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MAINTENANCE OF ANDRODIOECY IN THE FRESHWATER SHRIMP, *EULIMNADIA TEXANA*: ESTIMATES OF INBREEDING DEPRESSION IN TWO POPULATIONS

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Abstract.—Androdioecy is an uncommon form of reproduction in which males coexist with hermaphrodites. Androdioecy is thought to be difficult to evolve in species that regularly inbreed. The freshwater shrimp *Eulimnadia texana* has recently been described as both androdioecious and highly selfing and is thus anomalous. Inbreeding depression is one factor that may maintain males in these populations. Here we examine the extent of ''late'' inbreeding depression (after sexual maturity) in these clam shrimp using two tests: (1) comparing the fitness of shrimp varying in their levels of individual heterozygosity from two natural populations that differ in overall genetic diversity; and (2) specifically outcrossing and selfing shrimp from these same populations and comparing fitness of the resulting offspring. The effects of inbreeding differed within each population. In the more genetically diverse population, fecundity, size, and mortality were significantly reduced in inbred shrimp. In the less genetically diverse population, none of the fitness measures was significantly lowered in selfed shrimp. Combining estimates of early inbreeding depression from a previous study with current estimates of late inbreeding depression suggests that inbreeding depression is substantial ($\delta = 0.68$) in the more diverse population and somewhat lower ($\delta = 0.50$) in the less diverse population. However, given that males have higher mortality rates than hermaphrodites, neither estimate of inbreeding depression is large enough to account for the maintenance of males in either population by inbreeding depression alone. Thus, the stability of androdioecy in this system is likely only if hermaphrodites are unable to self-fertilize many of their own eggs when not mated to a male or if male mating success is generally high (or at least high when males are rare). Patterns of fitness responses in the two populations were consistent with the hypothesis that inbreeding depression is caused by partially recessive deleterious alleles, although a formal test of this hypothesis still needs to be conducted.

Key words.—Breeding systems, evolution, freshwater crustaceans, outcrossing, selfing.

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Androdioecy, in which populations are comprised of males and hermaphrodites with no pure females, is uncommon in plants and animals (Charlesworth 1984). This relative rarity is consistent with models that predict androdioecy to be unlikely to evolve, especially in self-fertilizing populations (Lloyd 1975; Charlesworth 1984; Charlesworth and Charlesworth 1987). Androdioecious mating systems appear to be anomalous because the benefits of being all-male in a primarily hermaphroditic population are unclear (Charlesworth 1984). Becoming all-male could have two potential benefits: reduced inbreeding depression for male-sired offspring and increased allocation to male function (relative to male allocation in hermaphrodites). Because fitness through male function is based on the availability of mates, any amount of self-fertilization in hermaphrodites reduces potential mates in an androdioecious population, making it difficult for the all-male strategy to be successful (Lloyd 1975; Charlesworth 1984). Therefore, if being all-male is beneficial because of reduced inbreeding depression, but all-male individuals cannot invade primarily selfing populations, the evolution of an androdioecious population becomes paradoxical (Charlesworth 1984). In fact, Charlesworth (1984) reviewed several species that were previously classified as androdioecious and found that most were functionally dioecious.

Nevertheless, several androdioecious systems have been documented since Charlesworth's (1984) review. In plants there are a handful of reported cases: the polyploid annual *Mercurialis annua* (Pannell 1997a,b); the shrubs *Phillyrea angustifolia* (Lepart and Dommee 1992) and *Phillyrea latifolia* (Aronne and Wilcock 1994); and the herbaceous perennials *Saxifraga cernua* (Molau and Prentice 1992) and *Datisca glomerata* (Liston et al. 1990). Of these, *D. glomerata* is the best studied example of a truly androdioecious species (Liston et al. 1990; Rieseberg et al. 1993). Sex appears to be controlled by two genetic loci, with female sterility being recessive at both loci (i.e., individuals with one or more dominant alleles at either locus are hermaphroditic; Wolf et al. 1997). Androdioecy is maintained due to a combination of factors: high outcrossing rates (65–92%; Fritsch and Rieseberg 1992), greater pollen production per flower in male-only plants (Philbrick and Rieseberg 1994), protogyny (Rieseberg et al. 1993), earlier male flowering (Spencer and Rieseberg 1995), and inbreeding depression in selfed offspring (Rieseberg et al. 1993).

In animals, there are two well-documented cases of androdioecy: the nematode *Caenorhabditis elegans* (Wood 1988) and the crustacean *Eulimnadia texana* (Sassaman and Weeks 1993; Zucker et al. 1997). *Caenorhabditis elegans* has an androdioecious mating system in which the hermaphrodites primarily self, but males are produced in a limited number of offspring by the nondisjunction of the X chromosome (Hodgkin et al. 1979). Outcrossing among hermaphrodites does not occur, and thus all outcrossing occurs via males. Hermaphrodites produce fewer sperm than eggs, and thus can only fertilize approximately 80% of their total eggs, unless outcrossed (Ward and Carrel 1979; Hodgkin and Barnes 1991). Outcrossing induces greater egg production and can increase, up to twofold, the overall reproductive output (Kimble and Ward 1988).

A second androdioecious system in animals has recently been described in crustaceans: the clam shrimp *E. texana* (Sassaman and Weeks 1993; Zucker et al. 1997). In this system, males coexist with hermaphrodites of two phenotypically similar but genetically different types: ''amphigenic'' and ''monogenic'' hermaphrodites. Sex appears to be controlled by a single locus (Sassaman and Weeks 1993), with a recessive allele coding for males (s) and a dominant allele for hermaphrodites (S). The homozygous dominants (SS) are monogenic hermaphrodites, the heterozygotes (Ss) are amphigenic hermaphrodites, and homozygous recessives (ss) are males (Sassaman and Weeks 1993). In this system, hermaphrodites cannot mate with one another (similar to *C. elegans*) because hermaphrodites lack the clasping appendages necessary for pairing. Thus, all outcrossing must involve males, which provides males with an advantage to the extent that selfing causes inbreeding depression. Except for one study on early inbreeding depression (Weeks et al. 1999), the factors allowing the persistence of androdioecy in this system remain to be discovered.

Because previous models of the stability of androdioecious populations (e.g., Lloyd 1975) are not appropriate for the *E. texana* populations (primarily because *E. texana* hermaphrodites cannot fertilize one another), Otto et al. (1993) developed a specific model to explore the conditions under which a mixed mating system, consisting of all three mating types (monogenics, amphigenics, and males), could be stable in *E. texana.* Their model consisted of four main parameters: α, the ability of males to fertilize hermaphrodites; β, the proportion of a hermaphrodite's eggs not fertilized by a male which are then self-fertilized; δ, inbreeding depression; and $(1 - \sigma)$, the relative survival of males to hermaphrodites. Otto et al. (1993) found that all three mating types would coexist if the following inequality was true:

$$\alpha(1 - \sigma) > 2\beta(1 - \delta). \quad (1)$$

If equation (1) was not true, then the system should evolve toward all selfing, which would eventually leave only monogenic hermaphrodites.

We continue our exploration of the dynamics of this mixed-mating system by estimating the inbreeding depression (δ) parameter of the Otto et al. (1993) model using two separate populations of these shrimp. We show that inbreeding depression in later life (egg production and survival after sexual maturation) is substantial in the more genetically diverse of the two populations, but not in the less diverse population. We combine these later-life estimates with previously collected early-life (hatching success and survival before sexual maturity) estimates to produce a lifetime estimate of inbreeding depression for both populations. We use these estimates to predict the necessary values of the remaining parameters in equation (1) that would allow the mixed-mating system observed in natural populations of these shrimp to be stable. We also discuss the observed patterns of inbreeding depression in relation to models for the genetic basis of inbreeding depression (Charlesworth and Charlesworth 1987).

Materials and Methods

Natural History of Eulimnadia texana

Eulimnadia texana inhabits temporary pools, ponds, ditches, and other ephemeral freshwater habitats throughout the southern United States, west of the Mississippi River, and into northern Mexico (Sassaman 1989). Hermaphrodites produce desiccation-resistant cysts that they bury within the top several millimeters of the soil. These cysts hatch rapidly following hydration under spring and summer conditions (Brendonck 1996), and release a nauplius larva. Larval and juvenile growth is extraordinarily rapid. Shrimp reach reproductive size in four to seven days in the laboratory at 27–30°C (Sassaman and Weeks 1993; Weeks et al. 1997) and in as little as four to six days in the field (Vidrine et al. 1987). The hermaphrodites produce thousands of eggs in their lifetime, generating clutches of up to 350 eggs one to two times a day (Knoll 1995; Weeks et al. 1997). Clutch size increases significantly with carapace length (Knoll and Zucker 1995; Weeks et al. 1997).

Sexual dimorphism is pronounced. The thoracic appendages of hermaphrodites are unmodified, but the first two pairs of thoracic appendages in males undergo differentiation into clawlike claspers that are used to hold on to the margins of a hermaphrodite's carapace during mating. *Eulimnadia texana* is omnivorous, able to filter feed as well as forage along pond floors.

Natural populations of *Eulimnadia* are typically hermaphrodite biased (Mattox 1954), with some populations completely lacking males (Zinn and Dexter 1962; Stern and Stern 1971). *Eulimnadia texana* populations range from 0% to 40% males (average = 25% males; Weeks and Zucker 1999), and inbreeding is positively correlated with female-biased sex ratios (Sassaman 1989, 1995; Weeks and Zucker 1999). Average inbreeding coefficients calculated from six natural populations ranged between 0.20 and 0.97, with an average of 0.49 (Sassaman 1989; Weeks et al. 1999; Weeks and Zucker 1999).

A recent genetic survey compared two *E. texana* populations (JT4 and WAL) for inbreeding level and overall genetic diversity (Weeks et al. 1999). The two populations did not differ substantially in inbreeding coefficient (JT4: $F = 0.39$; WAL: $F = 0.32$), but WAL had a greater number of polymorphic loci, more alleles per polymorphic locus, and a significantly greater number of heterozygotes than JT4. Thus, overall genetic diversity was higher in WAL than JT4, although the quantity of selfing appeared similar (at least in the few generations preceding the electrophoretic examinations; Weeks et al. 1999).

Rearing Conditions

Experiment 1: inbreeding depression measured from field-collected cysts

Soil containing clam shrimp cysts was collected from one site in New Mexico (JT4) located on the U.S. Department of Agriculture Jornada Experimental Range within Doña Ana County (south-central New Mexico) and one site in Arizona (WAL) near Portal in Cochise County, near the base of the Chiricahua Mountains. These samples were then transported back to the laboratory in Akron, Ohio. Subsamples of soil (250 ml) from each population were hydrated using dechlorinated tap water. Hydrations were partitioned among 37-L aquaria that had been separated in half using a piece of nylon screening (112-μ mesh), which allowed water, food, and oth-

er small particles to move between sides, but did not allow any clam shrimp nauplii or juveniles to cross the partition. Soil from JT4 was placed on one side of the partition and soil from WAL was placed on the opposite side. This ensured common-garden rearing environments for shrimp from both populations. Four such aquaria were hydrated per replicate. Three temporal replicates of these hydrations were conducted.

Standard rearing conditions consisted of the following. Aquaria were housed in an environmentally controlled room under continuous light (Durotest sunlight-simulating fluorescent bulbs) at 25–27°C and continuous aeration (see Sassaman and Weeks 1993; Weeks et al. 1997). Just prior to reaching sexual maturity (at approximately five to seven days) in these aquaria, hermaphrodites were randomly chosen for individual isolation in 500-ml plastic cups filled with dechlorinated tap water only (Marcus and Weeks 1997). The isolated hermaphrodites were removed from the rearing aquaria before males were capable of mating, and thus no outcrossing could have occurred before isolation. Shrimp in all cups were fed 1 ml of baker's yeast solution (1 g dried yeast per 100 ml water) per day. Across all three replicates, a combined total of 149 shrimp were isolated from these two populations (JT4 = 69 and WAL = 80).

Daily data collection consisted of gathering the previous day's eggs and measuring carapace length (for estimates of growth). Egg collections were made by shining a light on the bottom of the cup and using a small-bore pipette to extract the eggs (Marcus and Weeks 1997). Eggs were then stored in water in separate glass vials for later counting. Carapace length was measured by capturing a computer image of each shrimp, and using NIH Image to calculate size (see Weeks et al. 1997).

Data collection continued for seven days after isolation. After this time, all survivors were frozen for cellulose acetate electrophoresis. If a shrimp died before the end of the experiment, its age at death was recorded and it was immediately frozen for electrophoresis.

Electrophoretic assays were conducted using cellulose acetate electrophoresis (Richardson et al. 1986). Carapaces of each shrimp were removed, and whole animals were then ground in homogenizing solution (Richardson et al. 1986, p. 95) in centrifuge bullet tubes. Eight-microliter aliquots were then applied to cellulose acetate gels and were run at 200 v for 30–45 min. All shrimp were scored for five polymorphic loci: *Fum* (fumarate hydratase, EC 4.2.1.2); *Idh*-1, *Idh*-2 (isocitrate dehydrogenase, EC 1.1.1.42); *Mpi* (mannose-phosphate isomerase, EC 5.3.1.8); and *Pgm* (phosphoglucomutase, EC 5.4.2.2). All gels were run using buffer C from Richardson et al. (1986). Shrimp were scored as either homozygous (0) or heterozygous (1) at each of the above five loci, and these values were then totaled as an estimate of their individual heterozygosity.

Experiment 2: inbreeding depression measured from laboratory-reared cysts

Soil from JT4 and WAL was hydrated in partitioned aquaria under the standard conditions described above. After adding soil to aquaria, distilled water was added quickly to simulate flooding. The resulting shrimp were reared in these aquaria until just before sexual maturity. Six temporal blocks of these hydrations were conducted for each population (each population hydrated in each block).

From these six hydrations, a total of 341 hermaphrodites (JT4 = 181 and WAL = 160) were randomly chosen for isolation in 500-ml plastic cups with sieved water from their rearing aquarium approximately a day before sexual maturity. Again, care was taken to avoid outcrossing before isolation. One teaspoon of finely sifted soil (collected from the Jornada Experimental Range in New Mexico near JT4, but in an area devoid of all branchiopod eggs; henceforth referred to as ''clean soil'') was also added to each cup. Half of the hermaphrodites were mated by pairing them with a male and half were left to self-fertilize. Both sets of hermaphrodites were kept in these cups for two to three days. On the following day, the males were removed from the mated hermaphrodites and added to the cups with the unmated hermaphrodites. Immediately thereafter, both the new male/hermaphrodite pairs and hermaphrodites newly isolated from a previous pairing were placed into transition cups without soil until one batch of eggs had been shed to prevent any egg carryover from one mating treatment to the next. This typically took 24 h, but in some cases it took 36–48 h. After the transition batch of eggs was shed and discarded, hermaphrodites and males were moved to new isolation cups with clean soil for egg collecting. This process allowed half of the hermaphrodites to outcross first and then to self, while the other half were selfed first and then outcrossed to disassociate breeding treatment with the timing of egg production. After the hermaphrodites were left to mate or self-fertilize for another two to three days, all shrimp were frozen for gel electrophoresis.

Hermaphrodites and males were scored for *Fum, Pgm, Idh,* and *Mpi* to type mated pairs that were alternate homozygotes for at least one of these loci. Only egg banks collected from matings in which the parents were found to be alternate homozygotes were hydrated in the second part of this experiment so that outcrossing could be documented by genetically typing the resulting offspring (see below). Of the 341 scored isolations, 112 (JT4 = 48 and WAL = 64) were found to be alternate homozygous for one or more of the above four loci.

The egg banks were left to dry for more than 30 days before rehydration. After 30 days, 68 paired egg banks (selfed and outcrossed; hereafter termed ''hermaphroditic families'') were hydrated from both populations (JT4 = 41 and WAL = 27) in five temporal blocks, and the resulting nauplii were transferred into opposite sides of partitioned aquaria and raised under common-garden conditions as above. Of these 68 hermaphroditic families, only 28 (14 from each population) had sufficient numbers of offspring in both selfed and outcrossed treatments to continue testing.

After developing a carapace, up to 30 shrimp from each side of each aquarium in these 28 hermaphroditic families were measured for length and isolated in 500-ml cups without soil. Daily data collection was the same as that outlined in experiment 1 and continued for seven days. At death or when the experiment had run for seven days, the ''mated'' shrimp were frozen for gel electrophoresis and scored at the marker electrophoretic locus to determine which isolates were pro-

duced by outcrossing (some offspring can still be produced through selfing even in ''mated'' treatments; S. C. Weeks, unpubl. data). Of the 28 total hermaphroditic families, only eight families (four from each population) had sufficient outcrossing to be included in these analyses.

Statistical Analyses

All data were analyzed using the statistical program JMP (SAS Institute 1995).

Experiment 1

The distributions of individual heterozygosity for the two populations were compared using a chi-square analysis. Because of small sample sizes in JT4 for the higher heterozygosity classes (> 2), shrimp were grouped into three heterozygosity classes for the chi-square tests: 0, 1, and > 1 (i.e., 2, 3, and 4).

Total individual fecundity was measured by combining daily eggs counts. Adult growth in *E. texana* decreases in a logistic fashion with increasing age (Weeks et al. 1997). Therefore, lifetime growth was estimated by regressing carapace length on the log of age; the slope of this regression line was used as the measure of growth (see Weeks et al. 1997). Therefore, if any shrimp did not live for at least three growth measures (so that a regression line could be fit), its growth was not calculated. Other estimates of growth were also calculated (average daily growth increment and size at death standardized by age at death), but all gave similar results as the regression methods, which are reported here (see also Weeks et al. 1997).

Egg production and growth were analyzed using a blocked, two-way ANOVA, with population and heterozygosity class (0, 1, 2, or 3 of five electrophoretic loci heterozygous) as the main effects (due to small sample sizes, heterozygosity class 4 in WAL was included in class 3 for these analyses). Blocks were considered fixed effects. Growth and egg production data had to be square-root transformed to normalize residuals.

Because many of the shrimp were right censored, in that they did not die during the seven days of the experiment, the survival data were nonnormally distributed. Thus, a nonparametric survival analysis was used to compare treatments (proportional hazards model; SAS Institute 1995). A full model with replicates, populations, heterozygosity classes, and interactions among population and heterozygosity classes was initially run. Because heterozygosity classes and the population-by-heterozygosity classes interaction were not significant ($P > 0.50$), these effects were removed, and the final model was recalculated with the reduced factors of replicate and population only.

Life tables were constructed for each population-by-heterozygosity class combination to estimate net reproductive rates (daily survival rate multiplied by average daily fecundity). Because of low numbers of shrimp in heterozygosity classes 4 and 3 in WAL and JT4, respectively, shrimp in these respective classes were grouped with the next lower class for estimation of net reproductive rates.

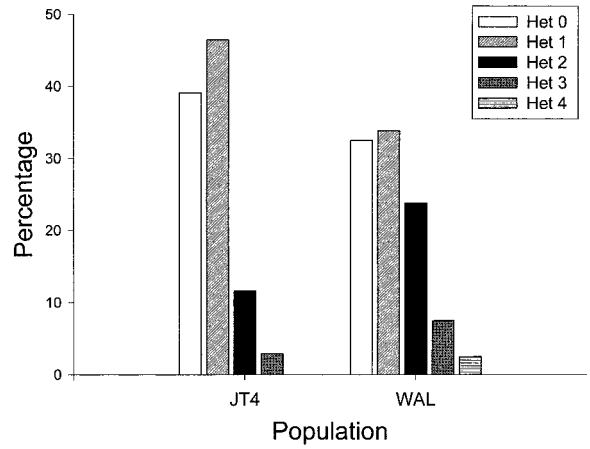

FIG. 1. Individual heterozygosity for the two shrimp populations. Het 0–4 refers to the number of loci (of five) that were heterozygous per individual.

Experiment 2

ANOVAs were performed for growth rate and total egg production (calculated as in experiment 1) using population and breeding treatment (selfed vs. outcrossed) as the two main, independent variables. Because the selfed and outcrossed treatments were performed on groups of related offspring from specific hermaphrodites, a nested analysis was used to adjust for family effects. The population main effect was then tested against the mean square error term for the nested factor (hermaphrodites) for calculation of *F*-ratios.

Blocks were confounded with hermaphroditic families, and therefore the ''hermaphrodite'' factor combines both block and family variation. Residuals for the growth rate analysis were normally distributed, whereas egg production data needed to be square-root transformed to normalize the residuals.

Survivorship was analyzed using a proportional hazards model, as in experiment 1 (SAS Institute 1995). The full model of population, breeding treatment, the interaction of population and breeding treatment, and hermaphrodites nested within population was run initially, but because the interaction of population and breeding treatment was not significant ($P > 0.50$), this term was removed from the final model.

RESULTS

Experiment 1: Inbreeding Depression Measured from Field-Collected Cysts

The distribution of heterozygosity among individuals showed a greater average heterozygosity among WAL compared to JT4 hermaphrodites ($\chi^2 = 6.839$, df = 2, $P = 0.0327$; Fig. 1). This is consistent with previous electrophoretic assays of these two populations, which indicate that JT4 has less genetic diversity than WAL (Weeks et al. 1999). Growth rates did not differ between populations or among heterozygosity classes (Table 1, Fig. 2). This lack of differences among heterozygosity classes was consistent within each of the two populations (Table 1, Fig. 2). In fact, growth only differed among the three blocks (Table 1).

TABLE 1. ANOVA results for the growth measures in experiment 1. Daily growth rates were square-root transformed for this analysis. Significant *P*-values are shown in bold.

Source	df	Sum of squares	*F*-ratio	*P*
Block	2	4.998	53.946	**0.0001**
Population	1	0.024	0.511	0.4761
Heterozygosity class	3	0.141	1.012	0.3899
Pop × Het	3	0.106	0.760	0.5187
Error	128	5.930		

TABLE 2. ANOVA results for lifetime egg production in experiment 1. Egg production was square-root transformed for this analysis. Significant *P*-values are shown in bold.

Source	df	Sum of squares	*F*-ratio	*P*
Block	2	6976	56.936	**0.0001**
Population	1	539	8.798	**0.0036**
Heterozygosity class	3	232	1.261	0.2903
Pop × Het	3	731	3.978	**0.0094**
Error	139	8516		

Lifetime egg production in JT4 was less than half that of WAL (Table 2, Fig. 3). When averaging across populations, there was no consistent differences in lifetime egg production among heterozygosity classes. However, when comparing heterozygosity classes within populations, the lowest heterozygosity class (0) had significantly lower lifetime egg production in WAL than the other three classes (Fig. 3). There were no differences in egg production among the other three classes within WAL. In JT4, there was no significant difference in egg production among any of the four heterozygosity classes (Fig. 3). This difference in performance of the heterozygosity classes between the two populations was significant (Table 2). Interestingly, the egg production of heterozygosity class 0 in WAL was similar to the average lifetime egg production among all classes in JT4 (Fig. 3).

Overall survival for WAL was significantly greater than that in JT4 ($\chi^2 = 4.70$, df = 1, $P = 0.0302$; Fig. 4). Survival was significantly affected by replicate ($\chi^2 = 37.34$, df = 2, $P < 0.0001$), but not by any other main effect.

Combining the survival and egg production data for each population and each heterozygosity class (averaging across replicates), net reproductive rates were calculated from life tables (Fig. 5). The overall patterns mirror those of daily egg production (Fig. 3), except that the differences between populations were accentuated. Again, the response of the heterozygosity classes differs between the two populations, with the lowest heterozygosity class (0) having the highest and lowest net reproductive rates in JT4 and WAL, respectively (Fig. 5). These differences translated to positive estimates of inbreeding depression for WAL, but negative estimates for JT4 (Fig. 5). Even though the inbreeding depression calculation for JT4 was negative, suggesting that homozygotes had higher fitness than heterozygotes, there was no evidence for significant differences among heterozygosity classes within JT4 for any fitness measure (Figs. 2, 3). In contrast, one of the two fitness components in WAL (egg production) revealed significantly lower performance of the lowest heterozygosity class ($F_{3,74} = 5.60$, $P = 0.0016$). Thus, the positive estimate of δ is robust for WAL, whereas the estimate of δ in JT4 should not be considered significantly different from zero.

Experiment 2: Inbreeding Depression Measured from Laboratory-Reared Cysts

There was no overall difference in growth rate between populations or breeding treatments (Table 3, Fig. 6). However, a significant interaction between these two factors was found: WAL outcrossed shrimp grew significantly more rapidly than WAL selfed shrimp, whereas JT4 selfed and outcrossed shrimp did not differ in growth rate (Table 3, Fig. 6).

Populations showed no significant difference for egg production (Table 4, Fig. 7). When averaged over populations, outcrossed shrimp produced significantly more eggs than selfed shrimp (Table 4, Fig. 7). However, when looking at within-population comparisons between outcrossed and selfed shrimp, it was clear that WAL outcrossed shrimp pro-

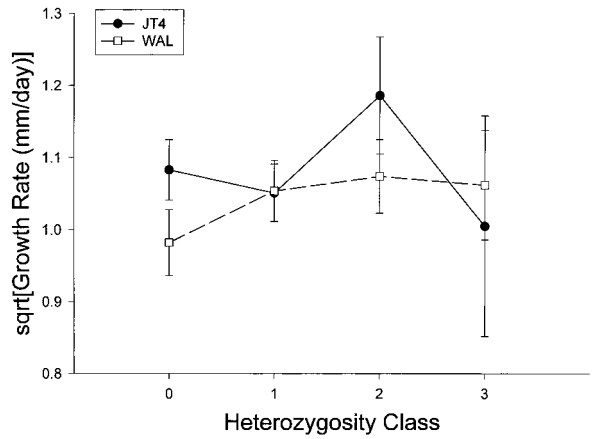

FIG. 2. Growth rates for shrimp in the four heterozygosity classes in the two populations in experiment 1. Growth rates were square-root transformed. Error bars show one standard error of the mean.

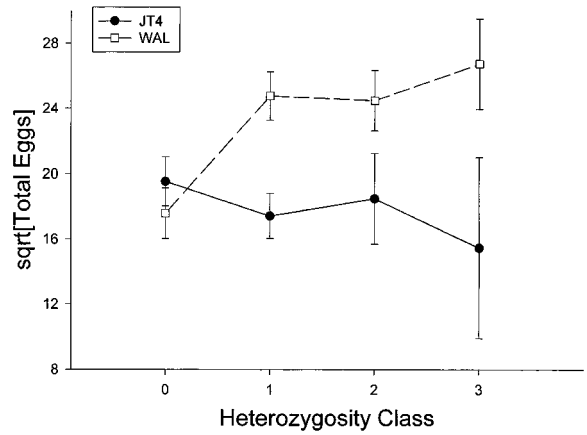

FIG. 3. Lifetime fecundity for shrimp in the four heterozygosity classes in the two populations in experiment 1. Fecundity was square-root transformed. Error bars show one standard error of the mean.

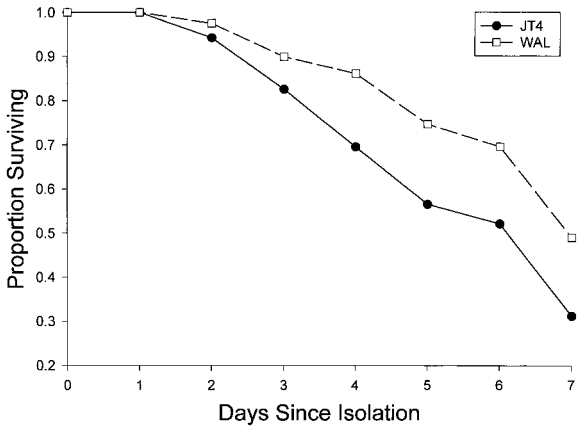

FIG. 4. Proportion of each population surviving over the course of experiment 1 (averaged across heterozygosity class).

duced significantly more eggs than WAL selfed shrimp, whereas again the two JT4 breeding treatments did not differ (Table 4, Fig. 7).

In contrast to experiment 1, the proportion of selfed and outcrossed JT4 shrimp surviving was significantly higher than for either WAL treatment (Table 5, Fig. 8). Averaged across populations, outcrossed shrimp lived significantly longer than selfed shrimp (Table 5, Fig. 8). There was no significant interaction between population and breeding treatment for this parameter, even though the survival curves indicate that there was little difference in survival for outcrossed versus selfed shrimp in JT4 and a large difference in WAL (Fig. 8).

Net reproductive rate was calculated by constructing life tables for each breeding treatment in each population (Fig. 9). WAL outcrossed shrimp had 73% higher net reproductive rates than WAL selfed shrimp, whereas JT4 outcrossed shrimp were only 12% more fit than selfed shrimp (Fig. 9).

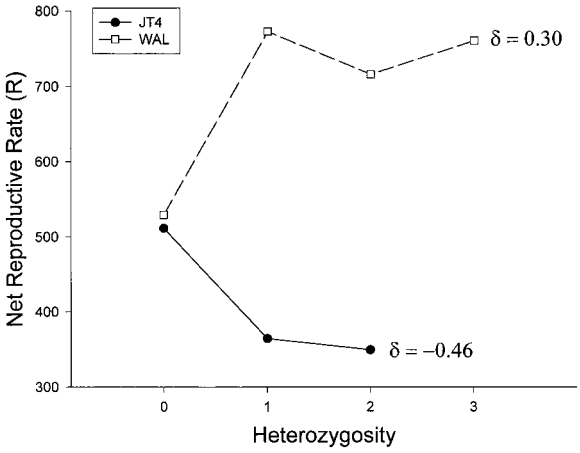

FIG. 5. Net reproductive rates (R) calculated from life tables for each heterozygosity class in each population (experiment 1). Because the highest heterozygosity class (3) in JT4 had so few individuals, no life table could be created; thus, those individuals were included in the life table for heterozygosity class 2. Inbreeding depression (δ) was estimated using the equation $\delta = 1 - (w_i/w_o)$, where w_i = heterozygosity class 0 and w_o = heterozygosity class 3 for WAL and heterozygosity class 2 for JT4. Differences in net reproductive rates among heterozygosity classes could not be statistically compared due to single estimates per heterozygosity class.

TABLE 3. Results of ANOVA for growth rate in experiment 2. Breeding treatments are selfed and outcrossed. Herm(population) refers to hermaphrodites nested within population. The *F*-ratio for population was calculated using herm(population) as the error term. Significant *P*-values are shown in bold.

Source	df	Sum of squares	*F*-ratio	*P*
Population	1	3.075	0.2307	0.6456
Breeding treatment	1	0.335	1.7264	0.1908
Population × breeding treatment	1	3.444	17.7585	**<0.0001**
Herm(population)	7	115.034	84.7473	**<0.0001**
Error	158	30.638		

DISCUSSION

Androdioecy is unlikely to evolve in highly selfing populations (Lloyd 1975; Charlesworth 1984). *Eulimnadia texana* populations, however, are both highly selfing (Sassaman 1989; Weeks et al. 1999; Weeks and Zucker 1999) and androdioecious (Sassaman and Weeks 1993; Zucker et al. 1997). One important difference between the *E. texana* mating system and androdioecious plant systems is that hermaphrodites are incapable of cross-fertilizing one another, and therefore outcrossing can only occur via matings with males (Sassaman and Weeks 1993). The importance of outcrossing is most likely its ability to reduce inbreeding depression. Thus, to begin to understand the stability of this androdioecious mating system, we need to estimate levels of inbreeding depression in populations of *E. texana* (Otto et al. 1993).

We used two approaches to estimate the effects of inbreeding depression: fitness comparisons among shrimp from differing heterozygosity classes (experiment 1) and direct comparisons of selfed versus outcrossed shrimp from the same family (experiment 2). Because self-fertilization should be accompanied by increased homozygosity (Wright 1977), in experiment 1 the lower heterozygosity classes should include higher proportions of self-fertilized offspring than the higher

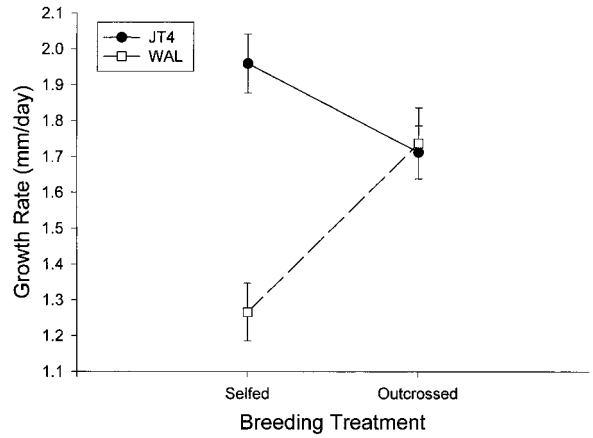

FIG. 6. Growth rates for both populations and breeding treatments in experiment 2.

TABLE 4. Results of ANOVA for egg production in experiment 2. Breeding treatments are selfed and outcrossed. Herm(population) refers to hermaphrodites nested within population. The *F*-ratio for population was calculated using herm(population) as the error term. Significant *P*-values are shown in bold.

Source	df	Sum of squares	*F*-ratio	*P*
Population	1	14.750	0.0200	0.8920
Breeding treatment	1	907.186	14.3253	**0.0003**
Population × breeding treatment	1	780.315	12.3219	**0.0007**
Herm(population)	6	6704.830	17.6459	**<0.0001**
Error	89	5636.152		

TABLE 5. Results of proportional hazards test for survivorship in experiment 2. Breeding treatments are selfed and outcrossed. Herm(population) refers to hermaphrodites nested within population. Significant *P*-values are shown in bold.

Source	df	χ^2	*P*
Population	1	12.088	**0.0005**
Breeding treatment	1	10.440	**0.0012**
Herm(population)	7	63.177	**0.0000**

heterozygosity classes. Thus, in experiment 1 we allowed selfing or outcrossing to occur in the natural ponds, and then took the cysts produced by these matings and reared them under common-garden conditions and measured the relative fitness of the hatched offspring in the laboratory. This first estimate of inbreeding depression assumes that low heterozygosity shrimp (0) tend to include a higher percentage of self-fertilized offspring relative to high heterozygosity shrimp (see also Weeks et al. 1999). In experiment 2, outcrossing and selfing were induced in the laboratory using a number of hermaphroditic sisters, and then the resulting offspring were reared and compared for various fitness measures.

Both estimates of inbreeding depression suggested low to no inbreeding depression in the more genetically homogenous population (JT4) and significant inbreeding depression in the more genetically diverse population (WAL). Comparisons of fitness components (growth rate, egg production, and survival) across heterozygosity classes in experiment 1 showed significantly lower fitness in the zero relative to the higher heterozygosity classes, but only in the WAL population ($\delta = 0.30$; Fig. 5). Similarly, in experiment 2, WAL outcrossed shrimp grew faster, produced more eggs, and had higher survivorship that WAL selfed shrimp, whereas there was little to no difference between outcrossed and selfed shrimp in these fitness correlates in the JT4 population. These data indicate that alleles that cause late inbreeding depression in homozygous form are still segregating in the WAL population, but may have been mostly purged or fixed in the JT4 population. This result is in contrast with an earlier study of inbreeding depression of these two populations that found significant early inbreeding depression for both populations in percent of eggs hatching, early survival (before reproductive maturity), and age to maturity (Weeks et al. 1999).

Testing the Otto et al. Model

Combining the current estimates of δ with those of Weeks et al. (1999), we can begin to address the stability of the mixed-mating system using the Otto et al. (1993) model (eq. 1). The cumulative estimates of early inbreeding depression (from hatching to age at maturity) were found to range from 0.41 to 0.47 (Weeks et al. 1999). Because no differences were found among JT4 and WAL, we can use the average value of $\delta = 0.44$ as an estimate of early inbreeding depression for both populations. Assuming fitness effects during the two periods are multiplicative (e.g., Husband and Schemske 1997), we can get a combined estimate of inbreeding depression: $1 - (1 - \delta_{early})(1 - \delta_{late})$. Because the inbreeding depression calculated in experiment 2 is a more direct estimate of δ (breeding state was only inferred from heterozygosity in experiment 1), we use the values of $\delta_{late} = 0.11$ and $\delta_{late} = 0.42$ for JT4 and WAL, respectively. To estimate lifetime inbreeding depression, we need to combine these estimates with the estimate of early inbreeding depression (before sexual maturity) from Weeks et al. (1999): $\delta = 1 - (0.56 \times w_{out}/w_{self})$, where w_{out} is the net reproductive rate (R) for outcrossed shrimp and w_{self} is R for selfed shrimp. Thus, the lifetime estimate of cumulative inbreeding depression in these populations is $\delta = 0.50$ for JT4 and $\delta = 0.68$

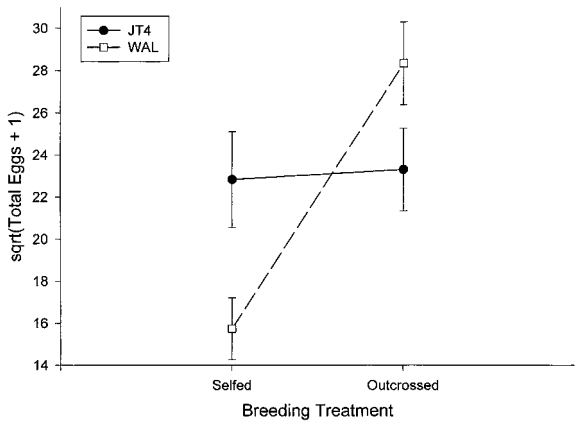

FIG. 7. Egg production for both populations and breeding treatments in experiment 2.

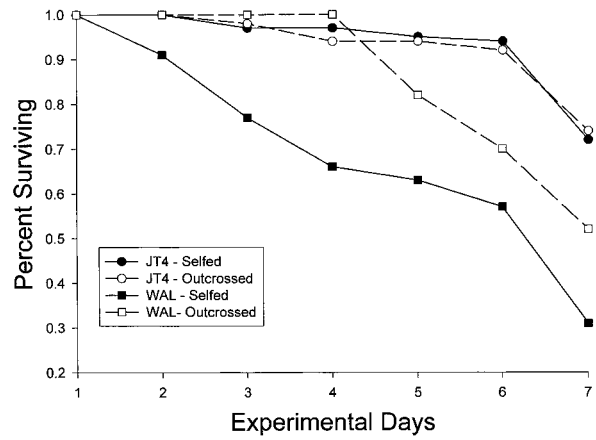

FIG. 8. Survivorship for both populations and breeding treatments in experiment 2.

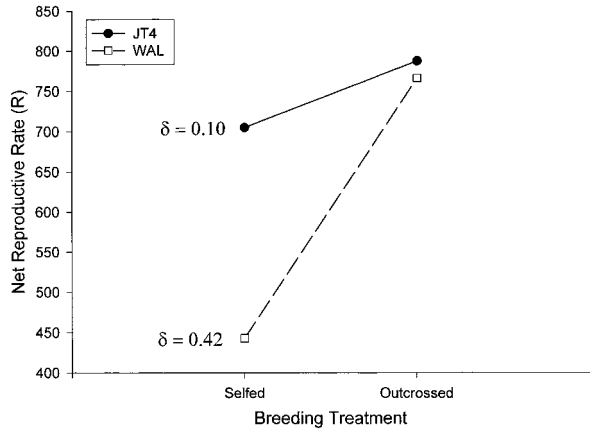

FIG. 9. Net reproductive rate for both populations and breeding treatments in experiment 2.

for WAL. Plugging these estimates into equation (1), we can suggest that for the mixed-mating systems found in these two populations to be evolutionarily stable, the following conditions must be met: $\alpha(1 - \sigma) > \beta$ (JT4) and $\alpha(1 - \sigma) > 0.64\beta$ (WAL). The parameter α can vary between 0 and ∞, with the constraint that $\alpha u \leq 1$ (u = male frequency in the population and αu is the proportion of eggs mated by males; Otto et al. 1993). If we assume that male fertility is not frequency dependent (i.e., α is constant, as modeled in Otto et al. 1993) and that male frequency is unlikely to exceed 50%, then the maximum value that α can take is 2.0. Therefore, the best-case scenario for the maintenance of males would be for $\alpha = 2$ (i.e., males, when rare, fertilize the equivalent of the lifetime egg production of two hermaphrodites) and $(1 - \sigma) = 1$ (i.e., males are as fit as hermaphrodites). Because β ranges from zero to one, the combination of the current estimates of inbreeding depression with this best-case scenario suggests that males can be maintained in either population even if 100% of all eggs not fertilized by males can be self-fertilized by hermaphrodites (i.e., $\beta = 1$). In *C. elegans,* it is estimated that hermaphrodites can only fertilize approximately 80% of their eggs when not mated to a male (Ward and Carrel 1979; Hodgkin and Barnes 1991), which, if true for *E. texana,* would make the maintenance of males even more likely (however, see below).

Preliminary estimates of the remaining three parameters suggests that the best-case scenario is not correct. Estimates of relative male survival $(1 - \sigma)$ in the two populations are: JT4 = 0.8–0.9 and WAL = 0.7–0.9 (N. Zucker and S. C. Weeks, unpubl. data) and preliminary estimates of β suggest that hermaphrodites can fertilize 100% of their eggs even when unmated to a male (i.e., $\beta = 1$). This suggests that in WAL, if $\alpha > \sim 0.9$, and in JT4, if $\alpha > \sim 1.3$, males can be maintained.

A second mechanism for the maintenance of males would be if α is not constant, but rather is frequency dependent (Otto et al. 1993). If male mating success is greater when males are rare, then males are more likely to be maintained. Mating systems where hermaphrodites do not cross-fertilize, like that of *E. texana* and *C. elegans,* are especially likely to exhibit such frequency-dependent selection, because rare males experience no competition when attempting to mate with a hermaphrodite. It may therefore not be coincidental that the only well-studied examples of androdioecy in animals involves hermaphrodites that cannot cross.

Clearly, further work needs to be conducted to better estimate the remaining parameters of the Otto et al. (1993) model. The current estimates of δ are substantial, but when viewed in the context of higher male mortality rates and high self-fertilization potentials (i.e., $\beta \sim 1$) are not great enough to be the primary factor maintaining males in these populations. Experiments in progress will provide better estimates of $(1 - \sigma)$ (N. Zucker and S. C. Weeks, unpubl. data) and β (S. C. Weeks, J. A. Hutchison, and N. Zucker, unpubl. data). Future studies are planned to estimate α and to determine the dependence of α on male frequency. With these data, we will be able to more fully assess the selective forces maintaining males in *E. texana.*

Genetic Causes of Inbreeding Depression

Inbreeding depression has been generally attributed to either of two genetic mechanisms: (1) the expression of recessive deleterious genes (partial dominance model); or (2) reduced heterozygote advantage (overdominance model; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). The currently observed fitness differences between the two populations has two components that require explanation: the relatively higher estimates of inbreeding depression in the more genetically diverse (WAL) population and the lower overall relative fitness of the more genetically homogeneous (JT4) population. These differences are consistent with either model of inbreeding depression, if we assume that JT4 has had either higher historical rates of selfing or more numerous population bottlenecks substantially lowering average effective population size (N_e) than in WAL. Higher historical rates of selfing in JT4 could occur either by shrimp inbreeding at higher rates at any one time or by inbreeding over longer periods of time than in WAL (Lande and Schemske 1985; Charlesworth and Charlesworth 1987). Previously collected genetic data (Weeks et al. 1999) do not bear out the former, suggesting that the lower genetic diversity of JT4 may be due to a longer history of inbreeding. This inference is supported by the fact that JT4 inhabits a natural depression, which would suggest that the population is very old (K. Havstad, pers. comm.), whereas WAL inhabits a human-made cattle tank built in the 1950s (W. Sherbrooke, pers. comm.).

Alternately, JT4 may have reduced genetic diversity due to recurring population bottlenecks, which lower levels of N_e. Currently, we have no information allowing estimates of N_e in either population. Clam shrimp densities are often quite high, with usually 500 individuals/m^2 (MacKay et al. 1990). Also, the egg bank would have a tendency to average out population bottlenecks that would otherwise greatly reduce N_e (Venable and Brown 1988). Nevertheless, clam shrimp life histories suggest that populations may often be founded by very few individuals, which would cause N_e to be low for several generations following a founding event (Nei et al. 1975).

Regardless of which mechanism created the reduced genetic diversity in JT4, the observation of reduced inbreeding

depression and decreased fitness in JT4 can be explained by either the overdominance or partial dominance models. First, if inbreeding depression is caused by reduced levels of overdominance, reduced heterozygosity at fitness-related loci should both lower average fitness (in direct proportion to the reduced proportion of heterozygotes in the population) and reduce inbreeding depression in a population that has higher historical selfing rates or lower average N_e (Charlesworth and Charlesworth 1987; Lynch et al. 1995). Second, if inbreeding depression is caused by partially recessive deleterious alleles, higher selfing rates or lower N_e should lower inbreeding depression by either purging these deleterious alleles (Charlesworth and Charlesworth 1987) or, in small populations, purging some alleles via selection while driving others to fixation via drift (Lynch et al. 1995). In either scenario, estimates of inbreeding depression would be lower in the population with higher selfing rates or lower average N_e, but only the second scenario (small populations) would also account for reduced average fitness in the more inbred population (Lynch et al. 1995). Thus, either model of inbreeding depression can explain the observed fitness results, but the partial dominance model requires the restriction that effective population size be small to account for the lower fitness of JT4 relative to WAL. As stated above, we have no data on the effective population size of either population and thus must await those data before settling these issues.

Interestingly, the current results contrast with previous results estimating early (before sexual maturity) inbreeding depression (Weeks et al. 1999). In that study, JT4 and WAL both exhibited significant early inbreeding depression, in contrast to the current findings of later inbreeding depression only in WAL. Additionally, Weeks et al. (1999) suggested that there was no sign of the operation of the partial dominance model, but rather a modified form of the overdominance model was thought to be operating: segregation of a large linkage group (containing the sex determining gene or genes) within which selective purging of individual loci is unlikely. Such a scenario explained the combined observations of tight linkage of three of eight variable electrophoretic loci to the sex-determining locus (or loci) and the observation of higher survival among amphigenic than monogenic offspring from the same selfed clutches. The latter observation could be caused by fitness effects of a single locus, but the former observation led Weeks et al. (1999) to infer a linkage group including the sex-determining locus and a number of fitness-related loci. If such a linkage group does exist, high selfing rates or low N_e would likely lead to fixation of one of the two linkage groups. If these linkage groups were large enough to contain a number of fitness-related loci, both linkage groups would likely contain a number of deleterious recessive alleles. Homozygosity of either linkage group would reduce fitness relative to the heterozygote, thus giving the appearance of overdominance (Weeks et al. 1999). It appears that alleles for early inbreeding depression are still segregating in JT4, but alleles for later inbreeding depression have been either purged or fixed in this population. If some alleles for later inbreeding depression have, in fact, been purged, these shrimp would be exceptions to the rule that alleles causing inbreeding depression in early life should be more quickly purged relative to those causing inbreeding depression in later life (Husband and Schemske 1996).

Currently, neither model of inbreeding depression is uniquely able to explain the observed fitness results, although the overdominance model (or a modified form based on large linkage groups) appears to be the more parsimonious of the two. To clearly distinguish between the two models, one must compare average fitness over many generations of selfing versus outcrossing (Barrett and Charlesworth 1991; Pray and Goodnight 1995; Carr and Dudash 1997; Dudash et al. 1997). Thus, to assess the underlying genetic causes of inbreeding depression in these shrimp, a multigenerational experiment needs to be conducted to quantify the changes in inbreeding depression over time. Such an experiment is currently in progress.

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