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A field test of a model for the stability of androdioecy in the freshwater shrimp, *Eulimnadia texana*

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Abstract

The evolution of hermaphroditism from dioecy is a poorly studied transition. Androdioecy (the coexistence of males and hermaphrodites) has been suggested as an intermediate step in this evolutionary transition or could be a stable reproductive mode. Freshwater crustaceans in the genus *Eulimnadia* have reproduced via androdioecy for 24+ million years and thus are excellent organisms to test models of the stability of androdioecy. Two related models that allow for the stable maintenance of males and hermaphrodites rely on the counterbalancing of three life history parameters. We tested these models in the field over three field seasons and compared the results to previous laboratory estimates of these three parameters. Male and hermaphroditic ratios within years were not well predicted using either the simpler original model or a version of this model updated to account for differences between hermaphroditic types ('monogenic' and 'amphigenic' hermaphrodites). Using parameter estimates of the previous year to predict the next year's sex ratios revealed a much better fit to the original relative to the updated version of the model. Therefore, counter to expectations, accounting for differences between the two hermaphroditic types did not improve the fit of these models. At the moment, we lack strong evidence that the long-term maintenance of androdioecy in these crustaceans is the result of a balancing of life history parameters; other factors, such as metapopulation dynamics or evolutionary constraints, may better explain the 24+ million year maintenance of androdioecy in clam shrimp.

Introduction

Elucidating the forces that select for combined (hermaphroditism) vs. separate sexes (dioecy or gonochorism) has been central in the quest to understand both evolutionary transitions between contrasting sexual systems and the evolution of sex allocation (Charnov *et al.*, 1976; Charlesworth & Charlesworth, 1978; Charlesworth, 1984; Schemske & Lande, 1985; Jarne & Charlesworth, 1993; Barrett, 2002; Wolf & Takebayashi, 2004; Weeks, 2012). In plants, dioecy has evolved from hermaphroditism on numerous occasions (Barrett, 2002), and a good deal of theoretical and empirical work has been published to explain these transitions (reviewed in Charlesworth, 2006). Much of this work has focused on the gynodioecious path, whereby male-sterile individuals (females) first invade and establish in hermaphroditic populations (Charlesworth & Charlesworth, 1978), and then, the remaining hermaphrodites gradually allocate increasingly more to male function and phenotype (Charlesworth, 1989; Delph, 2003).

Although the transition from hermaphroditism to dioecy has been thoroughly explored (reviewed in Barrett, 2010), the reverse transition, from dioecy to hermaphroditism, has received far less attention (Weeks, 2012). Ghiselin (1974) provided several verbal models

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outlining possible benefits for deriving hermaphroditism from dioecy, and Charnov (1982) outlined the conditions favouring hermaphroditism over dioecy using the concept of 'fitness sets' (male/female trade-offs). However, there have been few detailed accounts of how hermaphroditism could evolve from dioecy (Wolf & Takebayashi, 2004; Pannell, 2008), and even fewer that consider specifically the most likely paths via intermediate reproductive states (Weeks, 2012).

The dearth of detailed discussions about the evolution of hermaphroditism from dioecy does not reflect the frequency of this transition. Hermaphroditism in animals is, in fact, quite common and is often derived from dioecy. Indeed, with the exception of insects, up to one-third of animal species are hermaphroditic (Jarne & Charlesworth, 1993; Jarne & Auld, 2006). The distribution of hermaphroditism in animals is sporadic, with some higher taxa being primarily hermaphroditic (e.g. platyhelminthes, pulmonate molluscs) and others having few, largely unrelated hermaphroditic representatives (e.g. echinoderms, chordates; Ghiselin, 1974; Bell, 1982; Jarne & Charlesworth, 1993). This distribution points towards frequent transitions from dioecy to hermaphroditism, and indeed, Ghiselin (1974) argued that the majority of hermaphroditic animals are derived from dioecious ancestors (for an alternate interpretation, see Iyer & Roughgarden, 2008). Understanding the taxonomic distribution and evolution of sexual systems in animals thus demands a greater emphasis on the paths from dioecy to hermaphroditism than has hitherto been considered.

Androdioecy has been traditionally viewed as an intermediate evolutionary step between hermaphroditism and dioecy because it was believed that males would require a tremendous fitness gain to be selectively maintained in an otherwise hermaphroditic population (Lloyd, 1975; Charlesworth, 1984). Recently, however, androdioecy has been postulated as the most likely intermediate state in a transition from a sexually dimorphic dioecious reproductive system towards hermaphroditism (Weeks, 2012). Indeed, Weeks (2012) suggests that androdioecious species may be 'trapped' into males and 'female-biased' hermaphrodites that cannot effectively compete with males for outcrossing. Deriving hermaphroditism from a sexually dimorphic species, wherein each sex has been selected for generations to be effective as a single sex, the hermaphrodites may be constrained to only be effective as primarily one sex (most likely female) with only a marginal allocation to gametes of the other sex (usually sperm) for reproductive assurance (Weeks, 2012). In this scenario, males are maintained as the only unit of outcrossing among otherwise self-fertilizing, female-biased hermaphrodites, making a complete evolutionary transition to outcrossing hermaphroditism unlikely. Thus, studies of androdioecious species have been aimed at determining whether, indeed, these species are in a transitional state (Pannell, 2002; Weeks *et al.*, 2006a) and, if not, in exploring the mechanisms that can allow males to stably coexist with hermaphrodites (Otto *et al.*, 1993; Pannell, 1997, 2008; Wolf & Takebayashi, 2004).

One such species in which males commonly coexist with hermaphrodites is the crustacean ('clam shrimp') *Eulimnadia texana* (Sassaman & Weeks, 1993; Weeks *et al.*, 1999, 2000a, 2001a,b; Zucker *et al.*, 2001; Weeks & Bernhardt, 2004). These clam shrimp occupy small, temporary freshwater pools in primarily arid regions from the south-western USA to northern South America. The shrimp are filter feeders and consume detritus from the pool bottom. They grow quickly, reaching reproductive size in approximately 7 days, and have a shortened lifespan (3–4 weeks). They produce multiple clutches of desiccation-resistant cysts which can lie dormant in the pool sediment for years to decades before being rehydrated. In this system, androdioecy has been derived from dioecy (Hoeh *et al.*, 2006; Weeks *et al.*, 2009a) and males coexist with hermaphrodites of two phenotypically similar but genetically different types: 'amphigenic' and 'monogenic' hermaphrodites. Sex appears to be controlled by incipient sex chromosomes (Weeks *et al.*, 2010), with males homozygous at the sex-determining locus (ZZ) and hermaphrodites either heterozygous or homozygous for the alternative allele (ZW or WW, respectively; Weeks *et al.*, 2010); the two hermaphroditic genotypes are distinguished as amphigenics (ZW) and monogenics (WW). Outcrossing in *E. texana* requires a firm grip of the hermaphrodite's carapace by the males to allow sperm transfer during egg extrusion. Lacking claspers, hermaphrodites cannot outcross with other hermaphrodites, which distinguishes this androdioecious system from most plants, but which is similar to other androdioecious animal systems in which only males can sire outcrossed progeny (e.g. nematodes: Riddle *et al.*, 1997; Cutter *et al.*, 2003; fish: Weibel *et al.*, 1999; Mackiewicz *et al.*, 2006).

Androdioecy is rare in both plants and animals, occurring in a number of disparate groups scattered widely among plant and animal clades (Pannell, 2002; Weeks *et al.*, 2006a; Weeks, 2012). A notable exception to this pattern is seen in the *Eulimnadia* (Weeks, 2012), in which androdioecy is found in a number of species and appears to be the ancestral breeding system for the genus (Weeks *et al.*, 2006b, 2009a). Remarkably, phylogenetic reconstruction suggests that androdioecy has been maintained in *Eulimnadia* for at least 24 million years, and likely for much longer (estimated age of *Eulimnadia* = 24–180 million years; Weeks *et al.*, 2006b). The factors that combine to allow this unanticipated long-term persistence of androdioecy in these crustaceans are what we seek to delineate in our continuing studies of this system.

Because *Eulimnadia* hermaphrodites cannot cross with one another, previous models of androdioecy derived with plants in mind (e.g. Lloyd, 1975; Charlesworth,

1984) do not wholly apply to these shrimp. Otto *et al.* (1993) recognized this problem and developed a population genetics model to examine the dynamics of *E. texana*'s mating system. This model predicts the equilibrium frequencies of the three mating genotypes (ZZ males, and ZW and WW hermaphrodites) based on the relative male mating success (α); the proportion of eggs not fertilized by a male that are then self-fertilized by the hermaphrodite (β); the viability of males relative to that of hermaphrodites ($1 - \sigma$); and the inbreeding depression experienced by selfed offspring (δ).

The model assumes that outcrossing rate is related to male frequency, u. The parameters α and u are related in that α^*u is the proportion of eggs that a hermaphrodite fertilizes with male sperm (Otto *et al.*, 1993). While α^*u must lie strictly between 0 and 1 for all u, α is not similarly bounded. In the extreme case where all eggs are fertilized by male sperm if at least one male is present in the population, then $\alpha = 1/u$, which is much greater than one when males are rare (reflecting the fact that relative male mating success is enormous in this case). α is a function that includes several components of male mating success: (i) the number of encounters with hermaphrodites experienced by an average male during its reproductive lifetime, (ii) the probability of outcrossing per encounter and (iii) the proportion of eggs fertilized using male sperm given that mating has occurred (Hollenbeck *et al.*, 2002). The remaining proportion of eggs not fertilized by a male [i.e. $(1 - \alpha u)$] are then available for selfing. The model allows for some proportion, $(1 - \beta)$, of these nonoutcrossed eggs to remain unfertilized. This would occur if some eggs were specifically created for outcrossing, or if the hermaphrodites were unable to produce enough sperm to fertilize all of their eggs in the absence of males. In many clam shrimp, males are found to have a higher mortality rate than hermaphrodites (Strenth, 1977; Knoll, 1995; Zucker *et al.*, 2001). In the Otto *et al.* (1993) model, this mortality difference is defined as $(1 - \sigma)$. Finally, the model provides for the frequently observed decrease in viability (i.e. inbreeding depression, δ) in self-fertilized offspring (Johnston & Schoen, 1994; Byers & Waller, 1999; Crnokrak & Roff, 1999; Crnokrak & Barrett, 2002; Keller & Waller, 2002; Goodwillie *et al.*, 2005).

When the Otto *et al.* (1993) model was originally developed, one aspect of the biology of these crustaceans was not known: in laboratory experiments, WW hermaphrodites have lower fitness than ZW hermaphrodites (Weeks *et al.*, 2001a, 2010). This fitness difference has been explained assuming that expression of deleterious recessive alleles embedded in the nonrecombining W chromosome is expressed in WW hermaphrodites but are masked in ZW hermaphrodites (Weeks *et al.*, 2010). Pannell (2008) has thus modified the original Otto *et al.* (1993) model by adding a parameter (λ) that can account for this difference in hermaphroditic fitness. Specifically, λ describes the inbreeding depression expressed in WW hermaphrodites only due to the homozygosity of the W chromosome, which is masked by the Z chromosome in ZW hermaphrodites. Thus, WW fitness is reduced by the factor of $(1 - \lambda)$ in Pannell's model.

Herein, we report the results of a 3-year study that provides the most representative data set to date for assessing the likelihood of long-term coexistence of males and hermaphrodites under field conditions in these crustaceans. We have estimated three of the four parameters of the Otto *et al.* (1993) model (all but β which has been found in a previous test to be 1, and thus unimportant for the model; Weeks *et al.*, 2001b) in artificial and natural pools in New Mexico. We have used these estimates to test the validity of the Otto *et al.* (1993) model by comparing observed to predicted sex ratios of the ZZ, ZW and WW sex types. Additionally, we have used estimates of λ (J.R. Pannell, P. David & S.C. Weeks, in preparation) to compare how a newer version of the original model (Pannell, 2008) may better explain the observed sex ratios in this androdioecious system.

Materials and methods

We observed clam shrimp populations for 3 years, measuring three of the four parameters (α, δ and σ) of the Otto *et al.* (1993) model under both natural and semi-natural conditions at the USDA/ARS Jornada Experimental Range/NSF-LTER Jornada Site in New Mexico. Observations were made over a 2-month period in the summer of each of the three years: 2003, 2004 and 2005. Unfortunately, rain only occurred in one of those years (2004), and thus, the majority of the data were collected from artificially filled 'cattle tanks' – 2003: 9 cattle tanks; 2004: 10 cattle tanks and 3 natural pools; and 2005: 18 cattle tanks. The metal cattle tanks (eight total) ranged in size from 196 to 799 L (196, 224, 230, 494, 576, 590, 778 and 799 L). In each cattle tank, a plastic lining was added to eliminate the possibility of leaching of nickel. All tanks were placed adjacent to small natural pools from which soil (containing encysted eggs) was transferred inside the tanks (covering approximately 5 cm of the bottom of the tank). The tanks were then filled with well water using a water truck. The naturally filled pools were larger, earthen pools that were man-made, deep and large depressions designed to collect and maintain rainwater for cattle. The hatched clam shrimp were then monitored throughout the course of the hydration (see below) until their deaths, which occurred approximately 2–3 weeks after hydration, as is typical for these crustaceans (Weeks *et al.*, 1997). Because of the nature of their habitats (i.e. short-lived, small pools), these shrimp hatch synchronously 24–48 h after hydration (Simovich & Hathaway, 1997), and thus, only one cohort of shrimp were in these pools at any one time because of applying a single hydration event (cattle tanks) or the single rain event (natural pools).

The cattle tanks and natural pools were treated similarly, and thus, below, we refer to both only as 'pools'.

Estimates of relative male viability ($1 - \sigma$)

Relative male survival ($1 - \sigma$) was estimated by calculating sex ratios for each pool on five separate days post-flood (day 5, 8, 11, 14 and 17). In the pools, we collected shrimp from the entirety of each pool by sampling the perimeter and the middle sections using a large dipnet that was dragged through the water for a prescribed length of time at a steady rate. Total shrimp were then counted per sample, and sex was determined using a hand lens (sexual dimorphism is pronounced with males having claspers on their two anterior pair of trunk limbs which are readily observed with a hand lens). On day five, 250 shrimp were used for two projects (see below) with the remaining shrimp returned to the pool. On days 11 and 17, 50 shrimp were removed, and on day 8, all but 20 shrimp were returned to the pool. On day 14, all shrimp were returned. Because these pools typically contained multiple hundreds to multiple thousands of shrimp, removal of these few shrimp did not significantly affect population estimates.

From these data, we determined numbers of each sex per pool. We calculated average male and hermaphrodite lifespan by noting the numbers of shrimp dying per sampling period and their ages. Because in *E. texana* all shrimp hatch within 24–48 h after hydration (Weeks *et al.*, 1997), shrimp age is simply the number of days after hydration minus 1 day. The sum of the number dying times age divided by total dying gave the average lifespan (Farner, 1945). Relative male survival ($1 - \sigma$) was then calculated as the ratio of male to hermaphrodite average lifespan (Zucker *et al.*, 2001).

Additionally, we calculated population density of the artificially filled pools by measuring average depth of the pool and the known diameters of the cattle tanks and then using the formula for the volume of a cylinder. We could not estimate the population density of the natural pools because we could not easily measure volume nor did we do a thorough sampling for total population size.

Measuring male mating success (α) and inbreeding depression (δ) in the field

To estimate both α and δ from field-collected hermaphrodites, we compared size and fecundity with multilocus heterozygosity of shrimp collected at three time periods during the life of a pond (days 5, 11, 17). For each time period, 50 hermaphrodites were collected and measured, and eggs were counted. For the latter, the eggs were removed from the hermaphrodites' brood chambers and then counted under a microscope. The hermaphrodites were then frozen for cellulose acetate (CA) electrophoresis. Unfortunately, due to an ultra-low freezer failure, all of the frozen shrimp from 2003 and from several other pools in 2004 were lost; thus estimates of α and δ could not be determined for these pools.

Shrimp were scored for five electrophoretic loci: *Fum* (fumarate hydratase, EC 4.2.1.2), *Idh-1*, *Idh-2* (isocitrate dehydrogenase, EC 1.1.1.42), *Mpi* (mannose-phosphate isomerase, EC 5.3.1.8) and *Pgm* (phosphoglucomutase, EC 5.4.2.2). Electrophoretic analyses were performed using CA gel electrophoresis using 'buffer C' from Richardson *et al.* (1986). Alleles were scored from 'a' to 'e', based on relative mobility.

We used the program RMES (David *et al.*, 2007) to estimate selfing rate (s) using the maximum likelihood option. The RMES program was designed to estimate selfing rates without using heterozygote deficiencies, and therefore, in a way, that is insensitive to scoring biases (unlike F_{is}-based methods). However, the program does not take into account the peculiar genetic structure created by the androdioecious sexual system found in *Eulimnadia* spp. Therefore, we modified the program to account for these specificities (Appendix 1). Note that differential survival of amphigenic and monogenic hermaphrodites can bias the estimation of s; for this reason, we used the assumption $\lambda = 0.5$, which is a reasonable estimate given our previous data (Weeks *et al.*, 2001a, 2010; Pannell, 2008). Additionally, the likelihoods from the modified RMES were highest for $\lambda = 0.5$ than for $\lambda = 0$. For this analysis, only shrimp collected at day 5 of the experiment were used to estimate selfing rate. The selfing rate and male sex ratio (u) were used to determine α in the following way: $\alpha = (1 - s)/u$ (Otto *et al.*, 1993).

Individual shrimp were then assigned a 0 or 1 for homozygosity or heterozygosity, respectively, at each locus (Weeks *et al.*, 1999). The sum of these values for the five loci determined the 'individual heterozygosity' score for each shrimp. Shrimp were then divided into 'heterozygosity classes' (het classes) and were compared for relative size and fecundity, pooled across collection time (as in Weeks *et al.*, 1999). As detailed in an earlier study of these shrimp (Weeks *et al.*, 1999), the best estimate of inbreeding depression (δ_1) was calculated as one minus the ratio of fecundity of the most homozygous (0 het class) to the most heterozygous class (3+ het class) for each population. To estimate lifetime inbreeding depression (δ_2), we combined these estimates of δ_1 with the estimate of early inbreeding depression (before sexual maturity) from Weeks *et al.* (1999): $\delta_2 = 1 - (0.56 \times \delta_1)$ (see Weeks *et al.*, 2000a).

Field estimates of sex ratios

To test the Otto *et al.* (1993) and Pannell (2008) models, we estimated the proportion of males, monogenics and amphigenics among naturally and artificially filled

ponds. Proportion of males was determined by counts of males vs. hermaphrodites (based on appendage morphology) in the field. Distinguishing monogenic from amphigenic hermaphrodites is not as simple: there is no morphological distinction between the two forms. Therefore, a combination of allozyme surveys and progeny rearing was necessary to determine relative proportions of the two hermaphroditic forms (Sassaman & Weeks, 1993), and this was combined with the morphological estimates of males to determine overall sex ratios of the three forms (Weeks *et al.*, 1999).

For this more detailed sex ratio comparison, we collected and sexed approximately 200 shrimp on day 5. We preserved the males, and, on site, we individually isolated the hermaphrodites in 500-mL plastic cups. We allowed the hermaphrodites to produce eggs for 2 days within the cups. The hermaphrodite was then removed, and the cups were allowed to dry. We froze the hermaphrodites for CA enzyme analysis for later scoring of the three allozyme loci known to be located on the sex chromosomes (Weeks *et al.*, 2010): *Fum*, *Idh*-1 and *Idh*-2. Using this electrophoretic scoring, we identified amphigenics (heterozygous for one or more of these three loci; Weeks *et al.*, 1999). We then hydrated the cups of eggs from those shrimp found to be homozygous for all three loci. The resulting offspring were then sexed upon sexual maturity. These sex ratios were then used to determine which hermaphrodites were amphigenic (producing approximately 25% males when selfed) or monogenics (producing 100% hermaphrodites when selfed; Sassaman & Weeks, 1993), and these data were then combined with the allozyme scores and male proportions to calculate overall sex ratios of the three sex types (Weeks *et al.*, 1999).

Iterative predictions of the Otto *et al.* (1993) and Pannell (2008) models

Iterative predictions of the Otto and Pannell models were carried out using a Perl script. The iterative equations used for predicting the male, monogenic and amphigenic sex ratios were eqn.(1) from Otto *et al.* (1993) and eqns. (1b) to (1d) from Pannell (2008). A randomly selected set of initial sex ratios for the three sexes (males, u; amphigenics, v; monogenics, w) were generated, and then, values of α, δ and σ were selected among the estimates of these parameters for the two years separately (2004 and 2005). For the Pannell model, values of λ were specified, ranging from 0 to 1 in 0.1 increments (one entire run for each value of λ). After choosing these seven variables, the predicted values for u, v and w were generated using the appropriate model. These new estimates of u, v and w were then iterated (keeping the α, δ and σ values constant) until the predicted sex ratios either converged to a stable solution or when the number of iterations reached maximum threshold (100) (in our calculation, the maximum threshold was never reached). At this point, the values of u, v and w were recorded. This process was repeated 100 times, each time using a different draw of the α, δ and σ values from the pool of estimates for the 2 years. After these 100 estimates were completed, the program would record the mean and standard deviations for the 100 runs. We used five sets of initial sex ratios to examine whether the final predicted population sex ratios would depend on the randomly chosen initial sex ratios. For both models, the final predicted population sex ratios did not depend on the initial conditions.

Results

Lifespan

Average lifespan for males and hermaphrodites were similar, ranging from 8 to 10 days for the artificial pools and 8 to 14 days for the natural pools (Table 1). This difference of approximately 2 days between natural and artificial pools was significant (males: $F_{1,38} = 6.07$; $P = 0.0184$; hermaphrodites: $F_{1,38} = 7.02$; $P = 0.0117$). However, there was no significant difference among populations or among years in average lifespan for the artificial pools for either sex ($P > 0.15$ for all comparisons). Additionally, across all pools, there was no difference between males and hermaphrodites in average lifespan ($F_{1,78} = 1.10$; $P = 0.2981$). This lack of lifespan difference was reflected in the estimates of $1 - \sigma$ that averaged close to one (mean = 1.05; range 0.98–1.11; Table 1). Indeed, there were no differences in estimates of $1 - \sigma$ among the five populations raised in the artificial pools ($F_{4,32} = 1.49$; $P = 0.2277$) nor between the natural and artificial pools ($F_{1,38} = 0.08$; $P = 0.7774$). Additionally, there was no correlation between density and $1 - \sigma$ in the artificially filled pools ($F_{1,35} = 2.75$; $P = 0.1060$).

Table 1 Average lifespans (in days) for males and hermaphrodites in naturally (top rows) and artificially (bottom rows) filled pools.

Population	*N*	Males	SE	Hermaphrodites	SE	$(1 - \sigma)$	SE
Forsling	1	7.8		7.0		1.11	
Nelson	1	14.3		13.4		1.07	
Stablein	1	12.0		12.0		1.00	
Average		11.4		10.8		1.06	
JT4	9	8.6	0.4	8.8	0.5	0.98	0.05
SWP3	5	8.8	0.6	8.6	0.3	1.01	0.04
SWP4	10	8.8	0.5	8.3	0.3	1.06	0.04
SWP5	7	10.1	0.4	9.5	0.4	1.06	0.04
SWP5a	6	9.4	0.3	8.6	0.2	1.09	0.03
Average		9.1		8.8		1.05	
Overall average		10.0		9.5		1.05	

Relative lifespan ($1 - \sigma$ from Otto *et al.*'s model) is also reported.

Egg production

Egg production differed among heterozygosity classes and was affected by sampling year as well as age of the shrimp (Tables 2 and 3). The two naturally filling pools (Forsling and Nelson) had much higher average egg production (Tables 2 and 3), likely because 2004 (when we sampled these pools) had higher average egg production than 2005 and because clam shrimp lived longer in these two pools, which would translate into higher numbers of older (and thus larger) hermaphrodites in the later sampling dates (i.e. day 17). When age and year were accounted for, however, there was no significant difference between populations in egg production. On average, egg production was significantly higher for more heterozygous relative to homozygous hermaphrodites (Table 2). This difference among heterozygosity classes was the same across all six populations (Table 2), suggesting that the effects of inbreeding did not differ among any of the sampled populations. Average adult inbreeding depression (δ_1) ranged from −0.07 to 0.49 (average = 0.23), and average estimated lifetime inbreeding depression (δ_2) ranged from 0.40 to 0.71 (average = 0.57).

Sex ratios

Male proportion did not significantly differ among populations ($F_{4,30} = 2.07$; $P = 0.1095$) or across years ($F_{2,30} = 0.073$; $P = 0.9294$; Fig. 1). Average male sex ratios ranged from 20% to 27%, and the three naturally filled pools (Forsling, Nelson and Stablein) all fell well within the range of the five artificially filled pools (Fig. 1).

Sex ratios were estimated from an additional 18 populations from field-collected soil hydrated in the laboratory (Table 4). Although individual population data were spotty, the aggregate sex ratios were exceptionally close to those from both artificial and natural hydrations (Table 4). In fact, a contingency table analysis of the three methods of sex ratio estimation found no significant difference among the estimates (Likelihood Ratio $\chi^2 = 0.824$; $P = 0.6623$), even with counts in the tens of thousands, suggesting that these methods were very similar. The overall estimate of male percentage across all three estimation methods was 23.3%, which, as noted above, is similar to that reported for ten *E. texana* populations in a previous study (Weeks *et al.*, 2008).

Table 2 Results for restricted maximum likelihood, blocked, two-way ANOVA for egg production.

Source	d.f.	*F*-ratio	*P*-value
Year	1	76.37	< 0.0001
Age	4	44.47	< 0.0001
Population	5	2.20	0.1770
Heterozygosity	3	3.56	0.0138
Pop*Het	15	1.02	0.4278

The nested factor was pools nested within populations, and the total sample size (n) was 1181 sample shrimp. There were 1–3 pools per population per year.

The frequencies of all three genotypes at the sex-determining locus (monogenic hermaphrodites, amphigenic hermaphrodites and males) were estimated from a total of 14 pools, five each from JT4 and SWP3 and four from SWP4. There was no significant difference among populations for any of the three sexes ($P > 0.05$ for all comparisons; Fig. 2). There was, however, a significant difference between the 2 years for both the monogenics ($F_{1,8} = 7.33$; $P = 0.0268$) and amphigenics ($F_{1,8} = 5.38$; $P = 0.0490$). There was no difference between years for the males ($F_{1,8} = 0.08$; $P = 0.7813$; Fig. 2). There was no significant population by year interaction ($P > 0.40$ for all three sexes).

Selfing rates

Estimates of selfing rates ranged broadly from 0 to 0.9 (average = 0.47; Table 5). In the four populations where selfing rates were estimated over years (JT4, SWP3, SWP4 and SWP5), there was no significant difference in these estimates among populations ($F_{3,11} = 0.632$; $P = 0.6094$). Even though selfing rates in 2004 were substantially higher (0.58 ± 0.09) than in 2005 (0.33 ± 0.08; Table 5), this difference was only marginally significant ($F_{1,11} = 4.2748$; $P = 0.0630$). Selfing rates were not significantly correlated with per cent male or density, either when adjusting for differences between years (male %: $F_{1,13} = 1.9362$; $P = 0.1874$; density: $F_{1,13} = 2.1317$; $P = 0.1680$) or not (male %: $F_{1,14} = 0.8532$; $P = 0.3713$; density: $F_{1,14} = 1.0163$; $P = 0.3305$).

Testing the Otto *et al.* and Pannell models

To compare these model predictions, we examined an array of λ values for the Pannell (2008) model, ranging from 0 to 1 in 0.1 increments, and used an average estimate of $\lambda = 0.5$ (J.R. Pannell, P. David & S.C. Weeks, in preparation) for general comparisons. Because we consistently found no difference among populations for any of the three parameters estimated in this study (α, δ and σ), we combined all population estimates of these parameters to compare the two models. Also, because we found substantial differences between 2004 and 2005 for sex ratios (Fig. 2), egg production (Table 2) and selfing rates (Table 5), we compared the observed sex ratios to those predicted by the Otto *et al.* and Pannell's modification of that model using values of these parameters specific for 2004 and 2005 (Table 6). We used an iterative approach to produce the predicted proportions of the three sexes, which we then compared to the actually observed sex ratios for these two years (Fig. 3).

Table 3 Average egg production per hermaphrodite for each sampled pool, by heterozygosity class.

			Heterozygosity					
Population	Year	Pool	0	1	2	3+	δ_1	δ_2
Forsling	2004	1	805 (72)	969 (65)	987 (136)	932 (290)	0.136	0.516
Nelson	2004	1	967 (76)	925 (84)	935 (90)	1007 (141)	0.040	0.462
JT4	2004	1	54 (3)	57 (4)	60 (10)	67 (15)	0.194	0.549
JT4	2004	2	254 (37)	332 (44)	268 (50)	366 (69)	0.306	0.611
JT4	2004	3	29 (2)	38 (4)	30 (4)	41 (5)	0.293	0.604
JT4	2005	1	17 (2)	22 (2)	21 (3)	22 (17)	0.227	0.567
SWP3	2004	1	65 (7)	66 (7)	74 (6)	122 (17)	0.467	0.702
SWP3	2005	1	89 (10)	62 (7)	94 (14)	99 (16)	0.101	0.497
SWP3	2005	2	61 (7)	55 (4)	70 (7)	61 (10)	0.000	0.440
SWP4	2004	1	65 (14)	54 (9)	85 (13)	92 (17)	0.316	0.617
SWP4	2005	1	50 (6)	63 (6)	84 (9)	98 (9)	0.490	0.714
SWP4	2005	2	45 (6)	39 (3)	44 (4)	42 (9)	−0.071	0.400
SWP4	2005	3	132 (0)	204 (39)	206 (58)	211 (78)	0.374	0.650
SWP5	2004	1	212 (16)	233 (15)	273 (22)	306 (28)	0.307	0.612
						Total average	0.227	0.567
						2004 Average	0.238	0.573
						2005 Average	0.217	0.561

Inbreeding depression (δ) was calculated for only the adult state (δ_1) and estimated for the entire lifespan (δ_2) by assuming an inbreeding depression of 0.44 for early inbreeding depression (Weeks *et al.*, 2000a).

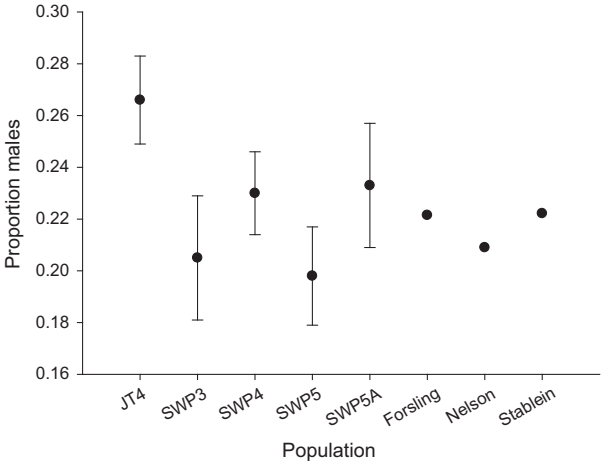

Fig. 1 Male proportion by population. Error bars portray one standard error of the mean over years. The three natural populations (Forsling, Nelson and Stablein) have no error bars because they were only filled in one year (2005).

Both versions of the model overestimated male proportion and underestimated amphigenic proportion, in both years (Fig. 3). In the current comparison, the original version of the model (Otto *et al.*, 1993) predicted a higher proportion of males (2004 = 30%; 2005 = 41%) than in a previous study (10–27%; Weeks *et al.*, 1999) and was at the high end of the estimates of a second study (19–36%; Weeks *et al.*, 2001a). Interestingly, the current estimates of monogenic vs. amphigenic ratios significantly changed between 2004 and 2005, with significantly higher estimates of amphigenics in 2004 than in 2005 (male proportion was relatively constant between years; Figs 2 and 3). The significant difference in reproductive output and selfing rate between years underscores the variation in estimates of the parameters of these models and suggests that equilibrium conditions may not have been met in these pools. If this is the case, then one could argue that the previous year's estimates of α, σ and δ are the best prediction of the following year's sex ratios (Otto *et al.*, 1993), which we have plotted onto the 2005 sex ratios displayed in Fig. 3 (bottom panel). Using this logic, the estimates of α, σ and δ from 2004 predict observed sex ratios more closely in 2005 using the Otto *et al.* model, whereas the 2004 parameter estimates (Table 6) still overestimate males and underestimate monogenics (amphigenic predictions are close to observations) percentages in 2005 when using the Pannell's modification of the model (Fig. 3).

Discussion

The maintenance of androdioecy in the genus *Eulimnadia* for at least 24 million years (Weeks *et al.*, 2006b) defies many notions of the 'short-term' nature of androdioecy (Lloyd, 1975; Charlesworth & Charlesworth, 1978; Charlesworth, 1984) and begs the question of what conditions in these crustaceans allow for such coexistence (Weeks, 2012). Otto *et al.* (1993) defined the conditions under which the maintenance of males and hermaphrodites could be stable in these shrimp, and recently, Pannell (2008) modified this original

Table 4 Aggregate sex ratio data for shrimp from artificially hydrated pools in the laboratory and field, and from naturally hydrated pools in the field.

Population	Males	Hermaphrodites	Male%
Artificially Filled in Laboratory			
Ares	22	84	20.8
Bromit	37	93	28.5
Cassy	40	98	29.0
CCC	0	3	0.0
Engho	2	6	25.0
Estel	27	73	27.0
Forsling	22	84	20.8
Hayden	13	52	20.0
Mesquite	0	3	0.0
Miller	1	25	3.8
Nelson	0	1	0.0
Price	23	78	22.8
Stablein	2	5	28.6
Walker	3	6	33.3
Tank 5	0	6	0.0
Tank 7	0	1	0.0
Tank 10	1	5	16.7
Tank 11	27	90	23.1
	220	713	23.6
Artificially Filled in Field			
JT4	4499	12 949	25.8
SWP3	2127	8231	20.5
SWP4	4389	14 627	23.1
SWP5	524	2228	19.0
SWP5A	434	1291	25.2
	11 973	39 326	23.3
Naturally Filled in Field			
Forsling	33	116	22.1
Nelson	28	106	20.9
Pearson	1	9	10.0
Stablein	2	7	22.2
	64	238	21.2

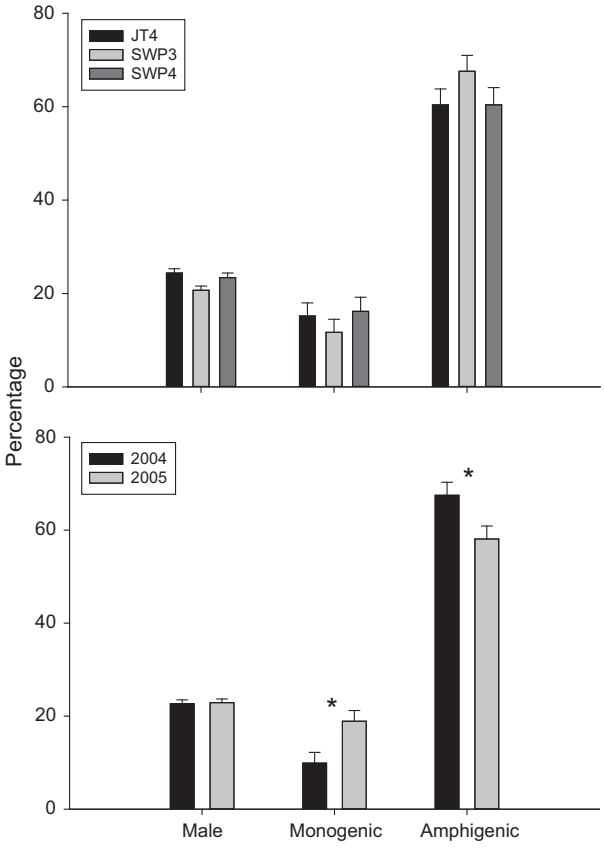

Fig. 2 Sex ratios for all three mating types, grouped by population and by year. Error bars portray one standard error of the mean. *Significantly different at the $P < 0.05$ level.

model by incorporating a known fitness difference between monogenic and amphigenic hermaphrodites (Weeks *et al.*, 2001a, 2011). The Otto *et al.* model has previously been tested in the laboratory (Weeks *et al.*, 2000a, 2001b; Zucker *et al.*, 2001) and in the field with laboratory-reared eggs (Weeks & Bernhardt, 2004), but not under wholly field conditions. Additionally, Pannell's update of the Otto *et al.* model has never been assessed with field or laboratory data. Herein, we report estimates of the original and updated models using data collected from shrimp reared in the field, either in artificially or naturally filled pools.

The biggest difference between the field- and laboratory-reared shrimp was in terms of relative lifespan. Average lifespan in the field ranged from approximately 9 to 11 days (Table 1) and was slightly higher for the naturally filled relative to the artificially filled field pools. These averages were lower than average lifespans reported from the laboratory (Zucker *et al.*, 2001): lifespans in the laboratory ranged from 11 to 18 days. This difference may indicate a more benign laboratory environment, which is not uncommon for laboratory vs. field comparisons (Crnokrak & Roff, 1999).

The most divergent finding in this study relative to previous findings is the parity of lifespans between males and hermaphrodites. In the Otto *et al.* (1993) model, relative lifespans are noted by the level of the value of $(1 - \sigma)$: values <1 suggest hermaphrodites survive longer than males, >1 the reverse and a value of 1 is lifespan parity. In the current study, males lived as long as or slightly longer than hermaphrodites (average $1 - \sigma = 1.05$; Table 1). In previous laboratory comparisons, $1 - \sigma$ ranged from 0.67 to 0.94 (Zucker *et al.*, 2001). Interestingly, in this previous work, males had greater parity in lifespan when they had greater mating opportunities. Zucker *et al.* (2001) proposed that males expend a great deal of energy searching for mates, while the mate guarding that occurs during paired mating requires less energy. They suggested that this relationship of higher energy expenditure with decreased mating opportunities might explain the differential survival they reported. A recent study supports this inference: males expend significantly more energy than

Table 5 Selfing rates by population and pool.

Population	Year	Pool	Selfing*	Male %	Density	α
Forsling	2004	1	0.68 (0.47, 0.79)	22.1	–	1.4
Nelson	2004	1	0.86 (0.83, 0.88)	20.9	–	0.7
JT4	2004	1	0.73 (0.60, 0.79)	22.4	11.6	1.2
JT4	2004	2	0.55 (0.31, 0.68)	25.5	0.5	1.8
JT4	2004	3	0.00 (0, 0.38)	24.3	23.4	4.1
JT4	2005	1	0.00 (0, 0.44)	34.3	0.6	2.1
JT4	2005	2	0.44 (0.11, 0.60)	25.5	16.5	2.2
SWP3	2004	1	0.81 (0.74, 0.84)	22.5	4.9	0.8
SWP3	2004	2	0.43 (0.25, 0.55)	20.7	1.8	2.7
SWP3	2005	1	0.50 (0.22, 0.63)	18.1	3.4	2.8
SWP3	2005	2	0.31 (0.06, 0.46)	19.5	9.4	3.5
SWP3	2005	3	0.08 (0.00, 0.35)	22.0	5.3	4.2
SWP4	2004	1	0.54 (0.31, 0.67)	31.7	1.6	1.5
SWP4	2005	1	0.38 (0.04, 0.55)	25.3	5.4	2.5
SWP4	2005	3	0.47 (0, 0.68)	18.8	1.3	2.8
SWP4	2005	4	0.43 (0.07, 0.61)	22.1	4.3	2.6
SWP5	2004	1	0.73 (0.64, 0.79)	20.6	0.8	1.3
SWP5	2005	2	0.33 (0, 0.56)	13.1	1.7	5.1
SWP5A	2005	1	0.35 (0, 0.61)	27.9	1.0	2.4
		Overall	0.47 (0.43, 0.51)	23.0	5.5	2.4
		2004	0.59 (0.42, 0.76)	23.4	6.4	1.7
		2005	0.33 (0.23, 0.43)	22.7	4.9	3.1

α are calculated as (1 − Selfing)/Male% (see Otto *et al.*, 1993). Density is numbers per litre.
*Parenthetical values are upper and lower 95% confidence intervals.

Table 6 Estimates of the parameters of the Otto *et al.* (1993) and Pannell (2008) models by year.

Parameter	2004	2005
α	1.7	3.1
$1 - \sigma$	1.03	1.05
δ	0.57	0.56

λ was estimated elsewhere at 0.5, and we used estimates from 0 to 0.9, at increments of 0.1, to assess the Pannell model.

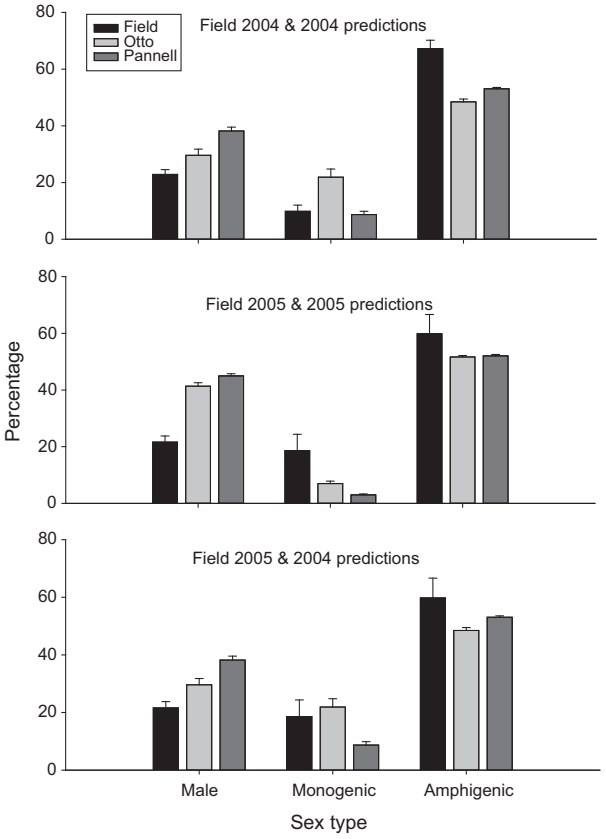

Fig. 3 Predicted and observed sex ratios for the three mating types by year. Black bars portray observed sex ratios, and the grey bars portray predictions from the Otto *et al.* (lighter) and the Pannell (darker) models. Error bars portray two standard errors of the mean.

hermaphrodites when the clam shrimp are in isolation (Weeks *et al.*, 2011), likely due to a much higher rate of swimming (up to 3 times as fast) for males in isolation relative to isolated hermaphrodites (Medland *et al.*, 2000). In the current study, although sex ratios were similar to previous estimates of sex ratios from laboratory studies (Weeks *et al.*, 2008), the densities in the artificially filled pools were higher than those of the laboratory populations. If those densities allowed males to find mates very easily, because of high encounter frequency, then the relative lifespans of the two sexes may have equalized, giving the observed $1 - \sigma$ values close to one. However, we did not find a positive relationship between $1 - \sigma$ and density. So, unless there is a threshold effect, wherein above a certain density, males have all the mating opportunities they can process (and we assume that the populations in this study were all above that threshold), there may be another explanation for the equivalency of lifespan for the males and hermaphrodites in these field estimates.

The current estimates of inbreeding depression (Table 3) were similar to previous reports from both laboratory and field estimates. The average estimate of adult inbreeding depression ($\delta_1 = 0.23$) is very similar to the average estimate of this parameter from the laboratory ($\delta_1 = 0.26$) (Weeks *et al.*, 2000a). The lifetime estimate of inbreeding depression ($\delta_2 = 0.57$) is close to a field estimate of $\delta_2 = 0.58$–0.69 (Weeks & Bernhardt, 2004) and a laboratory estimate of $\delta_2 = 0.47$–0.69 (Weeks *et al.*, 1999, 2000a). The lack of the heterozygote class by population interaction (Table 2) suggested that the effect of inbreeding depression was consistent across populations and that, although the naturally filled pools produced larger shrimp, there was no evidence of a difference in estimates of inbreeding depression between naturally and artificially filled pools. The lack of a difference in estimates of inbreeding

depression from the field relative to the laboratory (and in fact a slightly higher estimate from the laboratory) is counter to several other studies that have shown increased levels of inbreeding depression measured in field relative to laboratory studies (Crnokrak & Roff, 1999), although equivalent estimates from the laboratory and field have been documented in other species (Armbruster *et al.*, 2000; Keller & Waller, 2002; Henry *et al.*, 2003). It is commonly assumed that greater levels of inbreeding depression in the field signify greater stress under field conditions (Miller, 1994). Thus, the similarity of field to laboratory estimates may just signify that the laboratory settings closely simulate field conditions in this species.

To date, the majority of sex ratio data on clam shrimp have been derived from field-collected soil raised in the laboratory (Weeks *et al.*, 2008). Weeks *et al.* (2008) reared 14 *Eulimnadia* species in the laboratory, with an average male percentage of 20.6 ± 2.1%. There was one detailed examination of *Eulimnadia* in Australia in which two species (*E. feriensis* and *E. dahli*) were collected from the field (Weeks *et al.*, 2006c). In these collections, male percentage ranged from 0% to 31%, with an average of 14.5% males. Sassaman (1995) also reports point estimates of sex ratios from 13 *Eulimnadia* species collected in the field, which ranged from 0% to 17% male (average = 5.4 ± 1.7%). Thus, the current estimates from the laboratory and the field (Table 4, Fig. 2) are within the range of most previous estimates, but are generally higher than those reported in Sassaman (1995). The latter were from single collections in the field. In many instances, males have a higher mortality rate than hermaphrodites (Strenth, 1977; Sassaman & Weeks, 1993; Zucker *et al.*, 2001), which will skew sex ratios towards hermaphrodites as pools age (Strenth, 1977). It is quite possible that the reports outlined in Sassaman (1995) were biased towards hermaphrodites because they were not consistently measured over the course of the hydrations and therefore were likely to have misrepresented true primary sex ratios. For *E. texana*, the current data suggest that sex ratios are consistently in the 20–25% range and that field and laboratory data are consistent with one another (Table 4).

Estimates of selfing rates reported herein were lower than values previously reported for *E. texana*. Weeks & Zucker (1999) reported selfing rates ranging from 0.44 to 0.75 (average = 0.66). This may be due, in part, to a slight tendency to overestimate levels of selfing using the inbreeding coefficient (used in Weeks & Zucker, 1999) relative to the multilocus selfing estimates used herein (David *et al.*, 2007). In a direct estimate of selfing rates (i.e. not from genetic estimates but rather from collections of eggs from specific mating events; Weeks *et al.*, 2000b), selfing was estimated to be 0.65 in 30 clutches of offspring from two populations. These previous estimates were conducted under laboratory conditions, and thus, the field estimates in the current study may be a better indication of true selfing rates. Interestingly, the current data suggested no significant decrease of selfing with increased male proportion (Table 5). Male proportion was found to be negatively correlated with selfing in two previous studies of this species (Sassaman, 1989; Weeks & Zucker, 1999). The lack of a correlation herein may be due to the narrower range of male proportions relative to these previous studies: in the previous findings, male percentage ranged from 0% to 32% males, whereas herein, males only ranged from 18% to 32% (Table 5). The finding that density was not negatively correlated with selfing did not correspond with the above suggestion that males had a similar mortality rate to hermaphrodites because of greater mating opportunities. However, in *E. texana*, males can mate guard hermaphrodites and still have low to no outcrossing (Weeks *et al.*, 2004), possibly because many males are inbred and produce low-quality sperm (Weeks *et al.*, 2009b).

The reason for the large difference in selfing rates between years is unknown. Selfing was not affected by density or the sex ratio. There were significantly more amphigenics and fewer monogenics in 2004 compared with 2005 (Fig. 2), so if monogenics have a higher propensity to outcross, this could explain the lower selfing rate in 2005. If not, then some environmental factor other than density or percentage male must have influenced the propensity to outcross in the two years.

The current estimates of selfing rates, combined with estimates of the male proportion (u), allow us to estimate the effectiveness of males to outcross (α; Otto *et al.*, 1993). Herein, α ranged from 0.7 to 5.1 (average = 2.4 ± 0.3; Table 5). These estimates were less variable than the previous estimates of 0.7–7.7 estimated from behavioural observations (Hollenbeck *et al.*, 2002). Hollenbeck *et al.* (2002) did note that their higher estimates were likely overestimates because behaviour was only measured for an hour and extrapolated to daily activity, plus the higher fertilization rates assumed that males produced copious quantities of sperm with high replenishment rates. The current estimates should be a better indication of true male outcrossing success.

Amphigenic hermaphrodites had by far the highest frequency across the populations sampled (60–70%; Fig. 2). This finding is consistent with laboratory estimates of four *E. texana* populations, in which amphigenic frequencies ranged between 65% and 75% of the total population (Weeks *et al.*, 1999). The current estimates of monogenics between 12% and 16% of the population are somewhat higher than previous estimates, which ranged from 5% to 13% (Weeks *et al.*, 1999). As noted above, male percentages were within ranges of previous estimates (Weeks *et al.*, 1999, 2008). The decline in amphigenics and concomitant increase in monogenics in 2005 relative to 2004 cannot be

explained from the data that were collected. It is possible that the two hermaphroditic types may differentially hatch from the egg bank under different environmental conditions, but this possibility has not been addressed in any study to date. Another possibility is that the two different classes of hermaphrodite show different survival from hatching to sexual maturity (when hermaphroditic type was determined) in different years. Previous studies have shown a greater expression of inbreeding depression in the monogenics relative to the amphigenics (Weeks *et al.*, 2001a, 2010). If expression of inbreeding depression in this species is more pronounced under stressful conditions, as in many other species (Crnokrak & Roff, 1999), and 2004 was harsher than 2005, this might contribute to explaining the differences we found.

Testing the Otto *et al.* and Pannell models

The Otto *et al.* (1993) model requires estimates of the four parameters noted above (α, δ, β and σ) to predict sex ratios of the monogenics, amphigenics and males. Several previous estimates of these parameters have been made under primarily laboratory conditions. Those studies have shown that the hermaphrodites produce sufficient sperm to fertilize all of their own eggs if they are not fertilized by a male (Weeks *et al.*, 2001b), and thus, β has herein been assumed to be one for testing both the original and Pannell's (2008) update of the model. Combinations of estimates of the other three parameters have consistently found male and monogenic proportions to be overestimated and amphigenics to be underestimated by the Otto *et al.* model (Weeks *et al.*, 1999, 2001a; Weeks & Bernhardt, 2004). Pannell's (2008) update of the Otto *et al.* model was specifically created to help explain the much higher amphigenic relative to monogenic proportions among the hermaphrodites.

Herein, we have data that both allow a test of the Otto *et al.* (1993) model under field conditions as well as noting whether the addition of the λ parameter allows a better fit of the model to the observed data (Pannell, 2008). The original version of the model (Otto *et al.*, 1993) provided a generally better prediction of the monogenic proportion than did Pannell's update of the model (Fig. 3). Both versions provided a poor fit in 2004 (Fig. 3), whereas the original model provided a better fit in 2005, both when using within-year parameter estimates, but even more so when using the 2004 estimates to predict the 2005 sex ratios (Fig. 3). Additionally, no value for the estimated λ parameter (Pannell, 2008) provided a better fit of the observed sex ratios with those predicted by the Pannell model for either of the two years (data not shown). In fact, the best fit of the Pannell model to the observed sex ratios in 2005 was when $\lambda = 0$, which is when the Pannell model collapses to the Otto *et al.* model (Pannell, 2008).

These results were unexpected. Pannell's (2008) update of the model specifically adds the λ parameter to accommodate the observed difference in inbreeding depression between monogenic and amphigenic hermaphrodites (Weeks *et al.*, 2001a, 2010). We expected that this added level of realism would have allowed a closer fit of observed and predicted sex ratios, especially because the fit of the previous laboratory estimates of the three sex ratios to those predicted were primarily off by underestimating amphigenic and overestimating the monogenic proportions (Weeks *et al.*, 1999). One possible explanation for the lack of a better fit of the observed to the predicted sexual percentages in the updated model (Pannell, 2008) is that the differences in the expression of inbreeding depression between monogenics and amphigenics may not be as notable in the field as has been observed in the laboratory, possibly more so in 2005 than in 2004 (as suggested above). If this were true, it would run counter to previous research that has generally found greater expression of inbreeding depression in the field than in the laboratory (Crnokrak & Roff, 1999). Estimating the differential viability of monogenic and amphigenic hermaphrodites in the field is problematic because of the lack of morphological or genetic markers that differentiate these two types of hermaphrodites, so this possibility would be very difficult to test in the field.

Conclusions

The long-term maintenance of androdioecy is not predicted in evolutionary models of dioecy, androdioecy and hermaphroditism and the transitions among these breeding systems (Charlesworth & Charlesworth, 1978). Nevertheless, the *Eulimnadia* have maintained androdioecy for tens of millions of years (Weeks *et al.*, 2006b), a finding that has not been established in any other plant or animal taxon (Weeks, 2012). The equilibrium models of Otto *et al.* (1993) and Pannell (2008) provide one possible explanation of how androdioecy may be maintained in these shrimp. The differences between these models are the inclusion of a parameter (λ) to account for differences in expression of inbreeding depression in monogenic relative to amphigenic hermaphrodites. This added parameter did not improve the fit of the models to the observed sex ratios in this study, suggesting that this modification to the original model is unneeded for these populations. The observed year-to-year variability of the parameters of these two models does suggest that equilibrium conditions may not be in place in these populations and thus that nonequilibrium models for the maintenance of androdioecy (e.g. Pannell, 1997, 2002) may be more appropriate for these early-colonizing species than the original Otto *et al.* model. Another option is that these shrimp may be constrained from evolving full hermaphroditism from androdioecy and thus are 'trapped' in androdioecy even though full hermaphroditism may be the optimal reproductive system

for their ecological roles (Weeks, 2012). Future studies are required to explore these various options.

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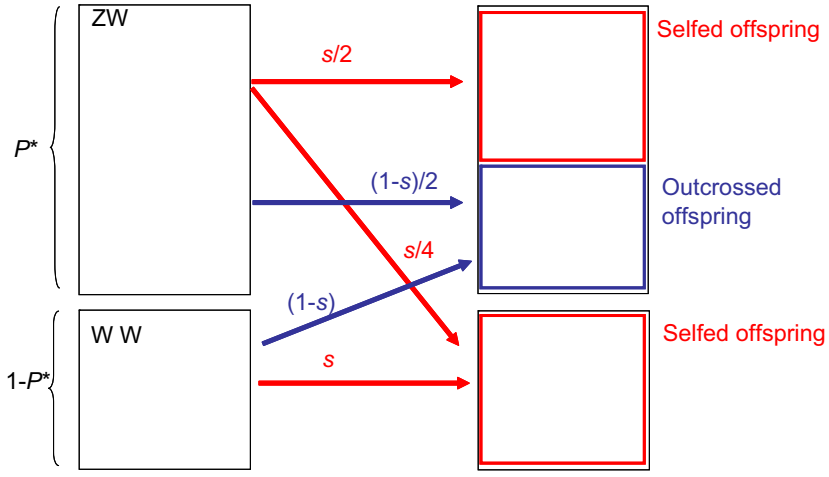
Appendix 1

Estimation of selfing rates by maximum likelihood in a ZZ/ZW/WW androdioecious system

The principle of the maximum likelihood estimation of selfing rates implemented in the RMES program is explained in David *et al.* (2007).

Traditional *Fis*-based estimates of selfing rates are based on the reduction in average heterozygosity compared with Hardy–Weinberg expectations; they are highly sensitive to genotyping biases (e.g. null alleles) because the latter also tend to reduce apparent heterozygosity and therefore mimic the effect of selfing. However, selfing does not affect only mean heterozygosity, it also increases the variance in multilocus heterozygosity (relative to its mean), and this effect is not expected from scoring biases. The RMES method relies on the shape of the multilocus heterozygosity distribution, not its mean, so it produces estimates that are free of scoring biases. Basically, RMES finds the selfing rate that maximizes the likelihood of this distribution, conditional on average observed heterozygosities at each locus.

However, the RMES method does not account for the specificities of androdioecious systems with sex-determining chromosomes such as found in *Eulimnadia* spp. We here develop a modified version to take these specificities into account. These specificities arise from the fact that the population is structured into different classes of individuals that behave differently with respect to selfing and outcrossing. We model these differences in the following ways:



Outcrossing only occurs with sperm provided by males (ZZ); hermaphrodites only use their own sperm to self-fertilize their eggs.

Two categories of hermaphrodites (amphigenics ZW and monogenics WW) coexist in the population. Amphigenics can produce males (ZZ) or amphigenics (ZW) through outcrossing; they can produce males and both kinds of hermaphrodites by self-fertilization. Monogenics (WW) can only produce monogenics by self-fertilization and amphigenics (ZW) by cross-fertilization.

We assume that gene flows from one generation to the next are as in the figure (s represents the selfing rate). We further assume that the population has reached a steady state in which the frequency of ZW hermaphrodites among all hermaphrodites (ZW + WW) is constant and equals P^* ($= v/(v + w)$ using the notations in the main text). We further assume that the selfing rate s is constant across generations and that males are sufficient to fertilize eggs and are not limiting the outcrossing rate (as exposed in the Results, we did not find correlations between estimates of selfing rates and sex ratios). We also assume that ZW and WW hermaphrodites self-fertilize the same proportion of their eggs (s) and that ZW have a viability advantage over WW such that the survival of WW to reproduction is $(1 - \lambda)$ times that of ZW (Pannell, 2008).

The aim of the following computations is to find a formula for the likelihood of a multilocus genotype of a hermaphroditic individual under a specified selfing rate s. For the sake of clarity, we decompose the set of locus into (i) a non-sex-linked set: loci are independent among themselves and with sex-determining chromosomes. (ii) a sex-linked set: loci are all completely linked to (and never recombine with) sex-determining chromosomal regions.

(i) Non-sex-linked loci

The distribution of multilocus heterozygosity in this case can be obtained by partitioning the hermaphrodite population into inbreeding classes; individual heterozygosity decreases by a factor 2 with each additional generation of selfing in its pedigree; an individual belongs to class k (i.e. k-times selfed) if it has been produced by outcrossing followed by k successive generations of selfing. Our problem is now to find the frequencies of these classes in the hermaphrodite population.

Let us consider z_k the proportion of ZW individuals that have k generations of selfing in their pedigree (the last outcrossing event is $k + 1$ generations in the past); y_k is the equivalent in WW individuals. At equilibrium, (assuming steady state of the distribution of k in both categories of individuals), the following recursions can be written

$$\begin{aligned} z_k &= z_{k-1}b \\ y_k &= z_{k-1}(1-c) + y_{k-1}c \end{aligned} \tag{A1}$$

Where $b = (s/2)P^*/[(1/2)P^* + (1-s)(1-P^*)]$ and $c = s(1-P^*)/[(s/4)P^* + s(1-P^*)]$ represent the proportion of ZW individuals that come from selfing of ZW individuals and the proportion of WW individuals that come from selfing of WW individuals, respectively.

The solution of the recursions is (for $k \geq 0$)

$$\begin{aligned} z_k &= (1-b)b^k \\ y_k &= \begin{cases} \frac{(1-c)(1-b)}{(c-b)}\left(c^k - b^k\right) & \text{if} \quad c \neq b \\ (1-b)^2 b^{k-1} k & \text{if} \quad c = b \end{cases} \end{aligned} \tag{A2}$$

Then, the overall frequency of individuals with k generations of selfing in their pedigree (among all hermaphrodites) is f_k

$$f_k = P^* z_k + (1 - P^*) y_k \tag{A3}$$

In this formula, not only b and c, but also P^* depend on the selfing rate. In addition, P^* also depends on the relative fitnesses of ZW and WW hermaphrodites. Using the notation in Pannell (2008), let us assume that the relative survival of ZW and WW is 1 and $1 - \lambda$, respectively. Then the recursion on P is

$$P[t+1] = \frac{(1/2)P[t] + (1-s)(1-P[t])}{(1/2)P[t] + (1-s)(1-P[t]) + (1-\lambda)((1/4)sP[t] + s(1-P[t]))} \tag{A4}$$

This gives the following equilibrium frequency (for any $\lambda < 1$)

$$P^* = \frac{3 - 4s + 2s(1-\lambda) - \sqrt{1 - 4s^2\lambda(1-\lambda)}}{2 - 4s + 3s(1-\lambda)} \tag{A5}$$

which can be plugged into the formula for f_k.

Now that the f_k's are known, the likelihood of a given selfing rate s for genotypes at loci unlinked to the sex-determining locus and with each other can be written. Each individual genotype j is characterized by its set of scored heterozygosities H_{ij}, $i = 1$ to L (number of loci). H_{ij} is 1 when individual j is scored as heterozygous at locus i, and 0 when it is homozygous. H_{ij} is the apparent (scored) heterozygosity, which may or may not equal the true heterozygosity (if there are null alleles or other scoring biases).

Conditional to k, the probability that locus i appears heterozygous is $d_i/2^k$, where d_i is the apparent gene diversity at locus i (gene diversity times detection rate of heterozygotes). The likelihood of a given genotype is therefore summing over all k:

$$L_{\text{nonsex-linked}}(s,j) = \sum_{k=0}^{\infty} f_k \prod_{i=1}^{L} \left(\frac{d_i}{2^k}\right)^{H_{ij}} \left(1 - \frac{d_i}{2^k}\right)^{1-H_{ij}} \tag{A6}$$

For computational reasons, this infinite sum can be approximated by the finite one, neglecting the proportion of heterozygotes that remain after $k_{\max}$ generations of selfing. In practice, for $k_{\max} > 10$, the approximation is not detectable:

$$L_{\text{nonsex-linked}}(s,j) \approx \sum_{k=0}^{k\max} f_k \prod_{i=1}^{L} \left(\frac{d_i}{2^k}\right)^{H_{ij}} \left(1 - \frac{d_i}{2^k}\right)^{1-H_{ij}} + \left(1 - \sum_{k=0}^{k\max} f_k\right) \prod_{i=1}^{L} (1 - H_{ij}) \tag{A7}$$

(ii) Sex-linked loci

Sex-linked loci (i.e. loci that do not recombine with the sex-determining loci on chromosomes Z and W) do not show the same behaviour as autosomal loci for several reasons.

First, all WW hermaphrodites must be completely autozygous for genes completely linked ($r = 0$) to the sex-determining locus. Indeed, ultimately, we can trace back the pedigree of all the WW individuals to a ZW ancestor. This ancestor selfed to produce a WW, which then may or may not have produced other WW through one or several generations of selfing. During the selfing event, the W-determining locus and all loci completely linked to it must have become autozygous.

Second, for ZW hermaphrodites, the situation is opposite. Whatever the number of generations of selfing in the recent pedigree of a ZW individual, these selfing events did not make the sex-determining loci (and all tightly linked loci) autozygous; otherwise, the offspring would have left the ZW category and would have become a male (ZZ) or a WW hermaphrodite.

Therefore, sex-linked genes are either fully autozygous or fully outbred. The likelihood computation only has to consider two possible conditions, without considering the number of generations of selfing in the pedigree: either the individual is WW with probability $(1 - P^*)$ (and then it must be homozygous) or the individual is ZW with probability P^* (and then it can be scored heterozygous at locus i with probability d_i, the apparent gene diversity at that locus). Multiplying over all sex-linked loci of individual j this gives:

$$L_{\text{sex-linked}}(s,j) = P^* \prod_{i=1}^{L} d_i^{H_{ij}} (1 - d_i)^{1-H_{ij}} + (1 - P^*) * \prod_{i=1}^{l} (1 - H_{ij}) \tag{A8}$$

(iii) Likelihood for a mix of non-sex-linked and sex-linked loci

We consider that U (unlinked) is the set of all non-sex-linked loci, unlinked to each other; while L (linked) is the ensemble of all sex-linked loci; the latter being all completely linked to the sex-determining locus. To compute the likelihood of a multilocus genotype at both U and L, we must first consider whether the individual is a ZW (probability P^*) or a WW (probability $1 - P^*$), and then, for each case, consider all possible values of k (the number of selfing generations in the pedigree), each weighted with its probability (z_k; for ZW, y_k for WW) and compute the likelihood of the genotype given k.

Using the formulae above, the likelihood of a genotype j conditional to its being a ZW hermaphrodite is obtained by multiplying the likelihoods for U and L loci; this is because the L loci have the same likelihood irrespective of k. Thus

$$L_{\text{mixed}}(s,j|ZW) = \left(\sum_{k=0}^{\infty} z_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}}\right) * \prod_{i\in L}(d_i)^{H_{ij}}(1-d_i)^{1-H_{ij}} \tag{A9}$$

Similarly, the likelihood conditional to WW is

$$L_{\text{mixed}}(s,j|WW) = \left(\sum_{k=0}^{\infty} y_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}}\right) * \prod_{i\in L}(1-H_{ij}) \tag{A10}$$

Combining the two cases (WW and ZW) gives the overall likelihood of genotype *j*.

$$L_{\text{mixed}}(s,j) = P^*\left(\sum_{k=0}^{\infty} z_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}}\right) \prod_{i\in L}(d_i)^{H_{ij}}(1-d_i)^{1-H_{ij}} + (1-P^*)\left(\sum_{k=0}^{\infty} y_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}}\right) \prod_{i\in L}(1-H_{ij}) \tag{A11}$$

As previously, infinite sums can be approximated by finite ones

$$L_{\text{mixed}}(s,j) \approx P^*\left[\sum_{k=0}^{k\max} z_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}} + \left(1-\sum_{k=0}^{k\max} z_k\right)\prod_{i\in U}(1-H_{ij})\right]\prod_{i\in L}(d_i)^{H_{ij}}(1-d_i)^{1-H_{ij}} + (1-P^*)\left[\sum_{k=0}^{k\max} y_k \prod_{i\in U}\left(\frac{d_i}{2^k}\right)^{H_{ij}}\left(1-\frac{d_i}{2^k}\right)^{1-H_{ij}} + \left(1-\sum_{k=0}^{k\max} y_k\right)\prod_{i\in U}(1-H_{ij})\right]\prod_{i\in L}(1-H_{ij}) \tag{A12}$$

in which expressions for z_k, y_k and P^* are given in eqns A2 and A5 above.

This has to be multiplied over all individuals to give the sample likelihood.

(iv) Maximum likelihood search for estimates of *s*

The RMES program (David *et al.*, 2007) implements an algorithm that explores the likelihood landscape to jointly maximize the likelihood of *s* and d_i's for all loci. We simply substituted the new likelihood formula (eqn A12) into this program to account for androdioecy.

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