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Can males successfully invade hermaphroditic populations of clam shrimp (*Eulimnadia texana*)?

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Androdioecy (mixtures of males and hermaphrodites) is distinguished by its rarity, being found in only ~40 animal species. Many of these species are clam shrimp in the genus *Eulimnadia*. A metapopulation model for the maintenance of androdioecy is tested herein by examining male success in aquaria with a single male-producing hermaphrodite introduced into an otherwise all-hermaphrodite population. This migration experiment did allow males to persist in these populations for seven generations, although at levels below those found in other populations of these shrimp. These results suggest that the maintenance of androdioecy via ‘reproductive assurance’ is unlikely by way of migration of male-producing hermaphrodites.

Keywords: Androdioecy, *Eulimnadia texana*, metapopulations, reproductive assurance, self-fertilization.

Introduction

IN our continuing attempts to understand the evolution of breeding systems, discerning the forces that select for a separation of the sexes (i.e. into pure males and pure females, termed dioecy) relative to a combination of the sexes (i.e. hermaphrodites or ‘co-sexuals’) has been a central theme^{1–5}. When selection favours a shift from one reproductive mode to the other (i.e. hermaphroditism to dioecy or vice versa), one of two temporary forms of reproduction is predicted to accompany the transition: gynodioecy (mixtures of females and hermaphrodites) or androdioecy (mixtures of males and hermaphrodites)^{1,6–9}. Of these, androdioecy is expected to be the least common^{6,9}. The observations that many androdioecious species are sporadically distributed within plants and animals appear to confirm this prediction^{7,10}.

Notwithstanding the above noted findings of androdioecy as recent evolutionary developments in a number of taxa, there is one group of freshwater crustaceans in which androdioecy has been repeatedly noted: the ‘large’ branchiopods^{10–13}. In fact, clam shrimp in the genus *Eulimnadia* have maintained androdioecy for tens to hundreds of millions of years¹⁴. In *Eulimnadia*, males coexist with hermaphrodites of two phenotypically similar but geneti-

cally different types: ‘amphigenic’ and ‘monogenic’ hermaphrodites¹¹. Sex determination is controlled by a linkage group that behaves as a single genetic locus, with males being the recessive sex: homozygous dominants (SS) are monogenic hermaphrodites, heterozygotes (Ss) are amphigenic hermaphrodites, and homozygous recessives (ss) are males¹¹. Monogenic hermaphrodites are always produced via selfing, whereas males and amphigenics can be produced via either selfing or outcrossing. There is no evidence of any environmental influences on sex determination in these shrimp¹¹. Androdioecy has been maintained in this mating system for many millions of generations¹⁴, even though self-fertilization is common (inbreeding coefficients = 0.20–0.97)^{15,16}, a trait which is commonly thought to make androdioecy unstable⁶.

The stability of this breeding system in these crustaceans is noteworthy because it implies that these shrimp have successfully struck a balance between bisexual (males + females) and unisexual (hermaphrodites) reproduction. Weeks and colleagues have been studying *Eulimnadia* in an attempt to understand what allows them to maintain this ‘unstable’ mating system^{14,17–26}. To date, these studies have explored a within-population, equilibrium model for the maintenance of androdioecy²⁷. This model has not, however, explained the dynamics of this system²⁸, and hence we propose that another model may better explain the maintenance of androdioecy in these clam shrimp.

A non-equilibrium, metapopulation model of androdioecy has been developed by Pannell^{7,29,30}, which suggests that androdioecy may be maintained because it confers ‘reproductive assurance’ (i.e. the ability to produce offspring through self-fertilization when population densities are too low to find mates) to female-biased, self-compatible hermaphrodites in landscapes with high rates of population turnover. Several aspects of Pannell’s model are consistent with the biology of the clam shrimp and thus may help explain the long-term persistence of androdioecy in these animals. In Pannell’s model, species experiencing high rates of colonization of new populations will support greater-than-expected frequencies of self-compatible hermaphrodites due to their superior colonizing abilities relative to females or males. If much of a species’ habitat is open for colonization (because of high sub-population extinction rates across the metapopulation), and if gene flow is low, a significant proportion of

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the metapopulation will consist of recently colonized subpopulations that have been initiated with only one or a few colonists²⁹. Under this scenario, males and females are at a disadvantage due to their inability to find mates in low-density environments. Thus, even though within-population factors (e.g. inbreeding depression and sexual specialization) may select for higher frequencies of single-sex individuals (i.e. males and females), between-population benefits of single propagule colonization can increase the frequency of hermaphrodites across the metapopulation in colonizing species²⁹.

There has only been one direct test of the Pannell model to date. Obbard *et al.*³¹ tested Pannell's metapopulation model using several populations of the androdioecious plant, *Mercurialis annua*. They hypothesized that when assessing genetic variation among all-hermaphroditic populations relative to male + hermaphrodite populations, the former would have low gene-flow estimates (suggesting recent colonization), whereas the latter would show genetic evidence of higher among-pool gene flow (suggesting multiple migration events). Indeed, Obbard *et al.*³¹ did find greater among-population genetic divergence (typical of low gene flow) in all-hermaphrodite relative to androdioecious (i.e. male + hermaphrodite) subpopulations, as predicted in Pannell's model. No other test of this model has been attempted.

Herein the results of the first test of Pannell's ideas in the clam shrimp *Eulimnadia texana* are reported. Pannell's metapopulation model²⁹ assumes that males which secondarily colonize an otherwise all-hermaphroditic, high-density population will be able to invade and persist because of their ability to outcross with hermaphrodites (in *Eulimnadia*, hermaphrodites cannot outcross with one another because they lack the appendages necessary for mating). The persistence of males in artificial populations of *Eulimnadia*, which have been started with eggs from either all-monogenics plus one amphigenic or only amphigenics, has been documented to see whether the all-monogenics plus one amphigenic populations will achieve stability in male proportion over seven generations and whether male proportion can actually increase to reach the levels of males found in the amphigenic-only treatments.

Materials and methods

Full details of the rearing methodology of the shrimp used for this study are given elsewhere¹⁹. Four populations of *E. texana* (JD1, JT4, SWP5 and WAL) were used in this study. The treatments herein are the result of six 'mistakes' from an earlier study¹⁹. In the previous investigation¹⁹, eggs from a single amphigenic hermaphrodite were mistakenly added to eggs derived from 10–14 monogenics to start a multi-generational study in six separate aquaria (each aquarium getting egg banks from a total of

10–15 hermaphrodites, all hermaphrodites being unrelated to one another). These six replicates were not evenly distributed across populations: one was from JD1, two were from SWP5, and three were from WAL. These replicates were therefore not used in the analysis of the previous study¹⁹ because of this mistake. However, these replicate populations allow for a test of Pannell's model by simulating the immigration of a single amphigenic hermaphrodite into an otherwise all-hermaphrodite population²⁹. Sex ratios (proportion male) in these six replicate 'amphigenic-migration' pools were compared with 23 replicate pools started with egg banks from 11 to 15 amphigenic hermaphrodites. All replicate populations were reared in 37 l glass aquaria (see Weeks¹⁹ for details) and reared under 'standard' conditions³².

Sex ratios were reported for the first population measurement after sexual maturity (day-4). Population estimates were made using fish-net sweeps of each aquarium: three sweeps of the aquarium were taken, each sweep being for a fixed length of time (30 s). Shrimp were removed from the net after each sweep, and added to a holding cup. After all three sweeps were made, the total shrimp in the cup were counted and sexed, and then returned to the population aquarium. This total count was then used to calculate the proportion male in each replicate. Sex ratio estimates were taken for a total of seven generations to note the relative performance of the lineages begun with the two types of hermaphrodites.

Shrimp in the replicate aquaria were allowed to mate or self at natural rates, and egg banks were then re-hatched after a 30-day drying period. Up to 200 shrimp were then moved to a new tank with new soil (known to be free of clam shrimp cysts) to start the next generation. These aquaria were fed and sampled as noted above. These procedures were repeated for a total of seven generations.

Proportion male was compared between these 'amphigenic-migration' treatments (egg banks from 10 to 14 monogenics + an egg bank from one amphigenic 'migrant') and the 'amphigenic-only' treatments (egg banks from 11 to 15 amphigenics) across seven generations of the experiment using weighted (proportion male weighted by total shrimp measured per replicate), two-way analysis of variance. For this analysis, proportion male needed square root transformation to normalize residuals. Because of the uneven sampling design among the original four populations, 'population' could not be considered as a main effect in the statistical design. Thus, population-to-population variation was subsumed into the residual variation in this statistical comparison.

Results

In the six 'amphigenic-migration' replicates, male proportion remained low, an average of 2–7%, throughout the course of the seven generations in this experiment

(Figure 1). In one replicate (WAL-M-2), male proportion grew to ~15% by generation-4 and then levelled-off at 10–15% throughout the remainder of the experiment. In all other replicates, males were <10% throughout the latter portion of the experiment. In one replicate (WAL-M-6), male proportions peaked at 18% in generation-4 and then fell to 0% males in generations 5–7. In all other amphigenic-migration replicates, males persisted throughout the seven generations of the experiment.

In the 23 ‘amphigenic-only’ replicates, average male proportion started at ~25% and then fell to between 12 and 18% in the latter three generations of the experiment (Figure 1). There was a variation around these per-generation averages, but overall proportion male remained fairly consistent from one generation to the next after generation-3.

The difference in male proportion between the ‘amphigenic migration’ and ‘amphigenic-only’ treatments was highly significant (Table 1). There was no consistent changes in male proportion across generations, but the

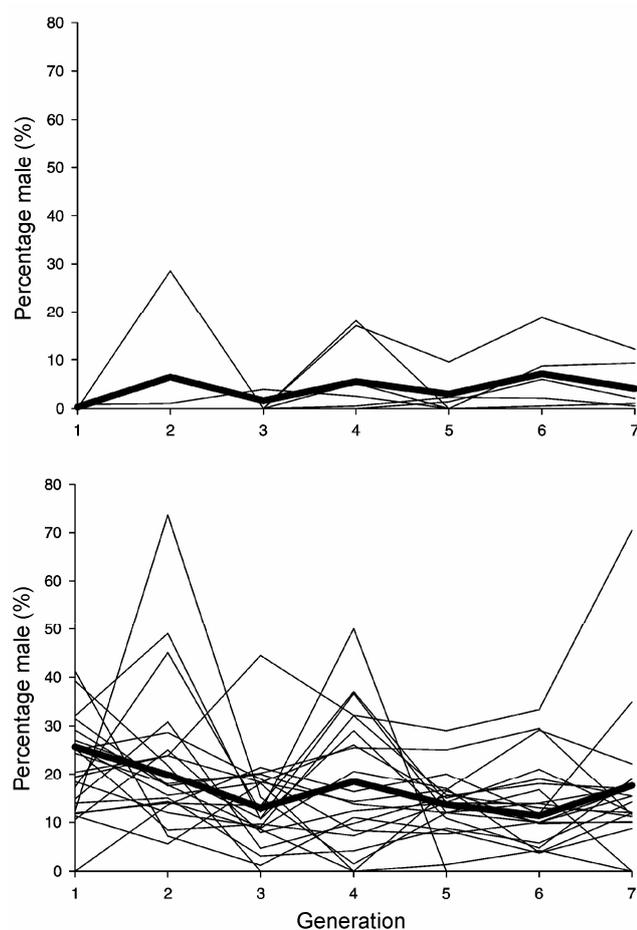


Figure 1. Male proportions in ‘amphigenic-migration’ (top panel) and ‘amphigenic-only’ (bottom panel) experimental treatments. Thin lines represent proportion males for each replicate per treatment and thick lines represent the weighted averages across all replicates per treatment.

difference between amphigenic-migration and amphigenic-only treatments did depend on generation (i.e. there was a significant ‘migration by generation’ interaction). However, this significant interaction only denoted a difference in the magnitude of the increased male proportion in the amphigenic-only relative to the amphigenic-migration treatments; for every generation, the average male proportion was higher in the amphigenic-only relative to the amphigenic-migration treatments.

Discussion

To maintain males and hermaphrodites in a metapopulation consisting of a landscape of pools of differing ‘evolutionary ages’, Pannell assumed that the youngest pools are initially colonized by single, self-compatible hermaphrodites and that population sizes are initially so low that finding mates is untenable²⁹. Pools that are only slightly older (in an evolutionary progression) than these earliest pools should then comprise all-hermaphrodites that are at densities high enough to allow successful cross-fertilization. These pools are then open for successful, secondary male migration²⁹. Thus, a key prediction of this model is that all-hermaphrodite pools at high densities should allow the successful establishment of males when they migrate into these pools^{7,29}.

In the *Eulimnadia* system, all-hermaphrodite pools would only comprise monogenic hermaphrodites¹¹. Male migration would then occur through the deposition of male or amphigenic cysts, the cysts being deposited either by abiotic (e.g. blown in with the wind) or biotic (e.g. being brought in on migrating birds) processes. In the case of male cysts, a male would hatch directly from the cyst and could start mating with hermaphrodites as soon as it matured. In the case of an amphigenic cyst, the hermaphrodite would self-fertilize, producing ~25% male cysts among the eggs¹¹. These eggs would then hatch in a subsequent hydration, wherein a number of males would then be able to mate with the hermaphrodites in that generation of offspring. Thus, both types of migration events would yield males; in the case of amphigenic migration, males (and cross-fertilization) would just lag the male-migration scenario by one generation.

Herein, we have simulated the second form of male migration: migration of amphigenics into an otherwise monogenic population. In five of the six replicates, this

Table 1. ANOVA results for weighted male proportion (square-root transformed)

Source	df	Sum of squares	F-ratio	Prob > F
Migration	1	167.98	129.35	<0.0001
Generation	6	6.53	0.84	0.5423
Migration × generation	6	23.67	3.04	0.0075
Error	180	233.75		

initial simulated migration event produced males that persisted throughout all seven generations. In the sixth replicate, males increased to 18% of the population but then disappeared, which seemed to be an extinction event for the male-determining genes in this replicate population. Thus, it appears that males can be commonly maintained in these initially monogenic-only populations via migration of a single amphigenic hermaphrodite, but that a low proportion of migration events does not maintain males.

Even though males were maintained in the majority of amphigenic-migration replicates, males were 3–5 times more common in the amphigenic-only relative to the amphigenic-migration populations. Only one of the six amphigenic-migration treatments had male proportions near the averages found in the amphigenic-only treatments. These observations can be explained if males in the amphigenic-migration treatments were not as effective in cross fertilization as in the amphigenic-only treatments. The most likely explanation for this would be if a diverse assemblage of male migrants (in this case, from a diverse array of amphigenic migrants) were more successful than a single migration event. If this is true, then the migration scenarios outlined in Pannell's model^{7,29,30}, which assumes initial and secondary migrations are of only one or a few migrants, will be difficult to achieve. If successful establishment of males requires a dozen or more simultaneous migration events (as in the amphigenic-only replicates in this experiment), then the processes outlined by Pannell^{7,29,30} are unlikely to explain the maintenance of androdioecy in *Eulimnadia*.

However, the above conjecture is not the only way in which males would do better in the amphigenic-only relative to amphigenic-migration treatments. Males produced by self-fertilizing amphigenics are prone to low sperm production³³. Therefore, a second explanation for the difference in male success is that many males produced by self-fertilizing amphigenic hermaphrodites are inefficient at cross-fertilization and thus do not effectively promulgate maleness when in low numbers. Given that one of the six amphigenic-migration replicates did result in males establishing themselves in the population at a level similar to the amphigenic-only replicates, we can estimate that approximately one-sixth of selfing amphigenics can produce males that are effective at cross-fertilization. If this is so, the amphigenic-only populations would have an average of two such amphigenics to start each replicate in this experiment. Once outcrossing was established, the males could effectively persist because of the benefits they confer to outcrossed offspring in this species^{19–21,23,28}. Under this scenario, there is yet again difficulty in maintaining androdioecy via the mechanisms outlined by Pannell²⁹, at least via migrations of amphigenics that then self-fertilize to produce males with low-sperm counts. Direct migrations of males (via male-producing cysts) may still be effective, assuming the cysts themselves were products of outcrossing.

A third possible explanation for the observed increased frequency of males in the amphigenic-only relative to the amphigenic-migration treatments has nothing to do with differences in outcrossing rates, but rather suggests that the relative proportion of males is directly proportional to the number of selfing amphigenics in these two treatments. Since selfing amphigenics produce ~25% males among their offspring, whereas selfing monogenics produce no males¹¹, it is possible that in both treatments hermaphrodites are primarily self-fertilizing and the lower proportion of males in the amphigenic-migration treatments merely reflects lower proportion of amphigenics in these treatments, relative to amphigenic-only treatments. This explanation would suggest that the single example of successful male establishment in the amphigenic-migration replicates was due to a higher-than-average production of eggs from this amphigenic migrant (or lower-than-average production of the 10–14 monogenics in that replicate), which then caused a higher proportion of initial hatchlings to be from the amphigenic relative to the monogenic clutches. Again, if this explanation is correct, and nearly all offspring were products of self-fertilization, the *Eulimnadia* system would appear to not conform to the predictions outlined in Pannell's model²⁹.

In summary, the current experiment verifies that migrating amphigenic hermaphrodites can bring males to otherwise hermaphrodite-only populations and that these males can commonly persist for many generations. However, they persist in low abundance and rarely attain the frequencies seen in replicate pools established with many migrant amphigenics, or seen in natural populations of *E. texana*^{16,23}. There are three possible explanations for this difference in male persistence, and all of them suggest that the metapopulation dynamics outlined by Pannell^{7,29,30} is unlikely to maintain androdioecy in these shrimp, at least via migrations of amphigenic hermaphrodites.

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