

Testing a behavioral model for the maintenance of androdioecy as a result of sexual conflict in the clam shrimp *Eulimnadia dahli*

Alissa Calabrese  | Stephen C. Weeks 

Department of Biology, The University of Akron, Akron, Ohio, USA

Correspondence

Stephen C. Weeks, Department of Biology, The University of Akron, Akron, OH, USA.

Email: scw@uakron.edu

Present address

Alissa Calabrese, Herzing University, Akron, Ohio, USA

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Abstract

Androdioecy is a rare mating system in which males and self-compatible hermaphrodites coexist. Such a system is proposed to be a temporary evolutionary transition between separate sexes and total hermaphroditism. Sexual conflict over occurrence of mating has been proposed as one crucial precondition for a behavioral mechanism that can maintain stable androdioecy. This work tests this hypothesis using *Eulimnadia dahli*, a species of clam shrimp that has maintained androdioecy for millions of years. Monitoring the behaviors of mating pairs, we examined the differences in antagonistic behaviors and propensity to mate between different types of hermaphrodites and their male counterparts. Contrary to predictions, inbred hermaphrodites from male-less populations did not have higher resistance to mating with males, were not found to be less attractive to males, and showed no increased tendency to self-fertilize than hermaphrodites from mixed populations.

KEYWORDS

androdioecy, clam shrimp, mating behavior, sexual conflict

1 | INTRODUCTION

Sexual conflicts originate from any disparity between the optimal strategies of the sexes (Parker, 1979). Investment differences between the sexes vary for several aspects of reproduction, for example, time spent in search of mates, competing for mates, provisioning of potential mates, guarding/defending mates, protecting a brood, and provisioning offspring (Benvenuto & Weeks, 2012; Chapman et al., 2003; Parker, 2006; Wachtmeister & Enquist, 2000; Westneat & Sargent, 1996). Although they are the focus of much research, sexual conflicts do not always follow stereotypical male–female patterns. Breeding systems other than dioecy are also susceptible to conflicts of interest between sexual types. Sexual conflicts have been noted in androdioecious (male and hermaphrodite) systems (Benvenuto & Weeks, 2011, 2012; Chasnov, 2010), gynodioecious (female and hermaphrodite) systems (Wang et al., 2021), and even in simultaneously hermaphroditic systems (Anthes & Michiels, 2007; Bedhomme et al., 2009).

Inter-locus sexual conflict can be described as when the ideal outcomes of interactions between individuals of different sexes do not align (Chapman et al., 2003; Parker, 1979). Some conflicts can be strong enough to promote the evolution of antagonistic traits, in which one sex displays an aggressive or defensive trait to either encourage or discourage mating. These traits can be morphological, behavioral, or both. In dioecious systems, water striders have been studied extensively for their displays of male persistence and female resistance, both anatomically and behaviorally (Arnqvist & Rowe, 2002). In simultaneously hermaphroditic systems, such as in the sea slug, there is antagonism to determine which partner will assume the female role (and thus the higher cost) of mating (Anthes & Michiels, 2007; Bedhomme et al., 2009).

Because mating behaviors are so critically linked to reproductive success, they are assumed to evolve relatively rapidly (Mayr, 1976). Changing antagonistic behaviors in one sex could promote quick development of resistance behaviors in the other sex. Conversely, if antagonistic behavior is reduced or removed altogether, the

corresponding resistance behavior may no longer be under any pressure to be maintained. For example, loss of female resistance over the course of several generations has been shown in *Drosophila* (Holland & Rice, 1999). Experimental removal of sexual selection in *Drosophila* has also been shown to result in anatomical changes in testis size and sperm production (Pitnick et al., 2001). Adjustments in relative densities of the sexes can also influence behavior and reproductive outcomes. In dung flies, an increase in male density results in increased aggressive behaviors toward females (Blanckenhorn et al., 2000), but some species show varying levels of mating behaviors between populations regardless of density effects. For example, in two geographically distinct populations of the two-spot ladybird beetle, females of one population showed higher levels of resistant behaviors against males no matter the male population of origin (Haddrill et al., 2013). Examples like these demonstrate how resistance behaviors in one sex, or even morphological characteristics, can be gained or lost in response to an initial change in mating behavior in the other sex.

Clam shrimp are a group of branchiopod crustaceans displaying a wide array of sexual systems, including dioecy (Sassaman, 1995), hermaphroditism (Weeks et al., 2005; Zucker et al., 1997), and androdioecy (Weeks, 2012; Weeks et al., 2006). Sexual conflicts that result from a misalignment in the mating outcomes of different sexes are found across these various systems in this group. In dioecious species of clam shrimp, a conflict in the optimal strategies of the sexes can be quantified by observing the length of time males spend guarding females to prevent their eggs being fertilized by another male (Benvenuto & Weeks, 2011). In general, female optimum mate guarding time would be less than that for males, as being guarded is costly for females (Benvenuto & Weeks, 2012; Jormalainen, 1998). In this male–female clam shrimp system, we can see evidence that the actual length of mate guarding reflects a compromise between both the female and male optima (Benvenuto & Weeks, 2011).

In androdioecious species of clam shrimp, there is also a conflict in ideal strategies between the males and hermaphrodites. Hermaphrodites of these species are capable of self-fertilization (Sassaman & Weeks, 1993); however, males need to outcross with hermaphrodites in order to reproduce (Weeks et al., 2004). The conflict here is even more evident than it is in dioecious groups: Hermaphrodites may be more resistant to mating with males because of the genetic costs that come with outcrossing (Williams, 1975) as well as the energetic costs to the animal being guarded (Benvenuto & Weeks, 2012). In a direct comparison of mate guarding durations between dioecious and androdioecious clam shrimp, length of time guarding was significantly longer in the dioecious species (Benvenuto & Weeks, 2011). There is evidence that when sexual conflict occurs, compromise between the sexes results, as neither sex is achieving their optimum result (Benvenuto & Weeks, 2012). Additionally, the strategies are not static over time and place. Lower density populations are costly for males who need to swim greater distances to find potential mates, while hermaphrodites can self-fertilize regardless of the population density (Weeks et al., 2011).

Androdioecious clam shrimp in the genus *Eulimnadia* occur in three genetic sexes: males, “amphigenic” hermaphrodites, and “monogenic” hermaphrodites (Sassaman & Weeks, 1993).

Amphigenic hermaphrodites will produce both male and hermaphroditic offspring when they self-fertilize while the offspring produced from monogenic self-fertilization will be entirely hermaphroditic.

A model developed by Chasnov (2010) highlights the sexual conflict that arises when optimal outcomes differ between self-compatible hermaphrodites and males. The model focuses on explaining androdioecy in two model species: *Caenorhabditis elegans* and *Eulimnadia texana*. According to Chasnov, the evolution of hermaphroditism is driven by the need for reproductive assurance (i.e., ability to produce offspring regardless of density of potential mates) even if their selfed offspring are less fit than outcrossed progeny. Many generations of inbreeding could then result in a purge of genetic load (Husband & Schemske, 1996), which is predicted to favor hermaphrodites that obligately self (Chasnov, 2010; Chasnov & Chow, 2002). Males face a strong pressure to outcross as this is their only means of reproduction, and in spite of hermaphroditic resistance, some males will successfully mate. These differential selective pressures are predicted to develop a sexual conflict over mating between males and hermaphrodites that then combine with the mechanism of sex determination to maintain the androdioecious mating system in these two species (Chasnov, 2010).

The sexual conflict model (Chasnov, 2010) predicts that monogenic hermaphrodites in all-hermaphroditic populations that have purged inbreeding depression will be actively resistant to copulation. In this case, there should be behavioral clues that suggest the hermaphrodite is averse to mating. Additionally, if the resistant behavior is assumed to be an evolved trait, then it should not be apparent in newly established populations, but only in older populations that have had adequate time to purge inbreeding depression and have subsequently adapted to increase the odds of selfing. The amount of time necessary to purge inbreeding depression and subsequently evolve a behavioral adaptation is unknown. In the *Drosophila* study mentioned above, behavioral changes were apparent after 47 generations (Holland & Rice, 1999). In nematodes, characters related to mating behavior (i.e., spicule insertion times) can evolve in under 60 generations (Palopoli et al., 2015). Comparable data for the evolution of behavioral change are not available for clam shrimp.

Clam shrimp in this family are found in discrete temporary freshwater pools, often scattered across the landscape in a metapopulation framework (Benvenuto et al., 2009; Calabrese et al., 2016). The populations occurring in these pools are of variable composition, with some containing true androdioecious populations of males and hermaphrodites and others containing completely hermaphroditic, purely monogenic populations (Sassaman, 1995; Weeks et al., 2006, 2008). Hermaphroditic pools can arise when a single monogenic hermaphrodite migrates to an uninhabited pool and proceeds to self-fertilize. Populations that originate in this way can remain completely hermaphroditic for multiple generations (Weeks, 2004), only including males via dispersal from neighboring pools (Weeks, 2009), potentially long enough to evolve behavioral changes rapidly. The clam shrimp in this study were from natural pools of unknown age.

Here, we test the sexual conflict model with assays comparing the behavioral difference between hermaphrodites in androdioecious versus all-hermaphroditic populations when mated with males

from a third population. *Eulimnadia dahlia*, a native, androdioecious clam shrimp of Western Australia (Weeks et al., 2006), was used for this study. Couples were isolated from these natural populations, monitored with time-lapse videography, and scored for the presence of certain mating behaviors. Behaviors that indicate resistance were used to assess whether there are any differences between the mating behaviors of hermaphrodites from androdioecious populations and those from monogenic populations, as predicted in Chasnov's (2010) model. Specifically, we tested the following predictions: Monogenic hermaphrodites that have purged inbreeding depression should be (1) resistant to outcrossing with males, (2) lose attractiveness to males, and (3) should prefer self-fertilization over outcrossing.

2 | METHODS

Sediment containing resting eggs of *Eulimnadia dahlia* was obtained from several locations in Western Australia: Tammin Rock, Wave Rock, The Humps, and Yorkrakine Rock (see Weeks et al., 2006 for location information). The locations chosen contained known androdioecious or all-hermaphroditic populations. *Eulimnadia* hermaphrodites from all-hermaphroditic pools are known to be monogenics (Weeks et al., 2005, 2008). However, androdioecious populations of *Eulimnadia* contain some levels of monogenic and amphigenic hermaphrodites, with the proportions of monogenics being ~10% or less of the total population (Weeks et al., 1999).

Sediment was hydrated in a temperature-controlled environment under continuous light. Post-hatching shrimp were fed an ad lib diet consisting of a liquid mixture of baker's yeast and powdered Tetramin™ fish food flakes (2.5 g of each suspended in 500 mL of water; see Weeks et al., 2008).

Seven days post-hatching, sexually mature male shrimp were removed from their hatching tank and individually placed in 40 mL beakers. To increase contrast for better visibility, black sand (commercially obtained) was layered across the bottom of each beaker. A hermaphrodite from either a monogenic (no males, designated M-) or an androdioecious (M+) population was placed with the male. Males were sourced from an androdioecious population, but not the same androdioecious population from which the hermaphrodite was procured. The beaker containing the pair was filmed for 24 h using time-lapse videography. After the 24-h recording session, shrimp used in the experiment were placed in a tank of "used" shrimp to avoid potentially re-using the same individuals in future trials. A total of 102 unique mating pairs were recorded. After eliminating videos in which one or both shrimp died and videos that were unable to be scored as a result of poor video clarity, 36 recordings remained to be scored.

Videos were scored for mating behaviors following methods outlined in Weeks and Benvenuto (2008). Such behaviors included the following: brief encounters (male grasps a hermaphrodite for more than 5 s but fewer than 30), mate guarding (male grasps a hermaphrodite for more than 30 s), and kicking (an attempt by the

hermaphrodite to free itself from the male by extending its telson toward the attached male). Mating behavior was grouped into three possible outcomes: (1) no fertilization, (2) self-fertilization, and (3) outcrossing. Hermaphrodites that did not move eggs into the brood chamber through the duration of the recording were recorded as being not fertilized. If a hermaphrodite moved eggs into their brood chamber while not being guarded by a male, the eggs were classified as self-fertilized (Weeks et al., 2004). If eggs were moved into the brood chamber while a male was guarding and actively inserting his telson (type II thrusting behavior as described in Weeks et al., 2004), the eggs were classified as outcrossed (Weeks et al., 2004).

Data were analyzed using SAS JMP Pro 15 (SAS Institute Inc., 2019). To achieve normality requirements for statistical analysis, the number of kicks, number of guarding attempts <30s, and total duration of mate guarding were square root transformed. The number of guarding attempts >30 s was natural log transformed. Number of kicks, number of guarding attempts <30 s, number of guarding attempts >30 s, and total duration of mate guarding were all analyzed in one-way ANOVAs with hermaphroditic type [monogenic pool (M-) or amphigenic pool (M+)] as the independent variable. The number of pairings in which hermaphrodites did not mate (= "None"), outcrossed, or selfed was compared between M- and M+ pools using a 2 × 3 contingency analysis with a Fisher's exact test.

3 | RESULTS

There was no significant difference in the number of kicks by hermaphrodites in monogenic versus androdioecious pools ($F_{1,33} = 0.78$; $p = .3846$; Figure 1A). Hermaphrodites from androdioecious pools were not found to significantly differ in brief encounters or bouts of mate guarding than hermaphrodites from monogenic pools ($F_{1,33} = 1.49$, $p = .2308$ and $F_{1,33} = 2.23$, $p = .1451$, respectively; Figure 1C&D). Hermaphrodites from both population types (monogenic and androdioecious) averaged nearly the same total amount of time being guarded by males ($F_{1,33} = 0.005$, $p = .9424$; Figure 1B).

Outcomes for mating behavior for hermaphrodites paired with males revealed that hermaphrodites from androdioecious pools either had no fertilization or self-fertilized in equal proportions (no fertilization $n = 10$, self $n = 10$, outcross $n = 0$; Figure 2). Monogenic hermaphrodites were significantly more likely to outcross than hermaphrodites from androdioecious pools (no fertilization $n = 10$, self $n = 3$, outcross $n = 3$; Fisher's exact test $p = .0168$).

4 | DISCUSSION

Sexual conflict results from the differing selection pressures between the sexes on behaviors and morphology associated with mating (Chapman et al., 2003; Parker, 1979). Differences in sex ratios

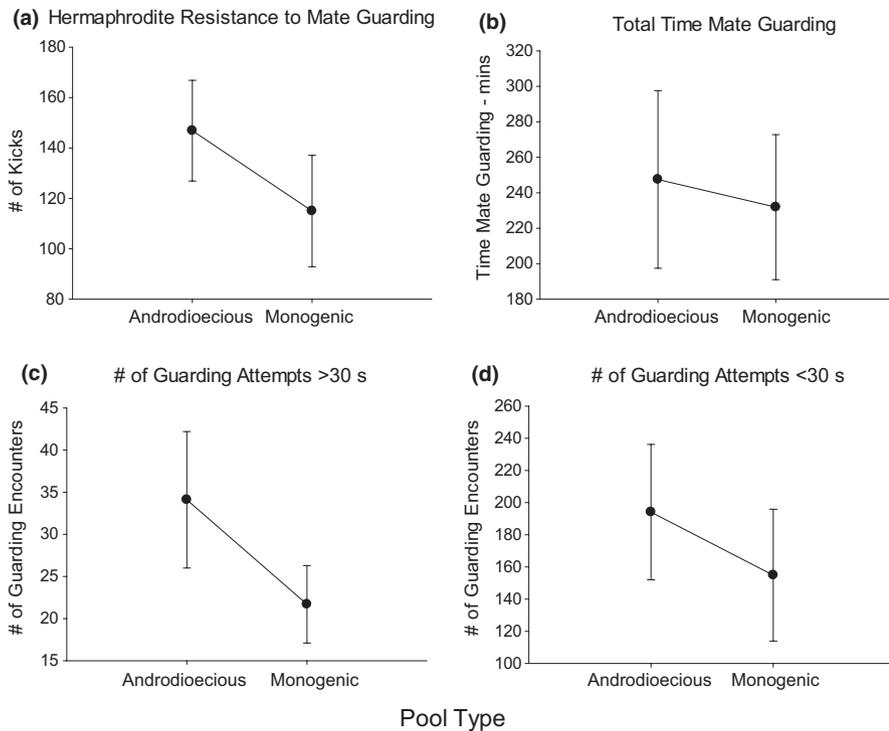


FIGURE 1 (a) Resistance to mate guarding by males as measured in “kicks” by hermaphrodite type. (b) Total time (minutes) spent mate guarding by hermaphrodite type. (c) Number of longer duration (>30s) encounters by hermaphrodite type. (d) Number of short duration (<30s) encounters with males by hermaphrodite type. Error bars represent one standard error of the mean

Mating Outcomes of Hermaphrodites Paired with Males

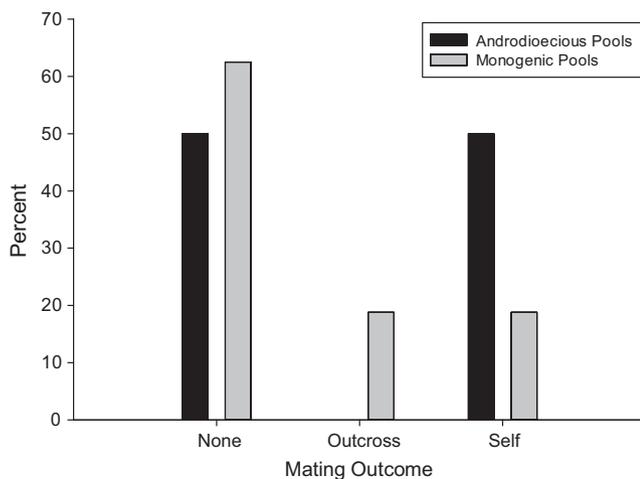


FIGURE 2 Percent of trials that resulted in self-fertilization, outcrossing with male, or no mating at all during the observation period by hermaphrodite source population type

and selective pressure to outcross between dioecious and androdioecious groups are predicted to result in variable receptivity of females/hermaphrodites to outcrossing (Chasnov, 2010). Male/female sexual conflict offers a greater likelihood of compromise than in male/hermaphrodite conflicts because neither the female nor the male can reproduce independently (Benvenuto & Weeks, 2011). This is not the case with self-fertilizing hermaphrodites (Baker, 1955). Thus, compromise should be reached in male/female interactions to ensure the production of offspring.

4.1 | Hermaphrodite resistance

Studies comparing androdioecious to dioecious nematodes provide evidence for differences in mating behavior related to hermaphrodite/female willingness to outcross. When dioecious male nematodes attempt to outcross, the females appear to encourage them by remaining still and allowing spicule insertion (Garcia et al., 2007). Hermaphrodites in closely related androdioecious nematode species adopt a pattern of rapid movements when approached by males, interpreted as avoidance behavior (Kleeman & Basolo, 2007). Hermaphrodites of *C. elegans* and *C. briggsae* appear to have lost their physiological response to male factors that promote outcrossing (Garcia et al., 2007). Additionally, hermaphrodites are more mobile and likely to avoid proximity to males than are dioecious females (Garcia et al., 2007). Thus, although it appears that male nematodes have retained their desire to outcross, receptivity of their potential hermaphroditic mates has changed drastically in these *Caenorhabditis* species.

In the clam shrimp, *Eulimnadia texana*, hermaphrodites paired with males over a three-day period produced 56% of the offspring via self-fertilization, suggesting a resistance to outcrossing with males even when paired in close proximity (Weeks et al., 2004). Results such as these support Chasnov's (2010) predictions that hermaphrodites should develop an unwillingness to outcross if inbreeding depression has been purged. Any fitness detriment caused by inbreeding would encourage outcrossing to avoid the fitness consequence. In the current experiment, *E. dahlia* hermaphrodites from all-monogenic pools are assumed to have purged genetic load associated with inbreeding. By comparison, the androdioecious pools should contain monogenic hermaphrodites,

amphigenic hermaphrodites, and males (Weeks et al., 2008) making a similar type of purge of genetic load unlikely. Therefore, amphigenic hermaphrodites should be more amenable to outcrossing, and monogenic hermaphrodites from long-established pools should have developed behaviors to actively resist mating with males to avoid the cost of outcrossing (Chasnov, 2010).

The results of the current experiment do not support the predictions of Chasnov's model (2010). Hermaphrodites from all-hermaphroditic populations (assumed to be monogenic) did not display any increase in resistance to mating when compared to hermaphrodites from androdioecious populations (Figure 1). According to the model, hermaphrodites from monogenic populations should evolve greater resistance to mating and/or reduced attractiveness to males than those from androdioecious populations. Chasnov's model specifically assumes that inbreeding in all-hermaphroditic populations will purge deleterious alleles that contribute to inbreeding depression. If there are no genetic costs to inbreeding, then monogenic hermaphrodites should avoid outcrossing with males. Over time, Chasnov predicts that phenotypes that reduce hermaphrodite attractiveness to males should be favored along with behaviors to actively resist mating. None of the data in this experiment support an increased resistance (as measured by kicking behavior; Weeks et al., 2004) in the monogenic as compared to the amphigenic hermaphrodites. It is possible that the resistance behaviors predicted by Chasnov are too costly to maintain in clam shrimp, or possibly too costly to maintain given the lower levels of outcrossing typically observed in these clam shrimp (Weeks et al., 2004). The current project is unable to assess this possibility.

4.2 | Hermaphrodite attractiveness

The attractiveness of hermaphrodites to males has also been explored in androdioecious versus dioecious nematodes. Females in two dioecious species (*Caenorhabditis remanei* and *C. sp.* strain CB5161) produce pheromones to attract males while hermaphrodites in two closely related androdioecious species (*C. elegans* and *C. briggsae*) do not (Chasnov et al., 2007). These female pheromones will attract males of both dioecious and androdioecious species alike. The assumption is that the hermaphrodites have lost the ability to produce pheromones to avoid outcrossing with males. This indicates that any attractiveness of the pheromone to androdioecious males is likely a hold-over from their dioecious ancestry (Chasnov et al., 2007).

In *Eulimnadia* clam shrimp, androdioecious males appear to have a more difficult time finding hermaphrodites when compared to closely related dioecious counterparts, suggesting the hermaphrodites prefer not to be found (Ford & Weeks, 2018). In the same study, hermaphrodites were observed actively avoiding proximity to males, while that avoidance behavior was not witnessed to the same extent in females. If male mate guarding attempts can be considered a proxy for hermaphrodite attractiveness, then attractiveness can be compared between monogenic hermaphrodites and hermaphrodites from androdioecious pools.

In this present experiment, there is no evidence that males approached and guarded monogenics at a lower frequency than they guarded amphigenics (Figure 1). Male clasping encounters, whether brief (<30 s) or long (>30 s), and the overall duration of clasping did not significantly differ in both groups. These data do not support Chasnov's (2010) model which predicts greater interaction between males and hermaphrodites from amphigenic pools than males and hermaphrodites from all-monogenic pools. It is unlikely that a loss of hermaphrodite attractiveness (e.g., through a loss of pheromone production) would be hampered by a cost to such loss, since a lack of pheromone production should actually reduce costs rather than increase them.

The differing conclusions on hermaphrodite attractiveness to males in this experiment relative to the findings in Ford and Weeks (2018) likely stem from the levels of comparisons in the two studies. Ford and Weeks compared relative male interest between two types of clam shrimp species: males and hermaphrodites in androdioecious species (*E. texana* and *E. dahlia*) and males and females in dioecious species (*Eocyzicus argillaquus* and *Paralimnadia stanleyana*). Males from the dioecious species guarded females at nearly twice the rate androdioecious males guarded hermaphrodites. Herein we are making a more subtle comparison of *E. dahlia* males to hermaphrodites derived from two types of pools (monogenic vs. androdioecious pools). Clearly, *E. dahlia* males show a pattern of relative "disinterest" in mate guarding compared with dioecious males, but the current data suggest that the finer-scale comparison of male interest in hermaphrodites from monogenic and androdioecious pools shows no significant difference.

4.3 | Mating outcomes

The comparison between self-fertilization and outcrossing is probably the most direct way to evaluate Chasnov's (2010) model. Outcrossing rates in the androdioecious *C. elegans* are typically low, on average less than 5% in most populations (Chasnov & Chow, 2002). However, outcrossing rates will increase if there are environmental stressors present (Morran et al., 2009). According to Chasnov's (2010) model, selfing rates should be high if there is little to no inbreeding depression present in the population. This appears to be the case for *C. elegans* (Chasnov & Chow, 2002; Johnson & Wood, 1982).

In the clam shrimp *Eulimnadia texana*, outcrossing rates have been measured between 1.3 and 22.5% based on a variety of factors, including age of the males and population density (Hollenbeck et al., 2002). Even when males successfully mate with hermaphrodites, the percentage of eggs per clutch that are fertilized by the male's sperm, as opposed to those selfed by the hermaphrodite, is only in the range of 25%–50% (Weeks et al., 2004). Clearly, self-fertilization is the more common mode of reproduction in this species, although not to the same extreme degree as is seen in *C. elegans*. Generally, long-term high selfing rates are associated with low levels of inbreeding depression (Barrett & Charlesworth,

1991; Husband & Schemske, 1996), as accumulated deleterious recessive alleles are likely to be purged within a few generations. However, inbreeding depression in the highly selfing *E. texana* has been reported as high as 0.7 in some populations (Weeks et al., 2000).

Outcrossing in the current experiment varied significantly between the androdioecious and all-hermaphroditic monogenic populations of *E. dahlia*. Hermaphrodites from monogenic pools had a significantly higher rate of outcrossing with males than their counterparts from androdioecious pools (Figure 2). This pattern contradicts the predictions of the Chasnov (2010) model that selfing should be favored in hermaphroditic populations with high selfing rates. This prediction, however, is based on the assumptions that all-hermaphroditic pools have purged deleterious alleles that contribute to inbreeding depression.

This concept of purging deleterious alleles that then reduces the negative consequences of inbreeding is one of the central assumptions in Chasnov's (2010) model. If the genetic load has not been purged, then pressure for monogenic hermaphrodites to outcross with males should remain to create offspring with higher fitness. Long-term inbreeding does not always lead to purging of genetic load in plants (Byers & Waller, 1999; Willis, 1999), and thus, reduced inbreeding depression with continued inbreeding is not a given for all species. Levels of inbreeding depression in *Eulimnadia dahlia* are unknown. However, the closely related *E. texana* has a demonstrated difficulty in purging deleterious alleles: Multiple generations of enforced self-fertilization resulted in no appreciable difference in inbreeding depression between selfed and outcrossed lineages (Weeks, 2004). The proposed mechanism for the lack of purging is that alleles for sex determination in *E. texana* are associated with other fitness-related alleles in a linkage group that is not subjected to frequent crossing over (Weeks, 2004). Typically, it is accepted that inbreeding depression is lower in hermaphroditic populations because continued self-fertilization will cause increased incidence of individuals expressing homozygous recessive deleterious mutations that will be subsequently purged from the population as a result of their low fitness compared with other genotypes. However, if recessive deleterious alleles are linked to the locus for sex determination, then purging would be unlikely (Weeks et al., 1999). Because both *E. texana* and *E. dahlia* share the androdioecious mating system (males, amphigenic hermaphrodites, and monogenic hermaphrodites; Weeks et al., 2008), it is possible that the samesex-determining linkage group exists in *E. dahlia* and similarly prevents purging of deleterious recessive alleles. If inbreeding levels in *E. dahlia* are indeed high in both monogenic and amphigenic populations, then this species does not meet one of the key assumptions of the sexual conflict model (Chasnov, 2010), which may explain why we see no reduction in outcrossing rates for monogenic versus amphigenic hermaphrodites.

A potential explanation for why monogenics might actually outcross at a higher rate than amphigenics concerns relative fitness differences between these two hermaphroditic types. Measurements of survival and net reproductive rates between amphigenic and monogenic hermaphrodites of *E. texana* have shown significant

differences (Weeks et al. 2001, 2010): Amphigenics have greater longevity and higher reproductive rates than monogenic hermaphrodites. This fitness difference was predicted to be due to a greater expression of inbreeding depression in monogenic relative to amphigenic hermaphrodites (Weeks et al. 2001). Measurements of the fitness differences between the hermaphroditic types in *E. dahlia* have not been undertaken. However, if the pattern is the same, then lower-fitness monogenics may be selected to outcross at a higher rate to reduce the expression of inbreeding depression. Fitness measurements comparing outcrossed to selfed offspring of monogenic hermaphrodites in this species would clarify whether this scenario could be correct.

For all of our comparisons, we are unlikely to have found results consistent with the Chasnov (2010) model if the monogenic pools we used were incorrectly identified. We believe this is unlikely for two reasons. First, we have never raised a male in repeated hydrations from soil sampled from these monogenic pools, which strongly suggests there are no males in these soil samples. Although there is always a possibility that the soil samples we collected are not representative of the pools' actual sex ratios, we attempted to minimize this possibility by taking soil samples from many locations within each pool. Second, the mating results found significant differences between monogenic and androdioecious pools, suggesting that these pool types are indeed distinct. Given the above, we believe that we have correctly identified these two pool types. If we have not, then we could have made comparisons of androdioecious to androdioecious pools, which clearly would not have been a valid test of the Chasnov (2010) model.

5 | CONCLUSIONS

When an androdioecious population arises from a dioecious ancestor, changes in behaviors and morphology of one or both sexes are to be expected (Weeks, 2012). This is the case in some species of nematode worms of the genus *Caenorhabditis* and in many aspects of clam shrimp of the genus *Eulimnadia* (Weeks, 2012). In the evolutionary transition from an ancestral dioecious species to the current system of androdioecy, sexual conflict takes on different characteristics. Hermaphrodites are less willing to mate with males when they can simply self-fertilize and avoid the genetic cost of outcrossing (Benvenuto & Weeks, 2012; Ford & Weeks, 2018). The model developed by Chasnov (2010) to explain the maintenance of androdioecy by sexual conflict is well supported in *C. elegans* and to some degree in *E. texana*. However, the model does not appear to function as well when applied to *E. dahlia*, where monogenic hermaphrodites outcross more frequently than their amphigenic counterparts. Reasons for this apparent incongruity are likely related to levels of inbreeding depression in this species: If inbreeding depression cannot be easily purged through selfing, then outcrossing with males should still be beneficial. Future research measuring the levels of inbreeding depression in both all-hermaphroditic and androdioecious populations of *E. dahlia* would be helpful in understanding the reasons this

species does not fully comply with the predictions of Chasnov's (2010) model.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

ORCID

Alissa Calabrese  <https://orcid.org/0000-0002-3295-236X>

Stephen C. Weeks  <https://orcid.org/0000-0001-8821-6612>

REFERENCES

- Anthes, N., & Michiels, N. K. (2007). Precopulatory stabbing, hypodermic injections and unilateral copulations in a hermaphroditic sea slug. *Biology Letters*, 3(2), 121–124. <https://doi.org/10.1098/rsbl.2006.0596>
- Arnqvist, G., & Rowe, L. (2002). Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, 787–789. <https://doi.org/10.1038/415787a>
- Baker, H. G. (1955). Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, 9(3), 347–349.
- Barrett, S. C. H., & Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature*, 352, 522–524.
- Bedhomme, S., Bernasconi, G., Koene, J. M., Lankinen, A., Arathi, H. S., Michiels, N. K., & Anthes, N. (2009). How does breeding system variation modulate sexual antagonism. *Biology Letters*, 5(5), 717–720. <https://doi.org/10.1098/rsbl.2009.0401>
- Benvenuto, C., Knott, B., & Weeks, S. C. (2009). Mate-guarding behavior in clam shrimp: A field approach. *Behavioral Ecology*, 20, 1125–1132. <https://doi.org/10.1093/beheco/arp106>
- Benvenuto, C., & Weeks, S. C. (2011). Mate guarding behavior in clam shrimp: the influence of mating system on intersexual conflict. *Behavioral Ecology and Sociobiology*, 65, 1899–1907. <https://doi.org/10.1007/s00265-011-1199-x>
- Benvenuto, C., & Weeks, S. C. (2012). Intersexual conflict during mate guarding in an androdioecious crustacean. *Behavioral Ecology*, 23(1), 218–224. <https://doi.org/10.1093/beheco/arr178>
- Blanckenhorn, W. U., Muhlhauser, C., Morf, C., Reusch, T., & Reuter, M. (2000). Female choice, female reluctance to make and sexual selection on body size in the dung fly, *Sepsis cynipsea*. *Ethology*, 106, 577–593.
- Byers, D. L., & Waller, D. M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics*, 30, 479–513. <https://doi.org/10.1146/annurev.ecolsys.30.1.479>
- Calabrese, A., McCulloch, C., Knott, B., & Weeks, S. C. (2016). Environmental characteristics of ephemeral rock pools explain local abundances of the clam shrimp, *Paralimnadia badia* (Branchiopoda: Spinicaudata: Limnadiidae). *Journal of the Royal Society of Western Australia*, 99, 9–15.
- Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003). Sexual conflict. *TRENDS in Ecology and Evolution*, 18(1), 41–47. [https://doi.org/10.1016/S0169-5347\(02\)00004-6](https://doi.org/10.1016/S0169-5347(02)00004-6)
- Chasnov, J. R. (2010). The evolution from females to hermaphrodites results in a sexual conflict over mating in androdioecious nematode worms and clam shrimp. *Journal of Evolutionary Biology*, 23, 539–556. <https://doi.org/10.1111/j.1420-9101.2009.01919.x>
- Chasnov, J. R., & Chow, K. L. (2002). Why are there males in the hermaphroditic species *Caenorhabditis elegans*? *Genetics*, 160, 983–994.
- Chasnov, J. R., So, W. K., Chan, C. M., & Chow, K. L. (2007). The species, sex, and stage specificity of a *Caenorhabditis* sex pheromone. *Proceedings of the National Academy of Sciences USA*, 104(16), 6730–6735. <https://doi.org/10.1073/pnas.0608050104>
- Ford, R. E., & Weeks, S. C. (2018). Intersexual conflict in androdioecious clam shrimp: Do androdioecious hermaphrodites evolve to avoid mating with males? *Ethology*, 124, 357–364.
- Garcia, L. R., LeBoeuf, B., & Koo, P. (2007). Diversity in mating behavior of hermaphroditic and male-female *Caenorhabditis* nematodes. *Genetics*, 175, 1761–1771. <https://doi.org/10.1534/genetics.106.068304>
- Haddrill, P. R., Majerus, M. E. N., & Shuker, D. M. (2013). Variation in male and female mating behavior among different populations of the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *European Journal of Entomology*, 110(1), 87–93.
- Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences USA*, 96, 5083–5088. <https://doi.org/10.1073/pnas.96.9.5083>
- Hollenbeck, V. G., Weeks, S. C., Gould, W. R., & Zucker, N. (2002). Maintenance of androdioecy in the freshwater shrimp *Eulimnadia texana*: sexual encounter rates and outcrossing success. *Behavioral Ecology*, 13(4), 561–570. <https://doi.org/10.1093/beheco/13.4.561>
- Husband, B. C., & Schemske, D. W. (1996). Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, 50, 54–70. <https://doi.org/10.1111/j.1558-5646.1996.tb04472.x>
- Johnson, T. E., & Wood, W. B. (1982). Genetic analysis of lifespan in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, 79, 6603–6607.
- Jormalainen, V. (1998). Precopulatory mate guarding in crustaceans: Male competitive strategy and intersexual conflict. *Quarterly Review of Biology*, 73(3), 275–304. <https://doi.org/10.1086/420306>
- Kleeman, G. A., & Basolo, A. L. (2007). Facultative decrease in mating resistance in hermaphroditic *Caenorhabditis elegans* with self-sperm depletion. *Animal Behavior*, 74, 1339–1347. <https://doi.org/10.1016/j.anbehav.2007.02.031>
- Mayr, E. (1976). Species concepts and definitions. *Topics in the Philosophy of Biology*, 353–371.
- Morran, L. T., Cappy, B. J., Anderson, J. L., & Phillips, P. C. (2009). Sexual partners for the stressed: Facultative outcrossing in the self-fertilizing nematode *Caenorhabditis elegans*. *Evolution*, 63(6), 1473–1482.
- Palopoli, M. F., Peden, C., Woo, C., Akiha, K., Ary, M., Cruze, L., Anderson, J. L., & Phillips, P. C. (2015). Natural and experimental evolution of sexual conflict within *Caenorhabditis* nematodes. *BMC Evolutionary Biology*, 15, 93.
- Parker, G. A. (1979). *Sexual selection and sexual conflict*. Sexual Selection and Reproductive Competition in Insects (eds Blum, MS, Blum NA), 123–166.
- Parker, G. A. (2006). Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society B*, 361(1466), 235–259.
- Pitnick, S., Miller, G. T., Reagan, J., & Holland, B. (2001). Males' evolutionary responses to experimental removal of sexual selection. *Proceedings of the Royal Society of London B*, 268, 1071–1080. <https://doi.org/10.1098/rspb.2001.1621>
- Sassaman, C. (1995). Sex determination and evolution of unisexuality in the Conchostraca. *Hydrobiologia*, 298, 45–65. <https://doi.org/10.1007/BF00033799>

- Sassaman, C., & Weeks, S. C. (1993). The genetic mechanism of sex determination in the conchostracan shrimp *Eulimnadia texana*. *The American Naturalist*, 141(2), 314–328. <https://doi.org/10.1086/285475>
- Wachtmeister, C. A., & Enquist, M. (2000). The evolution of courtship rituals in monogamous species. *Behavioral Ecology*, 11, 405–410. <https://doi.org/10.1093/beheco/11.4.405>
- Wang, H., Barrett, S. C. H., Li, X., Niu, Y., Duan, Y., Zhang, Z., & Li, Q. (2021). Sexual conflict in protandrous flowers and the evolution of gynodioecy. *Evolution*, 75(2), 278–293. <https://doi.org/10.1111/evo.14113>
- Weeks, S. C. (2004). Levels of inbreeding depression over seven generations of selfing in the androdioecious clam shrimp, *Eulimnadia texana*. *Journal of Evolutionary Biology*, 17, 475–484. <https://doi.org/10.1111/j.1420-9101.2004.00712.x>
- Weeks, S. C. (2009). Can males successfully invade hermaphroditic populations of clam shrimp (*Eulimnadia texana*)? *Current Science*, 96, 98–102.
- Weeks, S. C. (2012). The role of androdioecy and gynodioecy in mediating evolutionary transitions between dioecy and hermaphroditism in the Animalia. *Evolution*, 66(12), 3670–3686. <https://doi.org/10.1111/j.1558-5646.2012.01714.x>
- Weeks, S. C., & Benvenuto, C. (2008). Mate guarding in the androdioecious clam shrimp *Eulimnadia texana*: Male assessment of hermaphrodite receptivity. *Ethology*, 114, 64–74. <https://doi.org/10.1111/j.1439-0310.2007.01446.x>
- Weeks, S. C., Benvenuto, C., Sanderson, T. F., & Duff, R. J. (2010). Sex chromosome evolution in the clam shrimp, *Eulimnadia texana*. *Journal of Evolutionary Biology*, 23, 1100–1106.
- Weeks, S. C., Crosser, B. R., Bennett, R., Gray, M., & Zucker, N. (2000). Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: Estimates of inbreeding depression in two populations. *Evolution*, 54, 878–887. <https://doi.org/10.1111/j.0014-3820.2000.tb00088.x>
- Weeks, S. C., Crosser, B. R., & Gray, M. M. (2001). Relative fitness of two hermaphroditic mating types in the androdioecious clam shrimp, *Eulimnadia texana*. *Journal of Evolutionary Biology*, 14, 83–94.
- Weeks, S. C., Marcus, V., & Crosser, B. (1999). Inbreeding depression in a self-compatible, androdioecious crustacea, *Eulimnadia texana*. *Evolution*, 53, 472–483.
- Weeks, S. C., Marquette, C. L., & Latsch, E. (2004). Barriers to outcrossing success in the primarily self-fertilizing clam shrimp, *Eulimnadia texana* (Crustacea, Branchiopoda). *Invertebrate Biology*, 123(3), 146–155. <https://doi.org/10.1111/j.1744-7410.2004.tb00150.x>
- Weeks, S. C., Posgai, R. T., Cesari, M., & Scanabissi, F. (2005). Androdioecy inferred in the clam shrimp *Eulimnadia agassizii* (Spinicaudata: Limnadiidae). *Journal of Crustacean Biology*, 25, 323–328. <https://doi.org/10.1651/C-2555>
- Weeks, S. C., Sanderson, T. F., Wallace, B. F., & Bagatto, B. (2011). Behavioral cost of reproduction in a freshwater crustacean (*Eulimnadia texana*). *Ethology*, 117, 880–886. <https://doi.org/10.1111/j.1439-0310.2011.01942.x>
- Weeks, S. C., Sanderson, T. F., Zofkova, M., & Knott, B. (2008). Breeding systems in the clam shrimp family Limnadiidae (Branchiopoda, Spinicaudata). *Invertebrate Biology*, 127, 336–349. <https://doi.org/10.1111/j.1744-7410.2008.00130.x>
- Weeks, S. C., Zofkova, M., & Knott, B. (2006). Limnadiid clam shrimp biogeography in Australia (Crustacea: Branchiopoda: Spinicaudata). *Journal of the Royal Society of Western Australia*, 89, 155–161.
- Westneat, D. F., & Sargent, R. C. (1996). Sex and parenting: The effects of sexual conflict and parentage on parental strategies. *Trends in Ecology and Evolution*, 11, 87–91. [https://doi.org/10.1016/0169-5347\(96\)81049-4](https://doi.org/10.1016/0169-5347(96)81049-4)
- Williams, G. C. (1975). *Sex and Evolution*. Princeton University Press.
- Willis, J. H. (1999). Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. *Genetics*, 153, 1885–1898. <https://doi.org/10.1093/genetics/153.4.1885>
- Zucker, N., Cunningham, M., & Adams, H. P. (1997). Anatomical evidence for androdioecy in the clam shrimp *Eulimnadia texana*. *Hydrobiologia*, 359, 171–175.

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