Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: field estimates of inbreeding depression and relative male survival

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ABSTRACT

Androdioecy is a rare form of reproduction, only found in a few plant and animal species, wherein males co-exist with hermaphrodites. This particular form of mixed mating (mixtures of outcrossing and self-fertilization) is predicted to be evolutionarily unstable, with most androdioecious populations thought to be in a transition from hermaphroditism to dioecy, or vice versa. One well-studied androdioecious species is the freshwater clam shrimp *Eulimnadia texana*. A model by Otto *et al.* (1993), exploring the stability of this androdioecious system, predicts that males can co-exist with hermaphrodites when males fertilize an average of over twice the number of offspring that an average hermaphrodite produces in a lifetime. This value proportionally increases if males survive less well than hermaphrodites, and proportionally decreases with increased inbreeding depression. In the present study, we measured relative male longevity and inbreeding depression using laboratory-produced selfed and outcrossed eggs reared in the field. Males had lower survival than hermaphrodites in both mating treatments, but the survival difference was greater in the outcrossed relative to the selfed mating treatments (19 *vs* 9% difference). Inbreeding depression (δ) was estimated at 0.58–0.69, depending on the level of selfing among the parents in the outcrossed treatments. Both estimates of relative male viability and inbreeding depression corresponded well with earlier laboratory estimates of these parameters. Thus the within-pond dynamics outlined in the model of Otto *et al.* (1993), which are driven by high inbreeding depression and high relative male fertility, may still explain the maintenance of androdioecy in these shrimp. Field estimates of male mating effectiveness are required as a final test of the accuracy of this model.

Keywords: branchiopod crustaceans, evolution of mating systems, genetic load, hermaphrodites.

INTRODUCTION

Androdioecy (populations comprising males and hermaphrodites) is an exceptionally rare mating system (Charlesworth, 1984; Jarne and Charlesworth, 1993; Pannell, 2002), with only a maximum of 53 androdioecious species described in the plant and animal kingdoms

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(Pannell, 2002). Currently, there are two opinions on the rarity of androdioecious mating systems: (1) androdioecy is maintained at the within-population level due to the benefits of being all male, such as avoidance of inbreeding depression, reallocation of reproductive resources to male gamete production and increased mating advantages (Lloyd, 1975; Charlesworth, 1984; Otto *et al.*, 1993); or (2) hermaphrodites are maintained at the meta-population level in colonizing species due to the benefits of reproductive assurance, even if dioecy (separate males and females) is selectively advantageous within populations (Pannell, 1997b). In the within-population models, males and hermaphrodites can co-exist only in a narrow range of conditions, and thus androdioecy is thought to be a short-lived, transitory stage to full dioecy (Charlesworth, 1984). The metapopulation model also finds androdioecy to be difficult to maintain, with levels of immigration and extinction in the metapopulation dictating proportions of males and hermaphrodites (Pannell, 1997b, 2000, 2002). All models predict androdioecy to be less common than gynodioecy (mixtures of females and hermaphrodites).

Given that maintaining males and hermaphrodites appears to be so tenuous, several studies have attempted to determine the factors allowing androdioecy in these 53 purported cases. The best studied androdioecious systems are three plant species [*Mercurialis annua* (Pannell, 1997a,c,d), *Phillyrea angustifolia* (Lepart and Dommee, 1992; Pannell and Ojeda, 2000; Vassiliadis *et al.*, 2000) and *Datisca glomerata* (Liston *et al.*, 1990; Fritsch and Rieseberg, 1992; Rieseberg *et al.*, 1992, 1993)] and two animal species [*Caenorhabditis elegans* (Ward and Carrel, 1979; Hodgkin and Barnes, 1991; Barker, 1992; Van Voorhies, 1992) and *Eulimnadia texana* (Weeks *et al.*, 1999, 2000a, 2001a,b; Medland *et al.*, 2000; Zucker *et al.*, 2001; Hollenbeck *et al.*, 2002)]. Because of the small number of species examined to date, no overall pattern for the maintenance of androdioecy has become apparent (see Pannell, 2002, for a discussion of the emerging patterns).

We have been studying the mating system of the androdioecious clam shrimp, *Eulimnadia texana*, for the past 10 years. In this species, males co-exist with hermaphrodites of two phenotypically indistinguishable but genetically distinct types: 'amphigenic' and 'monogenic' hermaphrodites, the former type normally comprising 60–70% of the population, whereas the latter comprises 5–10% of the population (male frequencies range from 0 to 30%). Sex determination appears to be controlled by a single, two-allele, genetic locus (Sassaman and Weeks, 1993). The homozygous dominants are monogenic hermaphrodites, the heterozygotes are amphigenic hermaphrodites, and homozygous recessives are males (Sassaman and 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. We have focused our initial examinations of this system on testing the population genetics model developed by Otto *et al.* (1993), which predicts stable co-existence of males, monogenic and amphigenic hermaphrodites, given sufficient benefits to males via high outcrossing rates and avoidance of inbreeding depression. Because *E. texana* hermaphrodites cannot fertilize one another (Sassaman and Weeks, 1993), the advantages of outcrossing only accrue to males.

In these previous studies, we have determined that: (a) inbreeding depression ranges between 0.5 and 0.7 (Weeks *et al.*, 1999, 2000a, 2001b); (b) males survive at 70–90% the rate of hermaphrodites (Zucker *et al.*, 2001); (c) hermaphrodites do not experience sperm limitation in the absence of males (Weeks *et al.*, 2001b); and (d) a male can fertilize as

many as 10 times the number of eggs that a single hermaphrodite produces in a lifetime (Hollenbeck *et al.*, 2002). When we use the above ranges of estimates of the parameters in the model of Otto *et al.* (1993), we find that androdioecy is generally predicted to be stable, but that when predicting natural sex ratios, the model tends to under- and overestimate the proportions of amphigenic and monogenic hermaphrodites, respectively (Weeks *et al.*, 2001a,b). Specifically, the model predicts male frequencies to be 12–36%, monogenics to be 7–55% and amphigenics to be 33–57% of the shrimp populations, but we have found frequencies of 17–24%, 5–13% and 63–75%, respectively (Weeks *et al.*, 2001a).

Until now, all of our estimates of the parameters in the model of Otto *et al.* (1993) have been made from laboratory populations. More realistic estimates require us to examine these parameters from natural settings, and may allow better fits of the observed sex ratios to those predicted by the model of Otto *et al.* (1993). This is especially true for estimates of inbreeding depression, which have generally been shown to be greater in field relative to laboratory settings in other species (Crnokrak and Roff, 1999). Thus, we undertook the current field study to compare levels of inbreeding depression and relative male longevity in one population of *E. texana* reared in artificial pools in the Arizona desert.

METHODS

Eulimnadia texana is a small (carapace length up to 8 mm) branchiopod crustacean in the order Spinicaudata. The common name, clam shrimp, derives from the folding of the carapace around the body to resemble the shell of a bivalve mollusc. *Eulimnadia texana* inhabit temporary ponds, pools, ditches and other ephemeral freshwater habitats throughout the southwestern United States (Sassaman, 1989). Desiccation-resistant eggs are produced by hermaphrodites, which are buried within the top few millimetres of the soil. The eggs hatch rapidly (18–30 h) upon hydration and reach sexual maturity within 5–7 days (Weeks *et al.*, 1997). *Eulimnadia texana* are filter feeders and grow rapidly within the first few days after hatching, with growth slowing after offspring production begins (Weeks *et al.*, 1997). Eggs are produced in the ovotestes and moved to a 'brood chamber' located just under the carapace, where they sit for 12–20 h before being buried in the soil (Zucker *et al.*, 2002). The shrimp live for 1–2 weeks after reaching sexual maturity (Weeks *et al.*, 1997; Zucker *et al.*, 2001).

Soil containing *E. texana* eggs was collected from pools near Portal, Arizona (described as 'WAL' in previous publications) in Cochise County, near the base of the Chiricahua Mountains. These soil samples were taken to the University of Akron in Akron, Ohio, where subsamples (250 ml of soil per tank) were hydrated in two 37-litre aquaria using deionized water. The tanks were kept in environmentally controlled conditions at a constant temperature of 28°C under constant lighting from sunlight-simulating fluorescent bulbs (Durotest, Inc.), and subjected to light aeration. Fifty millilitres of a yeast solution (1 g dried baker's yeast per 100 ml water) was added to each tank daily. Directly before shrimp reached sexual maturity (~4 days), 120 hermaphrodites were individually separated into 500 ml isolation cups. Males were combined with hermaphrodites in one half (60) of the isolation cups to produce the 'outcrossed' egg banks, while the remaining 60 herma-phrodites produced the 'selfed' egg banks. Isolated shrimp were fed 3 ml of yeast solution on the first day and 1 ml per shrimp every day thereafter. Shrimp in the isolation cups were allowed to produce eggs for 3 weeks. Dead shrimp were replaced with live shrimp of the corresponding sex, making sure that only non-gravid shrimp were used to replace dead

'selfing' hermaphrodites [hermaphrodites cannot store sperm (Weeks *et al.*, 2000b), thus non-gravid shrimp will only produce selfed offspring]. The soil and eggs were allowed to dry for 30 days, and then the soil was combined by treatment type, mixed to distribute the eggs evenly throughout the soil and, finally, sealed in zip lock bags in three equal portions per treatment. Thus, each bag contained the eggs from ~20 hermaphrodites, three for each mating treatment.

The field experiment was conducted near Portal, Arizona, close to the site of original soil collection. Six artificial plastic pools (140 cm diameter, 18 cm deep) were sunk into sandy soil so that the upper rim was flush with the soil surface. The entire experimental area was encircled with a barbed wire fence and small mesh (2.5 cm diameter) chicken wire to limit the visitation of animals to the pools. The bottom of each pool was covered with 1 cm of soil free of branchiopod eggs (collected from an area known to be free of any clam shrimp eggs). Each pool was filled with 110 litres of rainwater collected in buckets at the Southwestern Research Station in Portal, Arizona. The water in the pools was allowed to sit for 6 days to make sure there were no branchiopods hatching. No pools had any branchiopods, so, after day 6, water was added to the pools (to bring the total volume once again to 110 litres) and the egg banks from the six bags were poured into one of the six pools (one bag per pool). The pools were then stirred to simulate flooding.

The first day of collection was 5 days after hydration, then days 8, 11, 14 and 17. No collections were done before day 5 because of the sensitive nature of the smaller shrimp (Weeks *et al.*, 1997). Shrimp were collected using a standard aquarium net (1 mm mesh size) in a sweeping motion back and forth across the pool. Pools were exhaustively sampled to remove all live shrimp. Males and hermaphrodites were counted separately and transferred to a bucket containing collected rainwater. The process was continued until all shrimp were collected and counted. Then, shrimp were returned to their respective pools. On collection days 5 and 11, a total of 20 hermaphrodites per pool were collected and individually preserved to estimate fecundity. On day 17, the remaining shrimp were collected and preserved for estimates of fecundity.

On the third day of the experiment, an unfortunate accident ruined pool 3 (an outcrossed treatment) and, therefore, this pool was not included in the experiment.

Carapace length was determined on the preserved shrimp using NIH Image Software. Carapace length was estimated by measuring maximal length on lateral images of the shrimp (Weeks *et al.*, 1997). All eggs per hermaphrodite were counted and recorded per individual.

Repeated measures multivariate analyses of variance were used to analyse population size using sex-specific population numbers per replicate pool at days 5, 8, 11, 14 and 17 as the dependent variables and sex (male *vs* hermaphrodite) and breeding treatment (selfed *vs* outcrossed) as the two main effects. Population size was square root transformed to normalize residuals. All data were analysed using JMP version 5.01 (SAS Institute, 2003).

Survival was also compared using a non-parametric Log-rank test (SAS Institute, 2003), which was performed on differences in survival between the two sexes within each of the breeding treatments (outcrossed and selfed). This test also calculates average time to death, which was used to calculate the $(1 - \sigma)$ parameter of the model of Otto *et al.* (1993); σ is the proportional decrement in survival of male shrimp relative to a value of 1.0 for hermaphrodites.

Size and egg production were measured on sub-samples of shrimp per replicate pool and thus were analysed using nested analyses of variance, one each for each of the 3 days

sampled (days 5, 11 and 17). Egg production at day 5 was square root transformed to normalize residuals, whereas egg production on days 11 and 17 did not require transformations. Because analyses on transformed and untransformed data yielded identical conclusions, analyses are presented for untransformed tests only. Size was normally distributed without transformations for all 3 days. In one of the three pools assigned to the self-fertilization treatment, no shrimp survived to day 17, and thus this pool was not included in the analyses of size and egg production on day 17.

Net reproductive rates (R) were calculated by multiplying the average egg production per hermaphrodite by total population size on days 5, 11 and 17, and then summing these values across all 3 days. Because the outcrossing treatments had a greater proportion of males than did the selfing treatments, we calculated R in two ways: (1) using total population size multiplied by average egg production, and (2) using only hermaphrodites multiplied by average egg production. The former allows a direct comparison between the two breeding treatments that is not biased by the reduced egg production capacity associated with increased numbers of males, whereas the latter is a comparison of actual egg production capability in the two types of treatments. The relative differences in net reproductive rate between breeding treatments using these two calculations only differed by 3%, and thus to avoid confounding breeding treatment differences with sex ratio differences, we report the results of method (1) only.

RESULTS

Population size over time was dependent on breeding treatment and sex (Fig. 1). There were significantly more shrimp in the outcrossed pools (Table 1), with total numbers being 3–4 times higher in the outcrossed than in the selfed pools (Fig. 1). These differences between breeding treatments were reflected in both sexes, but the relative magnitude of the differences depended on sex (Table 1).

Because the differences in the absolute numbers were so great, we re-plotted the population size data using proportions of the total populations surviving over the 17 days of the experiment (Fig. 2). Examination of proportion surviving over time shows that the relative differences between males and hermaphrodites was greater in the selfed than in the outcrossed treatments, with selfed males having the lowest proportional survival of the four treatments. Additionally, hermaphrodites lived significantly longer than males in both the outcrossed (Log-rank $\chi_1^2 = 44.6$, $P < 0.001$) and the selfed treatments (Log-rank $\chi_1^2 = 62.4$, $P < 0.001$). The overall relative male viability estimates [$(1 - \sigma)$ in Otto *et al.* (1993)] were 0.91 for outcrossed males and 0.79 for selfed males (Table 2).

The outcrossed shrimp were significantly smaller in carapace length than the selfed shrimp at sampling days 5 and 11 (Fig. 3, Table 3). Because size is positively correlated with fecundity (Weeks *et al.*, 1997), outcrossed hermaphrodites produced fewer eggs per individual than selfed hermaphrodites on both sampling days (Fig. 4, Table 3). The drop in average eggs per individual between days 5 and 11 (Fig. 4) may be attributable to sampling error. Only hermaphrodites containing eggs were sampled from the population for the fecundity measures. Because hermaphrodites become reproductive at about 4–7 days old (Weeks *et al.*, 1997), it is possible that only the most productive hermaphrodites were chosen for this early sample. By day 11, all hermaphrodites should be carrying eggs (Weeks *et al.*, 1997), and thus the sampling regime would include both highly productive and less productive individuals on days 11 and 17. Therefore, the sampling regime may have
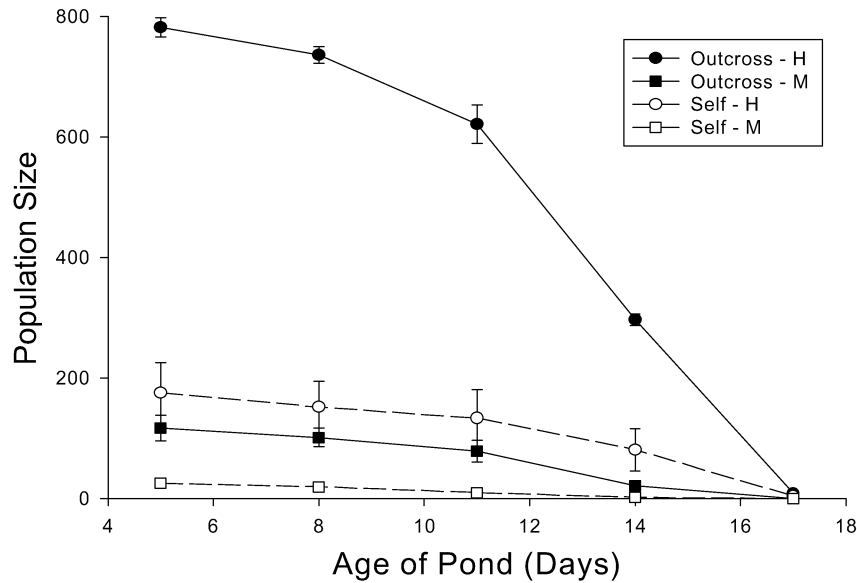
Fig. 1. Total population size of both selfed and outcrossed treatments and sex (H = hermaphrodites, M = male). Error bars represents one standard error.

Table 1. Results of repeated measures multivariate analysis of variance for population sizes of males and hermaphrodites

Source	Wilks' λ	F-ratio	Degrees of freedom		Prob > F
			Numerator	Denominator	
Treatment	6.1	36.3	1	6	0.0009
Sex	10.5	63.0	1	6	0.0002
Treatment × sex	1.2	7.2	1	6	0.0362
Time	791.1	593.3	4	3	0.0001
Time × treatment	117.9	88.4	4	3	0.0019
Time × sex	98.3	73.7	4	3	0.0025
Time × treatment × sex	29.9	22.4	4	3	0.0143

upwardly biased egg production per hermaphrodite on day 5 relative to days 11 and 17. Nonetheless, because sampling was consistent across treatments, this does not negatively affect the estimates of the relative differences between mating treatments.

Because of the different responses between treatments in population size relative to growth and egg production, we calculated net reproductive rates to compare overall productivities per pool. Outcrossed productivity was ~56% greater than selfed productivity (Table 4); however, this difference was not significant (Student $t_3 = 1.20$, $P = 0.1266$). As net reproduction is our best estimate of fitness, R was used to determine inbreeding depression using the following equation: $\delta = 1 - (\text{selfed } R/\text{outcrossed } R)$. Using this equation, inbreeding depression was estimated as $\delta = 0.36$.
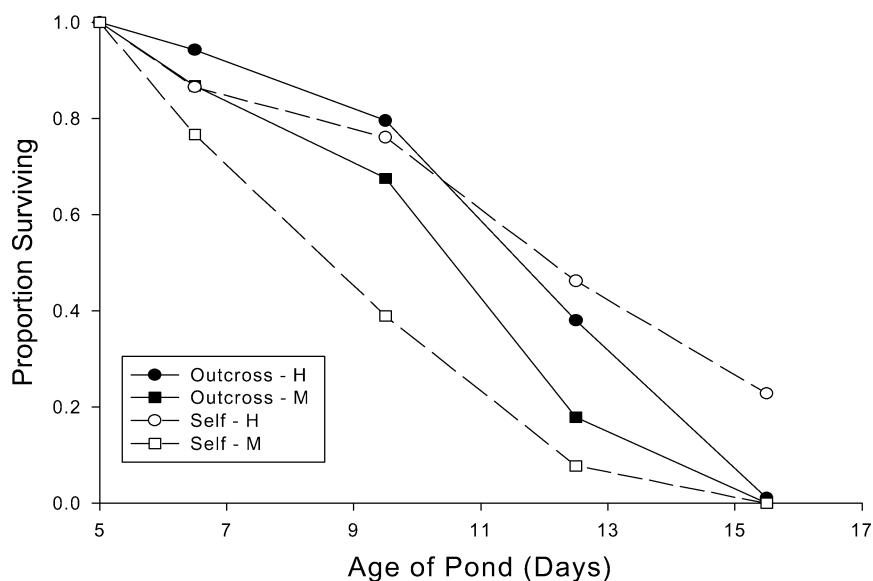
Fig. 2. Proportion of the total population surviving by day (H = hermaphrodites, M = male). Longevity estimates were started 5 days after hydration, and survival intervals were plotted as the mid-way point between successive sampling days.

Table 2. Mean longevity (in days) of males and hermaphrodites by treatment and the resulting parameter of $1 - \sigma$ from the model of Otto *et al.* (1993)

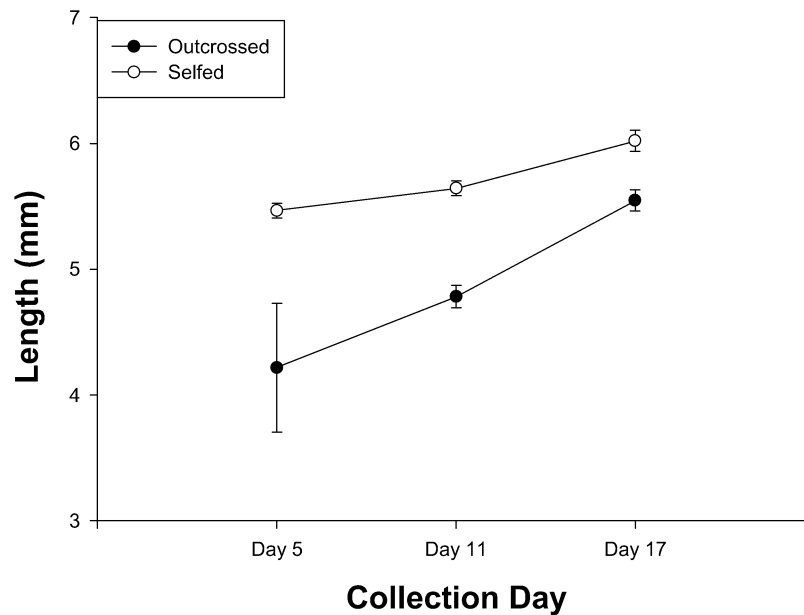|	Males	Hermaphrodites	$1 - \sigma$
Outcrossed	11.67	12.85	0.91
Selfed	10.20	12.76	0.79

Note: σ is the proportional decrement in survival of male shrimp relative to a value of 1.0 for hermaphrodites.

DISCUSSION

Androdioecy is thought to be an unstable, intermediate stage between all-hermaphroditic populations and dioecious populations. However, a few models have outlined scenarios in which androdioecy can be stable (Otto *et al.*, 1993; Pannell, 1997b, 2000). In the current study, two important parameters in one of these models (Otto *et al.*, 1993) were measured: relative male viability ($1 - \sigma$) and inbreeding depression (δ). Below, we consider each model parameter independently, and then discuss the ramifications of these results for interpreting the model of Otto *et al.* (1993).

Relative male viability ($1 - \sigma$)

Previous studies of *E. texana* have found evidence that males have higher mortality than hermaphrodites. Strenth (1977) found decreasing relative male proportion when sampling

Fig. 3. Total carapace length of hermaphrodites for three collection days. Error bars represent one standard error.

Table 3. Results of analysis of variance for egg production and size at the three sampling days (5, 11 and 17)

	Length				Eggs			
	d.f.	SS	*F*-ratio	*P*-value	d.f.	SS	*F*-ratio	*P*-value
Day 5								
Treatment	1	25.6	193.4	0.0008	1	28 760	11.6	0.0425
Pool (treatment)	3	0.4	1.1	0.3646	3	66 479	8.9	<0.0001
Error	96	12.8			96	238 797		
Day 11								
Treatment	1	36.76	210.7	0.0007	1	11 145	7.1	0.0766
Pool (treatment)	3	0.04	0.1	0.9702	3	80 151	16.9	<0.0001
Error	95	16.58			95	150 112		
Day 17								
Treatment	1	0.57	5.2	0.1501	1	1 380	0.6	0.5198
Pool (treatment)	2	0.27	1.2	0.3061	2	8 825	1.9	0.1682
Error	25	2.73			25	57 568		

Note: The effect 'pool' was nested within the two breeding treatments, and this nested term was used as the denominator sum of squares for testing the main effect of 'treatment' for all analyses.
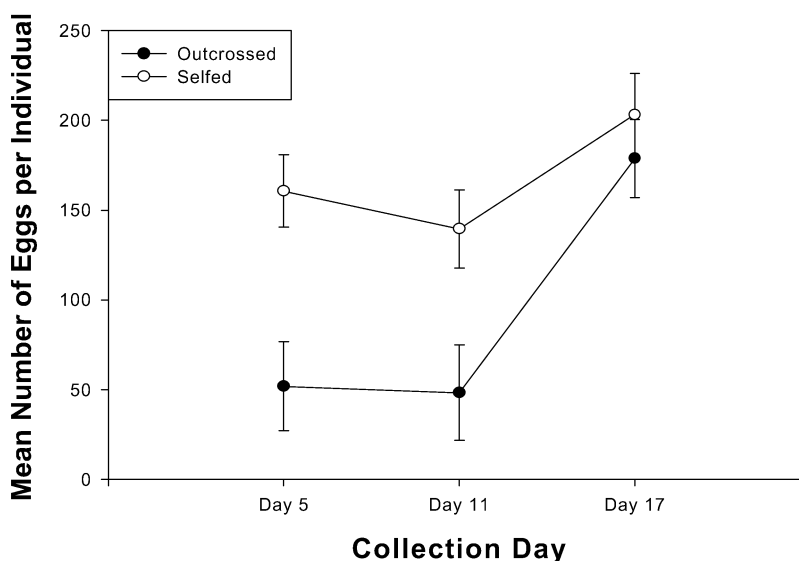
Fig. 4. Mean egg production per individual by treatment. Error bars represent one standard error.

Table 4. Net reproductive rate (R) for both selfed and outcrossed treatments

Level	R	Standard error
Outcrossed	81 654	18 814
Selfed	52 476	15 361

two naturally filling pools in Texas. Knoll (1995) found similar results in small laboratory populations. Zucker *et al.* (2001) set up a series of experiments measuring relative male viability at different densities of shrimp from two populations, and found that relative male viability $(1 - \sigma)$ ranged from 67 to 94%, with greater relative male viability at higher densities.

In the present study, we also found that hermaphrodites outlived males, with relative male viabilities of 91 and 79% for the outcrossed and selfed treatments, respectively. These estimates fall within the range reported by Zucker *et al.* (2001), and suggest that these previous estimates of relative male longevity correspond well with relative viabilities estimated from field-reared shrimp.

One observation that requires further comment is that the outcrossed pools yielded higher estimates of relative male viability than the selfed pools. Two factors may have played a role in this observed difference. First, the outcrossed treatments were at higher densities, which has produced similar survival rates between the sexes in a previous study. Zucker *et al.* (2001) found higher density populations exhibited increased male survivorship in *E. texana*, which they explained by postulating that increased mating opportunities led to increased relative male survivorship in this species. Zucker *et al.* found that males spent most of their time in the energetically costly activity of searching for mates (e.g. swimming and inter-

acting with other males), but that these behaviours were reduced when there were more mates available. They argued that if searching for mates is more energetically costly than actual mating, then increased mating opportunities may reduce the overall energetic cost of mating, thereby increasing male longevity. If this is true, then the cost of mating for males in *E. texana* is opposite to that found in other taxa: males with increased mating opportunities exhibit lower survivorship in nematodes (Van Voorhies, 1992), fruit flies (Partridge and Farquhar, 1981) and tsetse flies (Clutton-Brock and Langley, 1997). Thus, *E. texana* males may have had longer relative lifespans in the outcrossed treatments because of increased mating opportunities concomitant with the increased densities in these pools.

The second possible explanation for the observed differences in relative male viability in the two breeding treatments is that outcrossing may lead to a reduced viability difference between males and hermaphrodites. To understand this, one needs to recall the mating system of *E. texana*: selfing amphigenic hermaphrodites will produce ~25% males among their selfed offspring (Sassaman and Weeks, 1993), which explains the abundance of males in the 'selfed' treatments in the present study. Because all of the males were inbred in the selfed treatments, they may have had lower survival, on average, than the outcrossed males. The reason that the hermaphrodites did not suffer a similar fate in the selfed treatments may be explained by a large linkage group surrounding the sex-determining locus that 'protects' amphigenic hermaphrodites from some effects of inbreeding depression (Weeks *et al.*, 2001a). Weeks *et al.* (2001a) proposed that selfing amphigenics should produce amphigenic offspring with less inbreeding depression than their monogenic or male offspring due to the heterozygosity at many loci linked to the sex-determining locus. Such a scenario would cause a set of linked genetic loci to be 'permanently' heterozygous in these hermaphrodites, regardless of whether they were produced via selfing or outcrossing. This 'protected' class of hermaphrodites may cause a greater difference between the sexes in the selfed relative to the outcrossed treatments because the differences between the 'protected' amphigenic hermaphrodites and the males produced by selfing may be greater than the differences between the amphigenics and males produced by outcrossing. If this is the case, then this protection from inbreeding depression in selfed amphigenics may explain the greater proportion of amphigenics in natural populations than expected by theory (see discussion below).

Overall, the range of $(1 - \sigma)$ is consistent with previous estimates of this parameter in laboratory studies (0.67–0.94; Zucker *et al.*, 2001), suggesting that the true value of this parameter probably lies in the range of 0.7–0.9, with variation in this parameter likely to be due to effects of density and level of outcrossing.

Inbreeding depression (δ)

Level of inbreeding depression is considered one of the most important factors in the evolution of androdioecy (Charlesworth, 1984; Otto *et al.*, 1993), but has not been studied extensively in field settings (Crnokrak and Roff, 1999; Keller and Waller, 2002). Previous studies on this population (WAL) have found significant levels of inbreeding depression at various stages of the life cycle. Weeks *et al.* (1999) estimated early inbreeding depression to be between 0.4 and 0.5, whereas Weeks *et al.* (2000a) compared adult fitness of outcrossed and selfed shrimp and estimated $\delta = 0.68$. In a third study, Weeks *et al.* (2001b) found significant inbreeding depression in the embryonic stage, and estimated lifetime inbreeding depression to be between 0.6 and 0.7 in the WAL population. All of these studies were

conducted in laboratory settings, which may significantly underestimate true levels of inbreeding depression in the field (Crnokrak and Roff, 1999).

Here we extended these previous studies by examining inbreeding depression of *E. texana* in the field. A dramatic difference in overall population size was the most obvious effect of inbreeding in this experiment. By day 5, there were nearly four times as many clam shrimp in the outcrossed than in the selfed treatments. Because outcrossing versus selfing does not affect the total number of eggs produced in these shrimp (Knoll and Zucker, 1995), the total number of eggs in the outcrossed and selfed replicate pools should have been equivalent at the outset of this experiment. Thus, the lower early population size in the selfed pools was probably due to a combination of embryo mortality (Weeks *et al.*, 2001b) and reduced juvenile survivorship (Weeks *et al.*, 1999). The overall level of inbreeding depression, however, did not reflect the dramatic differences in population size because the larger populations in the outcrossed treatments caused greater competition, which resulted in reduced average adult size and, concomitantly, reduced average fecundity per hermaphrodite. Thus, overall inbreeding depression (δ) was estimated at 0.36 in this experiment.

The estimate of $\delta = 0.36$ assumes that there was 100% outcrossing in the laboratory production of the outcrossed eggs used to begin the outcrossed treatments. However, previous studies have shown that outcrossing rates in the laboratory actually range between 25 and 40% in the WAL population (Crosser, 1999; Weeks *et al.*, 2000b). Thus, the true level of inbreeding depression was likely to be underestimated by using the above comparison, and the true value should range between 0.58 and 0.69, assuming there was roughly 40 and 25% outcrossing, respectively, in the mating cups used to collect the eggs for this experiment (Table 5). These values largely agree with previous estimates of lifetime inbreeding depression in WAL, which ranged from 0.6 to 0.7 (Weeks *et al.*, 2001b).

Such similar levels of inbreeding depression in the laboratory and the field have been found in some comparisons, but are not consistent with most studies reviewed (Crnokrak and Roff, 1999), wherein higher inbreeding depression is regularly documented in the field relative to the controlled environment of the laboratory. Fluctuations in environmental factors, such as rainfall, drastic temperature fluctuations and resource limitation, are thought to cause the increased effects of inbreeding depression in wild populations. The lack of a difference between laboratory- and field-reared shrimp in the current study could have been due to the following: (1) our laboratory conditions were not benign and thus caused similar levels of 'stress' as found in natural populations; (2) the artificial pools used for this project were not as stressful as natural pools; or (3) the particular season in which inbreeding depression was measured in this field study was less stressful than usual. Another

Table 5. Definition and parameter estimates for the model of Otto *et al.* (1993)

Parameter	α	β	$(1 - \sigma)$	δ (40%)	δ (25%)
Definition	Relative male fertility	Sperm limitation in hermaphrodites	Relative viability of males to hermaphrodites	Inbreeding depression	
Estimates from WAL	1.0–7.7[a]	1[b]	0.79–0.91[c]	0.58[c]	0.69[c]

Note: For calculations of inbreeding depression, '40%' assumes 40% outcrossing and '25%' assumes 25% outcrossing (see text). [a] From Hollenbeck *et al.* (2002). [b] From Weeks *et al.* (2001b). [c] From current study.

possibility is that we underestimated inbreeding depression in the field in the current study by competing more fit individuals with one another in the outcrossed treatments, thereby stunting all the outcrossed shrimp's growth and egg production and, concomitantly, reducing the level of apparent inbreeding depression. A more realistic estimate of inbreeding depression might be to compete directly inbred and outcrossed shrimp to see which fares better in similar experiments, assuming that a marker could be developed to allow us to distinguish selfed from outcrossed shrimp (Christen *et al.*, 2002).

The observation of higher inbreeding depression in field than in laboratory (or captive) populations does not include invertebrate animals (Crnokrak and Roff, 1999). In two recent comparisons of field and laboratory estimates of inbreeding depression in invertebrates, neither showed the patterns described by Crnokrak and Roff (1999). In tree-hole breeding mosquitoes (*Aedes geniculatus*), no difference in level of inbreeding depression was noted in laboratory relative to field tests, even though overall fitness was greater in the laboratory setting, suggesting it was a more benign environment (Armbruster *et al.*, 2000). In the freshwater snail *Physa acuta*, inbreeding depression was actually lower in the field than in the laboratory environment (Henry *et al.*, 2003). Thus, our observation of no difference in estimates of inbreeding depression in laboratory and field populations of *E. texana* may indicate that invertebrates in general do not exhibit the same environmental dependence of inbreeding depression as found in captive versus field populations of mammals (Crnokrak and Roff, 1999).

The overall conclusion from the current study was that inbreeding depression was quite high (0.58–0.69), and was equivalent to previous laboratory estimates in this population. These high estimates of inbreeding depression suggest that the costs of self-fertilization are still important in this regularly selfing species, which could help explain the maintenance of males in this population.

Evaluating the model of Otto *et al.* (1993)

The model of Otto *et al.* (1993) predicts the stability and equilibrium frequencies of the three mating types found in androdioecious systems. The following inequality defines the condition for the stability of *E. texana*'s androdioecious system:

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

Using previously generated values for relative male fertility (α; Hollenbeck *et al.*, 2002) and proportion of eggs selfed (β; Weeks *et al.*, 2001b), and the current estimates of male viability ($1 - \sigma$) and inbreeding depression (δ; Table 5), we can predict the expected proportion of males, amphigenic and monogenic hermaphrodites for the WAL population (Table 6) using equations 2a–2c in Otto *et al.* (1993).

Evaluating the model of Otto *et al.* (1993) using 'high' estimates of the four parameters (i.e. using the estimates of the four parameters that allow the highest likelihood of the maintenance of males), the model predicts that androdioecy should persist in WAL, with populations consisting of 61% males, 39% amphigenics and no monogenics (Table 6). The 'low' estimates (using estimates of the four parameters that provide the lowest likelihood of the maintenance of males) suggest that androdioecy is unstable in the WAL population, and thus that WAL will become 100% monogenic over time.

Because the field estimates of $(1 - \sigma)$ and δ were so similar to the previous laboratory estimates, the predicted ranges of the three sex ratios are similar to previously reported

Table 6. Predicted and observed sex ratios for the three sexes from the model of Otto *et al.* (1993)

Sexes	Predicted		Observed[a]
	Low	High	
Males (*u*)	0.00	0.61	0.24
Amphigenics (*v*)	0.00	0.39	0.63
Monogenics (*w*)	1.00	0.00	0.13

Note: 'Low' and 'High' represent 'worst case' and 'best case' combinations of parameters (Table 5) for the maintenance of males, respectively. [a] From Weeks *et al.* (1999).

ranges (Weeks *et al.*, 2001b). The predicted ranges are broad, primarily due to the combination of the narrow range of parameters under which androdioecy is a stable strategy in this system (Otto *et al.*, 1993) (thus frequently leading to the conclusion of 'instability' in a population with only slight differences in parameter values) and our broad estimates of the relative male fertility parameter, α (Hollenbeck *et al.*, 2002), which range from 1.0 to 7.7. Otto *et al.* (1993) do note that their model is sensitive to α, and the upper extreme estimate of 7.7 for α results in the unrealistic prediction of 61% males and 39% amphigenic hermaphrodites. This high estimate of α was found when males were quite rare in the experimental treatments (Hollenbeck *et al.*, 2002), which is understandable if male fertility is frequency dependent. Incorporating such frequency dependence of male fertility would make sense for this model (see also discussion in Otto *et al.*, 1993), and we are currently estimating this frequency dependence in the laboratory. We are also conducting field projects to estimate α from field-collected shrimp.

From Table 6, it is obvious that the high proportion of amphigenic hermaphrodites continues to be unaccounted for using the model of Otto *et al.* (1993). In the four populations to date surveyed for the sex ratios of all three mating types, all populations have had > 60% amphigenics (Weeks *et al.*, 1999), and yet the highest predicted percentage of amphigenics using the model of Otto *et al.* (1993) has been ~40%. Some of this disparity may be attributed to the 'protected' nature of amphigenics from inbreeding depression (Weeks *et al.*, 2001a), which was not incorporated into the original model. The results of the current study have done nothing to ameliorate this problem, and the over-abundance of amphigenics remains one of the greatest challenges of the relevance of the model of Otto *et al.* (1993) to the *E. texana* system.

In conclusion, this first estimate of two of the four parameters of the model of Otto *et al.* (1993) from field-reared shrimp has indicated that previous laboratory estimates of inbreeding depression and relative male survivorship are realistic. Further refinement of this model, as well as better estimates of relative male fertility (both from field and laboratory studies), will hopefully result in more precise tests of this model to determine whether androdioecy can realistically be maintained by within-population factors. A continuing lack of fit of the predicted and observed sex ratios, such as the underestimation of amphigenic proportions, may mean that we need to explore other models of the evolution of androdioecy in this system (e.g. metapopulation models; Pannell, 1997b, 2000, 2002).

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REFERENCES

Armbruster, P., Hutchinson, R.A. and Livell, T. 2000. Equivalent inbreeding depression under laboratory and field conditions in a tree-hole-breeding mosquito. *Proc. R. Soc. Lond. B*, **267**: 1939–1945.

Barker, D.M. 1992. Evolution of sperm shortage in a selfing hermaphrodite. *Evolution*, **46**: 1951–1955.

Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. *Biol. J. Linn. Soc.*, **22**: 333–348.

Christen, M., Kurtz, J. and Milinski, M. 2002. Outcrossing increases infection success and competitive ability: experimental evidence from a hermaphrodite parasite. *Evolution*, **56**: 2243–2251.

Clutton-Brock, T. and Langley, P. 1997. Persistent courtship reduces male and female longevity in captive tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Behav. Ecol.*, **8**: 392–395.

Crnokrak, P. and Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity*, **83**: 260–270.

Crosser, B.R. 1999. Estimates of inbreeding depression in two populations of the ephemeral pond crustacean, *Eulimnadia texana*. Master's thesis, The University of Akron.

Fritsch, P. and Rieseberg, L.H. 1992. High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata*. *Nature*, **359**: 633–636.

Henry, P.Y., Pradel, R. and Jarne, P. 2003. Environment-dependent inbreeding depression in a hermaphroditic freshwater snail. *J. Evol. Biol.*, **16**: 1211–1222.

Hodgkin, J. and Barnes, T.M. 1991. More is not better: brood size and population growth in a self-fertilizing nematode. *Proc. R. Soc. Lond. B*, **246**: 19–24.

Hollenbeck, V.G., Weeks, S.C., Gould, W.R. and Zucker, N. 2002. Maintenance of androdioecy in the freshwater shrimp *Eulimnadia texana*: sexual encounter rates and outcrossing success. *Behav. Ecol.*, **13**: 561–570.

Jarne, P. and Charlesworth, D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Syst.*, **24**: 441–466.

Keller, L.F. and Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.*, **17**: 230–241.

Knoll, L. 1995. Mating behavior and time budget of an androdioecious crustacean, *Eulimnadia texana* (Crustacea, Conchostraca). *Hydrobiologia*, **298**: 73–81.

Knoll, L. and Zucker, N. 1995. Selfing versus outcrossing in the androdioecious clam shrimp, *Eulimnadia texana* (Crustacea, Conchostraca). *Hydrobiologia*, **298**: 83–86.

Lepart, J. and Dommee, B. 1992. Is *Phillyrea angustifolia* L (Oleaceae) an androdioecious species? *Bot. J. Linn. Soc.*, **108**: 375–387.

Liston, A., Rieseberg, L.H. and Elias, T.S. 1990. Functional androdioecy in the flowering plant *Datisca glomerata*. *Nature*, **343**: 641–642.

Lloyd, D.G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica*, **45**: 325–339.

Medland, V.L., Zucker, N. and Weeks, S.C. 2000. Implications for the maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana* Packard: encounters between males and hermaphrodites are not random. *Ethology*, **106**: 839–848.

Otto, S.P., Sassaman, C. and Feldman, M.W. 1993. Evolution of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am. Nat.*, **141**: 329–337.

Pannell, J. 1997a. Widespread functional androdioecy in *Mercurialis annua* L (Euphorbiaceae). *Biol. J. Linn. Soc.*, **61**: 95–116.

Pannell, J. 1997b. The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution*, **51**: 10–20.

Pannell, J. 1997c. Variation in sex ratios and sex allocation in androdioecious *Mercurialis annua*. *J. Ecol.*, **85**: 57–69.

Pannell, J. 1997d. Mixed genetic and environmental sex determination in an androdioecious population of *Mercurialis annua*. *Heredity*, **78**: 50–56.

Pannell, J.R. 2000. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. *Evol. Ecol.*, **14**: 195–211.

Pannell, J.R. 2002. The evolution and maintenance of androdioecy. *Annu. Rev. Ecol. Syst.*, **33**: 397–425.

Pannell, J.R. and Ojeda, F. 2000. Patterns of flowering and sex-ratio variation in the Mediterranean shrub *Phillyrea angustifolia* (Oleaceae): implications for the maintenance of males with hermaphrodites. *Ecol. Lett.*, **3**: 495–502.

Partridge, L. and Farquhar, M. 1981. Sexual activity reduces lifespan of male fruitflies. *Nature*, **294**: 580–582.

Rieseberg, L.H., Hanson, M.A. and Philbrick, C.T. 1992. Androdioecy is derived from dioecy in Datiscaceae: evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. *Syst. Bot.*, **17**: 324–336.

Rieseberg, L.H., Philbrick, C.T., Pack, P.E., Hanson, M.A. and Fritsch, P. 1993. Inbreeding depression in androdioecious populations of *Datisca glomerata* (Datiscaceae). *Am. J. Bot.*, **80**: 757–762.

SAS Institute, Inc. 2003. *JMP Statistical Software*. Cary, NC: SAS.

Sassaman, C. 1989. Inbreeding and sex-ratio variation in female-biased populations of a clam shrimp, *Eulimnadia texana*. *Bull. Mar. Sci.*, **45**: 425–432.

Sassaman, C. and Weeks, S.C. 1993. The genetic mechanism of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am. Nat.*, **141**: 314–328.

Strenth, N.E. 1977. Successional variation of sex ratios in *Eulimnadia texana* Packard (Crustacea, Conchostraca). *Southwest. Nat.*, **22**: 205–212.

Van Voorhies, W.A. 1992. Production of sperm reduces nematode lifespan. *Nature*, **360**: 456–458.

Vassiliadis, C., Lepart, J., Saumitou-Laprade, P. and Vernet, P. 2000. Self-incompatibility and male fertilization success in *Phillyrea angustifolia* (Oleaceae). *Int. J. Plant Sci.*, **161**: 393–402.

Ward, S. and Carrel, J.S. 1979. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev. Biol.*, **73**: 304–321.

Weeks, S.C., Marcus, V. and Alvarez, S. 1997. Notes on the life history of the clam shrimp, *Eulimnadia texana*. *Hydrobiologia*, **359**: 191–197.

Weeks, S.C., Marcus, V. and Crosser, B.R. 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. *Evolution*, **53**: 472–483.

Weeks, S.C., Crosser, B.R., Bennett, R., Gray, M. and Zucker, N. 2000a. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: estimates of inbreeding depression in two populations. *Evolution*, **54**: 878–887.

Weeks, S.C., Crosser, B.R., Gray, M.M., Matweyou, J.A. and Zucker, N. 2000b. Is there sperm storage in the clam shrimp *Eulimnadia texana*? *Invert. Biol.*, **119**: 215–221.

Weeks, S.C., Crosser, B.R. and Gray, M.M. 2001a. Relative fitness of two hermaphroditic mating types in the androdioecious clam shrimp, *Eulimnadia texana*. *J. Evol. Biol.*, **14**: 83–94.

Weeks, S.C., Hutchison, J.A. and Zucker, N. 2001b. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: do hermaphrodites need males for complete fertilization? *Evol. Ecol.*, **15**: 205–221.

Zucker, N., Stafki, B. and Weeks, S.C. 2001. Maintenance of androdioecy in the freshwater clam shrimp *Eulimnadia texana*: longevity of males relative to hermaphrodites. *Can. J. Zool.*, **79**: 393–401.

Zucker, N., Aguilar, G.A., Weeks, S.C. and McCandless, L.G. 2002. Impact of males on variation in the reproductive cycle in an androdioecious desert shrimp. *Invert. Biol.*, **121**: 66–72.