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# Is there sperm storage in the clam shrimp *Eulimnadia texana?*

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**Abstract.** Androdioecy is a rare form of mating system in which species comprise males and hermaphrodites. One recently described case of androdioecy is the freshwater crustacean *Eulimnadia texana.* A mathematical model of the mating system of this shrimp suggests that males and hermaphrodites should only coexist under limited circumstances. One possible factor not considered in this model would extend the conditions for coexistence: the possibility of sperm storage in the hermaphrodites. Here we use genetically marked matings between males and hermaphrodites to determine if hermaphrodites can store male sperms. Eggs were collected from hermaphrodites both in the presence of a male and after the male was removed. A total of 30 of these matings had successful hatches, but only 14 of these 30 could be used to test for sperm storage. In these 14 cases, an average of 35% of the eggs were outcrossed when males were present, but only 0.4% were outcrossed after males were removed. Thus, sperm storage by hermaphrodites was an insignificant factor in the production of offspring. These data suggest that sperm storage cannot help explain the coexistence of males and hermaphrodites in natural populations of this crustacean.

*Additional key words:* Conchostraca, Branchiopoda, Crustacea, mating system, androdioecy

Androdioecy is a rare type of mixed mating system in which males coexist with hermaphrodites, but there are no true females (Charlesworth 1984). There are only a few documented cases of androdioecy in plants, including *Datisca glomerata, Mercurialis annua, Saxifraga cernua,* and *Phillyrea augustifolia* (Swensen et al. 1998). Animals exhibiting androdioecy are also quite rare: the nematode *Caenorhabditis elegans* (Barker 1992), the barnacle *Balanus galeatus* (Gomez 1975), several branchiopod crustaceans (Sassaman & Weeks 1993; Sassaman 1995), and the killifish *Rivulus marmoratus* (Lubinski et al. 1995).

Charlesworth (1984) suggested that the rarity of androdioecy is related to the evolutionary instability of this mating system and that most such mating systems are probably evolutionarily transitory. Androdioecious mating systems appear to be anomalous because the benefits of being all-male in a primarily hermaphroditic population are unclear (Charlesworth 1984). Becoming all-male could have two potential benefits: (a) reduced inbreeding depression for male-sired offspring, and (b) increased allocation to male gamete production (relative to male allocation in hermaphrodites). Since fitness through male function is based on the availability of mates, any amount of self fertilization in hermaphrodites reduces potential mates in an androdioecious population, making it difficult for the all-male strategy to be successful (Lloyd 1975; Charlesworth 1984). Therefore, if being all-male is beneficial because of reduced inbreeding depression, but all-male individuals cannot invade primarily selfing populations, the evolution of an androdioecious population becomes paradoxical (Charlesworth 1984). In fact, Charlesworth (1984) reviewed several plant species that were previously classified as androdioecious and found that most were functionally dioecious. Therefore, studies that attempt to understand the maintenance of males in the few described androdioecious species are certainly warranted.

We have begun a series of such studies of one androdioecious species, the clam shrimp *Eulimnadia texana* (PACKARD 1871). Until quite recently, little was known about the reproductive biology of the *E. texana* and its relatives. For example, hermaphroditism was thought to be absent from eubranchiopods and individuals which could produce offspring without a mate being present were assumed to be parthenogenetic (Pennak 1989). In 1993, Sassaman and Weeks provided the first evidence of androdioecy in *E. texana* based

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on genetic studies, and anatomical support for the presence of hermaphrodites, rather than pure females, was obtained by Zucker et al. (1997). Sassaman & Weeks (1993) reported that hermaphrodites comprise two phenotypically similar but genetically different types: "monogenic" and "amphigenic" hermaphrodites. Sex appears to be controlled by a single genetic locus (Sassaman & Weeks 1993), with a recessive allele coding for males (s) and a dominant allele for hermaphrodites (S). The homozygous dominants (SS) are monogenic hermaphrodites, the heterozygotes (Ss) are amphigenic hermaphrodites, and the homozygous recessives (ss) are males (Sassaman & Weeks 1993).

Sexual dimorphism is pronounced. Males and hermaphrodites are of similar size (average adult carapace length, 5–8 mm), but the thoracic appendages of hermaphrodites are unmodified, whereas the first two pairs of thoracic appendages in males undergo differentiation into claw-like claspers. These claspers are used to hold on to the margins of a hermaphrodite's carapace during mating, and because hermaphrodites lack these appendages, they cannot pair with one other for mating. Pairing during mating can last from minutes to hours (Knoll 1995). Knoll (1995) noted that males often mate-guard the hermaphrodite for extended periods (up to 2 h) prior to sperm transfer.

The mechanics of sperm transfer and fertilization are not well known. Previous reports suggest that males transfer spermatophores to hermaphrodites (Strenth 1977; Scanabissi & Tommasini 1994). Strenth (1977) suggests that these spermatophores then enter the gonopore and thus eggs are fertilized internally. However, Knoll (1995) never observed the spermatophore-like structure described by Strenth (1977). According to her observations, sperm transfer occurs while the male thrusts his abdomen between the carapace "valves" of the hermaphrodite; simultaneously eggs are moved from the ovotestis into the phyllopods and then on to the brood chamber dorsally between the fold in the "valves." As soon as the clutch of eggs is in the brood chamber, the male releases the hermaphrodite. Molting was observed on average 11 min prior to male thrusting in 94 of 95 observations (Knoll 1995). After eggs are fertilized, they are kept in the brood chamber for 12–24 h (Weeks et al. 1997).

Populations of *Eulimnadia* are strongly hermaphrodite-biased (Mattox 1954; Zinn & Dexter 1962; Stern & Stern 1971; Sassaman 1989; Weeks & Zucker 1999), which becomes more pronounced as the populations age due to lower relative survival of males (Stern & Stern 1971). Electrophoretic evidence suggests that selfing is a common, but not universal form of producing offspring (Sassaman 1989; Weeks et al. 1999; Weeks & Zucker 1999). The mechanism of self fertilization is undescribed, although Zucker et al. (1997) report that only a small portion of the gonad is allocated to sperm production.

Otto et al. (1993) developed a specific model for the androdioecious reproductive system of *E. texana.* Their model explored the conditions under which a mixed mating system, comprising all 3 mating types (monogenics, amphigenics, and males), can be stable. The model consisted of 4 main parameters: $\alpha$ (the proportion of a hermaphrodite's eggs which are fertilized when a male encounters a hermaphrodite), $\beta$ (the proportion of a hermaphrodite's eggs that are not fertilized by a male but are self-fertilized), $\delta$ (inbreeding depression), and $(1 - \sigma)$ (the relative survival of males to hermaphrodites). Examination of the model found restrictive conditions under which all 3 types can coexist, with most combinations of the above parameters leading to monomorphic populations of monogenic hermaphrodites (Otto et al. 1993). Yet, most natural populations appear to comprise all 3 mating types, with only a few populations being primarily monogenic hermaphrodites (Sassaman 1989; Weeks & Zucker 1999).

One factor that was not considered in the model of Otto et al. (1993) is sperm storage. If hermaphrodites can store male sperms, then males can have a higher fertilization success rate and higher male mortality is less important than assumed in the model. The current experiment was designed to assess the ability of hermaphrodites of *E. texana* to store sperms, thereby allowing us to determine the importance of this function in these crustaceans.

## Methods

### Experimental organism

*Eulimnadia texana* inhabits temporary pools, ponds, ditches, and other ephemeral freshwater habitats throughout the southern United States, west of the Mississippi River, and into northern Mexico (Sassaman 1989). These shrimps produce desiccation-resistant cysts which they bury within the top several millimeters of the soil. The cysts hatch rapidly following hydration under spring and summer conditions (Brendonck 1996), each releasing a nauplius larva. Larval and juvenile growth is extraordinarily rapid. Shrimps reach reproductive size in 4–7 d in the laboratory at 27–30°C (Sassaman & Weeks 1993; Weeks et al. 1997) and in as little as 4–6 d in the field (Vidrine et al. 1987). Hermaphrodites produce thousands of eggs in their lifetime (which averages 14–21 d; Weeks et al. 1997), generating clutches in the range of 100–300 eggs, once or twice a day (Knoll 1995; Weeks et al.

1997). Clutch size increases significantly with carapace length (Knoll & Zucker 1995; Weeks et al. 1997).

## Rearing protocol

Soil containing clam shrimp eggs was collected from Arizona (hereafter AZ; previously reported as "WAL" in Weeks et al. 1999) near Portal in Cochise Co., near the base of the Chiricahua Mountains, and from New Mexico (hereafter NM; previously reported as "JT4" in Weeks et al. 1999) in Doña Ana Co. (south-central New Mexico). Samples were transported back to the laboratories in Akron and Las Cruces. Sub-samples of soil (250 ml) were hydrated using "standard conditions": dechlorinated tap water in 37-liter aquaria housed in an environmentally controlled room under continuous light (Durotest sunlight-simulating fluorescent bulbs), at 25–27°C, and with continuous aeration (see Sassaman & Weeks 1993; Weeks et al. 1997).

Just before reaching sexual maturity (at ~3–4 d) in these aquaria, shrimps were randomly chosen for individual isolation in 500-ml plastic cups filled with dechlorinated tap water and with ~12 g of finely sifted soil (<125 μm diameter) (Marcus & Weeks 1997; Weeks et al. 1999). The soil was collected from a site near the NM site, but in an area known to be free of branchiopod cysts. Shrimps in all cups were fed 1 ml of baker's yeast solution (1 g dried yeast per 100 ml water) per day. Directly before sexual maturation (4–6 d), the shrimps were sexed, and the males were discarded. The hermaphrodites were allowed to produce self-fertilized clutches for up to 7 d, after which the hermaphrodites were frozen for gel electrophoresis. The clutches were slowly dried and stored for future use (see below).

Hermaphrodites were assayed using cellulose acetate (CA) electrophoresis (Richardson et al. 1986). Shrimps were scored for 5 polymorphic loci: *Fum* (fumarate hydratase, EC 4.2.1.2), *Idh*-1 and *Idh*-2 (isocitrate dehydrogenase, EC 1.1.1.42), *Mpi* (mannose-phosphate isomerase, EC 5.3.1.8), and *Pgm*-1 (phosphoglucomutase, EC 5.4.2.2). Gels were run using "Buffer C" from Richardson et al. (1986). Since *Fum*, *Idh*-1, and *Idh*-2 are known to be linked to the sex-determining locus, shrimps heterozygous for any of these electrophoretic loci should be amphigenics (Weeks et al. 1999). Homozygotes for all three can be either monogenics or amphigenics. Both amphigenics and monogenics can be heterozygous or homozygous for the loci that are unlinked to the sex determining locus (*Mpi* and *Pgm*-1; Weeks et al. 1999).

From these electrophoretically-scored hermaphrodites, we chose pairs that were both heterozygous for one or more of the sex-linked loci (*Fum*, *Idh*-1, and *Idh*-2) but that were also alternate homozygotes for at least one of the 5 assayed loci. This pairing assured a choice of 2 amphigenic hermaphrodites (and thus the production of males among the selfed offspring of both egg banks), and that outcrossing between offspring from the two hermaphrodites would be genetically marked and thus distinguishable from a selfing event, using CA electrophoresis (Sassaman & Weeks 1993).

After drying for at least 30 d, egg banks generated from each hermaphrodite in the above pairs were hydrated. The resulting nauplii were transferred into 37-liter tanks, containing aged tap water and soil known to be free of all forms of branchiopod eggs, and reared under standard conditions. When shrimps in each tank grew to near sexual maturity, males from one family group were paired in 500-ml cups with hermaphrodites from the alternate family group (1 male and 1 hermaphrodite per cup). The hermaphrodites were allowed to produce eggs for 3 d; then the male was removed and the hermaphrodite was transferred to a holding cup for 24 h. Any eggs produced via mating and still held in the hermaphrodite's brood chamber would be dropped in the holding cups and these eggs were discarded. After this, the hermaphrodite was moved to a third cup and allowed to produce eggs for another 3 d. The two egg banks generated from each hermaphrodite were marked as "mated period" (for the 3 d when the males were paired with the hermaphrodites) or "post-mated period" (after the male had been removed), and again allowed to dry for 30 d.

Again, after drying, egg banks generated from each period were hydrated as above. When egg banks hatched, the resulting nauplii were transferred into 37-liter tanks containing aged tap water and soil, and again reared under standard conditions. When shrimps in each tank grew to near sexual maturity, they were frozen for gel electrophoresis. Up to 210 shrimps from these hatchings were scored for the marked locus. Homozygotes for this locus were categorized as "selfed" while heterozygotes were categorized as "outcrossed."

## Statistical analysis

Differences in the proportion outcrossed in mated and post-mated periods were analyzed using $\chi^2$ analyses in the JMP statistical package (SAS 1995). These analyses tested whether the proportion of eggs outcrossed was the same in the post-mated (i.e., experimental) relative to the mated (i.e., control) egg banks. These comparisons between mated and post-mated clutches were made at two levels: clutches from single hermaphrodites and totals across all sampled hermaphrodites. In smaller clutches where the expected numbers of offspring for either selfed or outcrossed was

**Table 1.** Outcrossing and selfing rates in mated and post-mated periods. % Out is the percentage of outcrossing in either period. Exp. Out is the expected number of outcrossed shrimps in the post-mated egg banks assuming the rate of outcrossing was the same as in the mated egg banks. Paired total is only those crosses in which both mated and post-mated treatments were successful. † .05<P<.10; *P<.05; **P<.001.

| | Offspring of mated period | | | Offspring of post-mated period | | | |
|---|---|---|---|---|---|---|---|
| Population | Selfed | Outcrossed | % Out | Selfed | Outcrossed | Exp. Out | $\chi^2$ |
| NM | 0 | 17 | 100 | 50 | 0 | 50 | 75.9** |
| NM | 0 | 26 | 100 | 3 | 1 | 4 | 15.0** |
| NM | 1 | 1 | 50 | 30 | 0 | 15 | 6.1* |
| NM | 16 | 14 | 46.7 | 3 | 0 | 1.4 | 3.5† |
| NM | 23 | 2 | 8 | 21 | 0 | 1.7 | 2.5 |
| NM | 14 | 1 | 6.7 | 54 | 0 | 3.6 | 3.1† |
| NM | 27 | 1 | 3.6 | 10 | 0 | 0.4 | 0.6 |
| NM | 0 | 10 | 100 | | | | |
| NM | 1 | 29 | 96.7 | | | | |
| NM | 13 | 0 | 0 | | | | |
| AZ | 12 | 46 | 79.3 | 15 | 0 | 11.9 | 37.1** |
| AZ | 35 | 15 | 30 | 48 | 0 | 14.4 | 22.8** |
| AZ | 10 | 4 | 28.6 | 27 | 1 | 8 | 5.3* |
| AZ | 8 | 3 | 27.3 | 209 | 0 | 57 | 18.8** |
| AZ | 24 | 3 | 11.1 | 5 | 0 | 0.6 | 1.1 |
| AZ | 48 | 6 | 11.1 | 10 | 0 | 1.1 | 2.2 |
| AZ | 48 | 2 | 4 | 4 | 0 | 0.2 | 0.3 |
| AZ | 19 | 0 | 0 | 80 | 0 | 0 | n/a[1] |
| AZ | 12 | 0 | 0 | 27 | 0 | 0 | n/a[1] |
| AZ | 10 | 0 | 0 | 23 | 0 | 0 | n/a[1] |
| AZ | 0 | 16 | 100 | | | | |
| AZ | 9 | 37 | 80.4 | | | | |
| AZ | 18 | 35 | 66 | | | | |
| AZ | 17 | 6 | 26.1 | | | | |
| AZ | 24 | 7 | 22.6 | | | | |
| AZ | 37 | 8 | 17.8 | | | | |
| AZ | 16 | 0 | 0 | | | | |
| AZ | 5 | 0 | 0 | | | | |
| AZ | 1 | 0 | 0 | | | | |
| AZ | 19 | 0 | 0 | | | | |
| Total | 467 | 289 | | 619 | 2 | 169.2 | |
| Paired | 266 | 141 | | 489 | 2 | 170.1 | |

[1] Crosses yielding no outcrossing in mated period.

less than 5, the $\chi^2$ values should be considered approximate (SAS 1995).

## Results

A total of 30 pairs of egg banks were hydrated (10 from NM and 20 from AZ). Of the 30 egg banks from the mated period, all 30 hatched and 22 (73%) displayed some outcrossing (Table 1). The overall outcrossing rate (for all 30 egg banks) was 38%. Considering only the 22 egg banks, the outcrossing rate was 44%. For the NM shrimps, only 1 of 10 egg banks displayed no outcrossing; the overall outcrossing rate for the 9 egg banks was 57%. For the AZ shrimps, 7 of the 20 egg banks displayed no outcrossing, and the overall outcrossing rate for the 13 egg banks was 37%.

Of the 30 egg banks from the post-mated period, only 17 (57%) hatched (Table 1), and 3 of these 17 hatches were from parents that displayed no outcrossing in offspring from either period (Table 1). Thus, 14 pairs of egg banks yielded useful predictions for outcrossed offspring during the post-mated period—offspring that would be evidence for sperm storage. In all 14 cases, there were fewer outcrossed offspring from the post-mated period than from the mated period, but in only 7 of these 14 cases was the degree of outcrossing significantly lower than expected (P<.05). Nevertheless, 170 outcrossed shrimps were

expected out of the 491 offspring that hatched and survived from the post-mated period in these 14 cases, but only 2 offspring were found to result from outcrossing ($\chi^2_{(1)} = 228.7$; $P<.001$; Table 1). For these 14 cases, the average outcrossing displayed in offspring from the mated period was 35%, whereas outcrossing in offspring from the post-mated period was only 0.4%.

## Discussion

Hermaphrodites of *Eulimnadia texana* appear to be unable to store appreciable numbers of male sperms. The lack of significant sperm storage has important ramifications for the evolution of the rare, androdioecious mating system of these shrimps. Early male mortality (Stern & Stern 1971; Sassaman & Weeks 1993) has been thought an important barrier to the maintenance of males in these mixed-mating populations (Otto et al. 1993). If hermaphrodites could store sperms, then even after the males have died, hermaphrodites could continue to use male sperms for outcrossing.

Because sperm storage appears not to be appreciable, the persistence of males in the face of early male mortality needs an explanation. Because hermaphrodites continue to grow with age, and because fecundity is positively correlated with size (Knoll & Zucker 1995; Weeks et al. 1997), it seems that longer survival in males would be selectively advantageous. Any long-lived male would have less competition for mates, and these mates should be larger with more eggs. Thus, the observation that males have shorter lifespans than hermaphrodites (Stern & Stern 1971; Sassaman & Weeks 1993) appears paradoxical.

One potential explanation for this apparent paradox concerns reproductive senescence in hermaphrodites. Egg production in hermaphrodites initially increases with age and size, but then decreases as the shrimps continue to age (Weeks et al. 1997). Also, there is some indication that the fewer eggs produced near the end of life are less likely to be viable (Weeks, unpubl. data; Zucker, unpubl. data). Thus, even though hermaphrodites may survive longer than males, mating quality (in terms of quantity and viability of eggs produced) may actually decline near the end of the hermaphrodite's lifetime. If this is true, then the relative male survival parameter ($1 - \sigma$) in the model of Otto et al. (1993) may overestimate the importance of survival differences between males and hermaphrodites in this species. What should be determined is the relative survival rates during periods of reproductive competence rather than absolute lifespan comparisons.

Another ramification of a lack of sperm storage is that each clutch produced by a hermaphrodite would need to be fertilized by a male for outcrossing to be successful. Because pairings between males and hermaphrodites can last up to 2 hours (Knoll 1995) and each hermaphrodite can have 1–2 clutches per day (Weeks et al. 1997), outcrossing for each clutch may be virtually impossible. Although outcrossed offspring hatch and survive better than selfed offspring, and thus mating with a male appears to be advantageous for hermaphrodites (Weeks et al. 1999), a mixed system of outcrossing and selfing may be maintained because of: (a) the low probability of outcrossing every clutch, (b) less than 100% outcrossing when mating does occur (average 44%; see Table 1), and (c) shorter lifespans for males than for hermaphrodites.

A note should be made of the relative hatching/survival of the egg banks from the mated and post-mated periods in this study. Many fewer post-mated clutches hatched and then survived relative to the mated treatments. This reduced performance is most likely due to two factors. First, as stated above, there is some evidence of reproductive senescence in hermaphrodites of *E. texana* (Weeks et al. 1997). Because the post-mated eggs were all produced later than the mated eggs, the lower quality of eggs produced later in life (see above) may partially explain the reduced performance of the post-mated class of eggs. Second, inbreeding depression has been found at several life-stages (hatching, survival to reproductive maturity, etc.) in these shrimps (Weeks et al. 1999). Because the post-mated eggs were almost entirely self fertilized, they may have been less viable than the outcrossed eggs produced in the mated egg banks.

The mechanism of egg fertilization in *E. texana* is unknown. No intromittant organ has been described in clam shrimps (Tommasini & Scanabissi 1992; Scanabissi & Tommasini 1994), and thus internal fertilization should be difficult, at best. Some male clam shrimps appear to transfer spermatophores to the female (Scanabissi & Tommasini 1994), but it is unclear whether these then enter the gonopore or are used to fertilize the eggs externally. Although transfer of spermatophores has been suggested in *E. texana* (Strenth 1977), we have never witnessed such a transfer, even after hours of video taping the mating process using a dissecting microscope (G. McCandliss & Weeks unpubl. data). From our observations, it appears that males may pass sperms directly into the opening of the folded carapace of the hermaphrodites, with the hermaphrodites then collecting the sperms with their phyllopod appendages (G. McCandliss & Weeks, unpubl. data; Zucker, unpubl. data). If enough sperms were transferred in this way, a balled-up mass could be misinterpreted as a spermatophore, which could ex-

plain the observations of Strenth (1977). Hermaphrodites do hold their newly expelled eggs for a few seconds in their phyllopods before moving them to their brood chamber (G. McCandliss & Weeks, unpubl. data). It is possible that at this point, they mix the male sperms with the eggs for external fertilization. Such a mode of fertilization would suggest that sperm storage is unlikely. However, as conchostracan sperms are ameboid (Scanabissi & Tommasini 1994), some sperms might remain in the brood chamber and thus be able to fertilize a second batch of eggs, under limited circumstances. Because hermaphrodites usually molt between egg clutches (Knoll 1995; Weeks et al. 1997), sperm carryover in the brood chamber is unlikely to occur.

A problem with assuming external fertilization is that the egg is already encapsulated in an egg shell by the time it is extruded from the gonopore into the brood chamber (Zucker et al. 1997). Thus, sperms would need to pass through this egg shell to fertilize the eggs if fertilization were indeed external. A similar situation occurs in many insects, in which the egg shell is laid down before fertilization occurs. In insects, 1–70 "micropyles" allow the sperms to pass through the egg shell to fertilize the eggs (Chapman 1998). Therefore, we suggest that external fertilization could occur in a similar fashion, with pores allowing sperm penetration. The outer shell (termed the tertiary membrane, Belk 1970, 1987) of clam-shrimp eggs is known to be porous, allowing the movement of water in and out of the inner embryo (Belk 1970). One mechanism by which this may occur is via micropyle-like pores, although evidence for this is currently lacking. A second possibility is that the sperms may chemically penetrate the egg shell to accomplish fertilization, although branchiopod sperms lack a well-defined acrosome (Jamieson 1991). Both of these options (pores or chemical penetration) are speculative and need to be tested.

In conclusion, mating experiments suggest that hermaphrodites of *E. texana* are unable to store significant numbers of male sperms after outcrossing. This could be due to the mechanics of fertilization in these shrimps, although the details of fertilization are unknown. A lack of sperm storage suggests that the higher male mortality rates observed in the laboratory and in the field are paradoxical. In future studies we will compare survival of males relative to hermaphrodites in terms of reproductive lifespan rather than absolute lifespan to see if earlier male mortality actually results in many eggs of hermaphrodites being self fertilized.

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## References

Barker DM 1992. Evolution of sperm shortage in a selfing hermaphrodite. Evolution 46: 1951–1955.

Belk D 1970. Functions of the conchostracan egg shell. Crustaceana 19: 105–106.

——— 1987. Embryonic cuticles of *Artemia* during diapause and hatching: insights from comparison with other branchiopoda. J. Crust. Biol. 7: 691–696.

Brendonck L 1996. Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). Hydrobiologia 320: 85–97.

Chapman RF 1998. The Insects: Structure and Function, 4th edition. Cambridge Univ. Press, New York. 770 pp.

Charlesworth D 1984. Androdioecy and the evolution of dioecy. Biol. J. Linn. Soc. 23: 333–348.

Gomez ED 1975. Sex determination in *Balanus (conopea) galeatus* (L.) (Cirripedia Thoracica). Crustaceana 28: 105–107.

Jamieson BGM 1991. Ultrastructure and phylogeny of crustacean spermatozoa. Mem. Queensland Museum 31: 109–142.

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

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

Lloyd DG 1975. The maintenance of gynodioecy and androdioecy in angiosperms. Genetica 45: 325–339.

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.

Marcus V & Weeks SC 1997. The effects of pond duration on the life history of an ephemeral pond crustacean, *Eulimnadia texana.* Hydrobiologia 359: 213–221.

Mattox NT 1954. A new *Eulimnadia* from the rice fields of Arkansas with a key to the American species of the genus (Conchostraca, Limnadiidae). Tulane Stud. Zool. 2: 1–10.

Otto SP, Sassaman C, & Feldman MW 1993. Evolution of sex determination in the conchostracan shrimp *Eulimnadia texana.* Am. Nat. 141: 329–337.

Pennak RW 1989. Fresh-water Invertebrates of the United States, Protozoa to Mollusca, 3rd edition. John Wiley, New York. 628 pp.

Richardson BJ, Baverstock PR, & Adams M 1986. Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies. Academic Press, New York. 410 pp.

SAS Institute Inc. 1995. JMP statistical software. SAS Institute Inc., Cary, NC.

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

——— 1995. Sex determination and the 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.

Scanabissi F & Tommasini S 1994. Functional morphology and ultrastructure of the male reproductive system in the Leptestheriidae (Branchiopoda, Conchostraca). Crustaceana 67: 362–370.

Stern DH & Stern MS 1971. Morphology and culture of *Eulimnadia diversa* (Crustacea, Conchostraca) found in Louisiana. Trans. Am. Microsc. Soc. 90: 483–486.

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

Swenson SM, Luthi JN, & Rieseberg LH 1998. Datiscaceae revisited: monophyly and the sequence of breeding system evolution. Syst. Bot. 23: 157–169.

Tommasini S & Scanabissi F 1992. Morphological and functional aspects of the female gonad of the conchostracan *Leptestheria dahalacensis* Ruppel, 1837 (Crustacea, Branchiopoda), and a comparison with the gonads of other branchiopoda. Can. J. Zool. 70: 511–517.

Vidrine MF, Sissom SL, & McLaughlin RE 1987. *Eulimnadia texana* Packard (Conchostraca: Limnadiidae) in rice fields in southwestern Louisiana. Southwest. Nat. 32: 1–4.

Weeks SC & Zucker N 1999. Rates of inbreeding in the androdioecious clam shrimp *Eulimnadia texana*. Can. J. Zool. 77: 1402–1408.

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 BR 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. Evolution 53: 472–483.

Zinn DJ & Dexter RW 1962. Reappearance of *Eulimnadia agassizii* with notes on its biology and life history. Science 137: 676–677.

Zucker N, Cunningham M, & Adams HP 1997. Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*. Hydrobiologia 359: 171–175.