

Rates of inbreeding in the androdioecious clam shrimp *Eulimnadia texana*

S.C. Weeks and N. Zucker

Abstract: Populations of the clam shrimp *Eulimnadia texana* exhibit androdioecy, which is a mixed mating system composed of males and self-compatible hermaphrodites. It has been suggested that such mating systems are evolutionarily unstable, and yet most populations of *E. texana* appear to exhibit both outcrossing and selfing (a mixed mating strategy). Genetic and sex-ratio features of seven populations of these clam shrimp confirm that the majority of these populations show a mixture of inbreeding and outcrossing modes of reproduction. Additionally, we suggest that the relationship of inbreeding rate with male frequency indicates that mating is not random, as was suggested in a previous model of the mating system of *E. texana*.

Résumé : Les populations d'*Eulimnadia texana* sont androdioïques, ce qui reflète un système d'accouplement mixte dans lequel il y a des mâles et des auto-hermaphrodites compatibles. Un tel système est généralement considéré comme instable du point de vue évolutif et pourtant, la plupart des populations d'*E. texana* se reproduisent aussi bien par fécondation croisée que par auto-fécondation (stratégie reproductive mixte). Les caractéristiques génétiques et les rapports mâles : femelles de sept populations d'*E. texana* confirment que la majorité de ces populations ont des modes de reproduction mixtes, ayant recours à l'auto-fécondation et à la fécondation croisée. De plus, nous croyons que la relation entre le taux d'auto-fécondation et la fréquence des mâles indique que les accouplements ne se font pas au hasard comme le suggérait un modèle antérieur de reproduction chez *E. texana*.

[Traduit par la Rédaction]

Introduction

In "mixed mating" populations, mating occurs via some combination of self-fertilization and outcrossing. Such mating systems are controversial, in that some believe that mixed mating is not evolutionarily stable but is merely a transitional phase in becoming either completely outcrossing or completely selfing (Fisher 1941; Nagylaki 1976; Lande and Schemske 1985; Schemske and Lande 1985). Others see mixed mating systems as evolutionarily stable (Maynard Smith 1977; Lloyd 1979; Charlesworth 1980; Holsinger et al. 1984; Charlesworth et al. 1990; Lande et al. 1994), and recent documentation of several species composed mainly of mixed mating populations lends support to this assertion (Husband and Schemske 1996).

There are three classes of mating systems in which mixed mating appears to be common: cleistogamy–chasmogamy, gynodioecy, and androdioecy (Jarne and Charlesworth 1993). In cleistogamous–chasmogamous mating systems (described only in plants; Lord 1981), a portion of the reproductive effort is devoted to small closed flowers that only self-pollinate (cleistogamous), while the remainder is devoted to open-pollinated outcrossed flowers (chasmogamous). In gynodioecious and androdioecious species, populations are

composed of mixtures of females and hermaphrodites and of males and hermaphrodites, respectively, and the hermaphrodites commonly produce offspring via some intermediate level of self-fertilization (Jarne and Charlesworth 1993). It remains unclear whether gynodioecious and androdioecious species are in a transitional phase between complete hermaphroditism and dioecy (separate sexes, and thus completely outcrossing), or if gynodioecy and androdioecy per se are evolutionarily stable end points (Charlesworth 1984).

Although gynodioecy and androdioecy have been well studied in plants, very little work has been done on analogous species of animals (Jarne and Charlesworth 1993). Those few animal reproductive systems studied to date have little in common, except that the species have one sex that is hermaphroditic and one that is pure (either female in gynodioecy or male in androdioecy). The specific details of each system (e.g., how sex is determined and who may mate with whom) have so far varied from species to species. One recently described androdioecious animal is the freshwater clam shrimp *Eulimnadia texana* (Sassaman and Weeks 1993; Zucker et al. 1997). In this species, males coexist with hermaphrodites of two phenotypically similar but genetically different types (Fig. 1): "amphigenic" and "monogenic" (Sassaman and Weeks 1993). Sex appears to be controlled by a single locus, with a recessive allele coding for males (*s*) and a dominant allele coding for hermaphrodites (*S*). The homozygous dominants (*SS*) are monogenic hermaphrodites, heterozygotes (*Ss*) are amphigenic hermaphrodites, and homozygous recessives (*ss*) are males (Sassaman and Weeks 1993). Populations of *Eulimnadia* spp. are strongly hermaphrodite-biased (Mattox 1954; Zinn and Dexter 1962; Stern and Stern 1971; Sassaman 1989), and electrophoretic evidence sug-

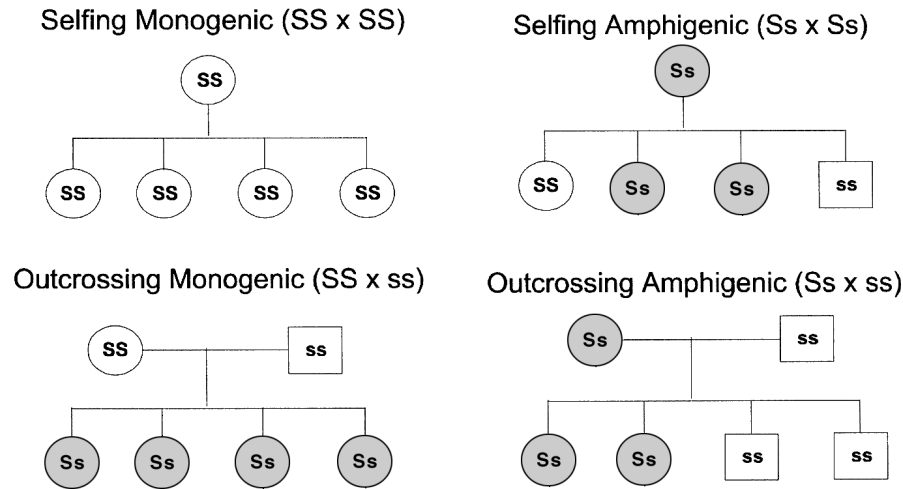
Received January 18, 1999. Accepted May 19, 1999.

S.C. Weeks.¹ Department of Biology, University of Akron, Akron, OH 44325-3908, U.S.A.

N. Zucker. Department of Biology, New Mexico State University, Las Cruces, NM 88003-0001, U.S.A.

¹Author to whom all correspondence should be addressed (e-mail: scweeks@uakron.edu).

Fig. 1. Outline of androdioecious sex-determining system in *E. texana*. Hermaphrodites are either monogenics (SS, open circles) or amphigenics (Ss, solid circles). Offspring production is either via self-fertilization or matings with males (ss, open squares). The figure is patterned after Sassaman and Weeks (1993).



gests that selfing is a common but not universal form of offspring production (Sassaman 1989; Weeks et al. 1999).

Otto et al. (1993) developed a specific model for the androdioecious reproductive system of *E. texana* that explored the conditions under which a mixed mating system, consisting of all three mating types (monogenics, amphigenics, and males), could be stable. Otto et al. (1993) assumed that the proportion of a hermaphrodite's eggs that are fertilized by a male is directly proportional to male frequency, and that mating occurs by "random" encounters between males and hermaphrodites. When males do encounter hermaphrodites, it is assumed that a constant proportion of eggs (α) is outcrossed. Of the proportion of eggs that is not fertilized by a male ($1 - \alpha$), a proportion (β) is then self-fertilized. These fertilization probabilities are then combined with two measures of relative fitness, inbreeding depression (δ) and the relative survival of males to hermaphrodites ($1 - \sigma$), to predict the proportions of the three mating types. Otto et al. (1993) found that all three mating types would coexist if the following inequality were true:

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

If inequality [1] is reversed, then the system should select for complete selfing, which would eventually produce only monogenic hermaphrodites.

In this paper, we examine the extent of inbreeding in natural populations of *E. texana*. We extend Sassaman's (1989) survey of six *E. texana* populations, by examining five additional populations from Arizona and New Mexico, and re-examine two populations from Sassaman's original survey. We report evidence that offspring production in most of the additional populations occurs through outcrossing and self-fertilization, indicating that mixed mating is common in this species. Additionally, we combine estimates of inbreeding with those made by Sassaman (1989), to test whether the level of inbreeding is negatively correlated with the frequency of males in the populations and whether this correlation is consistent with the "random encounter" rate assumed by the model of Otto et al. (1993).

Materials and methods

Natural history of *E. texana*

Until recently, *E. texana* was thought to be parthenogenetic (Pennak 1989). However, the genetic study of Sassaman and Weeks (1993) suggested that these shrimp are actually self-compatible hermaphrodites. The hermaphroditic nature of the formerly described "females" was recently confirmed histologically (Zucker et al. 1997). *Eulimnadia texana* inhabit 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). Hermaphrodites produce desiccation-resistant cysts that they bury within the top several millimetres of the soil. These cysts hatch rapidly following hydration under spring and summer conditions and release nauplii (Brendonck 1996). Larval and juvenile growth is extraordinarily rapid. Shrimp reach reproductive size in 4–7 days at 27–30°C in the laboratory (Sassaman and Weeks 1993; Weeks et al. 1997) and in 4–6 days in the field (Vidrine et al. 1987). Sexual dimorphism is pronounced. The thoracic appendages of hermaphrodites are unmodified, but the first two pairs of thoracic appendages in males undergo differentiation into clawlike claspers that are used to hold on to the margins of a hermaphrodite's carapace during mating. The hermaphrodites produce thousands of eggs in their lifetime, generating clutches ranging in size from 100 to 300 eggs, once or twice a day (Knoll 1995; Weeks et al. 1997). Clutch size increases significantly with carapace length (Knoll and Zucker 1995a; Weeks et al. 1997), but no difference in clutch size was detected between selfed and outcrossed clutches in the laboratory (Knoll and Zucker 1995a).

Rearing protocol and data collection

Soil containing *E. texana* eggs was collected in Doña Ana County, south-central New Mexico, from four sites located on the United States Department of Agriculture Jornada Experimental Range (JD1, JT4, LTER, and SWP5) and one site just east of New Mexico State University's main campus (AMT1). Two additional sites were in Arizona (WAL and YCOM; Portal, Cochise Co.) and correspond to the sites originally designated "Portal 1" and "Portal 2" by Sassaman (1989). These samples were then transported back to laboratories in Akron and New Mexico. Subsamples of soil from all populations were hydrated using dechlorinated tap water. In Akron, samples were hydrated in 37-L aquaria housed in an environmentally controlled room under condi-

Table 1. Percentage of males, location information, sample sizes, and allelic diversity among populations for the seven electrophoretically examined loci (*Pgm-1*, *Pgm-2*, *Mpi*, *Fum*, *Idh-1*, *Idh-2*, *Gpi*).

Location	Population	No. of males	No. of hermaphrodites	Total no. of individuals	Percentage of males	<i>n</i>	A_p	H_e
Las Cruces, N. Mex.	AMT1	18	46	64	28.1	64	2.50	0.20
	JD1	23	106	129	17.8	114	2.00	0.15
	JT4	767	2390	3157	24.3	183	2.00	0.13
	LTER	57	139	196	29.1	26	2.50	0.18
	SWP5	316	757	1073	29.5	169	2.20	0.19
Portal, Ariz.	WAL	333	1377	1710	19.5	229	2.83	0.34
	YCOM	0	35	35	0.0	35	2.00	0.11
	Total	1514	4850	6364	23.8	Mean	2.29	0.19

Note: *n*, genetic sample size; A_p , average number of alleles per polymorphic locus; H_e , average expected number of heterozygotes.

tions of continuous light (Durotest sunlight-simulating fluorescent bulbs) and continuous aeration and at 25–27°C water temperature (see Sassaman and Weeks 1993; Weeks et al. 1997). All aquaria were supplemented with 10 mL of baker's yeast solution (1 g dried yeast per 100 mL water) per day. After reaching sexual maturity in these aquaria (at approximately 5–7 days), shrimp were sexed and subsamples frozen for later electrophoresis. In New Mexico, hydrations of 100 mL of soil were made in plastic storage containers with 4.5 L of water. These tanks were kept in a room with natural daylight plus continuous lighting from incandescent bulbs, in order to bring the water temperature up to 28–30°C. All aquaria were supplemented with 0.5 mL of baker's yeast solution per day. The shrimp reared in New Mexico were used only for sex ratio determinations.

Electrophoretic assays were conducted using cellulose acetate electrophoresis (Richardson et al. 1986). Whole shrimp were ground in 20–50 µL of "homogenizing solution" (Richardson et al. 1986, p. 95). All shrimp were scored for seven 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); *Pgm-1* and *Pgm-2* (phosphoglucosyltransferase, EC 5.4.2.2); and *Gpi* (glucosephosphate isomerase, EC 5.3.1.9). All gels were run using "Buffer C" from Richardson et al. (1986). Allele designations were labeled sequentially, starting with the most anodally migrating allozymes (ranging from "a" to "g"). All alleles were compared across populations to provide uniform scoring designations.

Electrophoretic data analyses

Inbreeding coefficients (*f*), average number of alleles per polymorphic locus (A_p), and expected proportion of heterozygotes (H_e) were generated using the program Genetic Data Analysis (GDA), version 1.0 (Lewis and Zaykin 1997). Confidence intervals for the estimates of inbreeding coefficients were generated using the bootstrapping option in GDA (Lewis and Zaykin 1997).

Statistical analyses relating genetic measures to male frequency (*M*) were conducted using the JMP statistical package (SAS Institute Inc. 1995). Comparisons of numbers of alleles per polymorphic locus and genetic diversity (H_e) to male frequency were conducted using a Spearman's rank nonparametric correlation (SAS Institute Inc. 1995). Relationships between the inbreeding coefficient and male frequency were compared using a stepwise regression, with *M* and M^2 as possible independent factors, to test for linear and quadratic relationships between these two variables.

Results

In the seven *E. texana* populations, the percentage of males ranged from 0 to 30%, with an average of about 24%

(Table 1). Genetic variation was not significantly correlated with percentage of males, although the trends were in a positive direction (Table 1): percentage of males was positively correlated with both numbers of alleles per locus ($n = 7$; Spearman's $\rho = 0.430$) and H_e ($n = 7$; Spearman's $\rho = 0.464$). Since the loci examined were specifically chosen because they were likely to be polymorphic (Sassaman 1989), the proportion of polymorphic loci would not have been an informative statistic to calculate and thus it is not reported. The average number of alleles per polymorphic locus (A_p) ranged from 2.00 to 2.83 (Table 1), which is below the average reported for crustaceans (3.49 ± 0.42 ; mean \pm 95% confidence interval (CI)) but within the range reported for other branchiopods (1.50–2.43; Hedgecock et al. 1982; Innes 1991). In the all-female taxon *Limnadia lenticularis*, which represents the only other genetically analyzed limnadiid, $A_p = 2.00$ (Tinti and Scanabissi 1996). Overall, expected heterozygosity was low, ranging from 0.11 to 0.34 (Table 1), which again is below the average reported for crustaceans (0.41 ± 0.07 ; Hedgecock et al. 1982) but above that found in *L. lenticularis* (0.06; Tinti and Scanabissi 1996).

The inbreeding coefficient (*f*) calculated from all seven loci was significantly greater than zero in six of the seven populations (Table 2), ranging from 0.30 to 1.00. Because *f* assumes that the loci examined are not under selection, a second measure of *f* was calculated using the loci that segregate independently from the sex-determining locus (which, in turn, is likely to be under strong natural selection): *Pgm-1*, *Pgm-2*, *Mpi*, and *Pgi* (Weeks et al. 1999). In all but one population, this second estimate of *f* was equal to or greater than that calculated for all loci (Table 2). For both measures of inbreeding, five of the seven populations showed inbreeding coefficients significantly greater than 0.0 but also significantly lower than 1.0 (Table 2), indicating that these populations could be considered "mixed mating" populations. Assuming reproduction is through either outcrossing to unrelated individuals or selfing, the selfing rate (*s*) can be estimated using the equation $s = 2f/(1 + f)$ (Fyfe and Bailey 1951). Because this estimate of selfing only applies to loci that are not linked to the sex-determining locus, we used *f* values calculated for the unlinked loci to estimate that the selfing rate ranged from 0.44 to 1.00 (Table 2).

As previously suggested (Sassaman 1989), average inbreeding level was negatively correlated with male frequency in the population for both estimates of *f* (all loci, $n =$

Table 2. Inbreeding coefficients (f) and selfing rates (s) for each population studied.

Population	f (all loci)	f (unlinked loci only) ^a	s
AMT1	0.467 (0.124, 0.612)	0.581 (0.020, 0.643)	0.735 (0.039, 0.783)
JD1	0.301 (-0.043, 0.556)	0.556 ^b	0.715 ^b
JT4	0.305 (0.205, 0.475)	0.475 (0.398, 0.531)	0.644 (0.569, 0.694)
LTER	0.345 (0.057, 0.458)	0.283 (-0.040, 0.359)	0.441 (0.000, 0.528)
SWP5	0.369 (0.089, 0.619)	0.597 (0.000, 0.682)	0.748 (0.000, 0.811)
WAL	0.344 (0.039, 0.572)	0.509 (0.365, 0.624)	0.675 (0.535, 0.768)
YCOM	1.000 ^c	1.000	1.000

Note: Inbreeding coefficients are given for all loci and for only the sex-linked loci. Selfing rates are calculated using inbreeding coefficients from the unlinked loci. Values in parentheses are 95% confidence intervals (CI) estimated by bootstrapping across loci.

^aUnlinked loci are *Pgm-1*, *Pgm-2*, *Mpi*, and *Gpi*.

^bNo CI is given because only *Mpi* was polymorphic.

^cNo heterozygotes were found.

7, Pearson's correlation coefficient = -0.819, $p < 0.024$; unlinked loci, $n = 7$, Pearson's correlation coefficient = -0.829, $p < 0.021$; Fig. 2). These data, combined with those of Sassaman (1989), show that the level of inbreeding corresponds to the prediction that selfing will be negatively related to the percentage of males in the population, with the proportion selfed (s) being directly related to the equation

$$[2] \quad s = 1 - 2M$$

where M is the proportion of the population that is male. Substituting this value for s in Fyfe and Bailey's (1951) equation, Sassaman (1989) related f to M by the equation

$$[3] \quad f = (1 - 2M)/(1 + 2M)$$

which is shown as broken lines in Fig. 2. In the Otto et al. (1993) model, it is assumed that selfing is related to $1 - M$ rather than to $1 - 2M$ (assuming that males can fertilize 100% of a hermaphrodite's eggs when outcrossing occurs). Using this model, we would substitute M for $2M$ in [2] and [3], and this is shown as dotted lines in Fig. 2. From comparison of the two models, it emerges that Sassaman's model (1989) is a better predictor of the observed negative relationship between inbreeding and percentage of males in the population than the Otto et al. (1993) model. A quadratic component added to the model explained a significant proportion of the variability only when all loci were examined (Table 3).

Discussion

Inbreeding is clearly a predominant character in most *E. texana* populations. Sassaman (1989), who surveyed six *E. texana* populations, found that the level of inbreeding (f) ranged from 0.35 to 0.97. Our estimates of inbreeding ranged from 0.30 to 1.00, which is consistent with Sassaman's estimates and with that found for a related clam shrimp, *L. lenticularis* ($f = 0.97$; Tinti and Scanabissi 1996). Both the current study and that of Sassaman (1989) found that inbreeding coefficients were significantly greater than zero in 12 of 13 estimates. In this species, the self-compatibility of hermaphrodites and the mixed mating system (androdioecy; Sassaman and Weeks 1993; Zucker et al. 1997) suggest that the high levels of inbreeding arise from high rates of self-fertilization by the hermaphrodites

Table 3. Stepwise regressions for testing linear (M) and quadratic (M^2) relationships among selfing rates and male frequency (M).

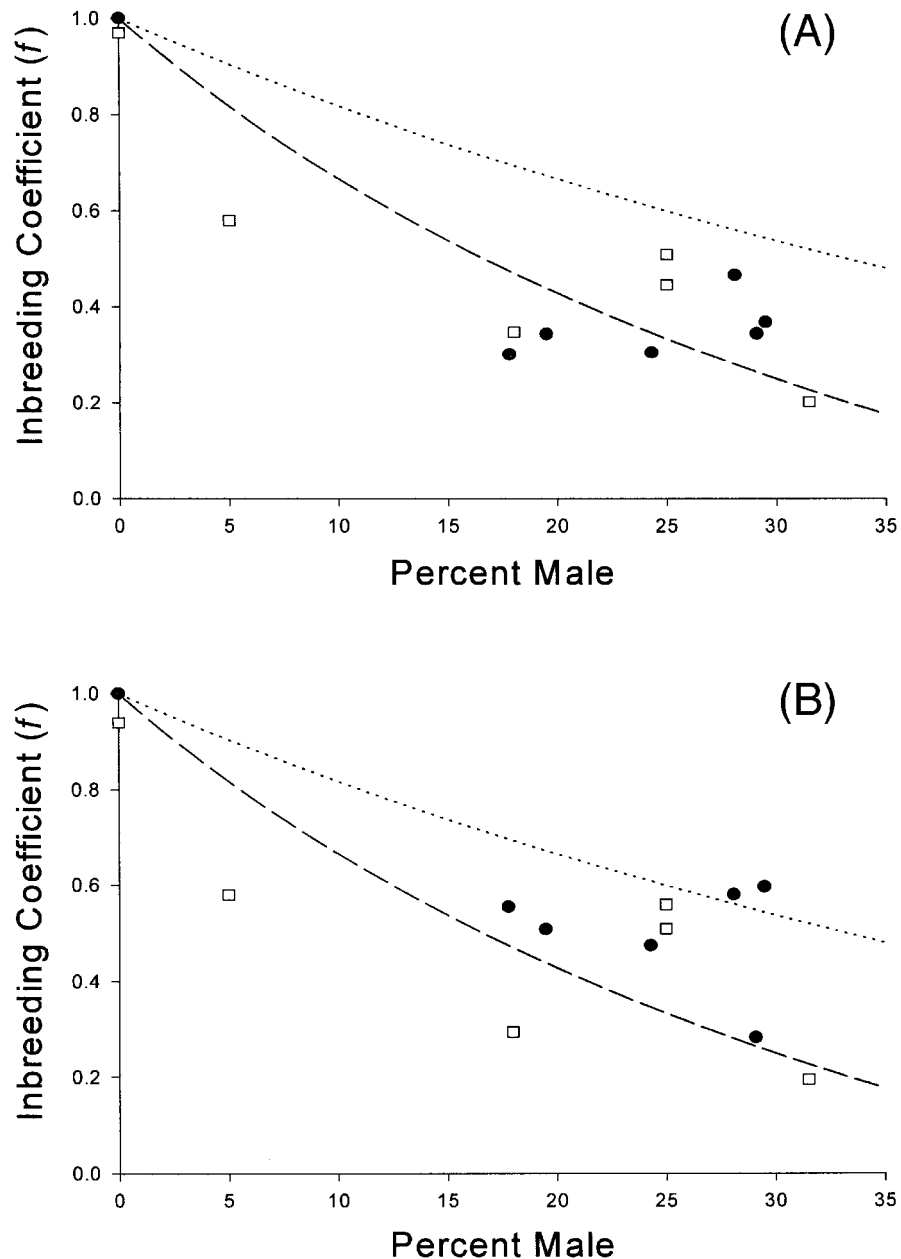
Factor	Estimate	df	Sum of squares	F ratio	p
All loci					
M	-0.049	1	0.238	19.55	0.0013
M^2	0.001	1	0.098	8.02	0.0178
Unlinked loci					
M	-0.016	1	0.362	14.86	0.0027

Note: For both tests, $n = 13$.

(Sassaman 1989). If we assume that offspring production occurs either by selfing or outcrossing with an unrelated male (i.e., that populations are large enough that outcrossing between close relatives is unlikely), then selfing appears to account for between 44 and 100% of the offspring produced in these populations. In fact, even when males are relatively abundant (~30%), estimated selfing rates remain quite high (45–75%). Such high levels of selfing, even with the higher levels of male frequency, suggest that either the hermaphrodites can choose to reject males even when males are abundant (Knoll and Zucker 1995b) or that males are not efficiently fertilizing hermaphrodites. Nonetheless, offspring production in most of the populations appears to be due to a mixture of self-fertilization and outcrossing. Either these populations are examples of a stable "mixed mating" system, or we must assume that they are all simultaneously in transition to dioecy or selfing hermaphroditism.

The fit of the inbreeding coefficients with that predicted by [3] (Sassaman 1989) suggests that outcrossing rates are likely higher than assumed in the Otto et al. (1993) model (i.e., the "random encounter" assumption). Thus, the level of inbreeding drops at a greater rate than one would expect under maximum outcrossing conditions (i.e., $\alpha = 1.0$) in the Otto et al. (1993) model, using either estimate of inbreeding (all or only unlinked loci). The former estimate (including loci both linked and unlinked to the sex-determining locus) should underestimate the level of inbreeding if natural selection is maintaining heterozygosity at the sex-determining locus (Weeks et al. 1999). In fact, most estimates of inbreeding were higher for the unlinked loci than for all loci combined (Table 2), and corresponded to a shallower decline in

Fig. 2. Correlation of inbreeding coefficient and percentage of males in the population for the seven populations in this survey (solid circles) and the six populations reported in Sassaman (1989) (open squares). (A) Inbreeding coefficients estimated from all seven loci. (B) Inbreeding coefficients estimated from only the four loci not linked to the sex-determining locus. In both A and B, the broken line is the expected relationship predicted by Sassaman (1989), whereas the dotted line is that predicted by Otto et al. (1993) (see the text for calculations of expectations).



inbreeding with increased male frequency (Fig. 2B). Nevertheless, even this level of decline of inbreeding with increased male frequency was primarily greater than expected by the Otto et al. (1993) model (Fig. 2B), thus suggesting that rates of outcrossing were higher than those expected using this model.

Behavioral observations also suggest that the assumption of "random encounters" between males and hermaphrodites

(Otto et al. 1993) underestimates true outcrossing rates. As assumed by the Otto et al. (1993) model, encounter rates are positively correlated with male frequency (Hollenbeck et al.).² However, swimming behavior in both males and hermaphrodites appears to allow greater than expected numbers of encounters (e.g., from "seeking" the opposite sex) than one would predict given random encounters based solely on relative frequency (Medland et al.).³

² V.G., Hollenbeck, N. Zucker, W. Gould, and S.C. Weeks. Maintenance of androdioecy in the freshwater shrimp *Eulimnadia texana*: sexual encounter rates and outcrossing success. Submitted for publication.

³ V.L. Medland, N. Zucker, and S.C. Weeks. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana* Parker: encounters between males and hermaphrodites are not random. In preparation.

It should be noted that the level of selfing outlined in the Otto et al. (1993) model is complex, being a combination of encounter rates between males and hermaphrodites, proportion of encounters that result in outcrossing (α), and the ability of the hermaphrodites to successfully self-fertilize eggs that are not fertilized by a male (β). However, even when these parameters are maximal (i.e., both α and $\beta = 1$, as assumed in Fig. 2), the Otto et al. (1993) model predicts outcrossing to be only 50%, even when male frequency is maximal (50%). The combination of the current data with those of Sassaman (1989) suggests that the rate of outcrossing is underestimated in the Otto et al. (1993) model, and that this rate may be better predicted by [3]. A reformulation of the Otto et al. (1993) model is thus warranted ((Hollenbeck et al., see footnote 2; Medland et al., see footnote 3), and such a reformulation is likely to provide a greater range of conditions under which mixed mating can be stable in this reproductive system.

Acknowledgements

We thank M. Kiringoda, C. McCurdy, M. Rucker, V. Singhatat, and D. Starcher for help in the electrophoresis laboratory; V. Marcus and B. Crosser for help in rearing the shrimp in Akron; L. Odom and K. Wilson for help rearing and sexing the shrimp in New Mexico; and B. Crosser, J. Hutchison, and J. Matweyou for critically reading a previous version of the manuscript. This study was funded, in part, by National Science Foundation grants DEB-9628865 and IBN-9614226 to S.W. and IBN-9631042 and BIR-942347 to N.Z.

References

- Brendonck, L. 1996. Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). *Hydrobiologia*, **320**: 85–97.
- Charlesworth, B. 1980. The cost of sex in relation to mating system. *J. Theor. Biol.* **84**: 655–671.
- Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. *Biol. J. Linn. Soc.* **23**: 333–348.
- Charlesworth, D., Morgan, M.T., and Charlesworth, B. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution*, **44**: 1469–1489.
- Fisher, R.A. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.* **11**: 53–63.
- Fyfe, J.L., and Bailey, N.T.J. 1951. Plant breeding studies in leguminous forage crops. I. Natural cross-breeding in winter beans. *J. Agric. Sci.* **41**: 371–378.
- Hedgecock, D., Tracey, M.L., and Nelson, K. 1982. Genetics. *In* The biology of Crustacea. Vol. 2. Embryology, morphology, and genetics. *Edited by* L.G. Abele. Academic Press, New York. pp. 284–404.
- Holsinger, K.E., Feldman, M.W., and Christiansen, F.B. 1984. The evolution of self-fertilization in plants: a population genetic model. *Am. Nat.* **124**: 446–453.
- Husband, B.C., and Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, **50**: 54–70.
- Innes, D.J. 1991. Geographic patterns of genetic differentiation among sexual populations of *Daphnia pulex*. *Can. J. Zool.* **69**: 995–1003.
- Jarne, P., and Charlesworth, D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Syst.* **24**: 441–466.
- Knoll, L. 1995. Mating behavior and time budget of an androdioecious crustacean, *Eulimnadia texana* (Crustacea: Conchostraca). *Hydrobiologia*, **298**: 73–81.
- Knoll, L., and Zucker, N. 1995a. Selfing versus outcrossing in the androdioecious clam shrimp, *Eulimnadia texana* (Crustacea: Conchostraca). *Hydrobiologia*, **298**: 83–86.
- Knoll, L., and Zucker, N. 1995b. Is sexual selection operating in the androdioecious clam shrimp, *Eulimnadia texana* (Crustacea: Conchostraca)? *Hydrobiologia*, **298**: 67–72.
- Lande, R., and Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution*, **39**: 24–40.
- Lande, R., Schemske, D.W., and Schultz, S.T. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution*, **48**: 965–978.
- Lewis, P.O., and Zaykin, D. 1997. Genetic data analysis: computer program for the analysis of allelic data. Version 1.0. Free program distributed by the authors. Available via <http://chee.unm.edu/gda/>.
- Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**: 67–79.
- Lord, E.M. 1981. Cleistogamy: a tool for the study of floral morphogenesis, function and evolution. *Bot. Rev.* **47**: 421–449.
- Mattox, N.T. 1954. A new *Eulimnadia* from the rice fields of Arkansas with a key to the American species of the genus. *Tulane Stud. Zool.* **2**: 3–10.
- Maynard Smith, J. 1977. The sex habit in plants and animals. *In* Measuring natural selection in natural populations. *Edited by* F.B. Christiansen and T.M. Fenchel. Springer-Verlag, Berlin. pp. 315–331.
- Nagyilaki, T. 1976. A model for the evolution of self-fertilization and vegetative reproduction. *J. Theor. Biol.* **58**: 55–58.
- Otto, S.P., Sassaman, C., and Feldman, M.W. 1993. Evolution of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am. Nat.* **141**: 329–337.
- Pennak, R.W. 1989. Freshwater invertebrates of the United States. 3rd ed. John Wiley, New York.
- Richardson, B.J., Baverstock, P.R., and Adams, M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, New York.
- SAS Institute Inc. 1995. JMP statistical software. SAS Institute Inc., Cary, N.C.
- Sassaman, C. 1989. Inbreeding and sex ratio variation in female-biased populations of a clam shrimp, *Eulimnadia texana*. *Bull. Mar. Sci.* **45**: 425–432.
- Sassaman, C., and Weeks, S.C. 1993. The genetic mechanism of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am. Nat.* **141**: 314–328.
- Schemske, D.W., and Lande, R. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution*, **39**: 41–52.
- Stern, D.H., and Stern, M.S. 1971. Morphology and culture of *Eulimnadia diversa* (Crustacea, Conchostraca) found in Louisiana. *Trans. Am. Microsc. Soc.* **90**: 483–486.
- Tinti, F., and Scanabissi, F. 1996. Reproduction and genetic variation in clam shrimps (Crustacea, Branchiopoda, Conchostraca). *Can. J. Zool.* **74**: 824–832.
- Vidrine, M.F., Sissom, S.L., and McLaughlin, R.E. 1987. *Eulimnadia texana* Packard (Conchostraca: Limnadiidae) in rice fields in southwestern Louisiana. *Southwest. Nat.* **32**: 1–4.

- Weeks, S.C., Marcus, V., and Alvarez, S. 1997. Notes on the life history of the clam shrimp *Eulimnadia texana*. *Hydrobiologia*, **359**: 191–197.
- Weeks, S.C., Marcus, U., and Crosser, B.R. 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. *Evolution*, **53**: 472–483.
- Zinn, D.J., and Dexter, R.W. 1962. Reappearance of *Eulimnadia agassizii* with notes on its biology and life history. *Science* (Washington, D.C.), **137**: 676–677.
- Zucker, N., Cunningham, M., and Adams, H.P. 1997. Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*. *Hydrobiologia*, **359**: 171–175.