Asexual freshwater snails make poor mate choice decisions

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Abstract

Once-useful traits that no longer contribute to fitness tend to decay over time. We address whether the expression of mating-related traits that increase the fitness of sexually reproducing individuals but are likely less useful or even costly to asexual counterparts seems to exhibit decay in the latter. *Potamopyrgus antipodarum* is a New Zealand freshwater snail characterized by repeated transitions from sexual to asexual reproduction. The frequent coexistence of sexual and asexual lineages makes *P. antipodarum* an excellent model for the study of mating-related trait loss. We used a mating choice assay including sexual and asexual *P. antipodarum* females and conspecific (presumed better choice) vs. heterospecific (presumed worse choice) males to evaluate the loss of behavioural traits related to sexual reproduction. We found that sexual females engaged in mating behaviours with conspecific mating partners more frequently and for a greater duration than with heterospecific mating partners, while asexual females seemed to lack the ability to make a choice. These results suggest that selection acting to maintain mate choice in asexual *P. antipodarum* is weak or ineffective relative to sexual females and that asexual reproduction likely contributes to the evolutionary decay of behavioural traits in this system.

Keywords: Asexuality, sexual reproduction, *Potamopyrgus antipodarum*, vestigial traits, parthenogenesis, trait decay

Introduction

Adaptations that were once important for survival that subsequently become useless or impose new fitness costs tend to decay over time in a process known as vestigialisation [1-3]. Many examples of this phenomenon can be seen throughout evolutionary history, including the loss of limbs in snakes [4], eye structures in cave-dwelling fish [5], and wings in many insect species [6].

While the genetic mechanisms underlying vestigialisation depend on evolutionary context, traits are most commonly lost by the accumulation of selectively neutral or deleterious mutations [7, 8]. The decay of presently useless characteristics exemplifies evolutionary tradeoffs: the decay of one trait frees up energy and resources to be used elsewhere by the organism [7]. For example, Roff [6] suggests that the evolution of flightlessness from flighted ancestors allows for allocation of resources for reproduction. This framework implies that trait loss could translate into increased fitness for organisms for which the lost trait is no longer useful.

The decay of traits specific to mating and/or sexual reproduction is one potential consequence of transitions from sexual to asexual reproduction. These traits are typically required for reproductive success in sexual organisms. By definition, asexual organisms do not need external genetic contribution to reproduce, with the exception of sperm-dependent forms of parthenogenesis (e.g., gynogenesis). Mating behaviours are themselves associated with numerous costs [9, 10]. Accordingly, vestigialisation of mating-related traits in asexual lineages – including those involved in mate choice – is expected, particularly in a scenario where such behaviours and their maintenance are costly [3, 11, 12]. Alternatively, these traits may be selectively neutral or maintained by selection in asexuals, but only in a context where the costs

do not outweigh the benefits of trait maintenance [13, 14]. Several studies have supported these ideas, finding abnormal or fully decayed traits involved in mating and reproduction (spermathecae in asexual *Timema* stick insects [13]; sperm in asexual *P. antipodarum* males [15]) in asexual organisms. These results suggest that these traits are not being maintained by selection, at least to the extent of the same traits in sexual counterparts. Our focus is on behaviours: assuming that mate quality contributes to fitness of sexual but not asexual females, do mating-related behaviours related to the likelihood that mate preferences are associated with mate quality [16-19] decay in asexual females relative to sexual counterparts?

Potamopyrgus antipodarum is a New Zealand freshwater snail characterized by the frequent coexistence of phenotypically and ecologically similar sexual and asexual lineages [20, 21], making this species an excellent model to study the vestigialization of traits involved in sexual reproduction. Previous studies have shown that mating behaviour in sexually reproducing male *P. antipodarum* does not differ from that of the presumed asexual males produced occasionally by asexual females [22]. Sexual males also show poor ability to discriminate between favourable and unfavourable mates, mating with equal frequency with sexual and asexual females and with parasitically castrated vs. healthy females [23]. Prior emphasis on investigation of male mating behaviour is largely a result of the assumption that males are physically in control of mating outcomes [24], despite a lack of studies assessing the female role. These previous results set the stage for a direct comparison between sexual and asexual *P. antipodarum* females. In a mating paradigm like that demonstrated for *P. antipodarum* where males seem to lack adequate mate discrimination, differences in mating between sexual and asexual females can be attributed to mating differences between these two female groups.

Therefore, we hypothesize that sexual female *P. antipodarum* are better able to choose a more favourable mate when presented with a choice than asexual counterparts.

We evaluated this hypothesis by comparing the mating behaviour of sexual and asexual *P. antipodarum* females with sexual male *P. antipodarum* and sexual male *Potamopyrgus estuarinus*. *Potamopyrgus estuarinus* is a closely related outgroup species with minimal habitat overlap with *P. antipodarum* [25]. Female *P. antipodarum* mating efforts with *P. antipodarum* males are presumed to be favourable relative to mating with *P. estuarinus* because of the possibility to generate offspring only with the former. Mating behavior (but not offspring production) has been shown to occur between male and female *P. antipodarum* and *P. estuarinus* [26], thereby providing *P. antipodarum* females with an adequate alternative choice.

Materials and Methods

Snail Selection and Care

We haphazardly selected three sets of 10 sexually mature (>3 mm long [27, 28]) sexual (N=30) and asexual (N=30) female *P. antipodarum* descended from one sexual and one asexual female, respectively, collected from Lake Mapourika, New Zealand, in 2018. These six sets of 10 female snails will hereafter be referred to as 'populations.' We also haphazardly selected three sets of 20 each sexually mature (possessing a visible penis [29]) sexual *P. antipodarum* males (N=60) and *Potamopyrgus estuarinus* males (N=60). All *P. antipodarum* males were chosen from sexual lineages established by females sampled from New Zealand Lakes Mapourika and Selfe in 2018. These lineages were separately derived from the lineages from which we drew females. We isolated each population for approximately 14 days prior to behavioural trials to control for previous exposure to males and possible recent mating. We painted female shells with light-

coloured nail polish to aid in differentiation between females and males when analysing video footage.

We housed each population in a 0.95 L plastic cup containing ~700 mL of water. Snails were fed *Spirulina* (a common *P. antipodarum* lab food) three times weekly and were given powdered chalk as a source of dietary calcium once weekly. Cups were maintained at 16°C on a 12:12 hour light:dark cycle (feeding and maintenance following [24]).

Mating trials occurred in standard four-inch petri dishes. We covered the exterior of each dish in electrical tape to control for external visual stimuli. To begin each trial for the sexual females, we placed one sexual *P. antipodarum* female, one sexual *P. antipodarum* male, and one *P. estuarinus* male equidistant from one another around the edges of the inside of the petri dish (Fig. 1). After the three snails were placed, we filled the petri dish to about 75% total volume with carbon-filtered tap water. We used this same setup for asexual *P. antipodarum* females, with the asexual female replacing the sexual female. No snail was used in more than one trial.

Observation of Mating Behavior

We conducted mating trials three times weekly at approximately 1500 hours, for three hours. As the maximum copulation duration for *P. antipodarum* is about two hours [23], we established this time frame to allow for multiple mating attempts to occur. We recorded trials using a USB webcam and time-lapse video software VideoVelocity (v3.7.2090 [30]). The three snails per trial were allowed to move and interact freely without physical barriers inside the petri dish.

We loosely followed previous studies of mating in *P. antipodarum* (e.g., [24]) to define attempted mating behaviour as any physical interaction resulting in one snail mounting the shell of another. It is relevant to note that it is otherwise impossible to otherwise visually confirm

sexual contact (joining of genitalia) between *P. antipodarum* because this phenomenon is obscured by the shells. Once this mounting behaviour was initiated, we arbitrarily established that any such interaction lasting 15 seconds or longer would be counted as an instance of mate choice; interactions shorter than 15 seconds were potentially too short to be discernible with our time-lapse software and were excluded. Fifteen seconds is also more than enough time for snails to reorient and move on following an incidental – versus mating – encounter. Both the frequency (number of mating interactions females had with either *P. antipodarum* or *P. estuarinus* males) and duration (length of time (seconds) that females and males were in physical contact) of mating interactions between females and either *P. antipodarum* or *P. estuarinus* males were recorded. Hereafter, mating pairs will be referred to with the female listed first as either SexF or AsexF, respectively, and the male referred to by species [*P. antipodarum* male = PAM; *P. estuarinus* male = PEM (e.g., SexF/PAM refers to a sexual female and male *P. antipodarum* pair)].

We also used this experimental framework to establish expected outcomes and determine how these outcomes would inform our hypotheses. First, a successful experiment would require that sexual females mate more frequently and for longer with *P. antipodarum* males than with *P. estuarinus* males. This outcome is critical in indicating that sexual females possess the ability to discriminate between preferential and poor mating partners. Absence of a difference in mating behaviour across male type for sexual females would indicate that these females are unable to differentiate between mates or that observed contact is reflective of behaviour outside of mating. Mating-related trait decay in asexual females should be evident in relatively weak or absent mate choice for *P. antipodarum* vs. *P. estuarinus* males relative to choices in sexual female *P. antipodarum*.

Statistical Analyses

We used the Shapiro-Wilk test to determine that both the mating frequency (sexual females: W = 0.769, p = 8.18x10^{-8}; asexual females: W = 0.810, p = 1.12x10^{-5}) and the mating duration (sexual females: W = 0.642, p = 1.05x10^{-9}; asexual females: W = 0.493, p = 3.17x10^{-8}) datasets violated normality assumptions of parametric analysis. Accordingly, we used nonparametric approaches to analyse the data. First, we used Wilcoxon rank-sum analyses to determine whether mating durations and frequencies of sexual and asexual females, respectively, with *P. antipodarum* males differed from mating durations and frequencies with *P. estuarinus* males. As another means of comparing mating behaviour across sexual and asexual females, we calculated discrimination scores - defined as the difference between mating frequency and mating duration, respectively, with male *P. antipodarum* versus male *P. estuarinus* – for each female. We then used Kruskal-Wallis tests to determine whether there was a significant difference in the mating frequency and mating duration discrimination scores, respectively, between sexual and asexual females. Finally, we used Kruskal-Wallis tests to compare the difference in mating frequency and duration between individual sexual and asexual females. All analyses were executed with R 3.6.2 [31], the ggplot2 (v3.3.3; [32]), ggpubr (v0.4.0; [33]) and dplyr (v1.0.4; [34]) packages.

Results

Sexual females mated significantly more often with *P. antipodarum* males (mean 1.41 ± 1.39 SD matings) than with *P. estuarinus* males (mean 0.67 ± 0.78 SD matings) (W = 483, p = 0.0303) (Fig. 2A). By contrast, asexual females mated with *P. antipodarum* males (mean 1.20 ± 1.50 SD

matings) with the same frequency as with *P. estuarinus* males (1.45 ± 1.43 SD matings) (W = 172, p = 0.427) (Fig. 2B).

Sexual females also mated for significantly longer with *P. antipodarum* males (405.56 ± 520.95 SD seconds) than with *P. estuarinus* males (129.92 ± 349.74 SD seconds (W = 520, p = 0.00608) (Fig. 2C). Asexual females did not differ in the duration of mating with *P. antipodarum* (138.73 ± 269.67 SD seconds) and *P. estuarinus* males (52.82 ± 54.88 SD seconds) (W = 202, p = 0.967) (Fig. 2D).

The discrimination score analysis revealed that sexual but not asexual females with a choice between *P. antipodarum* and *P. estuarinus* males tend to make mating behaviour decision with respect to mating frequency that favour the former (Kruskal-Wallis X^2 = 4.47, df = 1, p = 0.0344). By contrast, there was no significant difference in difference scores for mating duration between sexual and asexual females (Kruskal-Wallis X^2 = 1.79, df = 1, p = 0.181).

Discussion

Our experiment revealed that sexual female *P. antipodarum* exhibited significantly higher mating frequency and longer mating duration with conspecific vs. heterospecific males. These results are as predicted if sexual female *P. antipodarum* are able to discriminate between mates of higher vs. lower quality. By contrast, asexual *P. antipodarum* females showed no evidence for mate discrimination or preference. Together, these findings suggest that mate choice is exhibited by sexual but not asexual *P. antipodarum* females.

Evolutionary decay of mating-related traits in asexual contexts is a plausible explanation for these results and is consistent with a previous study demonstrating evidence for decay of sperm traits in the male offspring occasionally produced by asexual female *P. antipodarum* [15].

Despite absence of evidence for discriminatory mating behaviour, asexual females mated at a nearly identical frequency with *P. antipodarum* males as sexual females (Fig. 2). The high prevalence of mating behaviour combined with the lack of observable discriminatory behaviour in asexual females suggests that either copulatory behaviour is still necessary and/or beneficial, or simply that this behaviour has not fully decayed. That asexual females nevertheless engage in mating behaviour at all could be a function of the relatively recent derivation of most asexual female *P. antipodarum* from sexual counterparts [35]. With this in mind, it would be interesting to repeat this experiment with females from older asexual lineages (*sensu* [24] but departing from this prior study so that females vs. males have mate choice capabilities).

Another, non-mutually exclusive explanation is that copulatory behaviour could be required for maximal reproductive output in *P. antipodarum* ([36]; reviewed in [37]). Neiman [38] speculated that asexual females that have no direct use for sperm contributed by males might still benefit from mating if copulatory stimuli are required for maximization of reproduction, though Neiman [39], showed that this scenario is unlikely for *P. antipodarum*. Alternatively, male ejaculate may provide nutritive benefits to asexual females. This possibility is supported by the fact that sperm storage structures are maintained in asexual *P. antipodarum* females [40]; though evidence that these structures might also function for the digestion of waste in asexual *P. antipodarum* suggests the potential for a non-mating-specific or even unique function for this organ in asexuals [41]. Follow-up studies of whether and how sperm/ejaculate are used in asexual *P. antipodarum* and other asexual taxa will help clarify the prevalence and persistence of copulatory behaviour in asexual females.

Author Contributions

SS, JJ, and MN conceived of and designed the experiment. SS executed the experiment and collected the data. SS and MN analysed the data. SS created figures and drafted the manuscript. JJ and MN revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Figure Legends

Figure 1. One *P. estuarinus* male (A), one *P. antipodarum* male (B), and one sexual or asexual *P. antipodarum* female (C) were placed approximately equidistant from one another around the edges of the petri dish. Each female (C) was painted a light colour with nail polish.

Figure 2. Violin plots of mating frequency (A, B) and duration (C, D) of sexual (A, C; "SexF") and asexual (B, D; "AsexF") females with *P. antipodarum* ("PAM") and *P. estuarinus* ("PEM") males. Median values are indicated with black dots.

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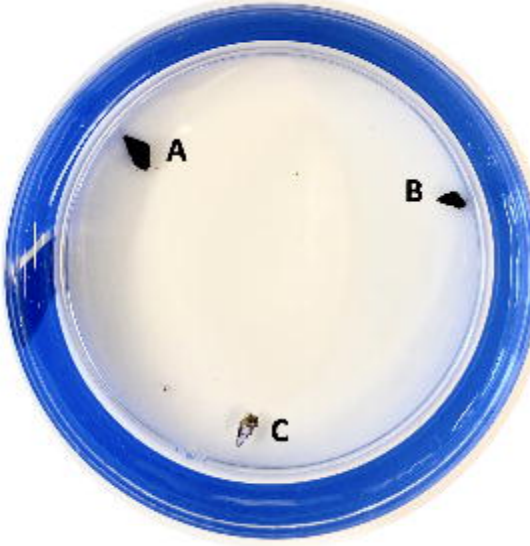
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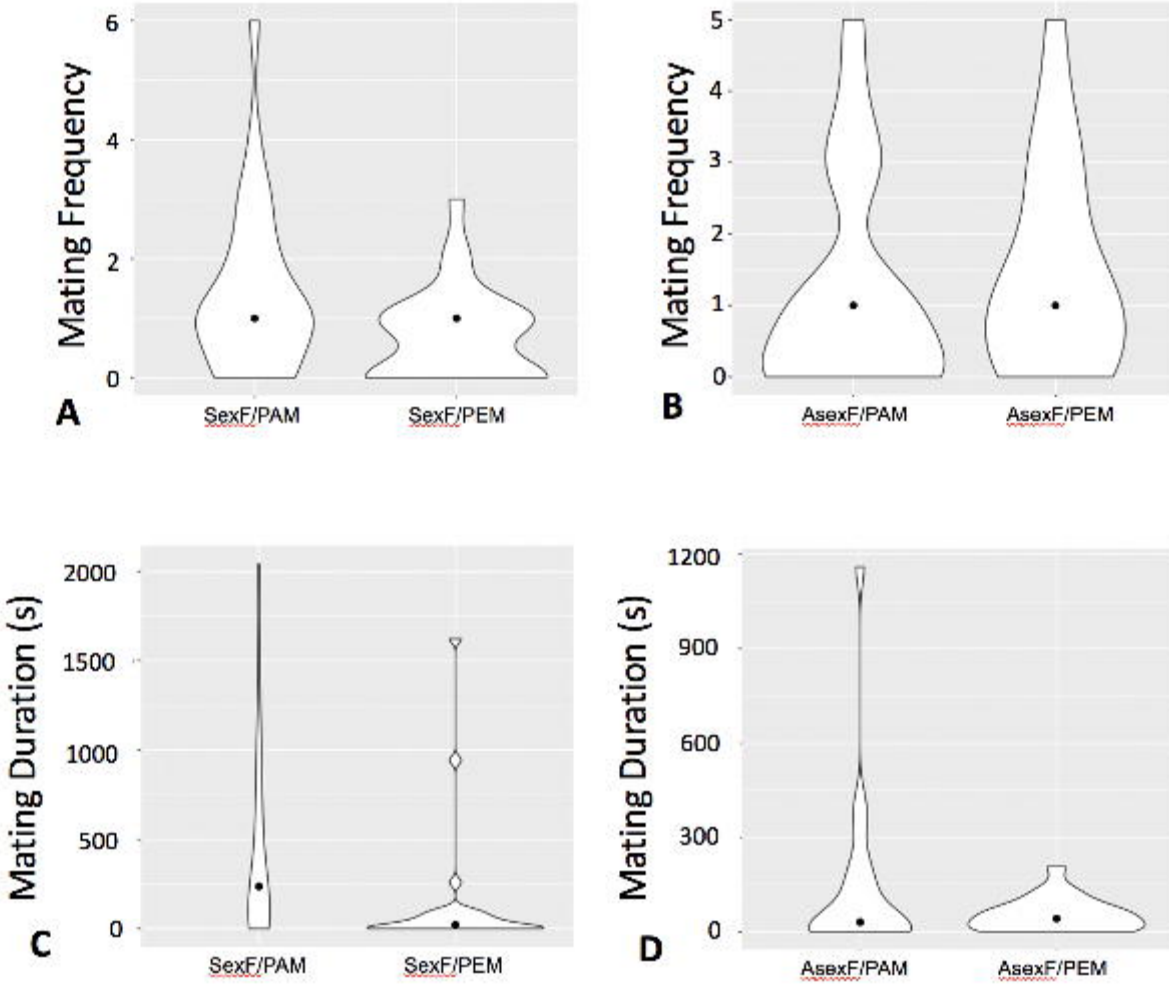
Mating Frequency
6
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A
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Mating Frequency
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AsexF/PEM
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SexF/PAM
SexF/PEM
Mating Duration (s)
1200
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AsexF/PAM
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