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Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: do hermaphrodites need males for complete fertilization?

STEPHEN C. WEEKS[1,*], JULIE A. HUTCHISON[1] and NAIDA ZUCKER[2]
[1]*Program in Evolution, Ecology, and Organismal Biology, Department of Biology, The University of Akron, Akron, OH 44325-3908, USA;* [2]*Department of Biology, New Mexico State University, Las Cruces, NM 88003-8001, USA*
*(*author for correspondence, tel.: +1-330-972-7156; fax: +1-330-972-8445; e-mails: scw@uakron.edu, scweeks@uakron.edu)*

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Abstract. Androdioecy (populations comprised of mixtures of males and hermaphrodites) is a rare mating system, found only in a few plants and animals. The rarity of this system stems from the limited benefits to males in an otherwise all-hermaphroditic population. One of the potential benefits to males is typified by the nematode *Caenorhabditis elegans*, in which hermaphrodites do not produce sufficient sperm to fertilize all of their eggs. Here we explore the possibility that males are needed for complete fertilization of hermaphrodites' eggs in a second androdioecious animal, the clam shrimp *Eulimnadia texana*. We compare the fertilization rate of outcrossed to selfed eggs to test whether the latter exhibit lower fertilization due to sperm limitation (as in *C. elegans*). Because this comparison confounds differences in egg fertilization due to sperm limitation with the potential for early inbreeding depression, we also used a third mating treatment, a brother/sister cross, to allow separation of sperm limitation from inbreeding depression. In both populations examined, the proportion of eggs that were fertilized decreased linearly with increasing relatedness: comparing eggs produced by outcrossing, brother/sister, and selfed matings, respectively. This pattern suggests that differences in fertilization among these three treatments were caused solely by inbreeding depression, and therefore that hermaphrodites are not sperm limited. These results are combined with previous data on this species to test whether the maintenance of males can be explained using a population genetics model specifically designed for this species.

Key words: conchostracan branchiopod crustaceans, inbreeding depression, mixed mating system, sperm limitation

Introduction

Androdioecy (populations comprised of males and hermaphrodites only) is rare in plants and animals (Charlesworth, 1984). Models predict that androdioecy should be rare because the benefits of being all-male in an otherwise hermaphroditic population are limited (Lloyd, 1975; Charlesworth, 1984). Males can benefit in two main ways in androdioecious populations: (1) by increased

allocation of reproductive resources to male function (relative to male allocation in hermaphrodites), and (2) by reduced inbreeding depression for male-sired offspring, because none of these offspring are produced by selfing. Since fitness through male function depends on the availability of mates, self-fertilization reduces mating opportunities in androdioecious populations, making it difficult for the all-male strategy to be successful (Lloyd, 1975; Charlesworth, 1984). Therefore, if being male is beneficial largely because of reduced inbreeding depression, but males have greatly reduced mating opportunities in primarily selfing populations, androdioecy should be quite rare (Charlesworth, 1984). It is thought that androdioecy should be a transitory stage, either in the evolution of hermaphroditism from dioecy (Charlesworth, 1984) or in the evolution of dioecy from hermaphroditism, especially in wind-pollinated or wind-dispersed, colonizing plants (Liston *et al*., 1990; Pannell, 1997b).

Androdioecy has been described in only five animals (Newman *et al*., 1969; McLaughlin and Henry, 1972; Wood, 1988; Turner *et al*., 1992; Sassaman, 1991; Sassaman and Weeks, 1993; Zucker *et al*., 1997) and five plants (Liston *et al*., 1990; Lepart and Dommee, 1992; Molau and Prentice, 1992; Aronne and Wilcock, 1994; Pannell, 1997a). One of these animals is the branchiopod crustacean *Eulimnadia texana* (Sassaman and Weeks, 1993; Zucker *et al*., 1997). In this species, males coexist with self-compatible hermaphrodites. Hermaphrodites are of two phenotypically similar but genotypically different types: monogenics and amphigenics. Sex appears to be controlled by a single genetic locus (Sassaman and Weeks, 1993), with a recessive allele for males (s) and a dominant allele for hermaphrodites (S). Hermaphrodites are either homozygous dominant (SS = monogenics) or heterozygous (Ss = amphigenics), while males are homozygous recessive (ss; Sassaman and Weeks, 1993). This mating system is intriguing because the androdioecious polymorphism is maintained in many populations, although self-fertilization is common in this species (inbreeding coefficients ranging from 0.20 to 0.97; Sassaman, 1989; Weeks and Zucker, 1999).

One of the crucial factors influencing the evolution and maintenance of this mating system is that (similar to the nematode *Caenorhabditis elegans*, Hodgkin, 1983) hermaphrodites cannot mate with one another because they lack the clasping appendages necessary for pairing. Thus, all outcrossing must involve males, which gives males an added advantage compared with that in other androdioecious systems, if selfing causes inbreeding depression. Therefore, the previous models for androdioecy (Lloyd, 1975; Charlesworth, 1984) are not appropriate for *E. texana*.

Otto *et al*. (1993) specifically modeled *E. texana*'s mating system to predict the equilibrium frequencies of the three mating types: males (u), amphigenics (v), and monogenics (w). They included four parameters in their population genetics model: δ, the inbreeding depression experienced by selfed offspring

(relative to a random cross), α, the relative male fertility (the number of male-sired offspring relative to offspring produced by an average hermaphrodite), $(1-\sigma)$, the relative male survival (relative to hermaphrodites), and β, the proportion of an hermaphrodite's eggs that remain unfertilized by males that the hermaphrodite fertilizes by itself. This last parameter would be less than 1 if hermaphrodites either do not produce enough sperm to fully fertilize all of its own eggs, or if certain eggs were 'earmarked' to be fertilized by males only.

Otto *et al.* (1993) found two sets of mating system equilibria using the above four parameters: (a) a stable polymorphism of the three mating types (u, v, and w) and (b) fixation of monogenics ($w = 1$). The following inequality defines the condition for the stable polymorphism:

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

To test this model, all four parameters must be estimated from natural populations, and then the mating frequencies observed in these populations can be compared to the predictions of the Otto *et al.* (1993) model.

Three of the four parameters have been estimated elsewhere: δ (Weeks *et al.*, 1999, 2000a), α (Medland *et al.*, 2000; Weeks *et al.*, 2000b; Hollenbeck *et al.*, in press), and $1-\sigma$ (Zucker *et al.*, 2001). We report estimates of the fourth parameter, β, in this paper. To accomplish this, we compared the numbers of fertilized eggs when a hermaphrodite selfs relative to the number when it mates with a male. However, a straight comparison of selfed to outcrossed eggs confounds potential inbreeding depression with sperm limitation (or 'earmarking' certain eggs for outcrossing; hereafter we refer to either possibility as simply 'sperm limitation'): reduced proportions of fertilized eggs in selfed vs. outcrossed matings can be due to either sperm limitation or inbreeding depression. Thus, to test for early inbreeding depression, we used a third mating: a brother/sister mating (Fig. 1). In this mating, sperm limitation was not a factor because the brother should supply sperm to its sister in the same quantities as in an unrelated mating. Additionally, inbreeding depression (if present) in the brother/sister mating would be approximately half the magnitude of a selfed mating (assuming that early survival is linearly related to the inbreeding coefficient of the progeny). Therefore, a comparison among the three mating treatments allowed us to distinguish the separate effects of sperm limitation and inbreeding depression.

We can distinguish sperm limitation from inbreeding depression by comparing the results of these matings to three potential outcomes: (a) proportion of eggs fertilized (scored as 'viable' eggs) declines linearly from outcrossed to brother/sister to selfed matings (this outcome suggests inbreeding depression only; Fig. 1A); (b) a male (related or not) provides sufficient sperm to fertilize all the eggs of an hermaphrodite, but when males are absent, many eggs are unfertilized (i.e., sperm limitation only; Fig. 1B); or (c) comparing outcrossed matings with males to matings with siblings shows a decline in viable eggs for the latter (due only to inbreeding depression), whereas a comparison between

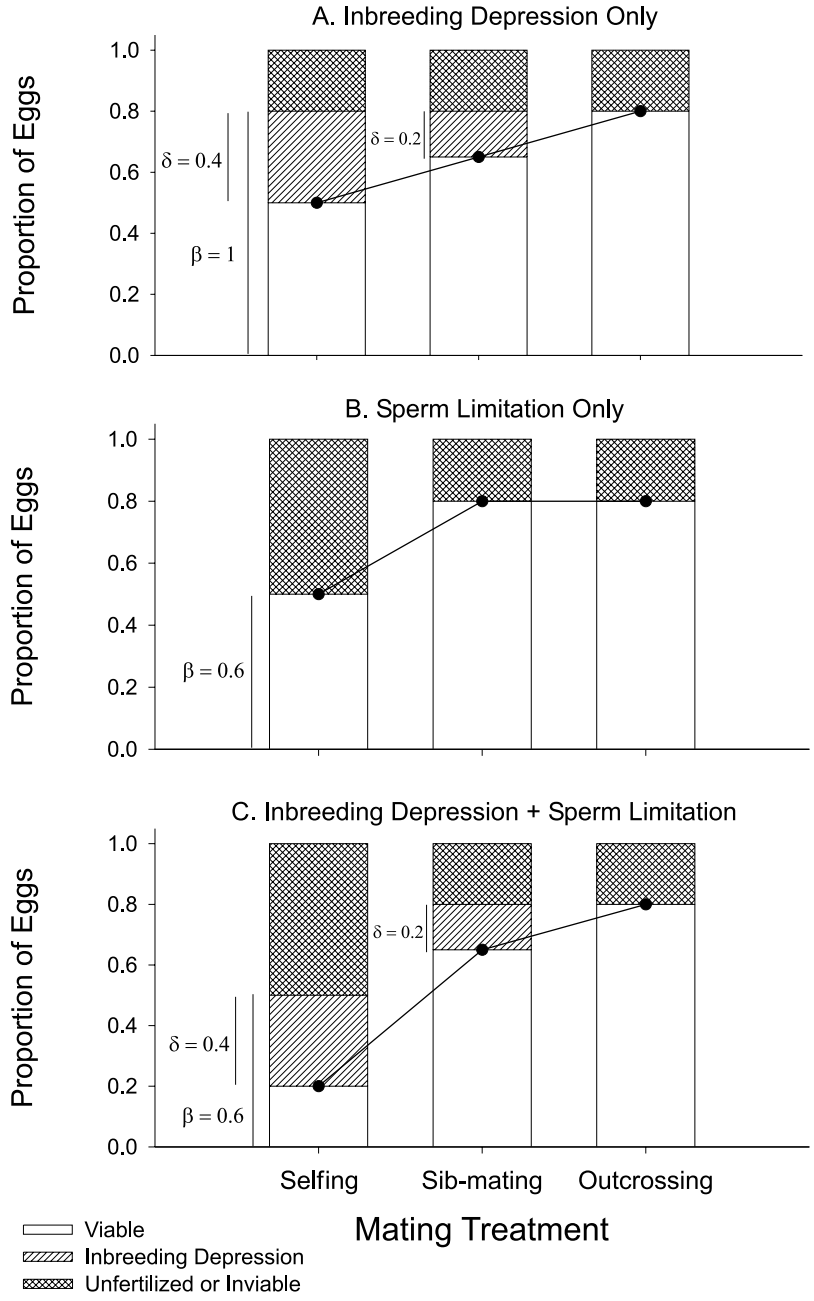

Figure 1. Hypothetical results of current experiment. (A) Outcome assuming inbreeding depression (e.g., $\delta = 0.4$) and no sperm limitation (i.e., $\beta = 1$). (B) Outcome assuming no inbreeding depression, and sperm limitation (e.g., $\beta = 0.6$). (C) Outcome assuming both inbreeding depression (e.g., $\delta = 0.4$) and sperm limitation (e.g., $\beta = 0.6$). All three scenarios assume ~20% of eggs are either unfertilized or experience early embryo mortality, no matter the mating treatment. For all graphs, filled circles and solid lines show the expected outcomes of the proportion of eggs fertilized under all three scenarios.

selfed and brother/sister matings shows a greater decline for the selfed eggs due to a combination of inbreeding depression and sperm limitation (i.e., inbreeding depression + sperm limitation; Fig. 1C). Note that it is assumed that some proportion of eggs will be inviable in all mating treatments, due to early embryo mortality and incomplete fertilization. Thus, using these three breeding treatments allows us to quantify both sperm limitation (β) and early inbreeding depression (δ) in this species.

The experiment was conducted on two populations of *E. texana* to estimate β, and these estimates were then combined with estimates of the other three parameters to test whether the Otto *et al.* (1993) model fits natural sex ratios.

Materials and methods

Natural history of E. texana

Eulimnadia texana is in the family Limnadiidae, order Spinicaudata (Fryer, 1987; Spears and Abele 2000). This crustacean is a small shrimp with a carapace length of 5–8 mm when mature (Weeks *et al.*, 1997) found in ephemeral pools of the deserts in the southwestern United States. *Eulimnadia texana* exhibits sexual dimorphism when mature: the first pair of male phyllopods are modified into claw-like claspers, allowing the male to grasp firmly the carapace of the hermaphrodite for mating (Sassaman and Weeks, 1993). Because hermaphrodites do not possess these claspers, they cannot cross-fertilize with one another. Hermaphrodites have 1–2 clutches of eggs per day, and are reproductively competent for 1–2 weeks (Weeks *et al.*, 1997). The fertilized eggs are carried underneath the carapace in a brood chamber on the back of the hermaphrodite for approximately 10–20 h (Weeks *et al.*, 1997). Hermaphrodites then shed the desiccation-resistant eggs at the bottom of the pool, and the eggs do not hatch until they undergo a period of drying. Once the eggs have been hydrated, nauplii hatch and the shrimp then grow to sexual maturity in as little as 4–7 days (Weeks *et al.*, 1997). The entire life cycle of *E. texana* is completed in 2–3 weeks.

Rearing protocol

Two populations of *E. texana* were used in this experiment. WAL originates from a shallow cattle tank (21 m long × 15 m wide × 1 m deep when filled) located 4 km north of Portal Road (Road 533) near Portal, Arizona, and has the greater genetic diversity of the two populations (Weeks and Zucker, 1999). The second population, JT4, originates from an oval depression (approximately 10 m long × 7 m wide × 0.5 m deep when filled) located near South Well on Jornada Road, near the Jornada Desert NSF-LTER site, 40 km north-

northeast of New Mexico State University in Las Cruces, Doña Ana County, New Mexico. Dry soil (containing eggs) from each population was collected in the field and transported back to the University of Akron.

For each population, 500 ml of field-collected soil was placed in the bottom of separate 38 l aquaria and hydrated with filtered tap water. Aquaria were maintained under 'standard conditions': 27 °C in a climate-controlled environment with constant aeration, 24 h lighting (Duratest Full Spectrum Lighting), and were fed 20 ml of a yeast suspension made by mixing 1 g of baker's yeast to 100 ml of filtered tap water (Weeks *et al.*, 1997, 1999). Under these conditions, eggs hatched within 24 h and nauplii reached sexual maturity within 5 days.

Directly before sexual maturity, hermaphrodites were isolated in 500 ml plastic cups containing approximately 5 ml of finely sifted (260 μm mesh) New Mexico soil (known to be free of branchiopod eggs) and filled with water from the above hatching tanks (strained using a 63 μm mesh screen). Isolated hermaphrodites were allowed to lay eggs for 4–7 days and were then frozen for later electrophoretic typing. Eggs in the cups were dried, sealed with lids and then placed in the dark where they remained for a minimum of 30 days.

Sassaman and Weeks (1993) and Weeks *et al.* (1999) have shown that three polymorphic electrophoretic loci are tightly linked to the sex-determining locus. These loci can therefore be used to determine hermaphroditic type (amphigenics are either homozygous or heterozygous at Fum, Idh-1 or Idh-2, while monogenics are always homozygous for all three loci, Weeks *et al.*, 1999). Only amphigenics are capable of producing males (necessary for outcrossing), and were therefore the only hermaphroditic type used to produce the individuals in this experiment. Electrophoretic typing was performed by running cellulose acetate gels on individual hermaphrodites to determine heterozygotes for one or more of the diagnostic loci.

Egg banks from these amphigenic hermaphrodites were hydrated with approximately 500 ml of filtered tap water and the hatching nauplii were transferred to 38 l 'rearing aquaria' containing 500 ml of New Mexico soil and filtered tap water. Aquaria were maintained under standard conditions (see above). Nauplii were fed the yeast suspension with amounts determined by the total number of nauplii in each aquarium: 20 ml for less than 20 nauplii, 30 ml for 20–50, and 40 ml for over 50. Just prior to reaching sexual maturity ($\sim$4 days), shrimp were individually isolated in 500 ml plastic cups, again using strained water from the rearing aquaria as outlined in the previous procedure.

Matings

Isolated individual hermaphrodites from each of the two populations were setup in three different mating treatments: (a) self, (b) brother/sister (mated

with sibling males), and (c) outcross (mated with males reared from another hatching cup; hermaphrodites raised from the original soil [see Rearing protocol above] were assumed to be unrelated). For each treatment, only those pairs in which successful copulation was observed (the male firmly clasping the hermaphrodite for 1 min or longer) were used. From each population nine separate 'families' (egg banks from nine separate amphigenic hermaphrodites) were used for each type of mating, with three to six replicates per family/mating combination (a total of 180 matings). Hermaphrodites were allowed to produce one clutch of eggs, which were then collected into individual glass vials (one clutch per vial), covered with water, labeled, and stored in the dark for later analysis.

Two sets of eggs were processed in both populations. In Set 1 [two families and 36 total clutches from population WAL; five families and 93 total clutches from JT4], eggs from each clutch were split into two equal groups: group 1 were left to develop for 7 days, and group 2 were left to develop for 30 days. After the developmental period, each group was then checked for embryos (see Analysis of eggs below) to test for effects of egg dormancy on our estimates of fertilization rates (see Duration of egg dormancy below). In Set 2, eggs were left to develop from 1 month [four WAL families; three JT4 families] to 1 year [three WAL families; one JT4 family], and were not split into age-based groups (proportion of eggs with embryos did not change for eggs dormant for $>$30 days, Hutchison, 1999).

Analysis of eggs

The proportion of eggs fertilized in each mating treatment was determined according to the presence or absence of a developing embryo within the egg shell. Eggs from each mating were removed from individual glass vials using a glass pipette and placed onto a gridded depression slide. The total number of eggs in each clutch was then counted using a compound microscope ($40\times$ magnification). After the eggs had been counted, bleach was used to dissolve the tertiary egg shell (Belk, 1987). The embryos remained after this treatment, and appeared as almost black masses when viewed using the light microscope (Belk, 1987). The eggs were then scored, and proportion fertilized was computed as the number of embryos divided by the total number of eggs scored for each clutch.

Statistical methods

Duration of egg dormancy

The effect of length of egg dormancy was tested across mating types (Set 1 eggs) using a blocked, two-way ANOVA (analyzed with JMP, version 3.2.1 SAS Institute Inc., 1997). Dormancy time (7 vs. 30 days) and mating type (self,

brother/sister, and outcross) were the two main effects, and family was a blocked effect. Each population was analyzed separately using arcsin, square-root transformed proportions. A Bartlett's test on the transformed data showed homogeneous variances and ANOVA residuals were found to be normally distributed.

Estimation of β

To test the effect of mating type, a nested two-way ANOVA was used, with mating type and population (WAL vs. JT4) as the two main effects, and family within population as the nested effect. The eggs analyzed for this test were the 30-day eggs from Set 1 and all of the eggs from Set 2 (see Matings above). Because differences among populations were tested using nine genetically distinct families in each population, the main effect of population was tested against the mean square error for the nested factor, which was considered a random effect. The interaction of the nested term with the mating treatment main effect was not significant ($p > 0.85$), and thus this interaction was not included in the final model. Two orthogonal contrasts were made within the population-by-mating type interaction term to test for a significant deviation from a linear decline in fitness with increasing inbreeding level. Each contrast (one for each population) tested whether the difference between outcrossing and brother/sister matings were equivalent to the difference between brother/sister and selfed matings. A significant contrast would suggest a non-linear decline in fitness with increasing inbreeding level. Proportions were also arcsin, square-root transformed, and a Bartlett's test on the transformed data found variances to be homogeneous. ANOVA residuals were found to be normally distributed.

Results

Duration of egg dormancy

A total of 22,908 eggs [7709 from WAL and 15,199 from JT4] were examined for this portion of the experiment. In both populations, 100% fertilization never occurred. In both populations the proportion of eggs fertilized was higher in the 7 day relative to the 30 day treatments ($F_{1,71} = 5.03$, $p = 0.028$ for JT4 and $F_{1,26} = 4.12$, $p = 0.052$ for WAL, Fig. 2). Maximum average fertilization rate was 60–70% at 7 days and only 40–50% at 30 days (Fig. 2). This lower estimate of fertilization rate in the 30 day mating treatments suggests that either (a) the unfertilized egg yolks were not fully degraded by day 7, or that (b) early embryonic mortality (possibly caused by early inbreeding depression) caused the difference in the two age treatments. These results, coupled with previous results of eggs left to develop for either 30 day vs. 1 year

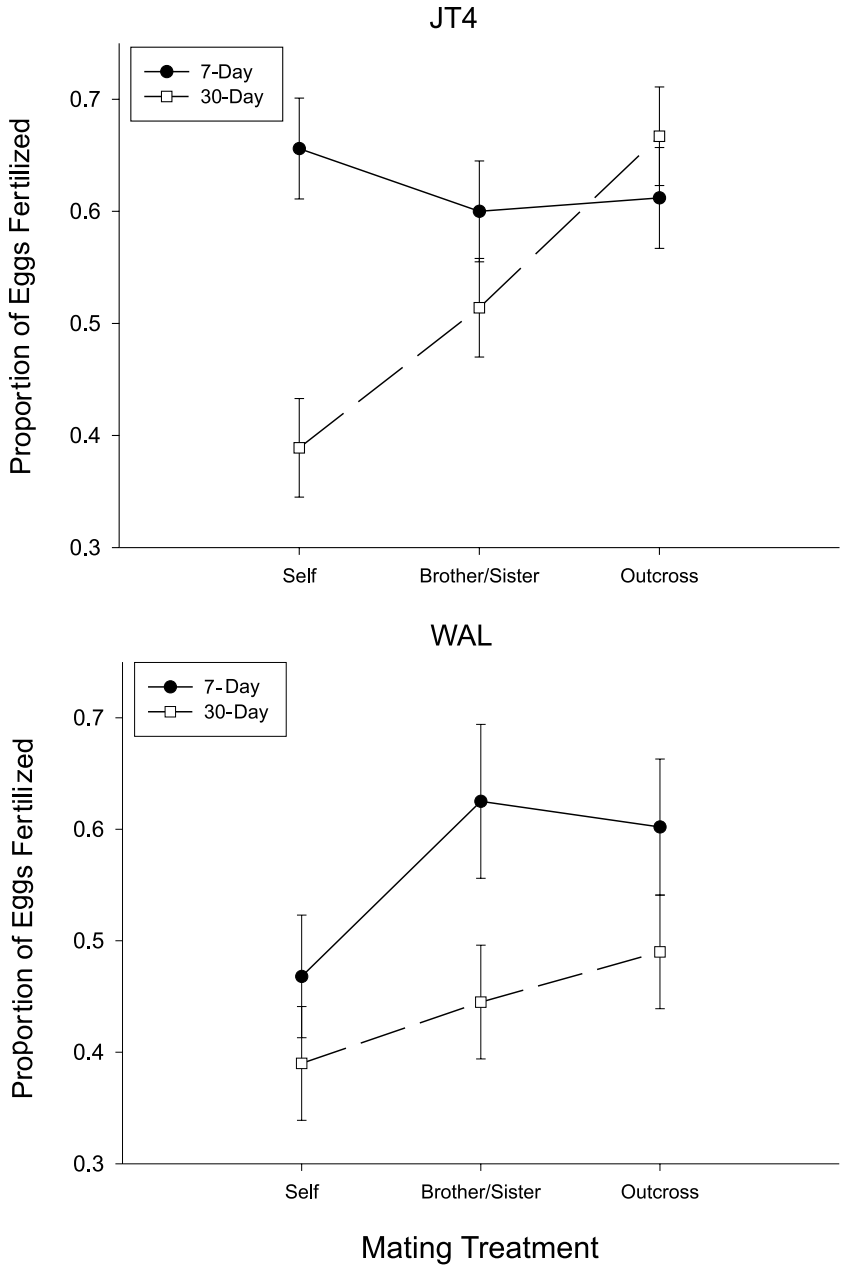

Figure 2. Egg fertilization in the three mating treatments from eggs stored for 7 days (●) or 30 days (□) in both populations. Error bars portray one standard error of the mean.

(Hutchison, 1999), suggested that unfertilized egg yolks degrade within 30 days. Alternatively, eggs may suffer a reduction in viability (possibly due to

early inbreeding depression) during the first 30 days of development and that after this initial period estimates of the proportion of eggs fertilized stabilize. We cannot distinguish between these two alternatives, and thus only ≥30 day old eggs (which included clutches stored for 30 days and 1 year; see Matings) were used in our analyses.

There were no significant differences in proportion fertilized among the three crossing treatments when eggs were left to develop for only 7 days, whereas significant differences in proportion fertilized began to appear when the eggs (from the same clutches as the 7 day eggs) were allowed to develop for 30 days (Fig. 2), although this difference was significant only in population JT4 ($F_{2,71} = 4.63$, $p = 0.013$).

Estimation of β

A total of 31,782 eggs (16,397 from WAL and 15,385 from JT4) were examined. When analyzing the nested, two-way ANOVA, a single outlier egg clutch in WAL was detected in the 0.5 percentile. ANOVAs run both with and without the outlier had virtually identical patterns and *p*-values. However, the residuals were normally distributed (Shapiro–Wilk *W* test) only when this outlier was excluded. Thus, the results reported below are from the analysis with the outlier removed.

In both populations, the proportion of eggs fertilized significantly declined from the outcrossed to the brother/sister to the selfed treatments (Table 1; Fig. 3). Maximal fertilization was ~85 and ~70% for JT4 and WAL, respectively, in the outcrossing treatments (Fig. 3). In both populations, the reduction in the proportion of eggs fertilized was essentially the same when comparing outcrossing to brother/sister matings, or sib-matings to selfing (Table 1, Contrasts 1 and 2). Therefore, the proportion of eggs fertilized in the selfed matings was not greater than predicted by assuming inbreeding de-

Table 1. ANOVA table for proportion of eggs fertilized (arcsin, square-root transformed); hermaphroditic families (Herm) were nested within population and thus the population main effect was tested using Herm[Pop] as an error term

Source	SS	DF	*F*	*p*-Value
Population	0.001	1	0.031	0.863
Mating treatment	1.403	2	46.527	< 0.0001
Pop × Mating	0.419	2	13.896	< 0.0001
(a) Contrast 1: JT4-nonlinear	0.006	1	0.384	0.536
(b) Contrast 2: WAL-nonlinear	0.000	1	0.005	0.945
Herm[Pop]	2.714	16	11.249	< 0.0001
Error	2.367	157		

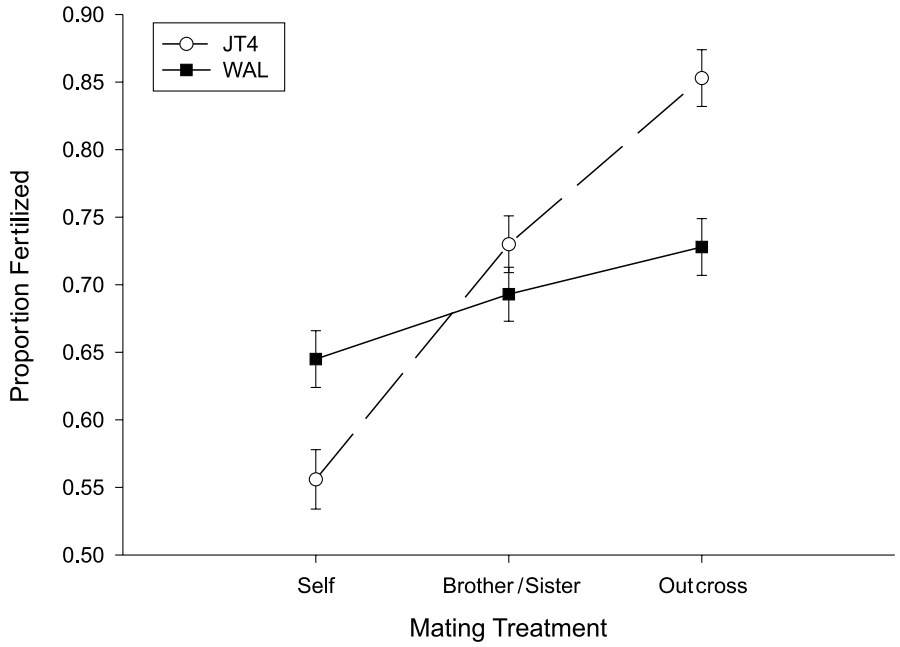

Figure 3. Egg fertilization in the three mating treatment from eggs stored for >30 days for both populations. Error bars portray one standard error of the mean.

pression only, indicating that hermaphrodites were not sperm limited nor did they 'earmark' a proportion of eggs to be fertilized by a male.

Although the overall pattern among the three mating types was essentially identical in the two populations, with the brother/sister eggs being almost exactly half-way between the selfed and the outcrossed eggs (Fig. 3), the magnitude of the decline differed between the two populations (Table 1), with JT4 having more eggs fertilized when outcrossed but less when selfed than WAL (Fig. 3).

Discussion

An important factor contributing to the maintenance of males in androdioecious systems is the ability of hermaphrodites to successfully fertilize all of their eggs with self-sperm (Otto *et al.*, 1993). If hermaphrodites either (a) produce two classes of eggs, those to be selfed and those to be outcrossed, or (b) cannot produce enough sperm to fertilize their own complement of eggs, then some fraction of eggs will remain unfertilized unless hermaphrodites mate with males. If either of these alternatives is true, then male frequency will be directly increased by the magnitude of the fraction of eggs that remains unfertilized (Otto *et al.*, 1993).

A similar androdioecious animal system in which alternative (b) holds true is the nematode *C. elegans* (Wood, 1988). Males coexist with hermaphrodites, but in rather low proportions (~0.5% of populations; Ward and Carrel, 1979). In this system, males are spontaneously produced via the non-disjunction of the sex chromosomes, resulting in XO males, which are approximately 0.5% of offspring produced (Hodgkin, 1983). Although these proportions suggest that males are strongly selected against, males appear to be effective at producing offspring when they do mate with the hermaphrodites (Ward and Carrel, 1979; Kimble and Ward, 1988). If a hermaphrodite does not mate with a male, there are only enough sperm to fertilize ~80% of its complement of eggs (Ward and Carrel, 1979; Hodgkin and Barnes, 1991). Also, when males fertilize hermaphrodites, the larger male sperm are more competitive than the self-sperm, and thus males are more successful at siring offspring (Ward and Carrel, 1979; LaMunyon and Ward, 1995, 1998). Overall, a hermaphrodite can produce up to twofold more offspring when mated to a male than when selfed (Kimble and Ward, 1988), which suggests that other factors are heavily biased against males, possibly such as mating opportunities or relative lifespan.

The observation of sperm limitation in the only other well-studied androdioecious animal species provided the motivation for the current study. In the Otto *et al.* (1993) model, the potential for either sperm limitation or 'earmarked' eggs in the hermaphrodites is modeled as the proportion β. If $\beta = 1$, a hermaphrodite can fully fertilize all eggs that are not fertilized by a male. If $\beta < 1$, a proportion $(1 - \beta)$ of the hermaphrodite's eggs will remain unfertilized if she does not mate with a male.

The current data suggest that hermaphrodites produce a sufficient quantity of sperm to fertilize all of their eggs, and do not produce a class of eggs that are required to be fertilized by males to be viable. Therefore β is estimated as unity, which contrasts with $\beta = 0.8$ in *C. elegans* (Ward and Carrel, 1979; Hodgkin and Barnes, 1991). Hermaphroditic *C. elegans* sperm are produced prior to egg production, and they apparently 'underestimate' the quantity of sperm required for complete fertilization (Ward and Carrel, 1979). This is not the case with *E. texana*, in which sperm and egg production occur simultaneously (Zucker *et al.*, 1997).

Our estimation of β assumes that inbreeding depression causes a linear decrease in fitness when comparing outcrossed to brother/sister to selfed offspring (i.e., inbreeding coefficients of 0:0.25:0.5). A linear decrease in fitness with increased inbreeding coefficient has been documented in many studies (Wright, 1977; Falconer, 1981; Willis, 1993), and thus our assumption is reasonable. However, synergistic epistasis among fitness-related loci can cause a greater fitness decline for higher inbreeding coefficients than that observed for lower rates of inbreeding (Charlesworth *et al.*, 1991; Willis, 1993). Such a synergistic relationship could have confounded our results had we found a greater decline

in the proportion of eggs fertilized between selfed and brother/sister matings, relative to outcrossed vs. brother/sister matings. In other words, we could have mistaken such relatively greater decline in proportion fertilized as indicative of sperm shortage when in fact it was due to synergism. Because we find no such difference between the three mating treatments, our conclusion that sperm limitation is not important in our study species remains valid.

The current results are the third data set in which significant inbreeding depression has been detected in *E. texana*. Interestingly, the pattern of inbreeding depression observed in the two populations in this study contrasts with that reported in two previous experiments. In one experiment, egg hatching and early survival (from hatching to reproductive maturity), were significantly reduced in inbred relative to outcrossed shrimp, and the overall difference was similar in the two study populations (Weeks *et al.*, 1999). In another experiment, inbreeding depression (after sexual maturity) was also detected in egg production and survival, but only in the WAL and not the JT4 population (Weeks *et al.*, 2000a). Thus, inbreeding depression seems to be greater in the JT4 population than in WAL in early developmental stages, whereas later in development it is absent in JT4. The JT4 population has lower genetic diversity than WAL (Weeks and Zucker, 1999; Weeks *et al.*, 1999), which could be caused by greater natural levels of inbreeding in this population (Weeks *et al.*, 2000a). If JT4 shrimp have purged deleterious alleles that affect post-maturity fitness (as suggested in Weeks *et al.*, 2000a), but have not purged early-acting deleterious alleles (current study), then this pattern would be opposite to that described in many plant species (Husband and Schemske, 1996), where alleles causing early inbreeding depression are more commonly purged relative to later-acting alleles.

Tests of the Otto et al. (1993) model

The Otto *et al.* (1993) model predicts the equilibrium frequencies of the three mating types in this system. The current experiment suggests that $\beta = 1$, which requires more restrictive conditions for males to be maintained in this androdioecious species than in *C. elegans* (Otto *et al.*, 1993). We have combined these estimates of β with earlier estimates of the other three parameters of the Otto *et al.* (1993) model in Table 2. Using these estimates of all four parameters, we can predict (using Otto *et al.*'s Equations (2(a)–(c)) the expected proportions of males, amphigenic, and monogenic hermaphrodites in these two populations (Table 3). Clearly, the ranges of predicted proportions for the sex-types are quite wide, mainly due to the wide range of relative male fertility, α (Table 2). The predicted ranges in frequencies for the three mating types are too broad to allow meaningful comparisons with sex ratios of field-collected animals.

Table 2. Definitions and estimates of the four parameters of the Otto *et al*. (1993) model

	α	β	$(1-\sigma)$	δ
Definition	Relative male fertility	Proportion of eggs not fertilized by a male that a hermaphrodite can self-fertilize	Relative viability of males to hermaphrodites	Inbreeding depression experienced by selfed offspring
Estimates-JT4	0.7–10.4[a]	1[b]	0.85–0.87[c]	0.47–0.53[d]
Estimates-WAL	1.0–7.7[a]	1[b]	0.67–0.94[c]	0.66–0.69[d]

[a] Hollenbeck, 1998; Weeks *et al*., 2000a.
[b] Current data.
[c] Zucker *et al*., 2001.
[d] Weeks *et al*., 1999; Weeks *et al*., 2000b.

Table 3. Predicted and observed sex ratios

Mating Type	Predicted	Observed
JT4		
Males (*u*)	0–0.82	0.21
Amphigenics (*v*)	0–0.18	0.69
Monogenics (*w*)	0–1.0	0.10
WAL		
Males (*u*)	0–0.62	0.24
Amphigenics (*v*)	0–0.38	0.63
Monogenics (*w*)	0–1.0	0.13

Nevertheless, if we assume that the laboratory-based estimates of these four parameters reflect conditions in the field, our data suggest a number of interesting conclusions. The model outlines three potential 'benefits' for males: sperm limitation in hermaphrodites, inbreeding depression for selfed offspring, and high relative male fertility. The current experiment reveals that the first of these potential benefits, reduced ability of hermaphrodites to fertilize all their own eggs if not fertilized by a male (β), is inconsequential (i.e., $\beta = 1$). The second, inbreeding depression (δ), appears to be quite important in these populations. Lifetime inbreeding depression was estimated at between 0.5 and 0.7 in these populations (Weeks *et al*., 1999, 2000a) which in most species would be sufficient to maintain outcrossing (Lande and Schemske, 1985). However, in this system these values alone are not great enough to select for complete outcrossing (Weeks *et al*., 2000a). Other important factors are lower male longevity and the inability of males to fertilize 100% of the hermaphrodite's eggs during an outcrossing event (Weeks *et al*., 2000b). Inbreeding depression >0.5 is therefore required for males to be maintained (Otto *et al*., 1993). Certainly, the levels of inbreeding depression detected in our studies may under-represent true values in the field (Schemske, 1983; Dudash, 1990;

Ramsey and Vaughton, 1998). If inbreeding depression is significantly greater in the field, then it may be sufficient to maintain males in both populations. Finally, although the predicted ranges of frequencies for males and monogenic hermaphrodites are wide enough to include observed sex ratios in both populations (Table 3), the observed proportion of amphigenic hermaphrodites is two to three times as high as the highest predicted proportions in both populations. This difference can partly be explained by fitness differences between these two hermaphroditic types that were not accounted for in the Otto *et al.* (1993) model (Weeks *et al.*, 2001). However, amphigenic proportions remain higher than expected even when these fitness differences are incorporated, suggesting that one or more additional factors may need to been added to the model.

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