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THE GENETIC MECHANISM OF SEX DETERMINATION IN THE CONCHOSTRACAN SHRIMP *EULIMNADIA TEXANA*

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Abstract.—We report the results of a laboratory pedigree analysis describing the unique sex-determining mechanism of the conchostracan shrimp, *Eulimnadia texana*. Natural populations of *E. texana* are mixtures of self-compatible hermaphrodites and males and represent one of the few known cases of androdioecy in animals. Hermaphrodites are of two types: amphigenic (producing both male and hermaphroditic offspring) and monogenic (producing only hermaphroditic offspring). We propose a simple genetic model to explain this polymorphism and show by genetic analysis that males, amphigenics, and monogenics can be interpreted as three alternative phenotypes of a one-locus system of sex determination. We discuss the implications of this novel system of sex determination for understanding the evolution of reproductive systems.

Animals and plants manifest a variety of reproductive mechanisms that range from asexuality to selfing hermaphroditism to bisexual outcrossing (Bell 1982). Such a spectrum of reproductive systems has motivated considerable research concerning the evolutionary forces responsible for this variation. Two related areas of inquiry have recently advanced: the evolution of self-fertilization (Jain 1976; Charlesworth and Charlesworth 1987) and the evolution of sex-determining mechanisms (Bull 1983; Wachtel 1989).

Consideration of the evolution of self-fertilization involves comparison of the relative advantages of selfing and outcrossing modes of sexual reproduction. A self-fertilizing organism gains an immediate 50% advantage relative to an outcrossing competitor because of the increased efficiency of gene transmission through selfed progeny, if selfing does not reduce fitness through male function and is not accompanied by inbreeding depression (Fisher 1941; Nagylaki 1976). Also, the ability to self-fertilize assures reproduction in habitats where the opportunities for outcrossing are low (Baker 1955). Nevertheless, since selfing is not the predominant mode of reproduction, selfed offspring must often be less fit than those produced through outcrossing. The reduced fitness associated with self-fertilization is termed inbreeding depression, which is generally attributed to reduced heterozygote advantage (overdominance model) or to expression of recessive deleterious genes (partial dominance model) (Charlesworth and

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Charlesworth 1987). Several theoretical treatments predict that the intensity of inbreeding depression is the critical parameter determining the evolutionary fate of a gene for selfing, with inbreeding depression greater than 50% favoring complete outcrossing and inbreeding depression less than 50% favoring complete selfing (Fisher 1941; Nagylaki 1976; Maynard Smith 1977; Lloyd 1979; Charlesworth 1980; Feldman and Christiansen 1984; Lande and Schemske 1985). In fact, Schemske and Lande (1985) suggest that this predicted bimodality of breeding systems exists in natural populations, though their conclusion has been contested (Waller 1986).

The second related topic concerns the evolution of the genetic mechanisms that determine various sexual systems (Bull 1983; Wachtel 1989). Within each of the above-mentioned reproductive systems is yet further variation in the underlying genetics controlling sexuality. Sexual differences can be regulated by numerous genetic systems, such as a single locus, heterogametic sex chromosomes, environmental sex determination, or haplodiploidy (for an excellent review, see Bull 1983). Understanding the mechanisms of sex determination of a particular system allows insight into the probable evolutionary path of that particular system.

The purpose of this article is to describe a newly discovered breeding system in a primitive crustacean, the clam shrimp *Eulimnadia texana* (Packard), that may facilitate our understanding of the evolutionary problems outlined above. We use a simple pedigree analysis to show that males, amphigenics, and monogenics are three alternative phenotypes of a genetically based system of sex determination. We propose a simple, one-locus genetic model to explain the results of our pedigree analysis, and then discuss the implications of this novel system of sex determination for understanding the evolution of reproductive systems.

MATERIALS AND METHODS

The Natural History of Eulimnadia texana

Eulimnadia texana is an inhabitant of temporary pools, ponds, ditches, small dry lakes, and other ephemeral freshwater habitats throughout western North America (Sassaman 1989). Clam shrimps are traditionally categorized as males and females on the basis of morphological dimorphism (see below), but we will show that *E. texana* "females" are actually functional hermaphrodites, as is the case in a closely related clam shrimp, *Limnadia lenticularis* (Zaffagnini 1969). Therefore, in what follows, we will characterize the two morphotypes as hermaphrodites and males.

Hermaphrodites bury desiccation-resistant eggs (about 220 μ in diameter) within the top several centimeters of the soil. Eggs hatch rapidly when hydrated under spring and summer conditions (generally at water temperatures above 18°C). Growth of nauplii and juveniles is rapid, leading to mature animals (ca. 6–7 mm in carapace length) within 7–8 d in the laboratory and in as little as 5–6 d in the field (Vidrine et al. 1987).

Sexual dimorphism is pronounced. The thoracic appendages of hermaphrodites

are unmodified phyllopods (flattened leaf-shape appendages), but the first two pairs of thoracic appendages in males undergo differentiation into clawlike claspers that they use to grasp the margins of the hermaphrodite's carapace during mating. Mating apparently consists of the transfer of a spermatophore-like package of sperm to the 11th pair of the hermaphrodite's phyllopods, with this package eventually migrating to the oviducal opening on the 11th thoracic segment (Strenth 1977). The need for modified appendages in the mechanics of fertilization eliminates the possibility of crossing between two hermaphrodites.

Populations of *E. texana* are characterized by hermaphrodite-biased sex ratios, with hermaphrodites typically constituting 70%–80% of the population (Sassaman 1989). Hermaphrodites may have higher survival in the field (Strenth 1977), which leads to more strongly biased sex ratios toward the end of the life span (about 1 wk after sexual maturity). Sex ratios vary spatially (Sassaman 1989), and some populations are composed entirely of hermaphrodites.

Rearing Methods

Experimental shrimp were reared from dry soil collected from the basin of a stock watering tank located about 6 km north of Portal, in the San Simon Valley of southeastern Arizona (designated elsewhere as the Portal 1 population; Sassaman 1989). Approximately 200 mL (by volume) of soil was hydrated in 30 L of demineralized water in 10-gal aquaria. Tanks were continuously aerated and were maintained on continuous light at a temperature of approximately 24°C. Tadpole shrimp (*Triops longicaudatus*) that hatched from the soil were removed to prevent predation, but the remaining fauna, consisting primarily of anostracans and cladocerans (and occasionally ostracods), was allowed to develop with the conchostracans. Cultures were fed ad lib. with a mixture of pulverized commercial goldfish food and baker's yeast.

Sex Determination

Eulimnadia can be distinguished from other genera of conchostracans by the fourth or fifth day at these temperatures, and early sexual differentiation of males is evident on about the sixth day. Sexual maturity (as indicated by pairing and by brooded clutches) begins on the eighth or ninth day, at which time *E. texana* can be distinguished from other species of the genus (Belk 1989). Sexes were determined on individuals at least 7 d old on the basis of differentiation of the modified claspers (males) and developing oocytes or brooded embryos (hermaphrodites).

Isolated Culture

Individuals to be reared in isolation were obtained from hatching tanks, beginning at day one to five. Juveniles were transferred to either 200- or 400-mL plastic cups filled with water (usually taken directly from the hatching tank) that was screened through a 63-μ sieve to exclude unhatched eggs or other small larvae. Juveniles and adults were fed ad lib. with the same foods used to supplement the

hatching tanks. The shrimp were reared to maturity, and hermaphrodites were kept alive for an additional 3 d of egg production.

Rearing of Laboratory Clutches

Clutches (egg banks) obtained from either outcrossed or selfed hermaphrodites were dried at room temperature and maintained in that state for several weeks to several years. Dried eggs were hatched by adding demineralized water and placing them near a light source to create temperatures of approximately 27°C. Hatched nauplii were then transferred to a rearing tank with 400 mL of steam-sterilized soil (further sieved to an exclusion diameter of 150 μ) that was hydrated with 30 L of demineralized water. The rearing tanks were maintained as described above. Segregation data were tabulated for clutches in which a minimum of 20 offspring were reared to sexual maturity.

Electrophoretic Methods

Individuals were prepared for electrophoretic assay by removing the carapace, the head and its associated digestive glands, the gut contents, and (in hermaphrodites) any eggs adhering to the epipodites. The carcass was then stored at −70°C in a microtiter plate well until further processing.

Individuals were homogenized in 70 μL of buffer (0.05 M Tris-HCl, pH 7.5) using a glass rod ground to fit tightly into the storage well. Samples of the extract were loaded into preformed wells in 12% starch gels for protein electrophoretic separations. Two buffer systems were used: a Tris-citrate/LiOH-borate discontinuous system (Selander et al. 1971) was used for the analysis of phosphoglucomutase (*Pgm,* E.C. #5.4.2.2) and mannose phosphate isomerase (*Mpi,* E.C. #5.3.1.8), and a citrate-aminopropylmorpholine continuous system at pH 8.5 (Clayton and Tretiak 1972) was used for fumarase (*Fum,* E.C. #4.2.1.2) and isocitrate dehydrogenases (*Idh,* E.C. #1.1.1.42). Protein positions were determined with conventional histochemical stains (Shaw and Prasad 1970). The genotypic interpretation of electrophoretic patterns was based on laboratory analyses of their inheritance.

Polymorphisms in the Portal 1 population were as follows (Sassaman 1989): *Pgm,* two alleles; *Fum,* two alleles; *Idh-2,* two alleles, *Mpi,* four alleles; all in frequencies greater than 0.05. Both homozygotes and heterozygotes at all four loci were observed in both sexual types.

Protocols for Crosses

Males and hermaphrodites to be used in crossing experiments were chosen by pedigree or were later determined to be homozygous for different alleles at one or more of the four allozyme marker loci. Thus, selfed offspring were homozygous and identical to the hermaphroditic parent at that locus, whereas outcrossed offspring were heterozygous for maternally and paternally derived alleles. All crosses were single-pair matings and were maintained for 3 d after hermaphrodites first reached maturity. All offspring reared from putative crosses were typed by electrophoresis for their genotypes at the marker loci.

RESULTS

Population and Clutch Sex-Ratio Polymorphism among Hermaphrodites

The sexual phenotype of several hundred individuals was monitored over their lifetimes without any cases of sex change or reversal. We reared 308 individuals to maturity from the Portal 1 soil samples, of which 20% were male.

Forty-nine Portal 1 hermaphrodites were isolated and progeny tested by rearing their offspring to maturity. There were two types of hermaphrodites in the sample. One type (hereafter called monogenic) produced only hermaphroditic offspring; a total of 574 hermaphroditic offspring were reared from 12 "isohermaphrodite" clutches of this type. The second type of hermaphrodite (hereafter called amphigenic) produced a mixed clutch of both hermaphroditic and male offspring in a characteristic ratio of 3:1. Thirty-seven of the hermaphrodites were of this type; they produced an aggregate of 618 males and 2,017 hermaphrodites (23.5% males). Four of the 37 isohermaphroditic clutches deviated from a 3:1 ratio at a probability criterion of $P < .05$, and only two did so at a level of $P < .01$, with deviations above and below 25% males being equally frequent.

Inheritance of Allozyme Markers during Selfing

The genotypic distributions of offspring produced by heterozygous hermaphrodites reared in isolation are shown in table 1. In each clutch, and for all four loci, the results were the same: heterozygous hermaphrodites produced an offspring distribution of 1:2:1 of the homozygous, heterozygous, and alternative homozygous classes, respectively. This pattern of allozyme inheritance was observed in both amphigenic and monogenic hermaphrodites.

The segregation of alleles and the binomial distribution of offspring genotypes indicated that gametes were produced meiotically and that eggs were not fertilized by reabsorbing polar bodies. The cytological basis of gamete production was not clear. Serial sections of *Eulimnadia texana* have not yet revealed gonadal tissue resembling the ovotesticular organization reported for *Limnadia* (Zaffagnini 1969). Nevertheless, the genetic consequence of the reproductive mechanism, whatever it may be, was operationally selfing (simultaneous) hermaphroditism.

The Inheritance of Clutch Sex Ratio

Inheritance of the offspring sex ratio was similar to the inheritance of codominant (allozymic) polymorphisms. Ten hermaphrodites isolated from a monogenic clutch were themselves typed by progeny testing. Each of the 10 produced an all-hermaphroditic clutch, leading to a total of 2,603 F_2 offspring from the original monogenic hermaphrodite. Thus, monogeny was a pure-breeding trait, an observation consistent with the interpretation that it represented a homozygous state in the genetic system of sex determination.

In contrast, the amphigenic hermaphrodites were not pure breeding. Not only did they produce clutches that were one-quarter male, but the hermaphroditic offspring of amphigenic hermaphrodites were themselves a mixture of monogenic and amphigenic hermaphrodites. We tested the ratio of these two categories of

TABLE 1

INHERITANCE OF CODOMINANT POLYMORPHISMS DURING SELFING OF HETEROZYGOTE HERMAPHRODITES

LOCUS AND CLUTCH NO.	SEX*	GENOTYPE DISTRIBUTION f/f	f/s	s/s	SAMPLE SIZE	χ^2†	*P*
Fum:							
58	M	0	0	20	64	2.59	.27
	H	11	33	0			
61	M	0	0	18	83	4.73	.09
	H	14	51	0			
66	M	0	0	21	80	1.07	.58
	H	16	43	0			
70	M	0	0	9	44	.86	.64
	H	10	25	0			
71	M	0	0	24	102	.35	.83
	H	24	54	0			
Idh-2:							
58	M	20	0	0	64	2.59	.27
	H	0	33	11			
61	M	18	0	0	83	4.73	.09
	H	0	51	14			
71	M	24	0	0	102	.35	.83
	H	0	54	24			
73	M	25	0	0	103	.96	.62
	H	0	56	22			
Mpi:							
79‡	M	4	13	1	55	1.36	.50
	H	11	17	9			
55-26§‖	H	19	59	22	100	3.42	.18
12-2-10‡‖	H	10	27	12	49	.67	.71
Pgm:							
58	M	7	7	6	61	4.08	.13
	H	7	18	16			
55-26‖	H	31	51	18	100	3.42	.18
12-2-10‖	H	12	27	11	50	.36	.83

* M, males and H, hermaphrodites.
† χ^2 tests and associated *P* values are for the expectation of a 1:2:1 segregation ratio.
‡ The *Mpi* genotypes are 1/1, 1/2, and 2/2, respectively.
§ The *Mpi* genotypes are 1/1, 1/3, and 3/3, respectively.
‖ Only hermaphrodites were electrophoretically sampled in these two clutches.

hermaphrodites among the offspring of five amphigenics by isolating each F_1 hermaphroditic "daughter," and then progeny testing each of these daughters. Forty-two of the hermaphrodites were monogenic, and their clutches totaled 5,233 hermaphroditic offspring (table 2). The remaining 95 hermaphrodites were amphigenic. Their clutches totaled 2,948 males and 9,814 hermaphrodites (23.1% males). Of the individual clutches, 12 deviated from a 3 hermaphrodite/1 male ratio at $P < .05$, and only 3 deviated from this ratio at $P < .01$. All except one deviation were deficiencies of males, however, which might suggest that male survival rate was lower in these experiments. Thus, the actual distribution of offspring types that arose from selfed amphigenic hermaphrodites was approxi-

TABLE 2

DISTRIBUTION OF OFFSPRING TYPES AMONG DAUGHTERS OF AMPHIGENIC HERMAPHRODITES

Family No.	No. of Amphigenic Hermaphrodites	No. of Monogenic Hermaphrodites	χ^2*	P
Natural isolates:				
55	25	10	.35	.55
58	7	3	.05	.82
70	16	8	. . .	1.00
Subtotal	48	21	.26	.61
Laboratory produced:				
12-1-1	19	2	5.35	.02
12-2-10	28	19	1.06	.30
Subtotal	47	21	.18	.66
Total	95	42	.44	.50

* χ^2 tests and associated P values are for the expectation of a 2:1 amphigenic to monogenic ratio.

mately 25% males/50% amphigenic hermaphrodites/25% monogenic hermaphrodites.

To test for the inheritance of sex ratio during crossing in the monogenics, nine matings were made between males reared from Portal 1 soil and hermaphrodites reared from a pure-breeding monogenic line. The outcrossing rate in these matings ranged from 0% to 100%, but every resulting offspring was hermaphroditic, including those that were the result of outcrossing. Forty-six of the hermaphroditic offspring that carried an electrophoretic marker (*Idh-2*) indicating their outcrossed origin were then progeny tested by rearing the clutches that they produced by selfing. Every hermaphrodite tested was amphigenic (table 3). Of the 46 clutches reared, eight deviated from a 3:1 ratio at $P < .05$ and five deviated at $P < .01$. Deviations more commonly involved deficits of males (five of eight cases) and were generally correlated with extremely high densities of shrimp in the rearing tanks. Nevertheless, the overall proportion of males from the 46 clutches was 23.6%. Thus, the offspring of a sexual cross between a male and a monogenic hermaphrodite was always an amphigenic hermaphrodite.

Finally, to test for sex-ratio inheritance in outcrossed amphigenics, amphigenic hermaphrodites (produced by marked matings of males to monogenic hermaphrodites) were in turn backcrossed to males marked at a second locus. The sex ratios of selfed and backcrossed offspring are summarized in table 4. Those individuals homozygous for the *Fum* f allele were the selfed offspring of amphigenic hermaphrodites (and exhibited the same 3 hermaphrodite/1 male ratio obtained by selfing amphigenics); the *Fum* f/s heterozygotes represented backcrossed offspring and exhibited a 1 hermaphrodite/1 male segregation ratio. Thus, the sex ratio in outcrossed clutches of amphigenics is 50% hermaphrodites and 50% males.

TABLE 3

CLUTCH SEX RATIOS OF SELFING AMPHIGENICS DERIVED FROM OUTCROSSED MONOGENICS

Cross	No. of Offspring Tested	No. Producing Males	No. of Males	No. of Hermaphrodites	Proportion Male
12-1	10	10	1,079	3,478	.237
12-2	10	10	496	1,714	.224
12-3	6	6	89	357	.200
12-7	10	10	844	2,743	.235
12-8	10	10	483	1,397	.257
Total	46	46	2,991	9,689	.236

TABLE 4

CLUTCH SEX RATIOS OF OUTCROSSED AND SELFED OFFSPRING FROM BACKCROSSED AMPHIGENIC HERMAPHRODITES

Cross and Mating Status	No. of Males	No. of Hermaphrodites	Proportion Male
12-7-3:			
Fum f/f (hermaphroditic)	38	109	.259
Fum f/s (backcross)	53	42	.558
12-1-6:			
Fum f/f (hermaphroditic)	121	342	.261
Fum f/s (backcross)	86	97	.470
12-1-7:			
Fum f/f (hermaphroditic)	91	268	.253
Fum f/s (backcross)	31	33	.484
Sum of hermaphroditic selfing	250	719	.258
Sum of backcrosses	170	172	.497

Linkage Relationships and Restriction of Recombination

The *Idh-2* and *Fum* loci were tightly linked to each other and to the sex-determining element. Hermaphrodites 58, 61, and 70 (table 1) were amphigenics and were also doubly heterozygous for *Idh-2* and *Fum*. The selfed progenies of each hermaphrodite contained only three two-locus genotypes: *Idh-2* f/f and *Fum* s/s, *Idh-2* s/s and *Fum* f/f, and *Idh-2* f/s and *Fum* f/s. There were no recombinant genotypes among the 249 offspring. Furthermore, all males were the *Idh-2* f/f and *Fum* s/s doubly homozygous genotype; the alternate double homozygotes and the double heterozygotes were hermaphrodites. Segregation of *Mpi* and *Pgm* genotypes (table 1) occurred independently of each other, of *Fum* and *Idh-2*, and of offspring sex during selfing of amphigenics.

Progeny testing of the daughters from amphigenics (table 2) also indicated linkage of *Idh-2* and *Fum* to the sex-determining element. Three selfed amphigenic hermaphrodites heterozygous for *Idh-2* collectively produced 53 amphigenic

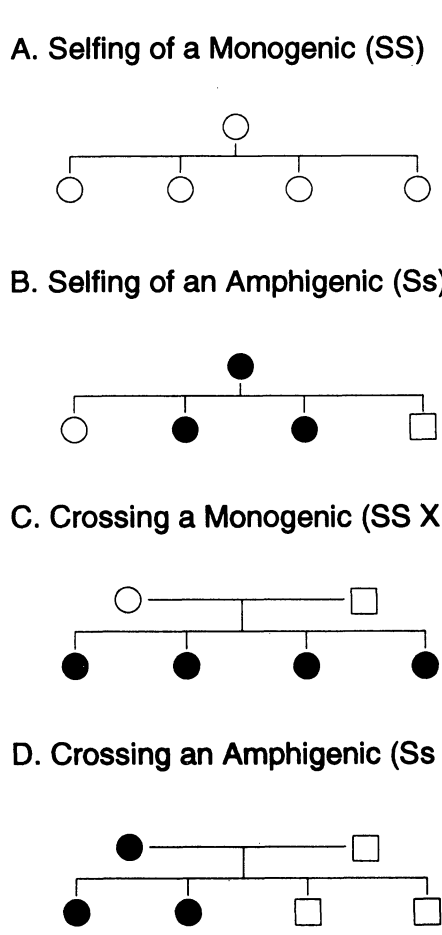

FIG. 1.—Pedigree predictions for selfing and outcrossing monogenic and amphigenic hermaphrodites. *A*, Selfing of a monogenic hermaphrodite (*open circles*) results in 100% monogenic hermaphroditic offspring. *B*, Selfing of an amphigenic hermaphrodite (*solid circles*) results in 25% monogenic hermaphroditic, 50% amphigenic hermaphroditic, and 25% male (*open squares*) offspring. *C*, Crossing a monogenic hermaphrodite with a male results in 100% amphigenic hermaphroditic offspring. *D*, Crossing an amphigenic hermaphrodite with a male results in 50% amphigenic hermaphroditic and 50% male offspring.

TABLE 5

SUMMARY OF LABORATORY INHERITANCE DATA

	No. of Crosses	Males	Hermaphrodites	Proportion Male	
				Observed	Expected
SS × SS (selfing)	10	0	2,603	.000	.000
SS × ss	8	0	367	.000	.000
Ss × Ss (selfing)	46	2,991	9,689	.236	.250
Ss × ss	3	170	172	.497	.500

daughters heterozygous for *Idh-2* as well as 23 monogenic daughters homozygous for *Idh-2*. Three selfed amphigenics heterozygous for *Fum* collectively produced 48 amphigenic daughters heterozygous for *Fum,* 17 monogenic daughters homozygous for *Fum,* and 1 monogenic daughter heterozygous for *Fum*. This last offspring represented the only apparent recombinant between either marker locus and the sex-determining element(s).

MODEL OF SEX DETERMINATION IN *EULIMNADIA*

The simplest model to explain our results is to suggest a single gene or genetic element with two allelic states, S and s, with s recessive to S. Under this model, males are genotypically ss, amphigenic hermaphrodites are Ss, and monogenic hermaphrodites are SS, respectively. The model incorporates the following inferences from the analysis of inheritance: selfing involves the combination of two pronuclei that are independently derived meiotic products (table 1), monogeny is a pure-breeding (homozygous) trait, amphigeny is a heterozygous condition (table 2), and males are homozygous recessive for the sex-determining element(s). The specific predictions for pedigrees of amphigenic and monogenic hermaphrodites during both unisexual reproduction and sexual outcrossing are diagrammed in figure 1. A summary of the genetic tests of the model (table 5) shows that all four predictions have been verified through laboratory crosses.

Our model of sex determination implies that the genotype of an amphigenic hermaphrodite simultaneously determines that it will produce both male and hermaphroditic offspring and that the frequency of males will be 25%. Although the majority of clutches reared from amphigenic hermaphrodites exhibited a ratio of hermaphrodites/males that was not significantly different from the predicted 3:1, there was a consistent pattern of a slight deviation from that expectation. Futhermore, the offspring distribution of these clutches was, in aggregate, less than the expectation of 25% males.

We do not believe that this discrepancy nullifies the genetic model that we have proposed (fig. 1). Sex-specific survival differences may explain the deficits of males in our studies. Higher male than hermaphroditic mortality was found in field studies of *E. texana* (Strenth 1977). Thus, the deviation from the expectation of 25% males could be because of the lower survival rate of males under laboratory rearing conditions and does not invalidate our proposed model of sex determination.

EVOLUTIONARY IMPLICATIONS

Consideration of *Eulimnadia texana*'s unique reproductive system may improve our understanding of three areas of reproductive biology: the evolution of sex-determining mechanisms, the evolution of androdioecy, and the maintenance of cross-fertilization. Though the sex-determining mechanism of *Eulimnadia* is different from any other so far described, it can be placed in context by considering its similarities and differences to other reproductive systems (Bull 1983). Its uniqueness derives from the ability of the sex derived from the dominant genetic

element to self-fertilize and thus produce the two forms of hermaphrodites. The recessive sexual element coding for maleness is less common than the converse in crustaceans but is not uncommon (Ginsburger-Vogel and Charniaux-Cotton 1982). Unisexual reproduction is also described in many crustaceans and is relatively common in branchiopods (Longhurst 1955; Zaffagnini 1969; for review, see Bell 1982).

The ability of the sex derived from the dominant genetic element to self-fertilize results in the phenotypic expression of all three sex-determining genotypes (i.e., population system = phenotype system) (Bull 1983), an impossible outcome if the males were heterogametic (as in the nematode *Caenorhabditis elegans* [McCoubrey et al. 1988] or in the polychaete *Capitella capitata* [Petraitis 1985*a*, 1985*b*, 1988]) or if the hermaphrodites were incapable of self fertilization. Although such a polymorphism can be created in other organisms by artificial manipulation of phenotypic gender (see, e.g., Humphrey 1945; Katakura 1984), it is a natural and common feature of *E. texana* populations.

Our data do not distinguish between sexual inheritance as a single-locus, two-allele trait or as a trait determined by a chromosomal segment bearing nonhomologous genes in its s and S forms. Two allozyme loci (*Fum* and *Idh-2*) were closely linked to the sex-determining element but were diploid in both sexes, which established that there was at least partial homology between s- and S-bearing chromosomes. Bowen (1963, 1965) established that male homogamety prevails in the brine shrimp *Artemia* and that sex-linked eye color genes were diploid in both males and females. Bowen proposed two possible models of the sex-chromosomal constitution of *Artemia*. The sex chromosome could simply be an autosome with a sex-determining gene embedded somewhere along its length. Alternatively, the sex chromosome could contain two regions, a "differential segment" on which nonhomologous genes specifying gender were carried and an "homologous segment" bearing other genes such as the white-eye marker locus. Bowen (1965) favored the second model because of karyological evidence of chromosomal heterogeneity among sexes in other *Artemia* populations. Recent evidence for pseudoautosomal inheritance in man and mice (see, e.g., Goodfellow and Goodfellow 1989) indicate a similar sex-chromosomal constitution in mammalian XY (male-heterogametic) sex-determining systems.

In *Eulimnadia*, both s- and S-bearing chromosomes, which by definition should be termed the sex chromosomes, are homozygously expressed in natural populations. Therefore, we would not expect deterioration of one or the other chromosome because of masked mutation (Muller 1914, 1918) or a Muller's ratchet phenomenon (Charlesworth 1978), nor any translocation of material from the s to the S segment (White 1973) that would result in reduced fitness accompanying homozygous expression. Furthermore, since there is no apparent phenotypic difference between Ss and SS hermaphrodites, the chromosomes are likely to differ primarily in sex-determining factors (Ohno 1967). Thus, we expect eventual chromosomal examination to reveal no evidence of heteromorphic sex chromosomes in *E. texana*.

Eulimnadia's reproductive mechanism also has interesting implications for the evolution of androdioecy. The presence of males and hermaphrodites in most

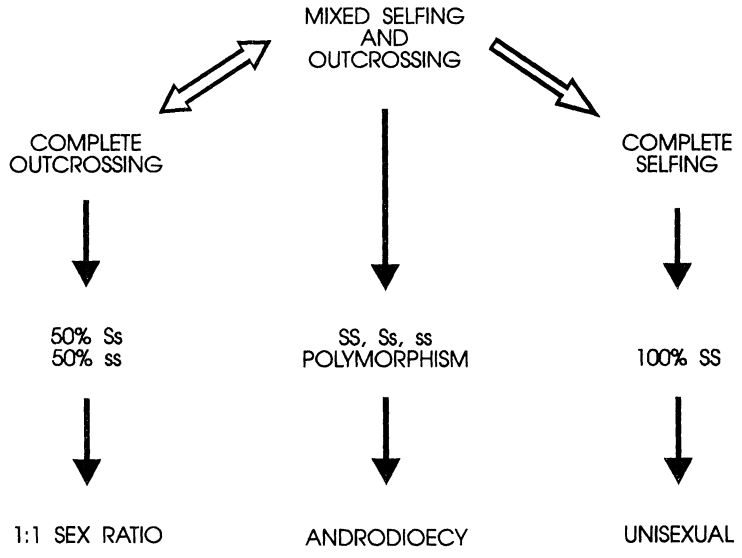

FIG. 2.—Possible evolutionary outcomes of the proposed sex-determining mechanism. *Eulimnadia texana* populations can alternate between complete outcrossing and a mixed strategy (transition depicted as a *two-sided open arrow*), whereas switching to complete selfing results in the loss of the s allele and thus the ability to outcross (transition depicted as a *single-sided open arrow*). Population characteristics associated with these three alternatives are shown under the *solid arrows*.

populations of *E. texana* (Sassaman 1989) represents one of the few reported cases of androdioecy in animals; it has been documented in barnacles of the order Thoracica (Newman et al. 1969; McLaughlin and Henry 1972; Crisp 1983). Androdioecy is thought to evolve by the spread of a mutant allele that causes a loss of female function in a hermaphroditic population, which results in a phenotypic polymorphism of males and hermaphrodites (Lloyd 1975; Charlesworth and Charlesworth 1978). Such a mutant allele is not predicted to spread in populations with even moderate levels of inbreeding. Since *E. texana* populations are typified by a significant level of selfing (Sassaman 1989), the existence of androdioecy in these shrimp appears to refute the prediction that androdioecy cannot evolve in such populations (Charlesworth 1984).

The ability of the *E. texana* hermaphrodites to self-fertilize makes the loss of males, and consequently cross-fertilization, a likely evolutionary end point. Since inbreeding confers an immediate fitness advantage (Fisher 1941), the maintenance of males in populations can only be the result of a concurrent reduction in fitness resulting from some type of inbreeding depression (Fisher 1941; Nagylaki 1976; Maynard Smith 1977; Lloyd 1979; Feldman and Christiansen 1984; Lande and Schemske 1985).

Theoretical analysis of this system (Otto et al. 1993) yields the qualitative interactions between populational outcrossing rates, heterozygosity of the sex-determining genes, and population sex ratios that are illustrated in figure 2. With

complete outcrossing, monogenic hermaphrodites would be eliminated (since monogenics are only produced by selfing hermaphrodites), and the system would be indistinguishable from any other system of obligate sexuality. With complete selfing, there would be rapid elimination of the s allele, which would eliminate both males and amphigenic hermaphrodites. The maintenance of both types of hermaphrodites in most natural populations (indicated by their largely hermaphrodite-biased sex ratios) suggests a mixed mating system of both outcrossing and selfing (Sassaman 1989). These shrimp clearly do not support the prediction that populations will be either completely selfing or completely outcrossing (Lande and Schemske 1985; Schemske and Lande 1985).

The maintenance of intermediate levels of selfing might result from temporally increasing inbreeding depression (Maynard Smith 1977), frequency-dependent fitnesses (Lloyd 1980), or reproductive assurance (Baker 1955). Considering the life history of clam shrimp, the ability to self-fertilize may be extremely important for the successful colonization of new, unoccupied habitats (Baker 1955). If we assume that outcrossing is advantageous, because of maintenance of heterozygosity, production of variability, or some related phenomena, the ability to self may be retained because of the advantage of successfully colonizing new habitats with a single propagule (Stebbins 1958; Baker 1963; Grant 1975; Jain 1976). Indeed, the ability of the amphigenic hermaphrodites to produce males imparts an added advantage to a colonizing species by permitting a single dispersed zygote to be the progenitor of a sexual population, which may increase the persistence of genetic variability in newly established populations. A trend of increased incidence of males with increased pond age would be evidence for the maintenance of selfing because of reproductive assurance.

In conclusion, the sex-determining mechanism proposed above is unique, yet simple. Further karylogical studies should establish the presence or absence of heteromorphic sex chromosomes, which would thus allow us to better understand the evolution of this intriguing sex-determining mechanism. Histological work in progress should establish the mechanics of self-fertilization, which may provide evidence for the secondary evolution of hermaphroditism proposed above. Also, exploiting *E. texana*'s unique combination of life-history traits, simple nutrient requirements, and short generation time provides a useful experimental system with which to study inbreeding depression in the laboratory.

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