

Maintenance of androdioecy in the freshwater shrimp *Eulimnadia texana*: sexual encounter rates and outcrossing success

Vicky G. Hollenbeck,^a Stephen C. Weeks,^b William R. Gould,^a and Naida Zucker^a

^aNew Mexico State University, Las Cruces, NM 88003-8001, USA, and ^bDepartment of Biology, The University of Akron, Akron, OH 44325-3908, USA

The clam shrimp *Eulimnadia texana* has a rare mating system known as androdioecy, in which males and hermaphrodites co-occur but there are no pure females. In this species, reproduction takes place by outcrossing between males and hermaphrodites, or by selfing within a hermaphrodite; this system provides a unique opportunity to examine the adaptive significance of outcrossing and selfing in animals. Our study examined mating behavior in hermaphrodites and males from two populations to understand the propensity of these shrimp to mate and to estimate a parameter of a model developed by Otto et al. (American Naturalist 141:329–337), which predicts the conditions for stability of the mixed mating system in *E. texana*. Here we present evidence that mating frequency is environmentally sensitive, with greater numbers of encounters and matings per male when males are rare and in younger males. However, the effects of shrimp density, relative male frequency, and shrimp age interact in a complex way to determine male mating success. Overall, mating frequency was determined by a combination of encounter rates between the sexes and the proportion of encounters resulting in mating. The mating rates were then used to estimate one of four parameters of the Otto et al. model, and these estimates were combined with previous estimates of the other three parameters to examine the fit of the predicted to the observed sex ratios in the two populations. *Key words*: androdioecy, branchiopod crustaceans, evolution of breeding systems, inbreeding, mating success, mating tactics, mixed mating system, self-fertilization. [*Behav Ecol* 13:561–570 (2002)]

In self-compatible hermaphrodites, questions arise as to when individuals should outcross (mate with an unrelated individual) or self-fertilize (Charlesworth and Charlesworth, 1987; Lande and Schemske, 1985). Outcrossing incurs a twofold cost compared to selfing (the fusion of gametes from the same individual) because gametes contain only half the genetic material of the parent (cost of meiosis; Williams, 1975). The genetic cost of meiosis can be recovered with selfing because all of the zygote's genetic material is from the same individual (Fisher, 1941; Maynard Smith, 1977; Naglyaki, 1976). However, despite the twofold cost, outcrossing is still prevalent in the majority of eukaryotic organisms (Bell, 1982). Many of the hypotheses put forth to explain the predominance of outcrossing can be lumped into adaptive variation hypotheses based on the premise that outcrossing leads to greater variation in progeny, which is advantageous in variable environments (Bell, 1982; Johnson et al., 1997; Wells, 1979).

Most theoretical models of mating systems predict evolutionary equilibria of complete selfing or complete outcrossing populations (Fisher, 1941; Holsinger et al., 1984; Lande and Schemske, 1985; Naglyaki, 1976; Wells, 1979). Yet other models predict that mixed mating systems (i.e., those with both outcrossing and selfing modes of reproduction) can be maintained (Charlesworth, 1980; Charlesworth et al., 1991; Holsinger et al., 1984; Lande et al., 1994; Latta and Ritland, 1994; Lloyd, 1979; Maynard Smith, 1977). Mixed mating systems provide excellent opportunities to address questions regarding selfing and outcrossing in a single system.

One form of mixed mating is androdioecy, an unusual system in which males and hermaphrodites co-occur, but there are no true females (Charlesworth, 1984). Many structurally androdioecious organisms have been found to be functionally dioecious: the hermaphrodites of these organisms function only as females (Charlesworth, 1984; Wolf et al., 1997). There have been only a few documented cases of functional androdioecy (or near androdioecy) in plants, including *Datisca glomerata* (Liston et al., 1990), *Mercurialis annua* (Pannell, 1997), *Saxifraga cernua* (Molau and Prentice, 1992), and *Phillyrea augustifolia* (Lepart and Dommee, 1992). The few animals exhibiting androdioecy include the nematode *Caenorhabditis elegans* (Wood, 1988), the barnacle *Balanus galeatus* (Gomez, 1975), the vertebrate killifish, *Rivulus marmoratus* (Lubinski et al., 1995), and several branchiopod crustaceans (Sassaman, 1995; Sassaman and Weeks, 1993).

One such androdioecious branchiopod is the clam shrimp *Eulimnadia texana* (Sassaman and Weeks, 1993; Zucker et al., 1997). Biparental reproduction occurs via outcrossing only between males and hermaphrodites; uniparental reproduction consists of selfing within a hermaphrodite. Hermaphrodites are of two types: monogenic and amphigenic. These terms refer to the genotypes for sex determination, which are hypothesized to be under the control of a single genetic locus with two alleles (Sassaman and Weeks, 1993). Monogenics are homozygous dominant for the sex-determining gene, and amphigenics are heterozygous. The homozygous recessive condition produces males (Sassaman and Weeks, 1993).

Various studies of androdioecious plants have addressed the stability of this breeding system. Fritsch and Rieseberg (1992) used outcrossing rates (determined via polymorphic DNA markers) to compare predicted and actual male frequencies in two populations of *D. glomerata*. They concluded that high pollen production by males (three times that of hermaphrodites) coupled with the high outcrossing rates in hermaphrodites appear to be sufficient to allow androdioecy to persist

Address correspondence to S.C. Weeks. E-mail: sweeks@uakron.edu. V.G. Hollenbeck is now at the Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752, USA.

Received 2 May 2001; revised 30 November 2001; accepted 11 December 2001.

in this species. In *M. annua*, it has been hypothesized that a balance between selection for reproductive assurance (i.e., ability to produce offspring without a mate) during colonization and selection favoring more males in established populations allows for the maintenance of androdioecy (Pannell, 1997). Lepart and Dommeé (1992) suggested that androdioecy in *P. angustifolia* is an intermediate state between hermaphroditism and dioecy. The ability of hermaphrodites to self-fertilize could be an adaptation for colonizing new habitats with few founders (Lepart and Dommeé, 1992).

Because of the unusual mating system of *E. texana* (e.g., two hermaphroditic types and hermaphrodites unable to fertilize one another), Otto et al. (1993) specifically developed a population genetics model for this species. The model describes conditions under which males can be maintained in the population given discrete generations and describes a life history that consists of mating, offspring production, and viability selection (Otto et al., 1993). Males can be maintained if the costs of sex (the reduction in gene copies due to meiosis) and reduced male longevity are offset by the costs of selfing (inbreeding depression and sperm limitation) and high relative male mating success. Specifically, the model predicts a stable polymorphism (maintenance of monogenics, amphigenics, and males) whenever the following inequality is true:

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

The parameter α is a measure of relative male mating success and is defined by the fact that αu is the proportion of eggs that a hermaphrodite fertilizes with male sperm. Although αu must lie strictly between 0 and 1 for all u , α need not. In the extreme case where all eggs are fertilized by male sperm if at least one male is present, then $\alpha = 1/u$, which is $\gg 1$ when males are rare (reflecting the fact that relative male mating success is enormous in this case). The variable α is a function that includes several components of male mating success: the number of encounters with hermaphrodites experienced by an average male during its reproductive lifetime; the probability of outcrossing per encounter; and the proportion of eggs fertilized using male sperm given that mating has occurred. In general, α may depend on the frequency of males in the population, although α would be constant for all u if encounters are rare and if they were governed by a Brownian motion process.

When an encounter does not result in sperm transfer, or when sperm transfer does not lead to fertilization of an entire clutch of eggs, a proportion, β , of the remaining eggs are self-fertilized by the hermaphrodite (Otto et al., 1993). The selfed offspring may suffer inbreeding depression, δ [$= 1 - (\text{fitness of selfed offspring}/\text{fitness of outcrossed offspring})$]. The model also incorporates the often-observed difference in viability between males and hermaphrodites (Sassaman and Weeks, 1993; Strenth, 1977) by reducing male fitness to $(1 - \sigma)$ relative to hermaphrodites. Equation 1 states that male relative mating success and relative viability [$\alpha(1 - \sigma)$] must be high enough to overcome the twofold cost of outcrossing, which can be offset by high inbreeding depression $(1 - \delta)$ or inability of the hermaphrodite to fertilize many of her own eggs (low values of β) in order for the mixed mating system to persist (Otto et al., 1993).

The objectives of this study were to (1) understand the likelihood of outcrossing in *E. texana* and how mating frequencies might be modified by environmental conditions and (2) quantify relative male mating success [α in the Otto et al. (1993) model]. An operational definition of α is that it is a product of encounter rates between males and hermaphrodites, the proportion of these encounters that result in sperm transfer from the male to the hermaphrodite, and the pro-

portion of eggs then successfully sired by the male's sperm. To make this operational definition a relative value, encounters need to be summed across the time between receptivity periods for the hermaphrodites (i.e., the time in which a male can mate with multiple hermaphrodites before an average hermaphrodite is again available for mating). The current study examined the first two of the above three components of α (encounter rates and the proportion of encounters resulting in sperm transfer); the third component (proportion of eggs then successfully sired by the male's sperm) has been estimated elsewhere (Weeks et al., 2000b). The current estimates of α were collected from two populations of *E. texana* that differ in their evolutionary histories. The results suggest that α does depend on environmental characters such as shrimp age, relative male frequency, and shrimp density. Therefore, a reliable test of the fit of the Otto et al. (1993) model to natural sex ratios will not be forthcoming until the model is reformulated to incorporate an environmentally sensitive α , and the parameters of the Otto et al. (1993) model are estimated from natural populations.

METHODS

Study organism

Eulimnadia texana is a freshwater clam shrimp in the crustacean subclass Branchiopoda and the order Spinicaudata (Spears and Abele, 2000). Although its higher taxonomy remains controversial, *E. texana* has been placed in the family Limnadiidae with two other genera and 15 congeners (Sassaman, 1995). Clam shrimp derive their common name from the bivalve-shaped carapace that covers the shrimp. Sexual dimorphism is evident. In males, the first two pairs of thoracic appendages are hooked and serve as claspers during mating. In gravid hermaphrodites, fertilized eggs are seen through the transparent carapace as a dorsal mass in the fold of the carapace. When not gravid, eggs lining the ovotestes are readily seen through the carapace.

Eulimnadia texana is a small clam shrimp, reaching only 8 mm in carapace length. This shrimp inhabits ephemeral habitats such as natural playas and human-made cattle tanks in the southwestern United States. Reproduction can take place by outcrossing between a male and a hermaphrodite or by selfing within a hermaphrodite. Fertilization between hermaphrodites does not occur because hermaphrodites lack the claspers necessary for outcrossing between two individuals (Sassaman and Weeks, 1993). *Eulimnadia texana* typically survive for 12–17 days (Marcus and Weeks, 1997; Weeks et al., 1997), although hermaphrodites typically live 25–50% longer than males (Knoll, 1995; Zucker et al., 2001). One or two clutches of eggs are produced daily once hermaphrodites reach sexual maturity, and eggs are continually produced until the hermaphrodites reproductively senesce at 14–21 days of age (Weeks et al., 1997). Because there is no sperm storage in hermaphrodites, a hermaphrodite must mate with a male every day if all the clutches are to be outcrossed (Weeks et al., 2000b). Eggs remain in the brood chamber for 10–20 h (Weeks et al., 1997) and are then buried in a small burrow in the pond bottom made by the hermaphrodite (Zucker et al., 2002). There is a period of about 3 h after the hermaphrodite buries the clutch of eggs before the next clutch is extruded into the brood chamber (Zucker et al., 2002). Eggs usually do not hatch until a period of desiccation has passed.

In natural populations, male frequencies are highly variable and range from 0 to 42% (Sassaman, 1995). Densities are also variable: MacKay et al. (1990) reported densities of *E. texana* in a desert tank of 250/m³, and Medland (1989) found branchiopod densities to be 3000–7000 individuals/m³ in similar

tanks, although densities for individual species were not reported. In natural conditions, *E. texana* exhibits a patchy distribution, concentrating around the rim and often the top of a pool (Hollenbeck, personal observations). In addition, small or drying pools often contain a nearly continuous layer of clam shrimp over the surface (Hollenbeck, personal observations; Pennak, 1989).

Study populations

Two populations of *E. texana* were used in this study: WAL is a population inhabiting a cattle tank (25.3 m × 26.2 m × 1 m deep when filled) constructed in the 1950s (Sherbrooke WC, personal communication) located 4 km north of Portal Road (Road 533) near Portal, Arizona, USA. It receives an average of 49 cm of rain annually, with approximately 60% falling during the monsoon season from June through September (Marcus and Weeks, 1997). JT4 is a population from an older, natural depression (Havstad K, personal communication; 32 m × 18.5 m × 0.3 m deep when filled), also used by cattle, located 5.8 km north of the entrance to the USDA Jornada Experimental Range on Jornada Road near Las Cruces, Doña Ana County, New Mexico, USA. Rainfall for JT4 averages 24 cm annually. As with WAL, approximately 60% falls from June through September via convection storms (Marcus and Weeks, 1997). Recent studies indicate that WAL has greater genetic diversity than JT4 (Weeks and Zucker, 1999; Weeks et al., 1999).

Rearing conditions

We collected samples of the top 2 cm of soil from various locations within each population and stored the samples in the lab for less than 1 year. On day 0 of an experimental observation period, a small amount of soil from a population was filtered through a 270- μ m sieve with dechlorinated tap water into 30 cm × 14 cm (5 cm water depth) plastic storage tanks. The filtering removed eggs of *Triops* sp., which, if allowed to hatch, prey on clam shrimp. Tanks were kept under constant incandescent light to maintain temperatures of 28–30°C. Many eggs hatch between 17 and 24 h after hydration, although some continue to hatch for up to 72 h. To ensure that all shrimp used in the experiment were approximately the same age, water with larvae was poured into a 35 cm × 22 cm (5 cm water depth) plastic rearing tank after 24 h, separating larvae from any unhatched eggs in the soil. Approximately 0.5 l of water from the alternate population was filtered through a 63- μ m sieve (small enough to remove any *E. texana* larvae) and added to each rearing tank. This allowed both populations access to food items (e.g., microorganisms) and minerals that may be present in the soil of one population but not the other. Larvae were supplemented with 0.25 g dry yeast in solution and 0.02 g TetraMin Baby “E” fish food upon transfer to rearing tanks and again 24 h later. Subsequently, clam shrimp were fed finely ground TetraMin fish flakes as needed until day 6, and frog tadpole food (Carolina Biological) as needed thereafter. We added dechlorinated tap water periodically to replace evaporated water. On day 6 or 7, we removed clam shrimp from their original rearing tanks and transferred them to fresh rearing tanks with new dechlorinated tap water to minimize algal growth. At this point, heat lamps were turned off and tanks were kept on a 15 h:9 h light:dark cycle at approximately 24°C.

Mating behavior

We observed mating behavior of groups of *E. texana* under various demographic conditions. A completely randomized

design with a 2⁴ factorial arrangement of treatments was used, resulting in 16 treatment combinations. The factors included (1) population, (2) age of shrimp, (3) density of shrimp within an observation cup, and (4) male frequency (percentage of males in a treatment population). Each factor was set at two distinct levels: (1) population: JT4 and WAL; (2) age: young (5–7 days) and old (9–11 days); (3) density: high (24 individuals/observation cup) and low (12 individuals/observation cup); and (4) male frequency: high (42%) and low (14–17%).

We established treatments 24 h before sampling. Shrimp were removed from rearing tanks, sexed, and placed in 500-mL (9 cm diam) clear plastic cups with 0.02 g finely ground TetraMin fish flakes and dechlorinated tap water to a depth of 6 cm. On the day of an observation, shrimp were marked with different colors of Testor’s enamel paint for individual identification.

An observation consisted of three 20-min periods, interrupted by 10-min rest periods to avoid observer fatigue. We used scan sampling (Altmann, 1974) to determine the number of encounters that took place and the number of those encounters resulting in outcrossing within the cumulative 60-min observation. An “encounter” was defined as clasping of a hermaphrodite by a male for at least 3 s. This disqualified random bumping and male–male interactions as encounters. “Mating” was defined as an encounter that continued uninterrupted for at least 5 min. Knoll (1995) found that if an encounter lasted longer than 1 min, subsequent copulation and movement of eggs into the brood chamber occurred (mean = 27 min; range = 2–120 min; $N = 95$ out of 95 observations). Therefore, a 5-min encounter has a high probability of resulting in mating. Scanning was continuous, and the amount of time spent on each individual lasted only a few seconds. Therefore, sampling approached a continuous, simultaneous sample on all males (Altmann, 1974). The order in which males were scanned was randomly chosen and retained throughout the 20-min observation. Scanning rounds continued until every male was observed an equal number of times in a particular 20-min period. We observed each male in an observation cup in turn, and any new encounters were noted. If a male was seen engaged in an encounter, the identities of the male and hermaphrodite, as well as the time, were recorded. If the encounter continued throughout the next 5 min, it was noted as an encounter that resulted in mating. Encounters that began during the last 4 min of an observation period were followed for a full 5 min to determine their outcomes. During the second and third 20-min observation periods, encounters that were noted as resulting in mating at the end of the previous period were considered to be continuous throughout the rest period and were not counted as new encounters. If more than one male clasped a hermaphrodite simultaneously, we recorded each encounter. However, if all males remained clasped for 5 min (rarely), we counted only one encounter as resulting in sperm transfer. At the end of the 60-min observation period, we tallied the total number of encounters and the total number of those likely resulting in sperm transfer. Each treatment combination was replicated eight times for a total of 128 observations.

Statistical analyses

We estimated mating frequency by dividing the numbers of encounters per tank in a 1-h period in which a male clasped a hermaphrodite for ≥ 5 min (see above) by the total number of males in that tank. These data were analyzed in a factorial, four-way ANOVA, with population, shrimp age, density, and male frequency as the four main effects (all considered fixed

Table 1
Analysis of variance of mating events per male per hour (square-root transformed)

Source	df	Sum of squares	F ratio	<i>p</i>
Population	1	0.23	2.51	.1163
Age	1	10.71	114.76	<.0001†
Population * age	1	0.01	0.074	.7865
Density	1	0.20	2.11	.1494
Population * density	1	0.15	1.64	.2028
Age * density	1	0.50	5.38	.0222†
Population * age * density	1	0.00	0.00	.9763
Male frequency	1	0.42	4.50	.0361†
Population * male frequency	1	0.01	0.15	.7006
Age * male frequency	1	0.06	0.60	.4394
Population * age * male frequency	1	0.00	0.01	.9099
Density * male frequency	1	0.38	4.04	.0467†
Population * density * male frequency	1	0.03	0.36	.5484
Age * density * male frequency	1	0.41	4.34	.0396†
Population * age * density * male frequency	1	0.00	0.00	.9810
Error	112	10.45		

† Statistically significant.

effects). The data were square-root transformed to normalize residuals.

Analysis of encounter rates was the same as for mating frequency, except the statistic was numbers of encounters per tank divided by numbers of males per tank (i.e., the number of encounters per male per hour). These data also required square-root transformation to normalize residuals.

To determine the effect of the four treatments on the proportion of successful encounters (i.e., the number of successful encounters divided by the number of encounters), we used a four-way weighted analysis of variance (treatment proportions weighted by the number of encounters in an observation period; SAS Institute, 1989). All four factors were considered fixed effects, with two levels per factor. Because encounters could be as short as 3 s, whereas matings were at least 5 min long, matings were more likely to be observed than encounters. Thus, the proportion of successful encounters was overestimated by the fraction of encounters missed in the scan sampling. All assumptions of ANOVA (normality and homogeneity of variances) were tested and met prior to analysis.

RESULTS

In this study, male mating success was estimated as the number of mating events per male per hour (i.e., mating frequency), which is composed of the number of male–hermaphrodite encounters per hour times the proportion of these encounters that resulted in actual mating. We first discuss our direct measure of male mating success (number of matings per male per hour) and then consider how encounters and the proportion of encounters resulting in mating combined to form this estimate of male mating success.

Mating frequency

The number of mating events per male per hour was significantly influenced by two main effects: shrimp age and frequency of males (Table 1). Three interaction effects were also noted: age by density, male frequency by density, and age by density by male frequency (Table 1). In the first interaction, mating events per male per hour did not differ between densities for young shrimp (high: 0.85 ± 0.07 ; low: 0.94 ± 0.07), but there were significantly more mating events per male per

hour under high relative to low density (0.30 ± 0.07 vs. 0.16 ± 0.07 , respectively) for older shrimp (Figure 1A). In the second interaction, matings did not differ between densities for high male frequency (high: 0.43 ± 0.07 ; low: 0.51 ± 0.07), but there were more matings at high male relative to low male density (0.73 ± 0.07 vs. 0.59 ± 0.07 , respectively) at low frequency (Figure 1A). The third interaction (three-way) was most informative: younger males had uniformly more matings under low male frequencies (regardless of density), whereas older males had the highest matings at low male frequencies and high density, intermediate success at high male frequency (regardless of density), and lowest success at low male frequency and low density (Figure 1A).

Clearly, mating frequencies were modified by environmental characteristics, and thus estimates of the behavioral components of α (encounter rates between males and hermaphrodites and the proportion of these encounters that resulted in sperm transfer) were also dependent on environment. Because α is a relative value, relating the number of offspring sired by a male relative to those produced by a hermaphrodite (see above), we needed to multiply the hourly mating frequency (measured here) by the average time required for a hermaphrodite to brood and release a fertilized clutch of eggs. The latter is the amount of time available for males to fertilize multiple hermaphrodites and thus allows a calculation of relative fertility of males to hermaphrodites, or α . The time between successive clutches has been estimated elsewhere to be approximately 20 h (Weeks et al., 1997). Thus, we estimated the behavioral components of α to range from 1.3 (old JT4 males at low frequency and low density) to 22.5 (young WAL males at low frequency and low density; Table 2).

Behavioral components of male mating success

To understand the behavioral components of male mating success, we also compared numbers of encounters per male per hour and the proportion of these encounters resulting in mating among treatments. The overall average encounters per male per hour was 5.6 ± 0.5 , whereas the number of mating events per male per hour was only 0.56 ± 0.05 (Table 2). Thus, approximately one-tenth of the encounters resulted in mating. Some treatments affected encounter rates and mating

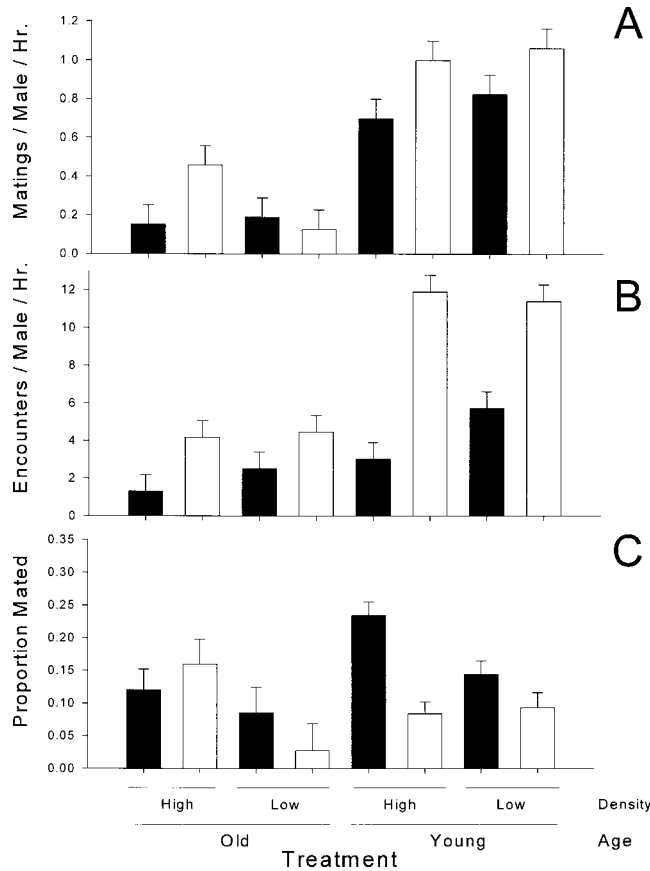


Figure 1
 (A) Mating success per male per hour; (B) encounters per male per hour; and (C) proportion of encounters resulting in mating in *E. texana*. All figures are averaged across population and are grouped by shrimp age, density, and male frequency (black bars = high male frequency; white bars = low male frequency). Error bars indicate 1 SEM.

frequencies similarly (Tables 1 and 3); for example, younger males had greater encounter rates (8.0 ± 0.4) and mating frequencies (0.90 ± 0.05) than older males (3.1 ± 0.4 and 0.23 ± 0.05 , respectively). Additionally, both encounter rates and mating frequencies were greater under low male frequency (8.0 ± 0.4 and 0.66 ± 0.05 , respectively) than under high male frequency (3.1 ± 0.4 and 0.47 ± 0.05 , respectively; Tables 2 and 3). Therefore, for these two main effects, encounter rates explained much of the differences seen in mating frequencies.

However, in general, mating frequencies were not a simple proportion of encounter rates (e.g., 10% of encounters), but rather, the relationship between these two measures was much more complex. The two populations significantly differed in encounter rates (JT4: 4.6 ± 0.4 ; WAL: 6.5 ± 0.4), but this difference was not significant for mating frequencies (JT4: 0.52 ± 0.05 ; WAL: 0.61 ± 0.05). The encounter rate difference between populations was driven by very low encounter rates for older JT4 shrimp (old: 1.5 ± 0.6 ; young: 7.7 ± 0.6). The magnitude of difference between young and old was not reflected in WAL (old: 4.7 ± 0.6 ; young: 8.4 ± 0.6). This difference caused the significant population-by-age interaction (Table 3). Again, this interaction did not translate into an interaction for mating frequencies (Table 1). Overall, the pattern for encounter rates was more encounters for younger shrimp, especially at low male frequency (Figure 1B).

Because mating frequencies were not simple proportions of encounter rates, we needed to analyze the second behavioral component of male mating success: the proportion of encounters resulting in matings. Higher density led to a significantly greater proportion of encounters resulting in mating (high: 0.15 ± 0.01 ; low: 0.09 ± 0.02 ; Table 4), but this effect was countered by a nonsignificant, lower number of encounters per male at high density (high: 5.1 ± 0.4 ; low: 6.0 ± 0.4 ; Table 3), resulting in no significant difference in mating frequency at higher density (high: 0.58 ± 0.05 ; low: 0.55 ± 0.05 ; Table 1). Although higher male frequency led to a significantly greater proportion of encounters resulting in mating (high: 0.15 ± 0.01 ; low: 0.09 ± 0.02 ; Table 4), this was countered by many more encounters per male at lower male fre-

Table 2
Estimates of average matings per male per hour, encounters per male per hour, proportion of encounters resulting in mating (proportion mating), and behavioral components of α for each treatment combination

Population	Male age	Density	Male frequency	Matings per male	Encounters	Proportion mating	α^a
JT4	Old	High	High	0.16 (0.07)	1.1 (0.2)	0.15 (0.06)	3.3
JT4	Old	High	Low	0.42 (0.10)	1.7 (0.4)	0.24 (0.08)	8.3
JT4	Old	Low	High	0.10 (0.05)	1.0 (0.2)	0.10 (0.09)	2.0
JT4	Old	Low	Low	0.06 (0.06)	2.4 (1.1)	0.03 (0.02)	1.3
JT4	Young	High	High	0.74 (0.19)	2.7 (0.2)	0.27 (0.05)	14.8
JT4	Young	High	Low	0.96 (0.17)	12.1 (2.0)	0.08 (0.02)	19.2
JT4	Young	Low	High	0.73 (0.22)	5.2 (0.8)	0.14 (0.04)	14.5
JT4	Young	Low	Low	1.00 (0.19)	10.8 (2.8)	0.09 (0.38)	20.0
WAL	Old	High	High	0.14 (0.05)	1.6 (0.3)	0.09 (0.03)	2.8
WAL	Old	High	Low	0.50 (0.14)	6.7 (1.7)	0.08 (0.03)	10.0
WAL	Old	Low	High	0.28 (0.09)	4.1 (0.9)	0.07 (0.02)	5.5
WAL	Old	Low	Low	0.19 (0.13)	6.5 (1.8)	0.02 (0.02)	3.8
WAL	Young	High	High	0.66 (0.08)	3.4 (0.3)	0.20 (0.02)	13.3
WAL	Young	High	Low	1.04 (0.26)	11.8 (1.1)	0.09 (0.03)	20.8
WAL	Young	Low	High	0.93 (0.12)	6.3 (0.3)	0.15 (0.02)	18.5
WAL	Young	Low	Low	1.13 (0.13)	12.1 (1.6)	0.09 (0.01)	22.5

Standard errors are given in parentheses ($n = 8$).

^a $\alpha = \text{mating events/male/hour} \times 20 \text{ h/hermaphroditic brood}$; first two components of α only (excluding proportion of mated eggs sired by male).

Table 3
Analysis of variance of encounters per male per hour (square-root transformed)

Source	df	Sum of squares	F ratio	<i>p</i>
Population	1	8.99	21.25	<.0001†
Age	1	41.35	97.73	<.0001†
Population * age	1	3.67	8.67	.0039†
Density	1	1.42	3.35	.0698
Population * density	1	0.56	1.32	.2537
Age * density	1	0.05	0.11	.7386
Population * age * density	1	0.04	0.10	.7533
Male frequency	1	26.95	63.70	<.0001†
Population * male frequency	1	0.44	1.03	.3118
Age * male frequency	1	4.17	9.86	.0022†
Population * age * male frequency	1	0.71	1.68	.1975
Density * male frequency	1	2.38	5.62	.0194†
Population * density * male frequency	1	0.20	0.47	.4930
Age * density * male frequency	1	0.43	1.01	.3174
Population * age * density * male frequency	1	0.56	1.33	.2515
Error	112	47.39		

† Statistically significant.

quency (high: 3.2 ± 0.4 ; low: 8.0 ± 0.4 ; Table 3), yielding a significantly higher mating frequency for males at low male frequency (high: 0.47 ± 0.05 ; low: 0.66 ± 0.05 ; Table 1). Younger males had a marginally greater proportion of encounters resulting in mating than older males (young: 0.14 ± 0.01 ; old: 0.10 ± 0.02 ; Table 4), which when added to the higher numbers of encounters (young: 8.0 ± 0.04 ; old: 3.1 ± 0.04 ; Table 3) explained the overall greater mating frequencies observed for younger males (young: 0.90 ± 0.05 ; old: 0.23 ± 0.05 ; Table 1). The greater numbers of encounters in WAL (WAL: 6.5 ± 0.4 ; JT4: 4.6 ± 0.4 ; Table 3) was countered by the fact that JT4 males had a marginally greater proportion of encounters that resulted in mating than did WAL males (WAL: 0.10 ± 0.01 ; JT4: 0.14 ± 0.02 ; Table 4), resulting in a nonsignificant

difference between the two populations in mating frequencies (WAL: 0.61 ± 0.05 ; JT4: 0.52 ± 0.5 ; Table 1).

Two-way and three-way interactive effects were also observed for the proportion of encounters that resulted in matings (Table 4). Again, the three-way interaction was most informative: for younger males, the proportion of successful mating events increased dramatically when going from low to high male frequency, more dramatically so at high density (Figure 1C). This was not reflected for older males: the proportion of successful mating events increased from low to high male frequency at low density, but this pattern was reversed at high density, with the greatest proportion of successful mating events occurring at low male frequency (Figure 1C). The combination of encounter rate with proportion of encounters

Table 4
Treatment effects on the proportion of successful matings using a weighted analysis of variance (weighted by the number of encounters)

Source	df	Sum of squares	F ratio	<i>p</i>
Population	1	0.684	3.45	.0661
Age	1	0.701	3.53	.0628
Population * age	1	0.285	1.44	.2333
Density	1	1.633	8.23	.0049†
Population * density	1	0.493	2.49	.1177
Age * density	1	0.193	0.97	.3264
Population * age * density	1	0.110	0.56	.4578
Male frequency	1	1.272	6.41	.0127†
Population * male frequency	1	0.001	0.00	.9510
Age * male frequency	1	0.875	4.41	.0380†
Population * age * male frequency	1	0.132	0.67	.4159
Density * male frequency	1	0.000	0.00	.9682
Population * density * male frequency	1	0.018	0.09	.7669
Age * density * male frequency	1	1.017	5.13	.0255†
Population * age * density * male frequency	1	0.355	1.79	.1836
Error ^a	111	22.019		

^a Note the number of error degrees of freedom is not 112 due to one trial in which no encounters occurred.

† Statistically significant.

that were successful resulted in the observed patterns of mating frequencies. Younger males had greater encounter rates at low male frequencies (Figure 1B) and also a greater proportion of mating events at high male frequencies (Figure 1C), which resulted in overall greater rates of mating for younger relative to older shrimp under both conditions, with low male frequencies slightly edging out high male frequencies for younger shrimp (Figure 1A). For older shrimp, the simple pattern of increased encounter rates at low male frequency (Figure 1B) was modified by a complex pattern of proportion of encounters resulting in successful mating (Figure 1C), resulting in the observed pattern of highest mating frequencies at high density and low male frequency, followed by intermediate rates of mating at high male frequency (regardless of density), with the lowest mating frequencies at low density and low male frequency (Figure 1A).

DISCUSSION

The current study was motivated by two goals: to understand how environmental factors may affect the likelihood of mating in *E. texana* and to understand the maintenance of androdioecy in this species by noting the relative effectiveness of males to fertilize the eggs of one or more hermaphrodites (α ; Otto et al., 1993). Below, we explore both of these issues.

Mating propensity

Successful outcrossing in *E. texana* should be a product of three components: (1) encounter rates of males with hermaphrodites, (2) proportion of encounters that result in sperm transfer from males to hermaphrodites (i.e., matings), and (3) proportion of eggs fertilized by male sperm once transfer occurs. The current study examined the first two of these three components; the third component has been estimated elsewhere (Weeks et al., 2000b).

The most straightforward combination of the above components would be if some invariant proportion of encounters resulted in successful sperm transfer and some invariant proportion of eggs were then sired by a male. These proportions might then be best explained by simple biological processes, such as differences in swimming speeds affecting encounter rates (Medland et al., 2000) or increased sperm production favoring one genotype over another (Parker, 1998; Rakitin et al., 1999). Some of the differences in mating frequencies measured herein did appear to be best explained by these types of straightforward processes. For example, the greater mating frequencies of younger males appeared to be largely driven by the higher encounter rates of young relative to older shrimp. This difference makes sense if we assume younger males are more vigorous swimmers and thus encounter hermaphrodites at a higher rate. Another straightforward example was found in the greater matings per male when males were in lower frequency, which again is easily interpreted when noting that lower male frequency led to greater numbers of encounters per male.

However, it is clear from the several other comparisons that differences in mating frequencies in *E. texana* were not only driven by underlying simple biological processes, but rather that most of the observed differences were due to apparent switches in male or hermaphroditic behaviors in the differing environments. The three-way interaction in mating frequencies of male frequency, shrimp age, and density was not explained by differences in encounter rates, but rather appeared to be a behavioral change by one or both sexes. In matings with younger shrimp, the proportion of encounters that resulted in successful mating was lowest when encounters were highest (at lower male frequency). This pattern could be ex-

plained if either sex were more selective when encounters were more common (Crowley et al., 1991; Hubbell and Johnson, 1987). For example, it is conceivable that males switch from a mate-guarding tactic (where males hold on to any hermaphrodite they encounter waiting until the hermaphrodite is receptive) when hermaphrodites are not much more plentiful than males, to a sampling tactic (where males encounter but immediately release hermaphrodites that are not receptive) when hermaphrodites are far more plentiful than males. Thus, the combination of higher encounter rates but an apparently more selective mating strategy led to more nearly equivalent mating frequencies at high and low male frequency than would be predicted on the basis of encounter rates alone.

In older shrimp, a different pattern was observed. Older males also had higher encounter rates at low male frequency, but the difference was not as dramatic as in younger males. However, in older shrimp, the proportion of encounters resulting in successful mating events was dramatically lower at low male frequency and low density, highest at low male frequency and high density, and intermediate at high male frequency. This pattern was significantly different from the younger shrimp, suggesting a different behavioral strategy for the older shrimp. A number of studies have shown independent effects of age, density, and male frequency on mating behavior. For example, different patterns of receptivity and mating attempts were documented in female and male (respectively) biting midges (*Culicoides nubeculosus*; Mair and Blackwell, 1998), and courtship behavior was age dependent in the cockroach *Diploptera punctata* (Woodhead, 1986). Relative male frequency has also been documented to affect female water striders, *Gerris odontogaster*: females were more receptive to mating when male frequency was high (Arnqvist, 1992). Population densities have been shown to affect mating behavior in male guppies (Jirotkul, 1999). Additionally, studies of water striders have shown that interacting environmental variables lead to complex mating dynamics. Sih and Krupa (1995) reported that complex interactions among male density, female density, and predation risk resulted in variations in male mating success, which may be explained by shifting mating behavior in both males and females due to intersexual conflicts (Krupa and Sih, 1993). Future studies in *E. texana* designed to study changes in specific mating tactics under differing conditions would help us understand these complex patterns of male mating success in these shrimp.

Tests of the Otto et al. model

The Otto et al. (1993) model attempts to predict the equilibrium frequencies of the three mating types in this system [males (u), monogenic (w), and amphigenic (v) hermaphrodites] based on four relevant parameters: α , the relative male mating success; 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 . Relative male mating success (α) can vary from 0 to ∞ , but is constrained such that $0 \leq \alpha u \leq 1$ (Otto et al., 1993). Thus, α can be viewed as the average number of hermaphrodites that can be fertilized by a single male. For example, if an average hermaphrodite's total egg production is 1000, and a male sires an average of 2000 eggs in its lifetime, then a male fertilizes an average of two hermaphrodites, and $\alpha = 2$ (Otto et al., 1993). Clearly, larger values of α can only occur when males are rare, because an average male cannot fertilize many hermaphrodites when males are common (e.g., $u = 0.5$; Otto et al., 1993).

Table 5
Estimates of the four parameters of the Otto et al. (1993) model
showing the worst and best values for the maintenance of males
(see text)

Parameter	JT4		WAL	
	Worst	Best	Worst	Best
Outcrossing rate ^a	1.3	20.0	2.8	22.5
% Fertilized ^b	52	52	34	34
α	0.7	10.4	1.0	7.7
β^c	1	1	1	1
$(1-\sigma)^d$	0.85	0.87	0.67	0.94
δ^e	0.47	0.53	0.66	0.69
$\alpha(1-\sigma)$	0.60	9.05 ^f	0.64	7.19 ^f
$2\beta(1-\delta)$	1.06 ^g	0.94	0.68 ^g	0.62
u (Predicted)	0	0.82	0	0.62
v (Predicted)	0	0.18	0	0.38
w (Predicted)	1	0.00	1	0.00
u (Observed) ^h	20.9%		24.2%	
v (Observed) ^h	69.1%		63.1%	
w (Observed) ^h	10.0%		12.6%	

^a Data from present study.

^b Weeks et al. (2000a).

^c Weeks et al. (in press).

^d Zucker et al. (2001).

^e Weeks et al. (1999, 2000b).

^f Mixed mating system stable.

^g Monogonics-only stable.

^h Observed sex ratios from Weeks et al. (1999).

The combination of male frequency in the population and relative male mating success dictates the expected proportion of hermaphroditic eggs that will be outcrossed (i.e., αu). The remaining proportion of eggs [i.e., $(1 - \alpha u)$] is then available for selfing. The model allows for some proportion, $(1 - \beta)$, of these non-outcrossed eggs to remain unfertilized. This would occur if some eggs were "earmarked" for outcrossing or if the hermaphrodites were unable to produce enough sperm to fertilize all of their eggs in the absence of males (as in *C. elegans*; Hodgkin and Barnes, 1991; Van Voorhies, 1992; Ward and Carrel, 1979). 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 (Husband and Schemske, 1996; Jarne and Charlesworth, 1993).

Estimates of relative male mating success (α) are divided into mating frequencies (measured here as a product of hourly encounter rates and proportion of encounters resulting in sperm transfer, extrapolated to a 20-h period) and the proportion of eggs actually fertilized by male sperm once sperm transfer has occurred (allowing for some proportion of eggs to be fertilized by hermaphroditic sperm). The current data reveal that male mating success is environmentally sensitive (Table 2), and thus we have chosen to use the highest and lowest estimates of α from both populations to represent the range of α that we expect in these two populations. We have combined these estimates with estimates of the other three parameters for both populations in Table 5. The "worst" column is the worst case scenario for the maintenance of males in the Otto et al. (1993) model, whereas the "best" column is the best case scenario (choosing the worst and best combinations of the four parameters for the maintenance of males, respectively). These values do not represent confidence limits per se, but rather represent the range of estimates that we have gathered while studying this species. The range of

relative male mating success (α) for JT4 was 1.3–20.0 and 2.8–22.5 for WAL (Tables 2 and 5). In a previous study, Weeks et al. (2000b) found that only a proportion of the eggs from a mating event are actually fertilized by male sperm (% fertilized in Table 5), and thus the current estimates of α need to be reduced by the previous estimates of percentage of a clutch sired by a male. Therefore, α ranges from 0.7–10.4 in JT4 and from 1.0–7.7 in WAL (Table 5).

We should note that our higher measurements of α are probably overestimates for two reasons. First, our extrapolation of the mating frequency in a 1-h observation period to a 20-h period will be clearly incorrect if it predicts that males successfully fertilize >100% of the available hermaphrodites. When sex ratios are more nearly equal, many encounters are likely to be between two males, and males may attempt to fertilize the same hermaphrodites more than once. Second, extrapolating the 1-h observations to 20 h also assumes that males are producing copious quantities of sperm. For the lower estimates of mating frequencies, sperm limitation is probably not an issue. However, it is unclear whether males can produce enough sperm to fertilize 20 hermaphrodites in 20 h. Without further information (e.g., sperm replenishment rates in males), we cannot correct the larger estimates of α . We therefore present the current values as our best estimates of male mating success to date.

The remaining three parameters ranged as follows. Hermaphrodites live significantly longer than males in both populations (Zucker et al., 2001), as has been reported in other studies (Knoll, 1995; Strenth, 1977), resulting in estimates of relative male survival $(1 - \sigma)$ between 0.85 and 0.87 for JT4 and 0.67 and 0.94 for WAL (Table 5). Estimates of the ability of hermaphrodites to self-fertilize eggs that are not fertilized by males (β) indicate that a similar number of eggs are fertilized in the presence or absence of males (i.e., $\beta = 1$; Table 5). Finally, inbreeding depression (δ) was noted at both early and late portions of the life cycle, resulting in estimates of lifetime δ ranging between 0.47 and 0.53 for JT4 and 0.66 and 0.69 for WAL (Table 5).

Using the current estimates of all four parameters, both best-case scenarios suggest that mixed sex-types should be stable in these two populations, whereas both worst case scenarios suggest that these populations should go to 100% monogonics (Table 5). Using Otto et al.'s equations 2a–c, we can predict the expected proportions of males, amphigenic, and monogenic hermaphrodites in these two populations (Table 5). Clearly, the ranges of predicted proportions for the sex types are quite wide, mainly due to the wide range in our current estimates of α (Table 2). At this time, the predicted ranges for all three mating types are too broad to allow any meaningful test comparing field-collected sex ratios to predicted sex ratios.

Nevertheless, if we assume that the laboratory-collected estimates of these four parameters are indicative of true conditions in the field, these data suggest a number of interesting results. The model outlines three potential benefits for males: sperm limitation in hermaphrodites, inbreeding depression for selfed offspring, and potential for high outcrossing rates. The first of these potential benefits, reduced ability of hermaphrodites to fertilize all their own eggs if not mated by a male (β), appears to be inconsequential (i.e., $\beta = 1$; Weeks et al., in press). 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); the high levels of inbreeding

depression are tempered, to some degree, by lower male longevity (Table 5), requiring even greater levels of inbreeding depression for males to be maintained (Otto et al., 1993). Certainly, the levels of inbreeding depression detected in these studies may underrepresent true values in the field (Dudash, 1990; Ramsey and Vaughton, 1998; Schemske, 1983). If inbreeding depression is significantly greater in the field, then this factor alone may be sufficient to maintain males in both populations.

Yet, even with high levels of inbreeding depression, relative male mating success, α , may truly be the determining factor for the relative abundance of males in this species (Otto et al., 1993; Weeks and Zucker, 1999) because the α parameter is larger (perhaps by an order of magnitude) than $(1 - \sigma)$, β , or $(1 - \delta)$, and thus dominates Equation 1. Our estimates of α suggest that relative male mating success can range widely (Table 2), and the current data suggest that α is negatively related to male frequency, u (Tables 1 and 2), indicating that α may not be a fixed value but, rather, is frequency dependent. The original formulation of α assumed that it was a fixed quantity (Otto et al., 1993). However, Otto et al. suggested that if α is high when males are rare, males can be maintained in the population under most combinations of the other three parameters.

This article was taken in part from a master's thesis by V.G.H. We thank Drs. Milligan, Howard, and Huenneke for their input during the course of this study. This work was supported by the New Mexico State University Department of Biology, as well as by awards from the New Mexico Commission on Higher Education and Sigma Xi to V.G.H. and from the National Science Foundation (IBN-9631042 to N.Z. and IBN-9614226 to S.C.W.).

REFERENCES

- Altmann J, 1974. Observational study of behavior: sampling methods. *Behaviour* 49:227–267.
- Arnqvist G, 1992. The effects of operational sex-ratio on the relative mating success of extreme male phenotypes in the water strider *Gerris-odontogaster* (Zett) (Heteroptera, Gerridae). *Anim Behav* 43: 681–683.
- Bell G, 1982. The masterpiece of nature. Los Angeles: University of California Press.
- Charlesworth B, 1980. The cost of sex in relation to mating system. *J Theor Biol* 84:655–671.
- Charlesworth B, Morgan MT, Charlesworth D, 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genet Res* 57:177–194.
- Charlesworth D, 1984. Androdioecy and the evolution of dioecy. *Biol J Linn Soc* 23:333–348.
- Charlesworth D, Charlesworth B, 1987. Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* 18:237–268.
- Crowley PH, Travers SE, Linton MC, Cohn SL, Sih A, Sargent C, 1991. Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *Am Nat* 137:567–596.
- Dudash MR, 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44: 1129–1139.
- Fisher RA, 1941. Average excess and average effect of a gene substitution. *Ann Eugen* 11:53–63.
- Fritsch P, Rieseberg LH, 1992. High mating frequencies maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata*. *Nature* 359:633–636.
- Gomez ED, 1975. Sex determination in *Balanus (conopea) galeatus* (L.) (Cirripedia Thoracica). *Crustaceana (Leiden)* 28:105–107.
- Hodgkin J, Barnes TM, 1991. More is not better: brood size and population growth in a self-fertilizing nematode. *Proc R Soc Lond B* 246:19–24.
- Holsinger KE, Feldman MW, Christiansen FB, 1984. The evolution of self-fertilization in plants: a population genetic model. *Am Nat* 124: 446–453.
- Hubbell SP, Johnson LK, 1987. Environmental variance in lifetime mating success, mate choice, and sexual selection. *Am Nat* 130:91–112.
- Husband BC, Schemske DW, 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70.
- Jarne P, Charlesworth D, 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu Rev Ecol Syst* 24:441–466.
- Jirotkul M, 1999. Population density influences male-male competition in guppies. *Anim Behav* 58:1169–1175.
- Johnson SG, Lively CM, Schrag SJ, 1997. Evolution and ecological correlates of uniparental and biparental reproduction in freshwater snails. In: *Evolutionary ecology of freshwater animals* (Streit B, Stadler T, Lively CM, eds). Boston: Birkhauser Verlag; 263–291.
- Knoll L, 1995. Mating behavior and time budget of an androdioecious crustacean, *Eulimnadia texana* (Crustacea: Conchostraca). *Hydrobiologia* 298:73–81.
- Krupa JJ, Sih A, 1993. Experimental studies on water strider mating dynamics: spatial variation in density and sex ratio. *Behav Ecol Sociobiol* 33:107–120.
- Lande R, Schemske DW, 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.
- Lande R, Schemske DW, Schultz ST, 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution* 48:965–978.
- Latta R, Ritland K, 1994. Conditions favoring stable mixed mating systems with jointly evolving inbreeding depression. *J Theor Biol* 170:15–23.
- Lepart J, Domme B, 1992. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Bot J Linn Soc* 108:375–387.
- Liston A, Rieseberg LH, Elias TS, 1990. Functional androdioecy in the flowering plant *Datisca glomerata*. *Nature* 343:641–642.
- Lloyd DG, 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am Nat* 113:67–79.
- Lubinski BA, Davis WP, Taylor DS, Turner BJ, 1995. Outcrossing in a natural population of a self-fertilizing hermaphroditic fish. *J Hered* 86:469–473.
- MacKay WP, Loring SJ, Frost TM, Whitford WG, 1990. Population dynamics of a playa community in the Chihuahuan desert. *Southwest Nat* 35:393–402.
- Mair M, Blackwell A, 1998. Affect of age and multiple mating on the mating behavior of *Culicoides nubeculosus*. *J Med Entomol* 35:996–1001.
- Marcus V, Weeks SC, 1997. The effect of pond duration on the life history traits of an ephemeral pond crustacean, *Eulimnadia texana*. *Hydrobiologia* 359:213–221.
- Maynard Smith J, 1977. The sex habit in plants and animals. In: *Measuring natural selection in natural populations* (Christiansen FB, Fenchel TM, eds). Berlin: Springer-Verlag; 315–331.
- Medland VL, 1989. Influence of terrestrial vegetation on the production and community structure of a desert playa (MS thesis). Las Cruces: New Mexico State University.
- Medland VL, Zucker N, Weeks SC, 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.
- Molau U, Prentice HC, 1992. Reproductive system and population structure in three arctic *Saxifraga* species. *J Ecol* 80:149–161.
- Naglyaki T, 1976. A model for the evolution of self-fertilization and vegetative reproduction. *J Theor Biol* 58:55–58.
- Otto SP, Sassaman C, Feldman MW, 1993. Evolution of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am Nat* 141:329–337.
- Pannell J, 1997. Widespread functional androdioecy in *Mercurialis annua* L. (Euphorbiaceae). *Biol J Linn Soc* 61:95–116.
- Parker GA, 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm competition and sexual selection* (Birkhead TR, Møller AP, eds). San Diego, California: Academic Press; 3–54.
- Pennak RW, 1989. Fresh-water invertebrates of the United States, Protozoa to Mollusca, 3rd ed. New York: John Wiley.
- Rakitin A, Ferguson MM, Trippel EA, 1999. Sperm competition and fertilization success in Atlantic cod (*Gadus morhua*): effect of sire

- size and condition factor on gamete quality. *Can J Fish Aquat Sci* 56:2315–2323.
- Ramsey M, Vaughton G, 1998. Effect of environment on the magnitude of inbreeding depression in seed germination in a partially self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae). *Int J Plant Sci* 159:98–104.
- SAS Institute, 1989. SAS/STAT users guide, version 6, 4th ed, vol. 2. Cary, North Carolina: SAS Institute.
- Sassaman C, 1995. Sex determination and evolution of unisexuality in the Conchostraca. *Hydrobiologia* 298:45–65.
- Sassaman C, Weeks SC, 1993. The genetic mechanism of sex determination in the Conchostracan shrimp *Eulimnadia texana*. *Am Nat* 141:314–328.
- Schemske D, 1983. Breeding system and habitat effects on fitness components in three neotropical *Costas* (Zingiberaceae). *Evolution* 37:523–539.
- Sih A, Krupa JJ, 1995. Interacting effects of predation risk and male and female density on male/female conflicts and mating dynamics of stream water striders. *Behav Ecol* 6:319–325.
- Spears T, Abele LG, 2000. Branchiopod monophyly and interordinal phylogeny inferred from 18S ribosomal DNA. *J Crustac Biol* 20:1–24.
- Strenth NE, 1977. Successional variation in sex ratios in *Eulimnadia texana* Packard (Crustacea, Conchostraca). *Southwest Nat* 22:205–212.
- Van Voorhies WA, 1992. Production of sperm reduces nematode lifespan. *Nature* 360:456–458.
- Ward S, Carrel JS, 1979. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev Biol* 73:304–321.
- Weeks SC, Crosser BR, Bennett R, Gray M, Zucker N, 2000a. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: estimates of inbreeding depression in two populations. *Evolution* 54:878–887.
- Weeks SC, Crosser BR, Gray MM, Matweyou JA, Zucker N, 2000b. Is there sperm storage in the clam shrimp *Eulimnadia texana*? *Invert Biol* 119:215–221.
- Weeks SC, Hutchison J, Zucker N, 2002. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: do hermaphrodites need males for complete fertilization? *Evol Ecol* 15:205–221.
- Weeks SC, Marcus V, Alvarez S, 1997. Notes on the life history of the clam shrimp *Eulimnadia texana*. *Hydrobiologia* 359:191–197.
- Weeks SC, Marcus V, Crosser B, 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. *Evolution* 53:472–483.
- Weeks SC, Zucker N, 1999. Inbreeding rates in the androdioecious crustacean, *Eulimnadia texana*. *Can J Zool* 77:1402–1408.
- Wells H, 1979. Self-fertilization: advantageous or deleterious? *Evolution* 33:252–255.
- Williams GC, 1975. Sex and evolution. Princeton, New Jersey: Princeton University Press.
- Wolf DE, Reisberg LH, Spencer SC, 1997. The genetic mechanism of sex determination in the androdioecious flowering plant, *Datisca glomerata* (Datisceae). *Heredity* 78:190–204.
- Wood WB, 1988. The nematode *Caenorhabditis elegans*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- Woodhead AP, 1986. Male age: effect on mating behavior and success in the cockroach *Diploptera punctata*. *Anim Behav* 34:1875–1879.
- Zucker N, Aguilar GA, Weeks SC, McCandless LG, 2002. Impact of males on variation in reproductive cycle in an androdioecious desert shrimp. *Invert Biol* 121:66–72.
- Zucker N, Cunningham M, Adams HP, 1997. Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*. *Hydrobiologia* 359:171–175.
- Zucker N, Stafki B, Weeks SC, 2001. Maintenance of androdioecy in the freshwater clam shrimp *Eulimnadia texana*: longevity of males relative to hermaphrodites. *Can J Zool* 79:393–401