Relative fitness of two hermaphroditic mating types in the androdioecious clam shrimp, *Eulimnadia texana*

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Abstract

Androdioecy (populations of males and hermaphrodites) is a rare reproductive form, being described from only a handful of plants and animals. One of these is the shrimp *Eulimnadia texana*, which has populations comprised of three mating types: two hermaphroditic types (monogenics and amphigenics) and males. In a recent study, the amphigenic hermaphrodites were found to be in greater abundance than that predicted from a model of this mating system. Herein, we compare the relative fitness of offspring from amphigenic and monogenic siblings, attempting to understand the greater relative abundance of the former. Populations started with offspring from selfed monogenic hermaphrodites had a net reproductive rate (R) 87% that of offspring from their amphigenic siblings. Additionally, within populations of amphigenic offspring (which included males, monogenics and amphigenics), amphigenics survived longer than monogenics. These differences help to explain the increased relative abundance of amphigenics in natural populations, but amphigenics continue to be more abundant than expected.

Introduction

Androdioecy (populations of males and hermaphrodites but no pure-females), is rare in plants and animals (Charlesworth, 1984), which is consistent with models that predict this reproductive form to be evolutionarily unstable, especially in mixed-mating (mating via both selfing and outcrossing) populations (Lloyd, 1975; Charlesworth, 1984). Androdioecy is predicted to be rare because the benefits of being all-male in an otherwise hermaphroditic population are constrained (Lloyd, 1975; Charlesworth, 1984). Becoming all-male could have two potential benefits: (a) reduced inbreeding depression for male-sired offspring (because males cannot self-fertilize) and (b) increased resource allocation to male function (relative to male allocation in hermaphrodites). As fitness through male function is defined by the availability of mates, any reduction in mating opportunities because of self-fertilization in hermaphrodites reduces relative male fitness 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 primarily because of reduced inbreeding depression, but all-male individuals have greatly reduced mating opportunities in primarily selfing populations, evolution of androdioecious populations should be uncommon (Charlesworth, 1984). In fact, Charlesworth (1984) reviewed several species that were previously classified as androdioecious and found that most were functionally dioecious.

Notwithstanding, several androdioecious systems have been documented since Charlesworth's (1984) review. In plants there are a handful of reported cases: *Mercurialis annua* (Pannell, 1997a, b), *Phillyrea angustifolia* (Lepart & Dommee, 1992), *P. latifolia* (Aronne & Wilcock, 1994), *Saxifraga cernua* (Molau & Prentice, 1992) and *Datisca glomerata* (Liston *et al.*, 1990). In animals, there are two well-documented cases of androdioecy: *Caenorhabditis elegans* (Wood, 1988) and *Eulimnadia texana* (Sassaman & Weeks, 1993; Zucker *et al.*, 1997). These 'exceptions to the rule' warrant further study to understand the factors which allow these species to maintain androdioecy

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although such a mating system is theoretically unlikely to evolve.

In *E. texana*, males coexist with hermaphrodites of two phenotypically similar but genetically different types: 'amphigenic' and 'monogenic' hermaphrodites. Sex appears to be controlled by a single genetic locus (Sassaman & Weeks, 1993), with a recessive allele coding for males (s) and a dominant allele coding for hermaphrodites (S). The homozygous dominants (SS) are monogenic hermaphrodites, the heterozygotes (Ss) are amphigenic hermaphrodites, and homozygous recessives (ss) are males (Sassaman & Weeks, 1993). Monogenics always produce 100% hermaphroditic offspring: 100% monogenics when selfed and 100% amphigenics when outcrossed. Amphigenics always produce a mixture of males and hermaphrodites: 25% monogenics, 50% amphigenics, and 25% males when selfed, and 50% amphigenics and 50% males when outcrossed. This mating system is intriguing because, although self-fertilization is common (inbreeding coefficients ranging from 0.20 to 0.97; Sassaman, 1989; Weeks & Zucker, 1999), androdioecy is still maintained.

We have been examining several behavioural, life history and genetic factors in an effort to explain the maintenance of androdioecy in this species. One of the most important factors is that hermaphrodites cannot mate with one another because they lack the clasping appendages necessary for pairing. Thus, all outcrossing must involve males, which allows males an advantage if selfing causes inbreeding depression. Other factors so far examined include inbreeding depression (Weeks *et al*., 1999, 2000a), rates of inbreeding (Weeks & Zucker, 1999), ability of hermaphrodites to self-fertilize (Hutchison, 1999), propensity of hermaphrodites to outcross with males (Hollenbeck, 1998) and ability of hermaphrodites to store sperm (Weeks *et al*., 2000b). All these factors have been used to test the predicted stability of this system using a population genetics model developed by Otto *et al*. (1993).

In the above studies of this mating system, one conspicuous result is that amphigenics are much more common than expected by the Otto *et al*. (1993) model (Weeks *et al*., 1999). Amphigenics were the most abundant mating type in four sampled populations, ranging from 63 to 75% of the population. These estimates were much greater than that expected by the Otto *et al*. (1993) model under a 'basic' scenario (i.e. males can mate with many hermaphrodites, no inbreeding depression and males and hermaphrodites have equivalent mortality schedules; see Weeks *et al*., 1999) or under a range of estimates of the four parameters of the model (Hollenbeck, 1998; Hutchison, 1999; Weeks *et al*., 2000a).

Two observations may partially explain this finding. First, individual heterozygosity was found to be positively correlated with egg hatching and early survival, and negatively correlated with time to reproductive maturity (Weeks *et al*., 1999), all three of which suggested that heterozygous shrimp were more fit in these populations. Because amphigenics are more heterozygous than monogenic hermaphrodites, on average, this would tend to favour amphigenics. Secondly, monogenic hermaphrodites from selfing amphigenic parents were found to have higher mortality rates than their amphigenic siblings (Weeks *et al*., 1999). Both of these results suggest that amphigenic hermaphrodites may be at a selective advantage relative to monogenic hermaphrodites, even within a selfed clutch, which could partially explain the observed bias towards amphigenics in these four populations.

The documented difference in survival between amphigenic and monogenic siblings (Weeks *et al*., 1999) is especially intriguing, as these siblings should, in theory, only differ at a single genetic locus (Sassaman & Weeks, 1993). If the previously measured survival differences are indicative of an overall difference in fitness between these two hermaphroditic types (rather than reflecting different life history strategies between these mating types), we have to conclude that either the sex-determining locus has these pleiotropic effects or that the sex determining gene is embedded in a linkage group of genes that cause this fitness effect (Weeks *et al*., 1999). In the current study, we further examined relative performances of monogenic and amphigenic hermaphrodites to determine whether the previously described survival differences truly reflect lower fitness of the former mating type. We made these comparisons using 'population aquaria' set up with egg banks produced by selfed amphigenics or monogenics from the four populations studied in Weeks *et al*. (1999). We extended the previous lifespan comparisons of these two hermaphroditic types by following fitness measures (survival, growth, fecundity and age at maturity) for 12 days post-maturity (most of the life span of *E. texana*), and by noting changes in proportion of the three mating types within aquaria begun with selfed amphigenic clutches. These data were then used to answer two related questions: (1) Are the previously documented differences in survival between hermaphroditic 'sisters' (amphigenics surviving longer than monogenics; Weeks *et al*., 1999) indicative of overall fitness differences of these two mating types? (2) Do amphigenics survive longer than monogenics within population aquaria begun with selfed amphigenic clutches? Answers to these two questions may allow us to understand the observation of higher than expected proportions of amphigenics in natural populations, and allow a better understanding of the maintenance of androdioecy in this species.

Materials and methods

Natural history of *E. texana*

Eulimnadia texana inhabit temporary playas, ditches and many other ephemeral freshwater habitats throughout

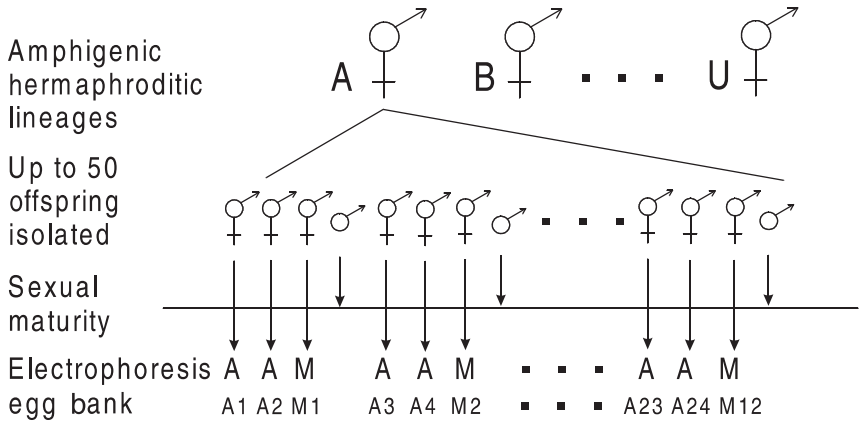
the southern United States, west of the Mississippi River and into northern Mexico (Sassaman, 1989). Hermaphrodites produce desiccation-resistant cysts which they bury within the top several millimetres of the soil. These cysts hatch rapidly at water temperatures above 18 °C. Larval and juvenile growth is extraordinarily rapid. Shrimp reach reproductive size in 4–7 days in the laboratory at 27–30 °C (Sassaman & Weeks, 1993; Weeks *et al.*, 1997) and in as little as 4–6 days in the field (Vidrine *et al.*, 1987). Total life span is 14–21 days (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 develop as claw-like claspers which are used to hold on to the margins of a hermaphrodite's carapace during mating. Hermaphrodites cannot store male sperm (Weeks *et al.*, 2000b) and thus males must mate with hermaphrodites repeatedly for high rates of outcrossing.

Natural populations of *Eulimnadia* are typically hermaphrodite-biased (Mattox, 1954) with some populations completely lacking males (Zinn & Dexter, 1962; Stern & Stern, 1971). *Eulimnadia texana* populations range from 0 to 40% males and inbreeding is positively correlated with hermaphrodite-biased sex ratios (Sassaman, 1989, 1995; Weeks & 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 & Zucker, 1999).

Rearing protocol and data collection

Four populations of clam shrimp were used in this study (see also Weeks *et al.*, 1999): three sites in New Mexico (JD1, JT4 and SWP5), all within Doña Ana Co. (south-central New Mexico), and one site in Arizona (WAL) in Cochise Co., near the south-east base of the Chiricahua mountains. Twenty amphigenic egg banks ('lineages') generated in a previous study (chosen on the basis of heterozygosity at either fumarate hydratase (*Fum*) or isocitrate dehydrogenase (*Idh*-1 or *Idh*-2); Weeks *et al.*, 1999) from each population were hydrated using filtered tap water and transferred to 37-L aquaria (Fig. 1). Hydrations occurred in 10 blocks of two lineage egg banks per population per block (eight hydrations per block for 80 total hydrations). Shrimp in these aquaria were raised under 'standard' conditions (aquaria were under continuous light using Durotest sunlight-simulating fluorescent bulbs, kept at 25–27 °C water temperature, and had continuous aeration; see Weeks *et al.*, 1999). Each aquarium was fed 40 mL of baker's yeast solution (1 g dried yeast 100 mL^{-1} water) per day. Just



	Amphigenic						Monogenic					
Lineage	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6
A	AA1	AA2	AA3	AA4	AA5	AA6	AM1	AM2	AM3	AM4	AM5	AM6
B	BA1	BA2	BA3	BA4	BA5	BA6	BM1	BM2	BM3	BM4	BM5	BM6
C	.	.	.	.	.	.	.	.	.	.	.	.
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U	UA1	UA2	UA3	UA4	UA5	UA6	UM1	UM2	UM3	UM4	UM5	UM6

Fig. 1 Experimental design. 'Amphigenic lineages' refers to the 20 original amphigenic egg banks hydrated per population from which up to 50 offspring were isolated. Males were discarded at sexual maturity and egg banks were collected from the remaining hermaphrodites. Hermaphrodites were electrophoretically scored and then assigned a hermaphrodite type (A – amphigenic; M – monogenic) on their allozyme patterns at three loci: *Fum*, *Idh*-1, *Idh*-2. Egg banks were then assigned a number, and stored for later hydration. Six replicates of each hermaphrodite treatment (monogenic and amphigenic) were started with the stored egg banks from each lineage as shown in the table.

prior to sexual maturity, up to 50 offspring from each lineage were isolated in 500 mL cups (Fig. 1) 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 (>1450 L of soil hydrated over 6 years has produced no clam shrimp). This 'shrimp-free' soil was used for all isolations. Offspring in all cups were fed 1 mL of baker's yeast solution per day. When the offspring matured, males were removed and discarded (Fig. 1), whereas the hermaphroditic offspring were allowed to produce selfed eggs for up to 1 week after isolation. A total of 1721 hermaphroditic offspring were isolated (JD1 = 381; JT4 = 433; SWP5 = 460; WAL = 447) from a total of 67 amphigenic lineages (JD1 = 15; JT4 = 16; SWP5 = 18; WAL = 18). After eggs were collected, hermaphroditic offspring were then frozen for enzyme electrophoresis (Fig. 1).

Hermaphroditic offspring were assayed for the heterozygous locus of their original hermaphroditic parent (i.e. lineage) using cellulose acetate electrophoresis [either *Fum* (EC 4.2.1.2), *Idh*-1 or *Idh*-2 (EC 1.1.1.42); for methods see Richardson *et al*., 1986]. All gels were run using 'Buffer C' from Richardson *et al*. (1986). Offspring heterozygous at the diagnostic locus were scored as amphigenics (A), whereas homozygotes were scored as monogenics (M; Fig. 1). Of a total of 1649 offspring that were successfully scored, 1161 were scored as amphigenics (70%) and 488 as monogenics (30%), which was significantly different ($\chi^2_{(1)} = 10.38$; $P < 0.01$) from the 2 : 1 ratio expected among hermaphroditic offspring from selfing amphigenics (Sassaman & Weeks, 1993). However, this 3% deviation from expectation is reasonable when considering the lower survival of monogenics relative to amphigenics (Weeks *et al*., 1999), and that electrophoretic typing was performed on fully grown adult shrimp.

Once electrophoretically typed, egg banks were numbered according to lineage and hermaphroditic type (A or M, Fig. 1), and stored until all 20 lineages per population were hydrated. The goal was to attain six egg banks of each hermaphroditic type from each of 20 lineages to allow genetic diversity within replicate aquaria and at the same time maintaining some consistency among aquaria (Fig. 1).

After all 20 lineages per population were hydrated, the resulting egg banks were dried for at least 30 days. After this period, egg banks from 11 to 15 lineages were combined into each replicate in 37-L aquaria (Fig. 1). Egg banks were combined by population and hermaphroditic type for a total of eight 'treatments' (four populations × two hermaphroditic types), all of which were replicated six times for a total of 48 replicate aquaria.

All 48 egg banks were hydrated (using filtered tap water) in three overlapping rounds of 16 (two replicates of each treatment), offset by 1 day each. Such a hydration strategy was used to avoid periods of extreme investigator activity (e.g. population counts, image analysis, etc.; see below) followed by periods of no activity. The hatching nauplii were reared under 'standard' conditions, with 40 mL of baker's yeast solution added daily. During day 3–4, up to 200 juveniles were randomly chosen to be transferred to a second 37-L ('population') aquarium with 500 mL of shrimp-free soil. These densities (5–6 individuals L^{-1}) are within the natural range reported for these shrimp (0.25–7 individuals L^{-1}; Medland, 1989; MacKay *et al*., 1990). The water from the 'hatching' aquarium was also transferred with the juveniles. Any remaining shrimp were frozen for later electrophoresis.

In the population aquaria, the shrimp were again raised under standard conditions, and were also fed 40 mL of baker's yeast solution daily. At sexual maturity, up to 100 shrimp were temporarily removed from the aquarium and were sexed to determine sex ratio. On days 4, 8 and 12, three additional measures were taken: (1) population estimates using three fish-net sweeps of each aquarium, (2) carapace length of males and hermaphrodites and (3) egg production in hermaphrodites. For the population estimates, three sweeps of the aquarium were taken, each sweep being for a fixed length of time (30 s). Shrimp were removed from the net after each sweep and added to a holding cup. After all three sweeps were made, the total shrimp in the cup were counted and used as the population size estimate. The latter two measures were made by taking images (using a computer-aided image analysis system running NIH Image software) of up to 10 shrimp per aquarium. Each gravid hermaphrodite had two images taken, one on each side of the shrimp. From these images, carapace length was measured, and because the carapace is clear, eggs could be counted directly through the carapace (see Weeks *et al*., 1997 for further details). At the end of the experiment (day 12), all remaining shrimp were captured and counted. All survivors were frozen for later electrophoresis.

In amphigenic treatments, hermaphrodites were electrophoretically typed for the three sex-linked enzyme loci (*Fum*, *Idh*-1 or *Idh*-2) in both the extra 'day 1' shrimp (those above the 200 used at the beginning of the experiment) and in those that survived the 12 days of the experiment (day 12 shrimp) using cellulose acetate electrophoresis. Hermaphrodites were scored as amphigenic if they were heterozygous for any one of the three sex-linked loci, otherwise they were scored as monogenic (Weeks *et al*., 1999).

The design of this experiment relies on the observation that three electrophoretically scored loci are tightly linked to the sex determining locus (Weeks *et al*., 1999). Homozygous offspring resulting from the selfing of an amphigenic heterozygous for either *Fum*, *Idh*-1 or *Idh*-2 should be either male or monogenic (Sassaman & Weeks, 1993; Weeks *et al*., 1999). However, some crossing over between these loci and the sex determining

locus can occur (Weeks *et al.*, 1999), thus allowing mistakes when using this method for scoring monogenics. In fact, six of the 24 'monogenic' aquaria (JD1: 1; JT4: 0; SWP5: 2; WAL: 3) had one or more amphigenic egg banks mistakenly added to the aquarium, resulting in male 'contamination' in these replicates. We, therefore, removed these replicates from all analyses because the presence of males made them neither monogenic-only nor amphigenic-only treatments. In the 24 amphigenic replicates, sex ratio was found to be 21.7 ± 2.6% (1 SE) males, which is within the range of the expected 25% males produced during the selfing of an amphigenic (a slightly lower proportion of males is expected because of higher male mortality; see Sassaman & Weeks, 1993).

Table 1 Growth (carapace length) and population size over the 12 days of the experiment. Values in parentheses are one standard error of the mean.

	Carapace length (mm)		Sqrt(population size + 1)	
Day	Monogenic	Amphigenic	Monogenic	Amphigenic
JD1				
1	2.31 (0.42)	3.51 (0.38)	8.2 (1.5)	11.0 (1.3)
4	5.17 (0.34)	5.11 (0.30)	4.2 (0.7)	4.9 (0.6)
8	6.00 (0.38)	6.32 (0.36)	3.1 (0.7)	3.1 (0.6)
12			2.8 (0.7)	2.7 (0.7)
JT4				
1	3.46 (0.41)	3.64 (0.38)	11.1 (1.3)	11.3 (1.3)
4	5.65 (0.30)	4.96 (0.30)	3.1 (0.6)	4.3 (0.6)
8	5.58 (0.38)	5.63 (0.37)	1.7 (0.6)	3.4 (0.6)
12			1.0 (0.7)	2.7 (0.7)
SWP5				
1	3.60 (0.46)	3.97 (0.41)	6.0 (1.6)	11.0 (1.4)
4	5.41 (0.36)	5.08 (0.32)	2.1 (0.7)	5.5 (0.6)
8	6.18 (0.46)	5.34 (0.36)	1.8 (0.7)	4.6 (0.6)
12			1.2 (0.8)	3.1 (0.7)
WAL				
1	3.95 (0.52)	3.60 (0.38)	8.4 (1.8)	10.1 (1.3)
4	5.22 (0.42)	5.05 (0.30)	3.8 (0.8)	4.4 (0.6)
8	5.52 (0.46)	5.59 (0.33)	3.0 (0.8)	4.0 (0.6)
12			3.0 (0.9)	3.0 (0.7)

Statistical procedures

It is important to note that the amphigenic aquaria were mixtures of males, monogenics and amphigenics (the products of selfing amphigenics). Therefore, the 'Hermaphroditic type' treatments herein (Tables 1 and 2) are actually comparisons between offspring of selfing monogenics vs. selfing amphigenics, not a direct comparison of monogenics to amphigenics. A direct comparison of the latter type is impossible, as monogenic and amphigenic hermaphrodites are morphologically indistinguishable, and the only method for specifying pure monogenic or amphigenic broods is to self or outcross monogenics, respectively (Sassaman & Weeks, 1993). This mating procedure would confound inbreeding depression with hermaphroditic type (i.e. monogenics – selfed, amphigenics – outcrossed), and would thus be a flawed approach. In the following, the appropriate level of comparison is between offspring produced from the selfing of two alternate parental types (monogenics vs. amphigenics), both parental types related to one another

Table 2 MANOVAS for size (carapace length) and estimates of population size. Bold *P*-values indicate significant tests at the $P < 0.05$ level.

	d.f.				
Test	Numerator	Denominator	Wilks' λ	*F*-ratio	*P*-value
Carapace length					
Block	2	27	0.971	0.401	0.6739
Population	3	27	0.963	0.344	0.7938
Hermaphroditic type	1	27	0.998	0.062	0.8047
Pop × herm	3	27	0.910	0.886	0.4610
Time	2	26	0.183	57.932	**0.0001**
Time × block	4	52	0.751	1.999	0.1084
Time × pop	6	52	0.712	1.607	0.1640
Time × herm	2	26	0.868	1.970	0.1598
Time × pop × herm	6	52	0.877	0.589	0.7379
Population size					
Block	2	32	0.651	8.580	**0.0010**
Population	3	32	0.971	0.317	0.8154
Hermaphroditic type	1	32	0.763	9.965	**0.0035**
Pop × herm	3	32	0.900	1.181	0.3324
Time	3	30	0.119	73.975	**0.0001**
Time × block	6	60	0.733	1.681	0.1413
Time × pop	9	73	0.651	1.566	0.1418
Time × herm	3	30	0.876	1.409	0.2594
Time × pop × herm	9	73	0.818	0.700	0.7068

as siblings (Fig. 1). Therefore, among the amphigenic offspring, measures of size, age at maturity and population will include males, monogenics and amphigenics, whereas egg production measures will include monogenic and amphigenic hermaphrodites. Among the monogenic offspring, all measures will only represent monogenic hermaphrodites.

Life-history data

All data were analysed using the statistical program JMP (SAS Institute, 1995). The hydration 'blocks' were included for all analyses. Size data were analysed using repeated measures MANOVA. For the size data, carapace length was averaged across samples per day to produce a single estimate for each aquarium for each day sampled (1, 4, 8 and 12). Because many treatments ended before day 12 (because of early mortality), only days 1, 4 and 8 were used in these analyses. Residuals of these analyses were normally distributed for each of the 3 days.

Population size estimates were also analysed using repeated measures MANOVA. Estimates from net sweeps were used with one exception: if population estimates were lower than the number of survivors caught at the end of the experiment, the actual number of survivors was substituted for the estimated number. Net sweeps were rarely exhaustive and thus population estimates were usually conservative. Population size values were square-root transformed to normalize residuals for the MANOVA test.

Because all shrimp within an aquarium essentially matured on the same day, age at maturity was compared on a per-aquarium basis using a two-way ANOVA. Residuals of the analysis were normally distributed.

Reproductive data were analysed using an ANOVA on average individual egg production during days 4 and 8. Day 1 was not used because none of the shrimp were mature at the start of the experiment. Day 12 was also not used because many of the treatments ended before this day and many of the hermaphrodites were reproductively senescing by this age (see Weeks *et al*., 1997). Thus, egg estimates per shrimp were averaged across days 4 and 8, resulting in a single measure per aquarium. These data were log-transformed to normalize residuals.

Net reproductive rates (R) were calculated per aquarium by constructing life tables of population estimates and average egg production at days 1, 4, 8 and 12. These two metrics were multiplied and then summed across days to calculate R. These data were log-transformed to normalize residuals.

Electrophoretic data

Changes in the distribution of males, monogenics and amphigenics among the offspring of selfed amphigenics were compared across the time span of the experiment by comparing the frequencies of these mating types at the beginning (day 1) and ending (day 12) of the experiment. To allow these comparisons, only replicates that had electrophoretically scored shrimp (see above) from both time periods (days 1 and 12) were used in these analyses. All four populations were represented in the analysis (JD1: two replicates, 86 total shrimp; JT4: two replicates, 57 total shrimp; SWP5: four replicates, 140 total shrimp; WAL: four replicates, 134 total shrimp). The proportion of males, monogenics and amphigenics was compared at day 1 relative to day 12 using a χ^2 contingency analysis (SAS Institute, 1995). Data were pooled across populations and replicates for this analysis.

Results

The size increased in a logarithmic fashion during the first 8 days of the experiment (Table 1), as is typical of this species (Weeks *et al*., 1997). It did not differ among populations or between offspring from the two hermaphroditic types nor did the pattern of change in size over time significantly differ among these independent variables (Table 2).

Population size declined in an exponential fashion over time (Table 1), again as is typical for this species (Weeks *et al*., 1997). Population size did not differ among populations (Table 2), but was significantly lower for monogenic relative to amphigenic offspring, indicating that monogenic offspring had lower survival than amphigenic offspring. This difference between offspring from the two hermaphroditic types was not significantly different among populations (Table 2).

The age at maturity did not significantly differ among populations (Tables 3 and 4) or between offspring from the two hermaphroditic types, although the time to maturity was slightly higher for monogenic relative to amphigenic offspring (Table 3).

Table 3 Age at maturity, egg production and R for the eight treatments. Values in parentheses are one standard error of the mean.

Treatment	Age at maturity (days)	ln (daily egg production)	ln(R)
JD1			
Monogenic	7.7 (0.6)	3.85 (0.26)	7.31 (0.54)
Amphigenic	6.4 (0.6)	4.77 (0.23)	8.89 (0.48)
JT4			
Monogenic	6.8 (0.5)	4.57 (0.23)	7.73 (0.48)
Amphigenic	6.9 (0.5)	4.46 (0.23)	8.00 (0.47)
SWP5			
Monogenic	7.9 (0.6)	4.22 (0.28)	7.01 (0.58)
Amphigenic	6.5 (0.5)	4.73 (0.24)	9.13 (0.51)
WAL			
Monogenic	7.0 (0.7)	4.37 (0.32)	8.16 (0.66)
Amphigenic	6.9 (0.5)	4.56 (0.23)	8.72 (0.47)

Table 4 Blocked, two-way ANOVAS for age at maturity, egg production and *R*. Egg production and *R* were log-transformed for analyses. Bold *P*-values indicate significant tests at the $P < 0.05$ level.

Test	d.f.	Sum of squares	*F*-ratio	*P*-value
Age at maturity				
Block	2	19.213	6.756	**0.0036**
Population	3	0.676	0.159	0.9234
Hermaphroditic type	1	3.997	2.811	0.1034
Pop × herm	3	4.324	1.014	0.3995
Error	32	45.504		
Egg production				
Block	2	1.389	2.320	0.1146
Population	3	0.267	0.298	0.8266
Hermaphroditic type	1	1.398	4.673	**0.0382**
Pop × herm	3	1.591	1.773	0.1722
Error	32	9.576		
Net reproductive rate				
Block	2	12.097	4.597	**0.0176**
Population	3	1.540	0.390	0.7609
Hermaphroditic type	1	12.608	9.583	**0.0041**
Pop × herm	3	5.473	1.387	0.2647
Error	32	42.102		

Egg production did not differ among populations, but was significantly reduced for monogenic relative to amphigenic offspring (Tables 3 and 4). Although there was no significant interaction between population and offspring from the two hermaphroditic types (Table 4), the JT4 population appeared to show an alternate response relative to the other three populations: a slight increase in egg production in monogenic relative to amphigenic offspring (Table 3).

When survival and fecundity data were combined into estimates of *R*, overall fitness was found to be significantly lower in monogenic relative to amphigenic offspring, but no differences were detected among populations (Tables 3 and 4). The interaction between population and hermaphroditic offspring was not significant (Table 4). Averaging across populations, monogenic offspring had an average fitness 87% that of amphigenic offspring.

At the beginning of the experiment (day 1), there was nearly a 1 : 2 : 1 ratio of monogenics (26%) to amphigenics (53%) to males (21%; Fig. 2) in the amphigenic treatments, which is consistent with expectations of selfing amphigenics (Sassaman & Weeks, 1993). Across the 12 days of the experiment, there was a significant increase ($\chi^2_{(2)} = 6.001$, $P = 0.0498$) in the relative proportion of amphigenics relative to either monogenics or males (Fig. 2). The decline in male proportion (−8%) was expected because of the higher relative mortality rate of males to hermaphrodites (Strenth, 1977; Sassaman & Weeks, 1993; Zucker *et al.*, in press). This lower proportion of males should have increased both proportions of hermaphrodites, if there was no difference in survival between monogenics and amphigenics. However, monogenic proportion actually declined over time (−4%), whereas the proportion of amphigenics substantially increased (+11%; Fig. 2), resulting in a ∼3 : 1 ratio of amphigenics to monogenics, rather than the expected 2 : 1 ratio.

Discussion

Clearly, offspring from amphigenic hermaphrodites are more fit than offspring from their monogenic counterparts. Amphigenic offspring survived longer and had

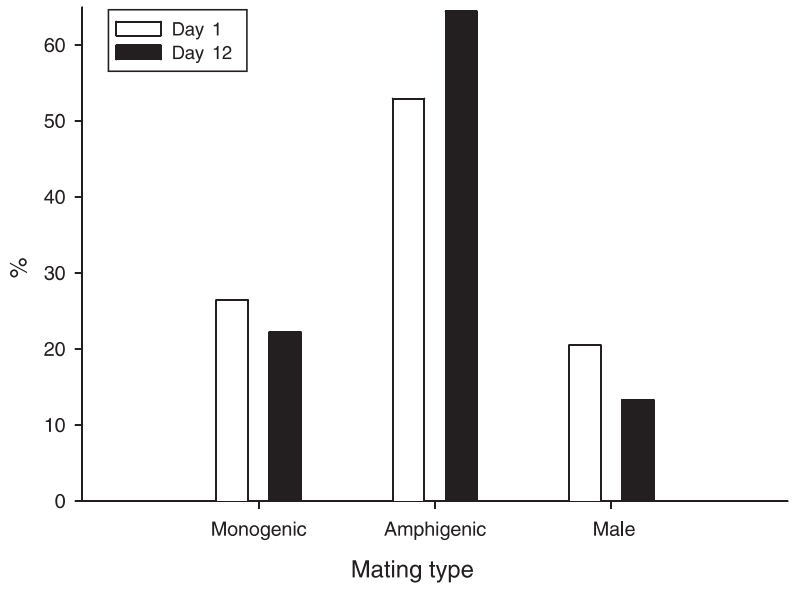

Fig. 2 Proportion of amphigenic offspring of each sex type at the beginning (day 1) and at the completion (day 12) of the experiment. An increase in the proportion of amphigenic hermaphrodites and a decrease in both monogenic hermaphrodites and males is noted over the course of the experiment.

greater reproductive output, on average, than monogenic offspring. These factors combined to produce a 13% fitness disadvantage for monogenic offspring. Note that this difference in fitness should be considered a lower bound, as the amphigenic treatments consisted of 25% monogenics, 50% amphigenics, and 25% males (i.e. the expected ratios produced by selfing amphigenics). Because the inclusion of 50% monogenics and males in the estimates of R will tend to lower the estimates of R (relative to amphigenic-only populations), the 13% fitness reduction measured herein should be considered conservative, and the true value of the fitness difference between monogenics and amphigenics could be as great as twice this value (see below).

Therefore, the answer to our first question, 'Are the previously documented differences in survival between hermaphroditic types (monogenic vs. amphigenic) found in isolation cups (Weeks *et al*., 1999) indicative of overall fitness differences when these two mating types are raised under population-level scenarios?' is a definitive 'yes'. Not only is the current data supportive of the previous survival differences, an additional relative advantage for amphigenics was seen in fecundity, and there was no evidence of any compensating advantages for monogenics in factors such as age at maturity or growth. These fitness differences between hermaphroditic mating types are meaningful because in the current comparison, overall genetic background was constrained to be similar by creating matched 'sibling' treatments of amphigenic vs. monogenic offspring whose parents only differed at the sex-determining locus and any loci linked to this locus. Thus, any fitness differences among offspring of the two hermaphroditic types should have been because of either pleiotropic effects of the sex determining locus or to effects of loci closely linked with this locus. As many of the electrophoretic marker loci examined in this species have been found to be genetically associated with the sex-determining locus (see also Sassaman, 1990), Weeks *et al*. (1999) suggested that the sex-determining locus (or loci) may be embedded in an extensive linkage group. If such a large linkage group exists in *E. texana*, then the observed fitness differences among mating types from the same clutch are easier to explain. Measurable fitness differences among individuals with different sets of large linkage groups (i.e. chromosomal inversions or 'supergenes'; Darlington & Mather, 1949) have been well documented in *Drosophila* (Beardmore *et al*., 1960; Dobzhansky, 1961, 1964; Dobzhansky & Pavlovsky, 1961). If *E. texana* has a similar 'supergene' associated with the sex-determining locus, then such a genetic complex could contain many fitness-related loci. If such a complex also harbours deleterious recessive alleles, then homozygous expression of this complex could result in lowered fitness for homozygotes, and the appearance of 'heterozygote advantage' for the sex-determining locus (Weeks *et al*., 1999).

The observation of increased fitness for amphigenic offspring, coupled with previously described survival differences among monogenic and amphigenic adults (Weeks *et al*., 1999) suggests that the fitness differences documented herein between monogenic and amphigenic offspring might be replicated within amphigenic treatments. In other words, if amphigenics truly outperform monogenics because of the linkage group suggested above, we should expect to see over-representation of amphigenics among the offspring in the amphigenic aquaria. This suggestion was confirmed when comparing starting relative to ending frequencies of all three mating types: monogenic hermaphrodites were ~4% less frequent after 12 days whereas amphigenic hermaphrodites were ~11% more frequent over this same period of time. Males dropped in frequency by ~8%, which is consistent with other studies which find increased mortality for males relative to hermaphrodites (Strenth, 1977; Sassaman & Weeks, 1993; Knoll, 1995; Zucker *et al*., in press). Thus, within the amphigenic treatments, a similar pattern of reduced relative fitness for monogenic compared with amphigenic hermaphrodites was apparent. This is to be expected, as the monogenic and amphigenic offspring within aquaria were generated in the same way as in the overall experiment (being siblings created from the selfing of an amphigenic parent). Thus, the within-aquarium results provide independent confirmation that amphigenics are more fit than monogenics.

The overall reduced relative fitness for monogenic offspring (87% that of amphigenic offspring) allows us to recalculate estimates of the three mating types using the Otto *et al*. (1993) model. This model predicts the equilibrium frequencies of the three mating types in *E. texana* [males (u), monogenic (w) and amphigenic (v) hermaphrodites] based on four relevant parameters: α – the ability of a male to fertilize hermaphroditic eggs; β – the proportion of eggs that are not fertilized by a male that are then self-fertilized by the hermaphrodite; $(1 - \sigma)$ – relative viability of males to hermaphrodites and δ – inbreeding depression experienced by selfed offspring. The model assumes that outcrossing rate is related to male frequency, u. The parameter α can vary from 0 to ∞, but is constrained such that $0 \leq \alpha u \leq 1$ (Otto *et al*., 1993). The combination of male frequency in the population and relative male mating ability (α) dictates the expected proportion of hermaphroditic eggs that will be outcrossed (i.e. αu). The remaining proportion of eggs [i.e. $(1 - \alpha u)$], are then available for selfing. The model allows for some proportion $(1 - \beta)$, of these nonoutcrossed eggs that will remain unfertilized. This would occur if some eggs were 'ear-marked' for outcrossing or if the hermaphrodites were unable to produce enough sperm to fertilize all their eggs in the absence of males (as in *C. elegans*; Ward & Carrel, 1979; Hodgkin & Barnes, 1991; Van Voorhies, 1992). A previous set of experiments has shown that hermaphrodites are capable of fertilizing all their eggs in the absence of males (i.e. $\beta = 1$;

Hutchison, 1999) and thus this factor is not included in any of the calculations below. The model also incorporates the commonly observed difference in viability between the sexes in conchostracan shrimp, defined as $(1 - \sigma)$. Finally, the model provides for the commonly documented decrease in viability observed in self-fertilized offspring (Jarne & Charlesworth, 1993; Husband & Schemske, 1996).

In two of the four populations (JD1 and SWP5), we used the Otto *et al*. (1993) model to predict mating type frequencies using a 'basic' scenario, as outlined in Weeks *et al*. (1999). This scenario assumes: (a) males can mate with many hermaphrodites when males are rare, (b) hermaphrodites regulate the amount of outcrossing (i.e. outcrossing rates, αu, are fixed by hermaphroditic preferences), (c) no inbreeding depression ($\delta = 0$) and (d) no difference in viability among males and hermaphrodites ($\sigma = 0$). Thus, this scenario produces baseline expectations, assuming males are maintained in the populations because of an elementary propensity of hermaphrodites to outcross (αu in Otto *et al*., 1993 and estimated as the outcrossing rates for both populations, *s*, given in Weeks *et al*., 1999) and similarly that monogenics are maintained because the propensity to outcross is not complete (i.e. $\alpha u < 1$; Otto *et al*., 1993). Herein, we modify these baseline expectations by incorporating reduced fitness for monogenic relative to amphigenic hermaphrodites. We have used both the conservative estimate of 87% relative fitness, as well as twice this fitness differential (74% relative fitness) in attempts to explain the observed high proportion of amphigenics in all four populations. These modifications do alter expected frequencies of the three mating types in each population relative to expectations generated when a hermaphroditic types were not considered separately (Weeks *et al*., 1999), but it is clear that monogenics remain lower than expected whereas amphigenics are more common than expected under this 'basic' scenario (Table 5). Males are within the range expected, being both below and above expected values depending on the scenario (Table 5).

The 'basic' scenario does not incorporate differences in inbreeding depression (which reduces relative monogenic fitness, Otto *et al*., 1993), nor does it account for observed reductions in male survival (Strenth, 1977; Zucker *et al*., in press). For two of these four populations (JT4 and WAL), we have detailed information on inbreeding depression (Weeks *et al*., 2000a) and on relative male viability (Zucker *et al*., in press). When incorporating those factors into our overall baseline expectations, we find a closer fit of observed to expected proportions of all three mating types (Table 5). The added parameter estimates allow closer fits of observed vs. expected frequencies in both populations, and in the case of the highest estimates of outcrossing rates and the

Table 5 Observed vs. expected sex ratios (%). Observed values (Obs.) were drawn from field-collected samples (Weeks *et al*., 1999). Bold expected values are above observed and italic values are below observed values. 'High' refers to the higher estimates of outcrossing whereas 'low' refers to the lower estimate, both from field collected soil (Weeks *et al*., 1999). 'Exp. 1' is the lower estimate of reduced fitness in monogenics (13% fitness reduction), while 'exp. 2' is the higher estimate (twice this value, 26%).

Sex-type	Obs.*	High-exp. 1	Low-exp. 1	High-exp. 2	Low-exp. 2
JD1†					
Monogenic	8.9	**37.0**		**30.1**	
Amphigenic	74.3	*44.1*		*47.6*	
Male	16.8	**18.9**		**22.4**	
	χ^2	42.4		31.4	
JT4‡					
Monogenic	10.0	**14.9**	**19.2**	**12.1**	**15.6**
Amphigenic	69.1	*56.7*	*55.3*	*57.6*	*56.7*
Male	20.9	**28.4**	**25.4**	**30.3**	**27.6**
	χ^2	6.3	8.7	5.6	6.4
SWP5†					
Monogenic	4.6	**17.6**	**54.6**	**14.2**	**45.7**
Amphigenic	75.1	*52.6*	*33.2*	*53.6*	*38.5*
Male	20.3	**29.8**	*12.2*	**32.1**	*15.8*
	χ^2	22.2	104.1	19.5	73.1
WAL‡					
Monogenic	12.6	*7.9*	**19.8**	*6.5*	**16.1**
Amphigenic	63.1	*57.7*	*55.2*	*57.9*	*56.6*
Male	24.2	**34.4**	**25.1**	**35.6**	**27.3**
	χ^2	6.3	3.8	9.8	1.8

*χ^2 values do not have associated *P*-values as these are for comparison only (small values = close to expected frequencies). †'Basic' scenario expected values, using parameters outlined in Weeks *et al.* (1999), which assumes no inbreeding depression ($\delta = 0$) and no difference in viability between males and hermaphrodites ($\sigma = 0$). ‡Expected values incorporating estimates of inbreeding depression (Weeks *et al*., 2000a) and relative male viability (Zucker *et al*., in press).

higher estimate of fitness differences between monogenics and amphigenics (Table 5, fourth column), the fit of observed and expected frequencies are quite close. More precise estimates of outcrossing rates (both behavioural and mechanistic) may allow a closer fit of observed sex ratios to those expected from the Otto *et al*. (1993) model.

A side issue that deserves comment concerns the relative performances of the four populations studied herein. Although the four populations did not significantly differ on any of the measures, there was a trend among populations in that JT4 consistently showed little to no difference between offspring from monogenic and amphigenic hermaphrodites. A different fitness response of JT4 relative to other populations has been noted in related studies: JT4 had no fitness difference among heterozygosity classes and there was only a small fitness reduction in selfed vs. outcrossed offspring (Weeks *et al*., 2000a). Homozygous shrimp from JT4 do show reduced hatching rates and early survival (Weeks *et al*., 1999). However, previous and current results suggest that JT4 shrimp have lower levels of inbreeding depression in later life traits (age at maturity, egg production and survival). These results may reflect a purging of deleterious alleles affecting these traits in the linkage group that contains the sex determining locus, although this remains to be determined.

The current study helps us to better understand the maintenance of males and hermaphrodites in one of the few well-studied androdioecious species. Because only a handful of androdioecious species have been examined to date, no general pattern explaining this mating system has been found. Of the androdioecious plants, *D. glomerata* is the best studied example of a truly androdioecious species. In this species, androdioecy is maintained by a combination of factors: high outcrossing rates (65–92%; Fritsch & Rieseberg, 1992), greater pollen production per flower in male-only plants (Philbrick & Rieseberg, 1994), protogyny (Rieseberg *et al*., 1993), earlier flowering of males (Spencer & Rieseberg, 1995) and inbreeding depression in selfed offspring (Rieseberg *et al*., 1993). The *D. glomerata* system primarily differs from *E. texana*'s in that hermaphrodites can cross with one another in the former species. This likely contributes to a second distinction between these systems: outcrossing rates are approximately two-fold higher in *D. glomerata* relative to *E. texana* (Fritsch & Rieseberg, 1992; Weeks & Zucker, 1999; Weeks *et al*., 1999).

Caenorhabditis elegans is a second androdioecious species that has been well studied, and which has a mating system much like that of *E. texana*: most individuals are hermaphrodites that can self-fertilize or receive sperm from males but do not exchange sperm with other hermaphrodites (Wood, 1988; Barker, 1992). Males are very rare in laboratory cultures, like *E. texana* (unfortunately, male frequencies in natural populations are not well known; Hodgkin & Barnes, 1991). However, *C. elegans* differs from *E. texana* in a number of ways. First, males are XO, and spontaneously arise from a meiotic loss of an X chromosome (Hodgkin & Barnes, 1991). In *E. texana*, males are homozygous recessive for either a single locus (Sassaman & Weeks, 1993) or the linkage group suggested herein. Secondly, the process of mating with a male induces hermaphroditic *C. elegans* to produce up to two-fold more eggs than if they were to self (Kimble & Ward, 1988). In *E. texana*, mating with a male does not affect hermaphroditic reproductive output (Knoll & Zucker, 1995). Thirdly, hermaphrodites in *C. elegans* produce fewer sperm than eggs, and thus can only fertilize approximately 80% of their total eggs, unless outcrossed (Ward & Carrel, 1979; Hodgkin & Barnes, 1991; Van Voorhies, 1992). Hermaphrodites of *E. texana* do not appear to be similarly sperm-limited (Hutchison, 1999). Finally, inbreeding depression appears to be an important component to the maintenance of androdioecy in *E. texana* (Weeks *et al*., 1999, 2000a) but appears to be unimportant in *C. elegans* (Johnson & Hutchison, 1993). Thus, although the two mating systems have several important similarities, the maintenance of androdioecy in each appears to be driven by diverse factors.

The greatest relevance of the current system is to that of other androdioecious branchiopod crustaceans. Androdioecy has been inferred in three other conchostracans (Sassaman, 1995) as well as a notostracan branchiopod (Sassaman, 1991). Additionally, sex ratios described in several conchostracan species in the family Limnadiidae suggest an additional nine species to be androdioecious (Sassaman, 1995), which would make androdioecy the most prevalent reproductive mode in this family. If these other conchostracans are also truly androdioecious, and if the genetic mechanism of sex determination in these species is found to be analogous to *E. texana*'s (genetic evidence from another conchostracan species suggests similar sex-linkage relationships as described in *E. texana*; Sassaman, 1990), then the current descriptions of fitness differences among hermaphroditic types may help to explain the preponderance of androdioecy in this crustacean family.

In conclusion, current comparisons of relative fitness between monogenic and amphigenic hermaphrodites suggests a minimum of 13% reduction in fitness for monogenics relative to their amphigenic siblings, although a more realistic estimate of this difference may be as much as twice this value. Such a fitness reduction can be explained if the sex-determining locus (or loci) is embedded in a large linkage group containing a number of fitness-related loci (Weeks *et al*., 1999). The observed fitness difference between the two sexual types can partially explain previous findings of greater-than-expected proportions of amphigenics in four natural populations, especially when inbreeding depression is also assumed (Weeks *et al*., 1999, 2000a). Future data on the magnitude of outcrossing need to be collected to fully understand the dynamics of this mating system.

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