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INBREEDING DEPRESSION IN A SELF-COMPATIBLE, ANDRODIOECIOUS CRUSTACEAN, *EULIMNADIA TEXANA*

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Abstract.—The observation that offspring produced by the mating of close relatives are often less fit than those produced by matings between unrelated individuals (i.e., inbreeding depression) has commonly been explained in terms of the increased probability of expressing deleterious recessive alleles among inbred offspring (the partial dominance model). This model predicts that inbreeding depression should be limited in regularly inbreeding populations because the deleterious alleles that cause inbreeding depression (i.e., the genetic load) should be purged by regularly exposing these alleles to natural selection. We indirectly test the partial dominance model using four highly inbred populations of an androdioecious crustacean, the clam shrimp *Eulimnadia texana*. These shrimp are comprised of males and hermaphrodites, the latter capable of either self-fertilizing or mating with a male (i.e., outcrossing between hermaphrodites is impossible). Hermaphrodites are further subdivided into monogenics (produced via self-fertilization) and amphigenics (produced via self-fertilization or outcrossing). Electrophoretic evidence suggests significant differences in heterozygosity among populations, but that selfing rates were not statistically different (average $s = 0.67$). Additional electrophoretic analyses reveal that three previously described sex-linked loci (*Fum, Idh-1*, and *Idh-2*) are all tightly linked to each other, with crossing over on the order of 1% per generation. Although selfing rates are clearly high, we present evidence that early inbreeding depression (hatching rates, juvenile survival, and age at sexual maturity) exists in all four populations. For all of these factors, inbreeding depression was inferred by the positive correlation of multilocus heterozygosity and fitness. Cumulative inbreeding depression (δ) is between 0.41 and 0.47 across all populations, which appears to be too low to limit the effects of purging via identity disequilibrium. Instead, we suggest that the maintenance of inbreeding depression in these populations is due to the observed linkage group, which we suggest contains a large number of genes including many related to fitness. Segregation of such a large linkage group would explain our observations of the predominance of amphigenic hermaphrodites in our field samples and of survival differences between monogenics and amphigenics within selfed clutches. We propose that a modified form of the overdominance model for inbreeding depression operating at the level of linkage groups maintains the observed levels of inbreeding depression in these populations even in the face of high rates of selfing.

Key words.—Conchostraca, heterozygosity, overdominance model, partial-dominance model, self-fertilization.

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One of the most common observations in controlled breeding programs is the lowered fitness of offspring produced by self-fertilization or mating between close relatives compared with outcrossed offspring (Thornhill 1993). This inbreeding depression has generally been attributed either to the expression of recessive deleterious genes (partial dominance model) or to reduced expression of heterozygote advantage (overdominance model; Charlesworth and Charlesworth 1987). If the partial dominance model is correct, historically inbred populations should show little inbreeding depression relative to outcrossed individuals because the former should have purged most of the highly deleterious recessive alleles during the population's history of inbreeding (Lande and Schemske 1985; Charlesworth and Charlesworth 1987). However, if inbreeding depression is due to overdominance, fitness should always decrease with continued inbreeding, as long as genetic variation at overdominant loci remains in the population (Charlesworth and Charlesworth 1987; Barrett and Charlesworth 1991). A number of recent studies have tested these ideas (Holtsford and Ellstrand 1990; Barrett and Charlesworth 1991; Agren and Schemske 1993; Dole and Ritland 1993; Eckert and Barrett 1994; Johnston and Schoen 1995; Husband and Schemske 1996), with most concluding that inbreeding depression is primarily caused by deleterious, partially recessive alleles (but see Pray and Goodnight 1995; Dudash et al. 1997).

Several theoretical treatments have predicted that inbreeding depression is the critical parameter determining the evolutionary fate of a gene for selfing (but see Baker 1955; Holsinger 1988a), with inbreeding depression greater than 50% favoring outcrossing, and inbreeding depression less than 50% favoring selfing (Fisher 1941; Nagylaki 1976; Maynard Smith 1977; Lloyd 1979; Lande and Schemske 1985; Charlesworth et al. 1990). Other models allow intermediate levels of selfing (mixed mating systems), when there is pollen discounting (Lloyd 1979; Charlesworth 1980; Holsinger et al. 1984; Holsinger 1988b), temporally increasing inbreeding depression (Maynard Smith 1977), high mutation rates to mildly deleterious alleles (Charlesworth et al. 1991), very high levels of inbreeding depression (Lande et al. 1994), or frequency-dependent fitness (Lloyd 1980).

Mixed mating systems provide a way to identify the selective advantage of both inbreeding and outcrossing modes of reproduction, because a balance must be struck for both reproductive modes to be maintained in such populations. Currently, most examples of mixed mating systems have been found among plant species. Many higher plants are self-compatible hermaphrodites and commonly show intermediate levels of selfing and outcrossing (Schoen 1983; Holtsford and Ellstrand 1990; Barrett and Charlesworth 1991; Agren and Schemske 1993; Dole and Ritland 1993; Eckert and Barrett 1994; Johnston and Schoen 1995). Recent investigations have also identified several animal groups with such systems (Chornesky and Peters 1987; Knowlton and Jackson 1993; Jarne et al. 1992; Sassaman and Weeks 1993), and it is likely that a better understanding of the biology of other animal

groups will reveal even more examples (Jarne and Charlesworth 1993).

A rare form of mixed-mating systems is androdioecy, in which species are comprised of males and hermaphrodites (Charlesworth 1984; Rieseberg, et al. 1993; Sassaman and Weeks 1993). The relative rarity of androdioecy is consistent with models that predict this mode of reproduction to be unstable (Charlesworth 1984; Charlesworth and Charlesworth 1987). Yet, androdioecious systems do persist in nature (Rieseberg, et al. 1992 1993; Sassaman and Weeks 1993). Because androdioecy is predicted to be unstable, examination of androdioecious systems might prove to be most informative to our understanding of biparental and uniparental reproductive tactics.

An androdioecious system has been recently described in the clam shrimp *Eulimnadia texana* (Sassaman and Weeks 1993). 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, 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).

An intriguing question raised by this reproductive system concerns the high frequency of pools with mixed outcrossing and selfing (Sassaman 1989): what factors maintain both modes of reproduction, when complete inbreeding is apparently a stable evolutionary endpoint (Otto et al. 1993)? The most obvious factor to explore initially is the level of inbreeding depression in *E. texana* populations (Otto et al. 1993). Here, we present evidence that early inbreeding depression is prevalent among shrimp from four separate populations, all of which have high levels of inbreeding. Additionally, we show that differences among amphigenic and monogenic offspring are sufficient to cause measurable differences in survival within a single, selfed clutch. These data provide an indirect test of the partial dominance model of inbreeding depression. The patterns of inbreeding depression shown in these populations indicate that inbreeding depression has not been eliminated through the purging of deleterious recessive alleles, contrary to the expectations of the partial dominance model. We suggest that a large linkage group may create a type of overdominance that produces the observed levels of inbreeding depression within and among clutches of these freshwater crustaceans.

Materials And Methods

Natural History of Eulimnadia texana

Eulimnadia texana inhabits temporary playas, ditches, and many 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 (at water temperatures above 18°C) 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 ranging between 100 and 300 eggs one to two times a day (Knoll 1995; Weeks et al. 1997). Knoll (1995) found that hermaphrodites often dig a shallow burrow into which eggs are released. Clutch size increases significantly with carapace length (Knoll and Zucker 1995; Weeks et al. 1997), but no difference in clutch size was detected between selfed and outcrossed clutches in the laboratory (Knoll and Zucker 1995).

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; the species is able to filter feed as well as forage along pond bottoms.

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 zero to 40% males, and inbreeding is positively correlated with hermaphrodite-biased sex ratios (Sassaman 1989 1995). Average inbreeding coefficients calculated from six natural populations ranged between 0.20 and 0.97, with an average of 0.49 (Sassaman 1989).

Rearing Protocol and Data Collection

Soil containing clam shrimp eggs was collected and brought back to the laboratory from three sites in New Mexico, (JD1, JT4, and SWP5), all within Doña Ana County (south-central New Mexico), and one site in Arizona (WAL; previously reported as Portal 1 by Sassaman 1989) in Cochise County, near the southeast base of the Chiricahua Mountains. Samples of soil (500 ml) were hydrated in 38-liter aquaria in an environmentally controlled room. Standard rearing conditions were that all aquaria were under continuous light (Durotest sunlight-simulating fluorescent bulbs), at 25–27°C, and continuous aeration (see Sassaman and Weeks 1993). After three days in these aquaria, a combined total of 1290 shrimp (JD1: 245; JT4: 279, SWP5: 389, and WAL: 377) was randomly chosen for isolation (Isolation 1) in 500-ml plastic cups with approximately 12 g of finely sifted soil ($<$ 125 μm diameter). The soil was collected from a site nearby the New Mexico sites listed above, but in an area known to be free of branchiopod cysts. This same soil was used for all isolations. Shrimp in all cups were fed 1 ml of baker's yeast solution (1 g dried yeast per 100 ml water) per day. A total of eight of the above hydrations for all four populations was required for the combined 1290 isolations. Upon sexual maturity (at approximately five to seven days), male isolates were frozen for cellulose acetate electrophoresis, whereas hermaphroditic isolates were allowed to produce self-fertilized eggs for four to seven days, after which they were also frozen for electrophoresis.

Both males and hermaphrodites were assayed using cellulose acetate electrophoresis (Richardson et al. 1986). A

TABLE 1. Percent males and allelic diversity among populations for the five electrophoretically examined loci (*Pgm-1, Mpi, Fum, Idh-1, Idh-2*). *N,* sample size; *P,* proportion of loci polymorphic at the 0.01 level; *Ap,* average number of alleles per polymorphic locus; H_{exp}, average expected number of heterozygotes.

Pop	% Males	*N*	*P*	*Ap*	H_{exp}
JD1	16.8	68	0.6	2.3	0.219
JT4	20.9	90	1.0	2.0	0.181
SWP5	20.3	89	1.0	2.2	0.273
WAL	24.2	213	1.0	2.6	0.404
Mean			0.9	2.3	

total of 996 surviving shrimp were scored for three polymorphic loci: *Fum* (fumarate hydratase, EC 4.2.1.2), *Idh-1*, and *Idh-2* (isocitrate dehydrogenase, EC 1.1.1.42). Two additional electrophoretic loci, *Mpi* (mannose-phosphate isomerase, EC 5.3.1.8) and *Pgm-1* (phosphoglucomutase, EC 5.4.2.2), were run for a subset of 460 of these shrimp. All gels were run using Buffer C from Richardson et al. (1986). Because *Fum, Idh-1*, and *Idh-2* are all known to be linked to the sex-determining locus (Sassaman and Weeks 1993), shrimp heterozygous for any of these electrophoretic loci should be amphigenics. Homozygotes for all three can be either monogenics or amphigenics. Both amphigenics and monogenics can be heterozygous or homozygous for the loci unlinked to the sex-determining locus (e.g., *Mpi* and *Pgm-1*)

After drying for at least 30 days, 374 of the above egg banks generated from the first isolations were hydrated. If the cups had hatchlings, the resulting nauplii were transferred into 38-liter tanks containing aged tap water and soil known to be free of all forms of branchiopod eggs and reared under standard conditions. If these isolates grew to sexual maturity, the age at maturity was recorded. For 67 broods from amphigenics heterozygous for *Fum, Idh-1*, or *Idh-2*, up to 30 hermaphrodites per brood were randomly chosen for a second round of isolations (Isolation 2) in 500-ml cups (1644 total isolates). These isolates were allowed to produce eggs for up to seven days, after which survivors were frozen for electrophoresis. Isolates that died were immediately frozen for later electrophoresis. All second-round isolates were scored for one or more of the sex-linked loci (*Fum, Idh-1*, and *Idh-2*). Because these loci are linked to each other and to the sex-determining element(s), they mark the isolated hermaphrodite as either monogenic (homozygous) or amphigenic (heterozygous), except for cases of crossing over (see below).

Statistical Procedures

Electrophoretic Data.—Inbreeding coefficients (*F*), proportion of polymorphic loci (*P*), average alleles per polymorphic locus (*Ap*), and observed and expected heterozygotes were generated using the program Genetic Data Analysis (GDA) version 1.0 (Lewis and Zaykin 1997). Confidence intervals (CIs) for the estimates of inbreeding coefficients were generated using the bootstrapping routine in GDA by bootstrapping across loci (Lewis and Zaykin 1997). Proportions of individuals within each population that were heterozygous for the five polymorphic loci were compared among populations using a chi-square contingency analysis (SAS Institute 1995). These comparisons were made across all four populations and in pairwise combinations of populations. In some of the pairwise analyses, the numbers of heterozygosity classes had to be collapsed from five (0–4) to three (0–2) because of low numbers of individuals in the higher heterozygosity classes.

Other Data.—All other data were analyzed using the statistical program JMP (SAS Institute 1995). The proportion of the hydrated cups that hatched and survived to maturity was analyzed using a logistic regression with population and heterozygosity class (parental hermaphrodite's level of heterozygosity) as the main effects. Average age at maturity for those hydrations where individuals survived to sexual maturity were compared using a two-way ANOVA, with population and heterozygosity class as the main effects. Because all shrimp within an aquarium essentially matured on the same day, age at maturity was compared on a per-hydration basis, as with hatching and surviving hydrations above. All three dependent variables were compared using two measures of heterozygosity: a larger set of hydrations in which *Fum, Idh-1*, and *Idh-2* (three-loci assays) were analyzed and a subset of this group in which *Mpi* and *Pgm-1* (five-loci assays) were also analyzed. This led to two groups of heterozygosity classes: heterozygous for zero, one, or two loci (three-loci assays) or zero to three loci (five-loci assays). In the five-loci analyses, interaction effects among population and heterozygosity class could not be tested because two of the four populations did not have a sufficient number of individuals that were heterozygous for the three polymorphic loci.

Survival of the offspring in the second isolation was compared using a two-way, nested analysis of variance on age at death. The two fixed factors were population of origin and hermaphroditic class (amphigenic or monogenic, as determined by electrophoresis). Because all three loci were linked and crossovers were rare (see below), such an analysis was more appropriate than identifying separate heterozygosity classes as in the previous analyses. Hermaphroditic parent was considered a random effect, nested within populations. Those shrimp frozen alive on day 7 were assigned a death age of 8 for this analysis.

RESULTS

Electrophoresis

Allelic diversity for the five electrophoretically examined loci (*Pgm-1, Mpi, Fum, Idh-1*, and *Idh-2*) was low for invertebrates (Table 1), especially considering that only known polymorphic loci were recorded in these populations. Polymorphic loci usually had only two alleles (Table 1). The WAL population had the highest overall genetic diversity, and JT4

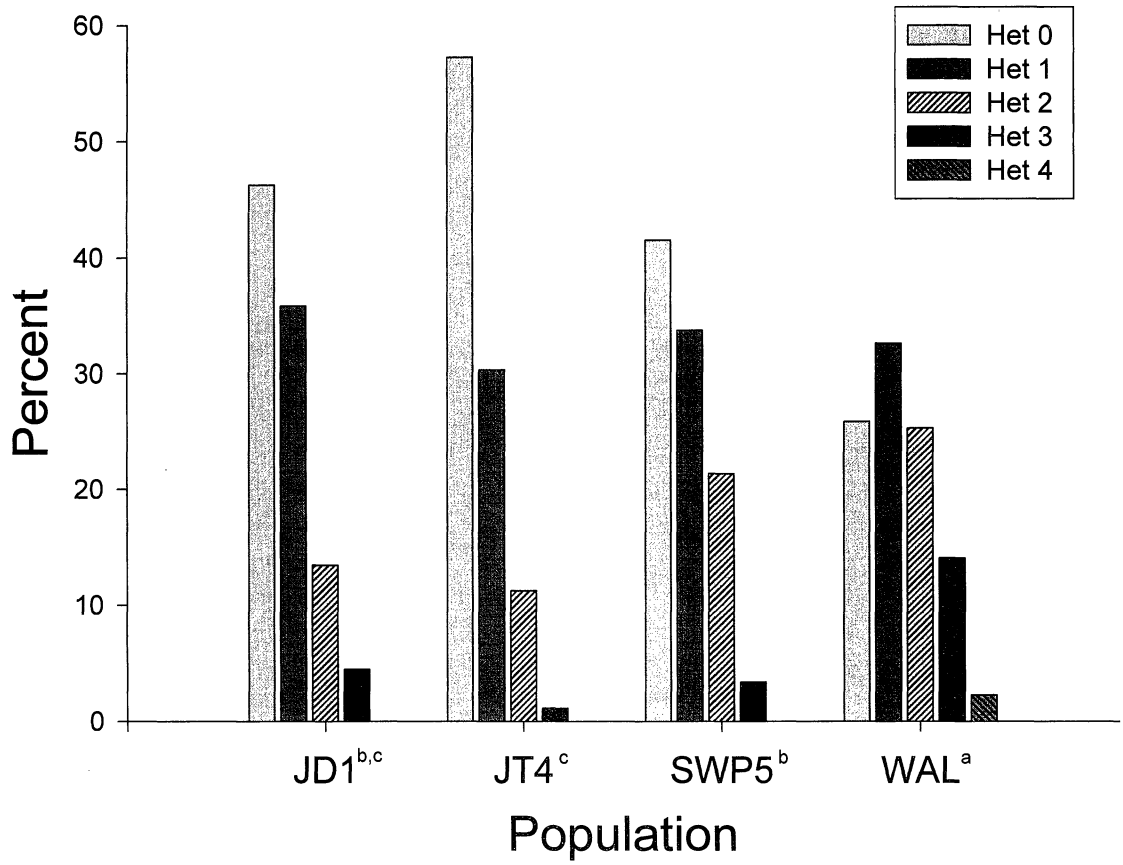

FIG. 1. Percentage of individuals in all four populations in different heterozygosity classes (of five loci). Populations with the same letters (in figure legend) were not significantly different at the 0.05 level in pairwise comparisons.

had the lowest. Population SWP5 had a greater proportion of polymorphic loci and a higher expected heterozygosity than JD1, but a lower number of alleles per polymorphic locus than JD1. These genetic differences were mirrored in the comparisons of individual heterozygosities within each population (Fig. 1). Proportions of individuals within each heterozygosity class (0–4) differed significantly among the populations ($\chi^2(12) = 51.1$, $P < 0.0001$), with WAL having the greatest proportion of individuals with higher heterozygosity (more than two of five loci heterozygous), followed by SWP5 and JD1. JT4 had the lowest heterozygosity, with 57% of the population found to be completely homozygous at all five electrophoretic loci.

Estimates of the inbreeding coefficient (F) were calculated in two ways (Table 2): estimates across all five polymorphic loci, and estimates across the two loci unlinked to the sex-determining element (*Pgm-1* and *Mpi*). For all estimates, the 95% CIs were great enough that differences among populations were not significant. Inbreeding coefficients ranged from 0.32 to 0.39 when all loci were considered, or between 0.42 and 0.61 when only *Pgm* and *Mpi* were considered. All estimates suggest significant levels of selfing for these four populations.

Estimates of selfing rates are commonly made using mother/offspring comparisons to note actual outcrossing per family (Ritland 1989). Currently, we do not have such familial estimates, and thus must rely on a less reliable method using our estimates of F. Assuming reproduction is either through outcrossing to unrelated individuals or selfing, the selfing rate (s) can be estimated using the equation $s = 2F/(1 + F)$

TABLE 2. Estimated inbreeding coefficients (F) and selfing rates (s) for each population. Estimates are given for all five scored loci (*Fum, Idh-1, Idh-2, Pgm-1,* and *Mpi*) and only the two unlinked loci (*Pgm-1* and *Mpi*). Selfing rates are estimated only from the inbreeding coefficients from the two unlinked loci. Values in parentheses are confidence intervals (estimated by bootstrapping across loci).

Population	F, all loci	F, *Pgm-1* and *Mpi* only	Selfing rates (s)
JD1	0.323 (−0.005, 0.514)	0.514*	0.68
JT4	0.387 (0.149, 0.442)	0.422 (0.385, 0.456)	0.59 (0.56, 0.63)
SWP5	0.360 (0.022, 0.609)	0.608 (0.290, 0.678)	0.76 (0.45, 0.81)
WAL	0.321 (0.035, 0.532)	0.482 (0.334, 0.579)	0.65 (0.50, 0.73)

* No confidence interval is given because only *Mpi* was polymorphic.

TABLE 3. Observed (H_{obs}) and expected (H_{exp}) heterozygosities and inbreeding coefficients (F) by electrophoretic locus and population. N, total sample size. The numbers of heterozygotes sampled ($\#H_{obs}$) is compared to those expected ($\#H_{exp}$) using a chi-square analysis (* = $P < 0.01$; ** = $P < 0.001$).

Locus	N	H_{obs}	H_{exp}	F	$\#H_{obs}$	$\#H_{exp}$
JD1						
Pgm-1	70	0.000	0.000	0.000	0	0
Mpi	70	0.243	0.498	0.514	17*	35
Fum	70	0.157	0.257	0.390	11	18
Idh-1	67	0.343	0.342	−0.005	23	23
Idh-2	60	0.000	0.000	0.000	0	0
JT4						
Pgm-1	89	0.090	0.146	0.385	8	13
Mpi	90	0.089	0.163	0.456	8	15
Fum	90	0.278	0.500	0.446	25*	45
Idh-1	90	0.078	0.075	−0.035	7	7
Idh-2	90	0.022	0.022	−0.006	2	2
SWP5						
Pgm-1	89	0.090	0.126	0.290	8	11
Mpi	89	0.191	0.587	0.676	17**	52
Fum	89	0.382	0.494	0.151	34	44
Idh-1	89	0.124	0.117	−0.060	11	10
Idh-2	89	0.090	0.086	−0.041	8	8
WAL						
Pgm-1	209	0.316	0.478	0.340	66**	100
Mpi	194	0.289	0.733	0.607	56**	142
Fum	220	0.473	0.453	−0.043	104	100
Idh-1	218	0.005	0.023	0.799	1	5
Idh-2	223	0.260	0.323	0.195	58	72
Mean	212.8	0.275	0.404	0.321		

TABLE 4. Logistic regression of proportion of hydrated cups that (a) hatched and (b) survived to sexual maturity, for (1) *Fum, Idh-1,* and *Idh-2* and (2) *Fum, Idh-1, Idh-2, Mpi,* and *Pgm-1*. Chi-square values were generated by a likelihood-ratio test, and significant P-values are given in bold. Total sample size was 374 hydrated cups for (1) and 214 hydrated cups for (2).

Source	df	Chi-square	P
(1) Three loci			
(a) Hatching			
Population	3	2.671	0.4451
Heterozygosity	2	9.228	**0.0099**
Pop × Het	6	3.682	0.7196
(b) Surviving to maturity			
Population	3	4.770	0.1895
Heterozygosity	2	22.397	**0.0001**
Pop × Het	6	9.042	0.1712
(2) Five loci			
(a) Hatching			
Population	3	3.671	0.2992
Heterozygosity	3	5.246	0.1546
(b) Surviving to maturity			
Population	3	0.915	0.8218
Heterozygosity	3	9.273	**0.0259**

(Fyfe and Bailey 1951). Because this estimate of outcrossing only applies to loci that are not linked to the sex-determining locus, we used the estimates of F for the unlinked loci (*Pgm-1* and *Mpi*) to estimate the selfing rate as ranging from 0.59 to 0.76 (Table 2).

In the five comparisons in which significantly fewer heterozygotes were found than expected (Table 3), only one (*Fum* for JT4) was among the three loci known to be linked to the sex-determining element. In fact, estimates of inbreeding coefficients per locus averaged across all four populations were greatest for the two unlinked loci relative to the three linked loci (Tables 2, 3).

Of the 806 offspring isolated from doubly heterozygous amphigenic parents (heterozygous for *Fum* and either *Idh-1* or *Idh-2*) in isolation 2, only 11 instances of crossing over were documented: eight cross-overs between *Fum* and *Idh-1* (2/267 in JD1; 3/135 in JT4; 3/108 in SWP5) and three cross-overs between *Fum* and *Idh-2* (0/37 in SWP5; 3/259 in WAL). Thus, crossing over between *Fum* and *Idh-1* and *Idh-2* was 1.6% and 1.0%, respectively. No double heterozygotes for both *Idh* loci were successfully tested, and thus ordering of these three linked loci was impossible.

Isolation 1

A total of 293 egg banks successfully hatched (an egg bank was scored as successfully hatching if at least one nauplius was seen in a hatching cup) of the 374 hydrated (78% of hydrated egg banks). Of these 293 hatchling cohorts, only 242 had individuals that survived to sexual maturity (65% of hydrated egg banks). There were no significant differences in either of these proportions among the four populations (Table 4). However, egg banks from hermaphrodites with greater measured levels of individual heterozygosity had significantly higher proportions of successful hatches as well as a higher proportion of tanks with survivors to sexual maturity (a tank was scored as successfully surviving if at least one adult was scored as sexually mature) than cohorts from hermaphrodites that were more homozygous (Fig. 2). This pattern was reflected in both the three- and the five-loci examinations (Fig. 2), although the differences in percent hatching was not significant in the five-loci analysis (Table 4). For both hatching and survival, increased individual heterozygosity was directly associated with increased performance. Differential survival after hatching tended to emphasize the differences among heterozygosity groups (Fig. 2). There was no difference among the four populations in the relationship of individual heterozygosity to hatching or survival (Table 4).

Of the 242 egg banks that had successful survival to sexual maturity, 152 were examined for sex ratios and were genetically assayed for the three sex-linked loci (*Fum, Idh-1,* and *Idh-2*). Of these 152, 128 were identifiably monogenic (no males produced) or amphigenic (approximately 25% males produced). Because identifying a cohort as monogenic was based solely on the absence of males, only all-hermaphrodite cohorts that contained 12 or more shrimp could be labeled monogenic (0.03 probability of getting no males out of 12 shrimp in an amphigenic cohort). Of the 52 surviving hatches that were heterozygous for at least one of the three sex-linked loci, all 52 cohorts had mixed males and hermaphrodites among the offspring (i.e., were offspring from a selfing amphigenic). There were an additional five cohorts that were produced by heterozygous parents that were comprised of

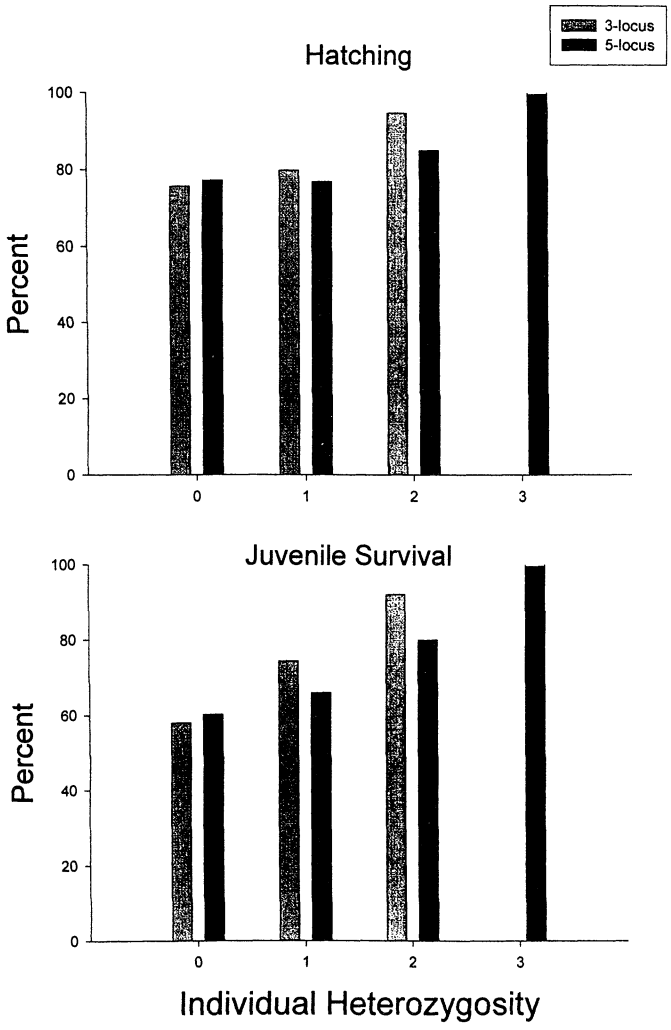

FIG. 2. Percent of hydrated cups (collected from Isolation 1) that successfully hatched (top) and survived to sexual maturity (bottom). Each cup was collected from isolates examined for three (light) and five (dark) electrophoretic loci, averaged across the four populations. Individual heterozygosity refers to the number of loci examined that were found to be heterozygous for each shrimp assayed.

only hermaphrodites, but had fewer than 12 survivors. Thus, between 91% and 100% of the Isolation 1 hermaphrodites that were heterozygous for either *Fum, Idh-1*, or *Idh-2* were amphigenics. Of the 76 surviving hatches that were homozygous for all three sex-linked loci, only 20 were truly monogenics (26%). There were an additional 19 cohorts that were produced by homozygous parents that were comprised of males and hermaphrodites but had fewer than 12 total survivors. Thus, between 22% and 26% of the Isolation 1 hermaphrodites that were homozygous for all three sex-linked loci were truly monogenics, with the remainder being homozygous amphigenics. Note that these are estimates across populations because sample sizes were too small to make within-population estimates. Combining the higher values for both estimates with the electrophoretic data and the counts of percent male (Table 1), estimates of the three mating types were constructed (Fig. 3). Each percentage was estimated using the following formulas: % males = observed numbers of males hatching per population averaged across hydrations; % monogenics = numbers of hermaphrodites per population scored as homozygous for *Fum, Idh-1*, and *Idh-2* multiplied by 26%; and % amphigenics = (numbers of hermaphrodites per population heterozygous for *Fum, Idh-1*, and *Idh-2*) plus (numbers of hermaphrodites per population homozygous for *Fum, Idh-1*, and *Idh-2* multiplied by 74%). In all four populations, amphigenic hermaphrodites were the predominant mating type, being five to 16 times more abundant than the monogenics (Fig. 3).

Expected equilibrium frequencies of the three mating types was estimated using a modification of the Otto et al. (1993) model, assuming that the mating frequencies were dictated only by the rate of inbreeding (Table 5). This modification assumes: (1) males can mate with many hermaphrodites when males are rare; (2) hermaphrodites regulate the amount of outcrossing; (3) no inbreeding depression; and (4) no difference in viability among males and hermaphrodites. Thus, this modified model produces null expectations, assuming that the rate of outcrossing (using the inbreeding rates calculated in Table 2) is the only factor controlling mating-type frequencies. In all populations, there were more amphigenics and fewer monogenics than expected on the basis of the level of outcrossing (Fig. 3). In the three populations from which 95% CIs could be calculated for inbreeding rates (JT4, SWP5, and WAL), the percent males estimated from the field-collected soil fell within the range estimated by the modified Otto et al. (1993) model. In contrast, for all of these populations, the amphigenics were overrepresented while the monogenics were underrepresented (Fig. 3). In the single population where no 95% CIs for inbreeding rate was obtained, the single estimates for all three mating types follows the trends found in the other three populations.

Proportion of hydrations that survived to maturity was also analyzed by electrophoretic genotype (Table 6). Of the 15 possible comparisons among genotypes, seven had more than five egg banks from each electrophoretic category, and thus the proportions could be compared using a chi-square analysis. In six of these seven comparisons, the heterozygotes survived better than either homozygote, with four of these six being significant at the $P < 0.05$ level (Table 6). Two of these four significant cases were found for the Fum locus, with one each for *Idh-1* and *Idh-2*. Note that all three of these loci are linked to each other and to the sex-determining element, and that one of the two homozygous classes (BB) is primarily found among males because the B allele is linked to the male-determining genetic element. Therefore, one of the two homozygous classes (BB) is usually rare among hermaphrodites (with the one exception of *Fum* in JT4). In the two unlinked loci (*Mpi* and *Pgm-1*), there was no consistent evidence for heterozygote superiority (Table 6).

Average age at maturity was approximately seven days after hatching. Age at maturity differed among populations and heterozygosity classes, with the SWP5 population having a lower age at maturity than either JD1 or WAL (Table 7, Fig. 4). No other differences among populations were significant. Three of the populations (JD1, SWP5, and WAL) showed a consistent pattern among heterozygosity classes: similar ages at maturity for heterozygote classes 0 and 1 but significantly shorter ages to maturity for heterozygote class

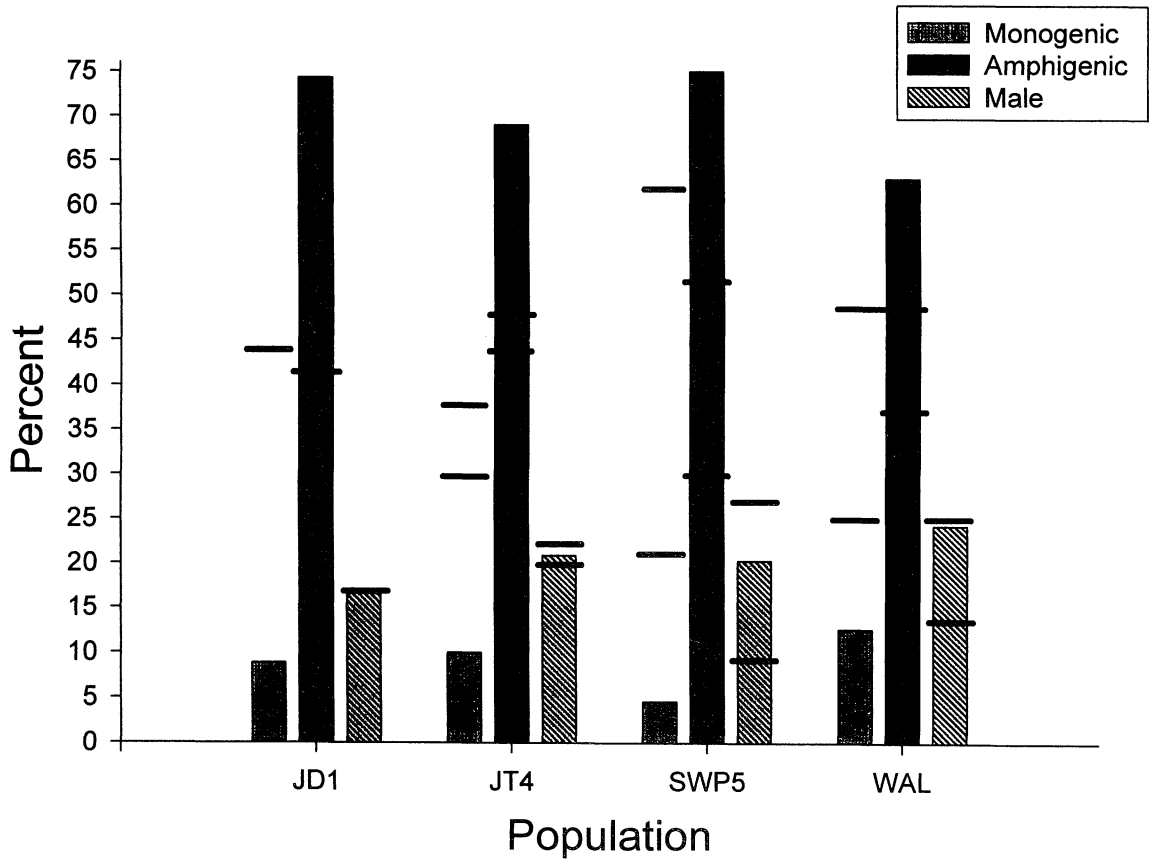

FIG. 3. Estimated percent of total hatched shrimp of the three mating types. Estimates were based on electrophoretic examinations of surviving hatchlings combined with progeny testing of a subset of the hermaphroditic hatchlings to determine sex type (monogenic vs. amphigenic). Dark, horizontal lines for both hermaphroditic types reflect the expected proportions of these two mating types if the electrophoretic estimates correctly reflects outcrossing rates (see text).

2. In the JT4 population, the lowest age to maturity was in heterozygote class 1. Nevertheless, when again averaging across populations, there was also a tendency for greater performance with increased heterozygosity (Table 7, Fig. 5).

Isolation 2

A total of 1644 hermaphroditic offspring were isolated from 67 amphigenic hermaphroditic parents (JD1: 15, JT4: 16, SWP5: 18, and WAL: 18) from Isolation 1 that were heterozygous for at least one of the three sex-linked loci (*Fum, Idh-1*, or *Idh-2*). Of these, 1108 (67.4%) were electrophoretically typed as heterozygous (i.e., 99% likely to be amphigenics) and 536 (32.6%) were homozygous (i.e., 99% likely to be monogenics). This sex ratio was not significantly different from the 2:1 ratio of amphigenics to monogenics ($\chi^2_1 = 0.394$; $P > 0.50$) expected from the selfing of an amphigenic hermaphrodite (Sassaman and Weeks 1993), eliminating the possibility of early mortality skewing the sex ratios of these two classes of hermaphrodites. Nevertheless, survival for the first seven days after sexual maturity was significantly different between these two hermaphroditic classes (Table 8), with all four populations having reduced survival of the monogenic relative to the amphigenic hermaphrodites (Fig. 6). Survival was not different among populations, and the difference in survival between the two hermaphroditic classes did not depend on population origin (Table 8).

TABLE 5. Recurrence equations for expected mating-type frequencies. Symbols are defined as follows: SS, monogenic hermaphrodites; Ss, amphigenic hermaphrodites; ss, males; *x, y,* and *z,* initial mating type frequencies of SS, Ss, and ss, respectively; *s,* selfing rate; Out, outcrossed; In, inbred (selfed).

Mating type	Out-SS	Out-Ss	In-SS	In-Ss	Total
SS			sx	$sy/4$	$s[x + (y/4)]$
Ss	$(1 - s)x$	$[(1 - s)y]/2$		$sy/2$	$x(1 - s) + y/2$
ss		$[(1 - s)y]/2$		$sy/4$	$(y/2)(1 - s/2)$

DISCUSSION

Two lines of evidence suggest that inbreeding depression is important in this androdioecious crustacean. First, offspring from selfed hermaphrodites differing in heterozygosity consistently displayed increased performance with increased heterozygosity. All three measures of early fitness (percent hatching, survival to maturity, and age at maturity) were significantly greater in clutches produced by more hetero-

TABLE 6. Percentage of hydrated egg banks from hermaphroditic parents of each genotype that hatched and survived to reproductive maturity. Comparisons are shown only for cases in which all electrophoretic categories had five or more hydrated egg banks. Numbers in parentheses are the total hydrated in each genotypic class. The largest percentages are marked in bold, and the subset of these in which statistical heterogeneity among groups were found are marked with asterisks.

Source	AA	AB	BB
Pgm-1			
WAL	**74** (34)	67 (15)	58 (19)
Idh-1			
SWP5*	53 (97)	**89** (9)	
Idh-2			
WAL***	60 (55)	**95** (20)	67 (9)
Fum			
JT4	69 (54)	**75** (20)	55 (31)
SWP5**	48 (73)	**78** (32)	60 (5)
Wal**	57 (51)	**89** (26)	67 (6)
Mpi			
JDI	55 (11)	**73** (11)	55 (20)

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

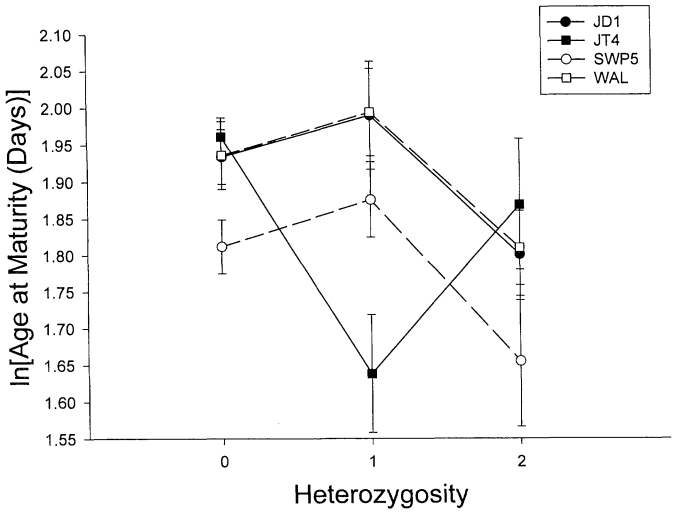

FIG. 4. Average ages at maturity for each heterozygosity class in each population (from shrimp reared from Isolation 1). Error bars portray one standard error of the mean. Individual heterozygosity refers to the number of loci examined that were found to be heterozygous for each shrimp assayed.

zygous hermaphrodites. If level of heterozygosity is negatively correlated with level of inbreeding (Wright 1977), then these results indicate that significant early inbreeding depression exists in all four of these populations. Second, the estimated proportion of individuals from each population that were monogenics (estimated using combinations of electrophoresis and progeny testing) was consistently lower than expected and amphigenics higher than expected on the basis of estimated outcrossing rates (because selection may be operating on one or more of loci in the linkage group containing *Fum, Idh-1*, and *Idh-2*, we used the estimates of F from *Pgm-1* and *Mpi* to calculate the selfing rate, s). The lower than expected proportion of monogenics could be due to: (1) incorrect estimates of true outcrossing rates; (2) lower hatching of monogenic eggs; (3) lower survival of monogenic offspring; or (4) greater production of eggs by amphigenics than by monogenics. Because our estimates of the selfing rate correctly predicted the proportion males in all populations, it seems likely that the lower than expected proportions of monogenics was not due to underestimating outcrossing rates. To obtain as few monogenics as observed, true selfing rates would need to be between 0.10 and 0.28 across all four populations, which is three to six times lower than our estimates. If we assume that our estimates of selfing rates in these ponds are accurate, then the lower-than-expected proportion of monogenics must indicate some lowered fitness for these hermaphrodites due to either (2), (3), or (4) above. Because monogenics can only be produced by selfing, this again suggests that inbreeding depression is important in these populations.

The combined evidence suggests that early inbreeding depression continues to be important in these four populations,

TABLE 7. ANOVA for ln(age at maturity) for broods from hermaphrodites scored for either (1) *Fum, Idh-1,* and *Idh-2* or (2) *Fum, Idh-1, Idh-2, Mpi,* and *Pgm-1*. Significant P-values are given in bold.

Source	df	Sum of squares	F-ratio	P
Three loci				
Population	3	0.334	3.496	**0.0171**
Heterozygosity	2	0.294	4.624	**0.0112**
Pop × Het	6	0.563	2.948	**0.0094**
Error	156	4.964		
Five loci				
Population	3	0.070	0.603	0.6148
Heterozygosity	3	0.286	2.455	0.0674
Error	101	3.920		

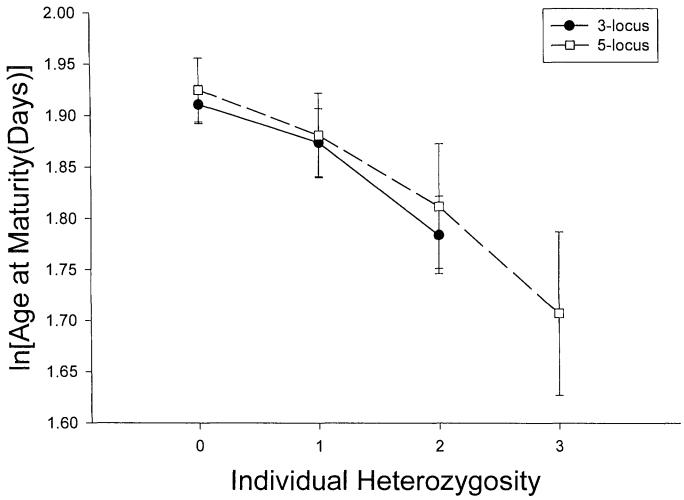

FIG. 5. Age at maturity for a subset of the surviving hydrations that survived to sexual maturity. Each cup was collected from isolates (from Isolation 1) examined for three (filled circles) and five (open squares) electrophoretic loci, averaged across the four populations. Individual heterozygosity refers to the number of loci examined that were found to be heterozygous for each shrimp assayed.

TABLE 8. ANOVA results for age at death for hermaphrodites from Isolation 2. Significant *P*-values are given in bold.

Source	df	Sum of squares	*F*-ratio	*P*
Population	3	56.920	1.300	0.2816
Sex_Type	1	11.902	7.975	**0.0048**
Pop × Sex_Type	3	0.766	0.513	0.6733
Parent(Pop)	63	19.790	13.260	**0.0001**
Error	1558	2325.294		

even though all populations appear to have significant levels of selfing in the wild (see also Sassaman 1989). These data imply that alleles affecting inbreeding depression are still segregating in these populations. This is at odds with the partial dominance model, which assumes that moderate levels of inbreeding should purge populations of many of the deleterious recessive alleles that cause inbreeding depression (Lande and Schemske 1985; Charlesworth and Charlesworth 1987). In fact, selfing rates as low as 0.10 should purge populations of recessive lethals (Lande and Schemske 1985). Several recent comparisons of inbred and outcrossed populations have found less inbreeding depression in historically inbred populations (Barrett and Charlesworth 1991, Dole and Ritland 1993; Latta and Ritland 1994, Husband and Schemske 1996), which indicates that the expected purging of inbreeding depression is in fact important in these species. In this study, there was no evidence of past purging for hatching or survival to maturity for any of the four populations, all of which would be classified as primarily selfing populations (i.e., $s > 0.55$) by Husband and Schemske (1996). In fact, populations with estimated selfing rates as high as 0.76 (SWP5) continued to show reduced fitness in offspring derived from homozygous hermaphrodites. These observations are even more unusual because they are from early measures of fitness. Husband and Schemske (1996) have shown that in most plant species, characters that affect early fitness measures are the most likely to be purged of deleterious recessives.

Inbreeding depression expressed later in life (age at maturity) also did not show a consistent pattern indicative of purging. Because early maturity is strongly advantageous in temporary-pond organisms (Wilbur 1980), any delay in maturity can dramatically affect fitness. Age at maturity did differ among populations, but the earliest maturity was found in SWP5 (intermediate levels of heterozygosity), whereas the latest was in WAL (highest heterozygosity). Thus, no population-level purging was obvious. There was a significant population-by-heterozygosity class interaction, caused by a significantly different response of the heterozygosity classes within JT4 relative to the other three populations: the earliest maturation was in heterozygosity class 1 relative to both classes 0 and 2. One could argue that this suggests the beginning of a higher-fitness, low-heterozygosity group, which would indicate the early signs of purging in this low-heterozygosity population. However, this a posteriori explanation is weak. Overall, there is little evidence of purging of deleterious alleles that cause inbreeding depression at any life stage in these populations.

Although we have no evidence of past purging in any population in this study, we cannot completely discount this process in these populations for two reasons. First, because we have not done a longitudinal study to observe the effects of selection over time (see Barrett and Charlesworth 1991; Carr and Dudash 1997; Dudash et al. 1997), we have no way of knowing whether some form of inbreeding depression may have been purged earlier in these four populations. Second, although we did not find purging at the population level, purging may be operating within families (Pray and Goodnight 1995; Dudash et al. 1997; Carr and Dudash 1997). If there are heritable differences among families for purging, among-family selection could lead to long-term, population-level purging under continued inbreeding. Several recent studies have found little evidence for populationwide purging, but did find evidence that purging occurred within some selfing families (Pray and Goodnight 1995; Carr and Dudash 1997; Dudash et al. 1997). Among-family differences in purging can lead to population-level purging with continued selfing, even when this purging is not detected at the population level (Pray and Goodnight 1995; Carr and Dudash 1997; Dudash et al. 1997). Because families capable of purging will produce higher-quality offspring than those that cannot purge, purging families should be overrepresented as inbreeding continues, thereby associating higher-fitness genotypes with the propensity to inbreed (Holsinger 1988b). Therefore, the lack of purging at the population level does not rule out the potential importance of purging under continued inbreeding. Currently, we are conducting a long-term project comparing selfed and outcrossed populations over many generations to better discern the importance of purging in these *E. texana* populations.

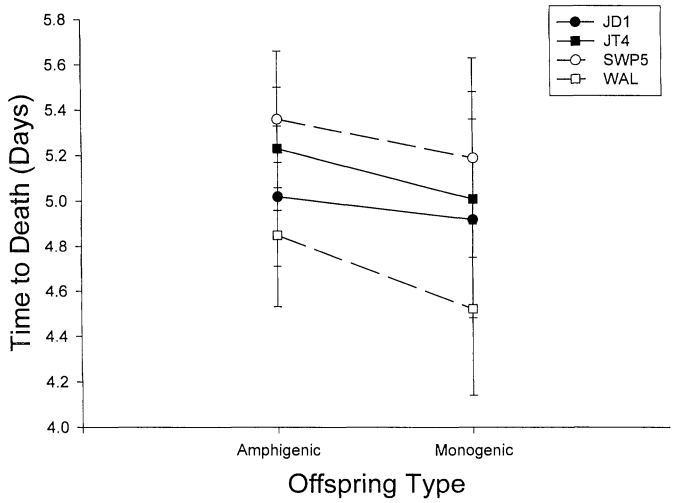

FIG. 6. Average time to death for monogenic and amphigenic offspring from selfed amphigenic parents, by population (Isolation 2). Error bars show one standard error of the mean.

Another scenario in which inbreeding depression due to partially recessive deleterious alleles could be maintained even though primary selfing rates are quite high is when the inbreeding depression is substantial and the mutation rate to deleterious alleles is high (Lande et al. 1994). For example, inbreeding depression as high as $\delta = 1.0$ has been estimated in a gynodioecious plant (*Cucurbita foetidissima*) even though selfing rates were as high as $s = 0.70$ (Kohn and

TABLE 9. Cumulative inbreeding depression estimates ($\delta = 1 - w_i/w_o$) for each fitness measure using w_i = zero heterozygosity class and (A) w_o = double heterozygotes and (B) w_o = triple heterozygotes.

Fitness measure	δ
(A) Three-loci	
Hatching	0.20
Juvenile survival	0.37
Age at maturity	0.41
(B) Five-loci	
Hatching	0.23
Juvenile survival	0.40
Age at maturity	0.47

Biardi 1995). In the current study, it is unclear whether the scenario suggested by Lande et al. (1994) is operating in *E. texana*. Assuming genomewide mutation rates are less than or equal to one mutation per individual per generation, the extreme inbreeding depression necessary to drive this process appeared to be lacking in these populations (Table 9). Estimates of the effects of inbreeding depression were generated by comparing relative performance of the zero heterozygosity class to the highest heterozygous class 0 (two or three heterozygous loci). Using a cumulative measure of inbreeding depression (e.g., Husband and Schemske 1997), inbreeding depression is estimated to be as high as 0.5 in these populations (Table 9). Nevertheless, this value is well below that necessary to retard purging via the mechanism outlined in Lande et al. (1994). However, because we did not measure lifetime fitness for these shrimp, we cannot discount the possibility that lifetime δ may be high enough to create the conditions that block purging. Experiments in progress will allow us to estimate inbreeding depression at the later stages of life (e.g., lifetime egg production, growth, and survival).

An alternate hypothesis for the cause of inbreeding depression, the overdominance model (Lande and Schemske 1985; Charlesworth and Charlesworth 1990), suggests that inbreeding lowers heterozygosity at one or more overdominant (or marginally overdominant) loci, thus causing inbreeding depression. There is some experimental evidence on naturally inbreeding species that is consistent with this model (Pray and Goodnight 1995; Carr and Dudash 1997), although the importance of overdominance in most inbreeding species is not clear (Husband and Schemske 1996). Fitness differences in these four populations suggest that a modified version of the overdominance model may account for the observed early inbreeding depression. In six of seven comparisons of early survival among allozyme classes, the heterozygotes had greater early survival than either homozygote class (significant in four of six cases). The standard explanation for such an observation (e.g., Prout 1971) would be that either these electrophoretic loci show overdominance or that they are linked to overdominant loci. In fact, the four significant cases of greater heterozygote survival were all sex-linked loci (one each for *Idh-1* and *Idh-2* and two for *Fum*). Thus, all four apparent overdominant cases may reflect a single, large linkage group that includes the sex-determining locus (or loci). If such a linkage group exists, then heterozygotes for one locus would tend to be heterozygous for many loci as a block. Homozygotes for this linkage group might exhibit lower fitness because of either expression of a number of deleterious recessives or lack of overdominance at one or more loci. In the former case, purging of deleterious alleles would be retarded because such alleles might be linked to other, beneficial alleles that are being positively selected. (This is a more extreme version of the linkage envisioned in Lande et al. [1994] in that the loci are directly linked rather than exhibiting identity disequilibrium.) Such a scenario would lower the effectiveness of purging by allowing selection to only operate on the average effect of the alleles within the linkage group (Dobzhansky 1970). Therefore, such a linkage group could have profound effects on inbreeding depression, including greatly lengthening the time needed to purge the deleterious recessive alleles within the linkage group.

The existence of such a linkage group could explain the unusual observation of detectable differences in survival among offspring within a selfed clutch. The segregation of a large linkage group could create differences in inbreeding within a single clutch by creating offspring that differ at blocks of loci that are inherited as either all homozygotes or all heterozygotes (on average). This could explain the survival differences observed among monogenics (homozygous for the linkage group) and amphigenics (heterozygous for the linkage group) within clutches found in our study. This could also explain the lower estimates of inbreeding from the three sex-linked loci (*Fum, Idh-1*, and *Idh-2*) relative to the other two loci (*Pgm-1* and *Mpi*). Differential survival between homozygotes and heterozygotes for the linkage group could maintain heterozygosity for those loci within the linkage group while allowing other, unlinked loci to become increasingly homozygous (Cockerham and Weir 1983).

An alternate explanation for both of the above results is that segregation of alleles at the loci examined (i.e., *Fum, Idh-1*, or *Idh-2*) were the direct cause of the observed survival differences, a possibility we cannot currently reject. However, consideration of the genetic mechanism of sex determination may suggest an explanation for the existence of the hypothesized linkage group, making this a more plausible explanation of the Isolation 2 survival results than a single locus of large effect. Sassaman and Weeks (1993) proposed that sex was determined by a single locus with two alleles. However, their findings are also compatible with a model of sex being determined by a suite of loci that are all tightly linked. If sex is determined by more than one locus (such as in *Drosophila* and *Caenorhabditis,* Bull 1983), then crossing over between these loci would produce intersexes (mixtures of sex-specific traits) that might have low or no fitness (Bull 1983). This idea has been used to explain the reduced crossing over observed in the heterogametic sex of many species (Nei 1969; Charlesworth and Charlesworth 1980; Bull 1983). Thus, if sex is determined not by one, but by many, linked loci in *E. texana*, there might be selection for reduced crossing over between these loci, which could in turn generate a large linkage group. The existence of a large linkage group could explain both the lack of purging of deleterious alleles in the four populations (assuming the linkage group were large enough to contain many, fitness-related loci) and the observed

fitness differences among offspring within the same selfed clutch.

In conclusion, all measures of fitness suggest that all four populations retain segregating alleles that cause inbreeding depression, even though all populations undergo significant levels of natural inbreeding (Sassaman 1989). There is little evidence that inbreeding depression has been purged in these populations, and thus little support for the partial dominance model for inbreeding depression (Charlesworth and Charlesworth 1987). We cannot rule out the purging of deleterious alleles early in the history of these inbred populations, nor have we estimated the potential importance of among-family differences in purging. Nevertheless, we currently have little evidence that the fitness differences observed herein were caused by independently assorting deleterious recessives. In contrast, the results are consistent with the overdominance model in that fitness generally increases with heterozygosity in all populations and that heterozygotes often survive better than either class of homozygotes. Detection of linkage among three, sex-linked enzyme loci (*Fum, Idh-1*, and *Idh-2*) suggests that overdominance may be caused by a large linkage group that includes the sex-determining locus or loci. Selection for reduced recombination among multiple sex-determining loci could be the cause of such a linkage group. However, the current data do not rule out the possibility of a few, overdominant loci of large effect that could be driving the observed fitness patterns. A long-term project now in progress will allow us to detect the relative importance of the partial dominance and overdominance models in the manifestation of inbreeding depression in *E. texana*.

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