.08	0.09	0.05	0.04	0.00	0.46	0.46	0.45	0.46	0.46	0.45
P3			—	0.08	0.15	0.09	0.08	0.08	0.09	0.05	0.04	0.00	0.46	0.46	0.45	0.46	0.46	0.45
P4				—	0.06	0.01	0.00	0.00	0.06	0.08	0.02	0.08	0.15	0.15	0.14	0.15	0.15	0.15
P5					—	0.07	0.06	0.06	0.07	0.15	0.09	0.15	0.08	0.08	0.08	0.08	0.08	0.08
P6						—	0.01	0.01	0.00	0.09	0.03	0.09	0.16	0.16	0.16	0.16	0.15	0.15
P7							—	0.00	0.01	0.08	0.02	0.08	0.15	0.15	0.14	0.15	0.15	0.15
P8								—	0.01	0.08	0.02	0.08	0.15	0.15	0.14	0.15	0.15	0.15
P9									—	0.09	0.03	0.09	0.16	0.16	0.16	0.16	0.15	0.15
P10										—	0.01	0.05	0.38	0.38	0.37	0.38	0.38	0.37
P11											—	0.05	0.25	0.25	0.24	0.25	0.25	0.24
S12												—	0.46	0.46	0.45	0.46	0.46	0.45
S13													—	0.00	0.00	0.00	0.00	0.00
S14														—	0.00	0.00	0.00	0.00
S15															—	0.00	0.00	0.00
S16																—	0.00	0.00
S17																	—	0.00

survey, the probability seems minuscule that two sexual species would not differ at these loci.

It has been postulated that there was strong selection for the spontaneous origin of obligate parthenogenesis in *C. decisum* in response to male sterility caused by the unencysted metacercariae of a digenetic trematode, *Leucochloridiomorpha constantiae*. The presence of fixed homozygotes in North Carolina and Wisconsin is consistent with this spontaneous hypothesis. The presence of highly heterozygous individuals from northern populations may indicate dual origins of parthenogenesis in *C. decisum*. However, a complementary hypothesis involving male sterility by the digenetic trematode and secondary hybridization (*sensu* Cuellar, 1977) by genetically distinct males is possible.

The critical question is whether parthenogenesis arose as a by-product of impaired meiosis due to hybridization. The pattern of heterozygous genotypes can be readily explained by the fertilization of a diploid parthenogenetic female, which arose by parasitic castration, by males with the opposite alleles fixed. Incorporation of the divergent paternal genome and maintenance of parthenogenetic reproduction would produce heterozygous, triploid genotypes. There is no convincing evidence from the intensity of protein staining to indicate polyploidy. The hypothesis assumes that dispersal of parthenogenetic diploid females, probably from the southeastern populations, results in contact with sexual individuals from the southern states. This hypothesis predicts a hybrid zone between parthenogenetic and sexual populations. Flow cytometry could be used to address the hypothesis that heterozygous individuals are triploid, and may be rejected if heterozygous individuals are diploid. Varying ploidy levels occurred in *C. parthenum* (Dougherty, 1982); parthenogenetic *C. rufum* were diploid (Mattox, 1937). An analysis of mtDNA can address the prediction from the secondary hybridization hypothesis that the maternal genome should be derived from southeastern parthenogens.

Modes of parthenogenesis and the origin of clonal diversity

A surprising result of this study was the relatively low clonal diversity within and among parthenogenetic populations. The gene combinations in the five homozygous and heterozygous clones of *C. decisum* are all found in the sexual lineages in the southeastern and southern United States and, therefore, mutation has not generated significant clonal diversity. That most of the clonal variability is derived from sexual lineages also suggests a relatively recent origin of these parthenogens. Recent work on cladocerans (Crease *et al.*, 1989; Hebert *et al.*, 1989), ostracods (Havel & Hebert, 1989), grasshoppers (Honeycutt & Wilkinson, 1989), and a variety of vertebrates (Dawley & Bogart, 1989), revealed much higher levels of clonal diversity than that of *C. decisum*, with clonal variability being generated by repeated hybridization between sexual lineages. The low clonal diversity in *C. decisum* is probably attributable to two causes: parthenogenesis arising in the absence of hybridization and, where hybridization has occurred, the sexual ancestral lineages in the southeastern and southern state having low levels of genetic variation.

Different modes of parthenogenesis (automictic versus apomictic) in *C. decisum* are implicated by the presence of homozygous clones (North Carolina and Wisconsin) and highly heterozygous clones (Indiana, Michigan, and Wisconsin). The only likely mechanism of automictic parthenogenesis which preserves heterozygosity is central fusion of two polar nuclei providing that the initial mother is heterozygous and there is no recombination between the locus and the centromere (Suomalainen *et al.*, 1987). Apomictic reproduction occurs in *C. rufum* (Mattox, 1937), *C. decisum* in New York (Selander *et al.*, 1977) and *C. parthenum* (Karlin *et al.*, 1980).

Cryptic species of Campeloma

The taxonomic status of many species of *Campeloma* remains unclear (Vail, 1979; Burch, 1989). Recent revisions have resulted in grouping many northern species into *C. decisum*, presumably because the morphological characters used to differentiate these taxa were phenotypically plastic (Burch, 1989). The allozyme analysis in this study revealed genetic distances of 0.45–0.46 between the southeastern and southern sexuals. These populations have diverged considerably, and probably represent distinct species. Clearly, a thorough revision of the whole genus using molecular analysis should resolve the uncertain status of *Campeloma* species.

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Appendix

Table A1 Collection localities for parthenogenetic (1–11) and sexual (12–18) populations of *C. decisum*. All sample sizes are 20 individuals except for Diamond Lake and Chippewa River ($n = 19$). P and S refer to parthenogenetic and sexual populations, respectively

Sample Number	Population
P1	Waccamaw Lake, Columbus Co., NC
P2	Flat River, Person Co., NC
P3	South Fork New River, Ashe Co., NC
P4	Potato Creek, St Joseph Co., IN
P5	Wolf Creek, Marshall Co., IN
P6	Thunder Bay River, Alpena Co., MI
P7	Black Mallard River, Presque Isle Co., MI
P8	Burt Lake, Cheboygan Co., MI
P9	Carp Lake River, Emmet Co., MI
P10	Diamond Lake, Vilas Co., WS
P11	Chippewa River, Sawyer Co., WS
S12	Black River, Sampson Co., NC
S13	Tangipahoa River, Pike Co., MS
S14	Old River, LaSalle Parish, LA
S15	Iatt River, Grant Parish, LA
S16	Bayou Bartholomew, Morehouse Parish, LA
S17	Ouachita River, Ashley Co., AR
S18	Saline River, Bradley Co., AR

Table A2 Enzyme systems and buffers used in horizontal starch gel electrophoresis of *Campeloma decisum*. Running and voltage for each buffer were TC-EDTA (6 h at 120 V) and LiOH (5 h at 160 V).

Buffer	Enzyme system
Tris-citrate EDTA, PH 7.2 (135 mM) Tris, 45 mM citrate, 1.3 mM EDTA; gel 1:15 dilution)	Aconitase (Acon-1, Acon-2) Creatine kinase(Ck) Glycerol-3-phosphate dehydrogenase (G3pdh) Isocitrate dehydrogenase (Icdh-1, Icdh-2) Malate dehydrogenase (Mdh-1, Mdh-2) 6-Phosphogluconate dehydrogenase (6-Pgd) Phosphoglucomutase (Pgm)
Lithium hydroxide (Selander *et al.*, 1971)	Esterases (Est-1, Est-2) Leucine aminopeptidase (Lap-1, Lap-2) Leucine–glycine–glycine (Pep-B) Phosphoglucose isomerase (Pgi) Pro–leu (Pep-A, Pep-C) Superoxide dismutase (Sod-1)