RESEARCH ARTICLE

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Sexually but not parthenogenetically produced females benefit from mating in a stick insect

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Abstract

1. In facultatively parthenogenetic populations, the prevalence of sexual reproduction depends on whether females mate and therefore produce sons and daughters or avoid mating and produce daughters only.
2. The relative advantage of mating in such species may depend on a female's own reproductive origin (i.e. development from a fertilised or unfertilised egg) if parthenogenesis reduces heterozygosity similar to sexual inbreeding, or if it inhibits mating, sperm storage or fertilisation. But effects of reproductive origin on development and performance are poorly understood.
3. Using the facultatively parthenogenetic stick insect, *Extatosoma tiaratum*, we quantified morphology, mating probability, and reproductive success in mated versus unmated females of sexual versus automictic (parthenogenetic) origin.
4. We found strong evidence that increased homozygosity negatively impacted some traits in parthenogenetically produced females: compared to sexually produced females, parthenogenetically produced females were smaller and more prone to deformities in vestigial wings, but not more prone to fluctuating asymmetry in their legs.
5. Parthenogenetically produced females received fewer mating attempts and avoided mating more often than sexually produced females. Yet, contrary to the expectation that sex should rescue parthenogenetic lineages from the detrimental effects of increased homozygosity, parthenogenetically produced females gained no net reproductive benefit from mating, suggesting that physiological constraints limit fitness returns of sexual reproduction for these females.
6. Our findings indicate that advantages of mating in this species depend on female reproductive origin. These results could help to explain spatial distributions of sex in facultatively parthenogenetic animals and evolutionary transitions to obligate asexuality.

KEYWORDS
automixis, facultative parthenogenesis, life-history, mating behaviour, maternal effect, paradox of sex, Phasmatodea

1 | INTRODUCTION

Numerous animal lineages have undergone evolutionary transitions from sexual to asexual reproduction, but many questions remain about how such transitions occur. In facultatively parthenogenetic animals, female individuals are capable of both sexual and asexual reproduction: females that mate produce both sons and daughters from fertilised eggs (although mated females can also produce some offspring parthenogenetically, as occurs in *Timema* stick insects; Arbuthnott et al., 2015; Schwander et al., 2010), while females that avoid mating usually produce only daughters that develop partheno-genetically from unfertilised eggs (although it is possible for virgin females to occasionally produce sons parthenogenetically through the rare loss of an X chromosome, as occurs in a number of stick insect species; Brock et al., 2012; Morgan-Richards et al., 2019; Pijnacker, 1969). In facultatively parthenogenetic organisms, all-female populations can be established if females do not mate. Indeed, many such species exhibit geographical mosaics where mixed-sex, sexually reproducing populations and all-female, par-thenogenetically reproducing populations coexist alongside each other (Buckley et al., 2009; Burns et al., 2018; Law & Crespi, 2002; Morgan-Richards et al., 2010), and all-female populations can, in some cases, evolve to become obligately asexual species (Schwander et al., 2010). Facultatively parthenogenetic systems thus offer valu-able opportunities to investigate the factors and processes that en-able evolutionary transitions between reproductive modes.

Because facultatively parthenogenetic females typically have to mate to produce sons, the prevalence of sexual versus asexual re-production in facultative parthenogens is likely to depend in part on females' propensity to mate, store sperm and use sperm, as well as on the fitness costs of mating arising from sexual conflict (Burke & Bonduriansky, 2017). Sexual conflict over whether to mate could be particularly important in facultative systems if females that do not mate perform better, in at least some circumstances, than those that do (Burke & Bonduriansky, 2017). Such females will be selected to avoid mating and reproduce parthenogenetically, which could have flow-on effects for spatial and temporal distributions of sex (Burke & Bonduriansky, 2018b). However, since facultatively parthenogenetic females can be produced either sexually or asexually, the relative benefits of mating versus not mating could depend on the sexual versus asexual reproductive origin of mothers (i.e. whether mothers developed from a fertilised or unfertilised egg).

Reproductive origin could affect the costs and benefits of mat-ing for several reasons. First, the mode of reproduction could affect heterozygosity and developmental stability if parthenogenesis oc-curs via automixis. Under automictic parthenogenesis, diploidy is re-stored by the fusion of meiotic products which can result in zygotes with substantially reduced heterozygosity (Engelstaedter, 2017; Goudie et al., 2012; Nougué et al., 2015; Pearcy et al., 2006; Pertoldi et al., 2001; Stenberg & Saura, 2009; Suomalainen et al., 1987). As with inbreeding in obligately sexual populations, lower heterozy-gosity due to automictic parthenogenesis can depress fitness by increasing the expression of recessive, deleterious alleles previously

masked by dominance effects. If heterozygosity per se confers a fitness advantage, automixis could reduce fitness by increasing the overall number of homozygous loci (Crnokrak & Barrett, 2002). These effects can cause automictic organisms to exhibit larger per-turbations from the normal morphology (Leary et al., 1985; Pertoldi et al., 2001). If the expression of functional traits deteriorates in asexual lineages as a result of reduced heterozygosity, then such lineages could be 'rescued' by sexual recombination, as predicted for inbred genotypes produced by mating between close relatives (Charlesworth et al., 1993; Lynch et al., 1990; Maynard Smith, 1978; Muller, 1964; Zeyl et al., 2001), leading to the prediction that sex-ual reproduction should be particularly beneficial for females with a parthenogenetic history. Nonetheless, because automictic popu-lations can theoretically have higher neutral genetic diversity and lower genetic load compared to sexual populations due to differ-ences in how selection and drift operate in these populations (Engelstaedter, 2017), lineages that regularly reproduce via auto-mixis might show little functional trait decay and may even benefit by continuing to reproduce automictically.

Reproductive origin could also affect the likelihood and fitness consequences of mating versus not mating via maternal effects. If parthenogenesis is fairly frequent but its occurrence varies tem-porally or spatially (e.g. because of variation in the abundance of males), maternal reproductive origin could predict daughters' repro-ductive environment, such that daughters of parthenogenetically produced females are themselves likely to reproduce parthenoge-netically. Selection could thus favour maternal effects that opti-mise the physiology or behaviour of females for the reproductive mode that matches their mother's, such that females that developed from fertilised eggs are optimised for mating and sexual reproduc-tion, whereas females that developed from unfertilised eggs are optimised for parthenogenetic reproduction. If asexually produced females produce eggs that are optimised for parthenogenetic de-velopment but subsequently encounter males, mating could lead to high rates of fertilisation failure or embryo death because sperm (or seminal products) could interfere with parthenogenetic develop-ment. Parthenogenetically produced females could therefore exhibit reduced propensity to mate and reduced reproductive performance via mating than via parthenogenesis.

Stick insects are ideal organisms for investigating effects of re-productive origin as many species are facultatively parthenogenetic. Facultative parthenogenesis has evolved multiple times in this group (Bradler & Buckley, 2018), and sexual reproduction has been lost and regained on numerous occasions (Morgan-Richards et al., 2019; Schwander et al., 2010). Here, we ask how females' reproductive origin affects their morphology and reproductive performance fol-lowing mating versus no mating in the facultatively parthenogenetic spiny leaf stick insect, *Extatosoma tiaratum*, a sexually dimorphic phasmid endemic to tropical rainforests in north-eastern Australia (Brock & Hasenpusch, 2007). Whereas females of this species have reduced wings and are incapable of flight, males have functional wings and readily fly in search of mates (Brock, 2001). Females start laying eggs approximately 1 month after their final moult and

oviposit eggs one at a time continuously throughout their lives (Burke et al., 2015; Carlberg, 1983). The high rate of transition to homozygosity (between 0.66 and 1, based on 15 polymorphic loci) of parthenogenetically produced daughters (Alavi et al., 2018) suggests that the mechanism of parthenogenesis in *E. tiaratum* is automixis by terminal fusion. The ability of facultative parthenogens to reproduce both sexually and asexually is thought to provide reproductive assurance in situations where mating is particularly challenging, such as when density is low (Kramer & Templeton, 2001; Schwander et al., 2010; Stalker, 1956), although the presence of male harassment can complicate such density effects (Gerber & Kokko, 2016). Little is known about the density of wild populations of *E. tiaratum*, but it has been suggested that sexual conflict over mating in this species could favour the ability to reproduce asexually by helping females to avoid mating (Burke & Bonduriansky, 2017; Burke et al., 2015). Sexual conflict in *E. tiaratum* is suggested by sexual behaviour and chemical signalling: males use their genitalic clasping structure (vomer) to grip onto the ventral lamella of the female sub-genital plate to facilitate copulation (Bedford, 1978), but virgin females typically curl their abdomens to avoid being clasped by males (Burke et al., 2015). Pre-reproductive females also secrete repugnatorial scents that repel males, and mature females alter their pheromonal signals when reproducing asexually, making themselves less detectable to males (Burke et al., 2015). Whether the intensity of sexual conflict over mating depends on female reproductive origin remains unknown.

Starting with a sexually propagated laboratory population of *E. tiaratum*, we obtained fertilised eggs from females that had been allowed to mate and unfertilised eggs from females that had been isolated from males and reared the resulting offspring to produce focal females of sexual and asexual origin. We asked whether focal females' reproductive origin affected the developmental stability of their morphological traits and whether functional traits (legs) or non-functional traits (vestigial wings) were similarly impacted. If functional traits are well-canalised against deformities, or if recessive mutations that adversely affect such traits are rare, then asexually produced females may suffer little reduction in performance, whereas deformities may be more common in non-functional traits due to weaker selection on those traits (Crespi & Vanderkist, 1997). The first generation of asexual reproduction may be especially relevant for gauging the magnitude of morphological effects of parthenogenetic reproduction because, as with inbreeding, purging of deleterious recessive alleles could reduce mutation load in subsequent generations (Charlesworth et al., 1990; Hedrick, 1994; Hedrick & Garcia-Dorado, 2016). To assess the effect of females' sexual versus asexual origin on their own mating behaviour and reproductive performance with versus without mating, focal females in each reproductive origin treatment group were paired with males until mating occurred (mated treatment group) or with males that were incapable of mating (virgin treatment group). In females from the mated treatment group, we asked whether female attractiveness (quantified as the number of mating attempts delivered by males), resistance (quantified as a composite score of the frequency of kicking, walking away, playing dead and abdomen curling during mating attempts) and mating probability (quantified as the proportion of females that mated during a 72-hr pairing with males capable of mating) depended on reproductive origin. We also assessed the effects of reproductive origin and mating status on focal females' reproductive performance by quantifying oviposition latency, fecundity, egg hatching rate, offspring sex ratio and expected total offspring.

2 | MATERIALS AND METHODS

2.1 | Experimental design and animal maintenance

We performed a fully factorial laboratory experiment using mated and virgin *E. tiaratum* females that were either sexually or parthenogenetically produced (parth-mated: $n = 33$; parth-virgin: $n = 32$; sex-mated: $n = 38$; sex-virgin: $n = 30$). The nocturnal lifestyle, cryptic morphology and rainforest canopy habitat of *E. tiaratum* makes sourcing wild-caught individuals in large numbers challenging. Our laboratory population was therefore founded using several dozen individuals of both sexes obtained from professional insect breeders. While our laboratory population may have been highly heterozygous as a result of crossing individuals originally sourced from different locations (see Section 4), there is no reason to believe that natural populations of this species are typically inbred since *E. tiaratum* males can fly and may therefore be able to disperse over substantial distances, and heterozygosity did not differ from Hardy–Weinberg expectations in a wild population (Alavi et al., 2018). Our stock population was propagated for two generations prior to the start of the experiment by housing males and females together in large groups and allowing them to mate freely throughout their lives. From these laboratory stocks, we then obtained focal females of the parthenogenetic origin treatment as 2nd and 3rd instar nymphs from eggs laid by unmated females that had been isolated from males their whole adult life. We obtained focal females of the sexual origin treatment as 2nd and 3rd instar nymphs from eggs laid by mated females that had been housed with males since hatching and allowed to freely mate. Focal females were housed individually in cylindrical enclosures (20 cm diameter × 40 cm high), whereas males that were used for the experiment were kept in groups in 90 L tubs. Females and males were both fed *Agonis flexuosa* leaves ad libitum. The leaves were sprayed with water every other day for the stick insects to drink. No ethical approvals were required to study this stick insect.

Approximately 2 weeks after adult ecdysis, female focals were randomly assigned to either a 'mated' or 'virgin' treatment group. Females in the mated group were each paired with a male and could freely mate. Females in the virgin group were each paired with a male that had a skirt of waxed paper taped around the end of their abdomens which prevented copulation but ensured females experienced male pheromonal and tactile stimuli. This design allowed effects of mating to be isolated from other effects of male exposure. Because high mortality limited male numbers, some males were used more

than once in the unmated treatment (with male identity accounted for statistically in our analyses). Pairings lasted 3 days and 3 nights and took place in shallow enclosures ($13 \times 12 \times 22$ cm) so that mating behaviours could be captured on video. Only pairings of the mated treatment group were recorded. Females in the mated treatment that failed to mate during the 72-hr observation period were kept with the same male until mating took place up to 4 days later.

E. tiaratum females store sperm from a single mating for several weeks (Carlberg, 1983), enabling our mated females to produce offspring sexually throughout the experiment. Nonetheless, like other facultatively parthenogenetic stick insects (e.g. Arbuthnott et al., 2015; Schwander et al., 2010), mated *E. tiaratum* females can produce some offspring parthenogenetically (Alavi, 2016: chapter 5), and it is possible that production of such offspring contributed to the effects that we report (see Section 4).

2.2 | Effect of reproductive origin on morphology

To investigate the effect of reproductive origin on developmental stability, we photographed the fore, mid, and hind femurs and tibias of focal females after death and assessed non-directional deviations from bilateral symmetry (i.e. fluctuating asymmetry; Palmer & Strobeck, 1986) for each of these traits. We initially subtracted right-side lengths from left-side lengths (Figure 1A) and assessed confirmation to normality of these signed differences using Jarque–Bera normality tests from the moments package in R (Komsta &

Novomestky, 2015). Since distributions of asymmetry for all leg lengths were strongly non-normal ($76.53 \leq JB \leq 11,601$; $p < 0.001$) and showed very high kurtosis ($6.72 \leq \kappa \leq 49.65$), we used a nonparametric method for assessing fluctuating asymmetry that maximises power and reduces type I errors when kurtosis is high (Leung et al., 2000). Briefly, this method obtains a composite measure of fluctuating asymmetry by ranking the absolute values of length differences for each trait and summing the ranks for each individual (Leung et al., 2000). For females with missing legs, we excluded the matching leg from the opposite side of the body. For summary statistics, we report the absolute value of the difference between the sum of the left-leg segments and the sum of the right-leg segments (Table 1). Because legs are functional traits used in locomotion, high asymmetry in leg length is likely to impede locomotory ability and reduce fitness (Gummer & Brigham, 1995; Palmer & Strobeck, 1986). We also photographed the forewings of focal females and quantified the distance between them along the border of the dorsal sclerites of the mesothorax and metathorax (see Figure 1B). Because overlapping forewings (i.e. no distance between wings) is the normal morphology for *E. tiaratum* (Brock, 2001), we interpreted larger distances between forewings as evidence of increased developmental instability. However, because the wings are vestigial and nonfunctional in *E. tiaratum* females (Brock & Hasenpusch, 2007), wing deformity is unlikely to be strongly, causally linked to fitness (see Crespi & Vanderkist, 1997).

Measurements from images were made using ImageJ software (Eliceiri et al., 2012). Each measurement was replicated three times
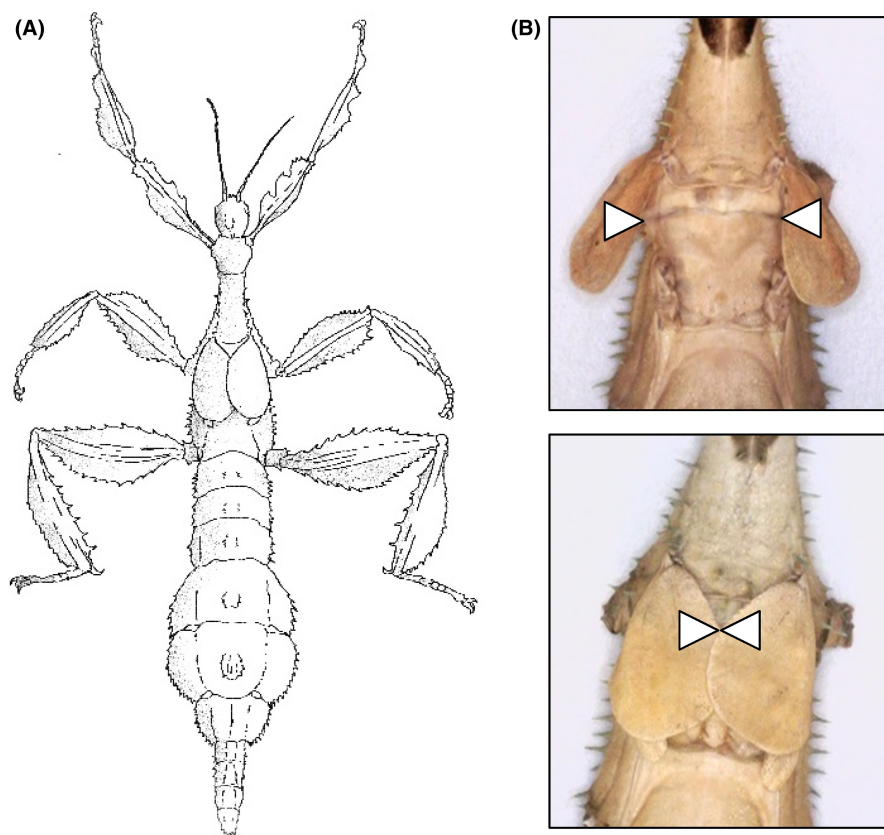

FIGURE 1 Images of *Extatosoma tiaratum* females. Leg-length asymmetry was calculated by taking the absolute value of the difference between left-hand femoral and tibial lengths and right-hand femoral and tibial lengths (A). Distances between forewings were measured along the border of the mesothoracic and metathoracic sclerites (i.e. the distance between the white arrows in B). Drawing courtesy of David Sindel

TABLE 1 Summary statistics

	Sexual origin (S) (n = 68)		Parthenogenetic origin (P) (n = 65)	
Leg asymmetry	1.03 (0.15) *61*		1.82 (0.35) *60*	
Distance between forewings	1.40 (0.24) *61*		4.89 (0.34) *60*	
Body size	119.28 (1.08) *68*		114.68 (0.78) *65*	
	Mated (S:M) (n = 38)	**Virgin (S:V)** (n = 30)	**Mated (P:M)** (n = 33)	**Virgin (P:V)** (n = 32)
Mating attempts	19.29 (2.77) *38*	NA	16.31 (5.04) *32*	NA
Resistance by abdomen curling	0.73 (0.04) *38*	NA	0.59 (0.06) *32*	NA
Resistance by kicking	0.02 (0.02) *38*	NA	0.04 (0.02) *32*	NA
Resistance by playing dead	0.01 (0.01) *38*	NA	0.07 (0.03) *32*	NA
Resistance by walking away	0.23 (0.04) *38*	NA	0.17 (0.05) *32*	NA
Composite resistance score	1.00 (0.06) *38*	NA	0.86 (0.08) *32*	NA
Proportion of females that mated	0.68, *38*	NA	0.39, *33*	NA
Latency to oviposit	32.05 (1.16) *38*	35.50 (2.18) *30*	41.69 (1.59) *32*	40.28 (2.04) *25*
Egg output	66.00 (2.94) *38*	65.30 (3.80) *30*	55.55 (2.99) *33*	45.97(5.28) *32*
Hatching rate	0.52 (0.05) *38*	0.29 (0.03) *30*	0.27 (0.04) *32*	0.34 (0.04) *25*
Expected offspring count	35.16 (3.66) *38*	19.93 (3.01) *30*	15.83 (2.62) *33*	20.62 (2.35) *32*
Offspring sex ratio	0.23 (0.04) *38*	0.02 (0.02) *30*	0.19 (0.05) *33*	0 (0) *32*

Note: Statistics are means, *SE*s (in brackets), and sample sizes (in italics). NAs indicate responses which were not measured for certain treatment levels, and therefore for which summary statistics do not apply. Note that the mating behaviours for a single female in the P:M treatment could not be assessed because the video of that female was corrupted. Note that the smaller sample sizes for morphological measurements are due to lost specimen samples.

Abbreviations: P:M, mated females of parthenogenetic origin (these females successfully mated within 7 days, and egg collection began after mating); P:V, virgin females of parthenogenetic origin; S:M, mated females of sexual origin (these females successfully mated within 7 days, and egg collection began after mating); S:V, virgin females of sexual origin.

and the mean of the replicates was used in subsequent calculations to minimise effects of measurement error. We analysed composite leg asymmetry scores (calculated as described above) using a linear model (LM) in which female reproductive origin (sexual vs. parthenogenetic) was the fixed effect. Day of ecdysis (i.e. days since the first female moulted) was included as a scaled covariate to account for potential seasonality effects, since *E. tiaratum* eggs are oviposited continuously and therefore hatch and develop into adults over a period of months (Carlberg, 1983), and also because focal females entered the mated/virgin treatment over a period of 4 months. To account for potential body-size effects, which are common in insects (Honěk, 1993), body size (i.e. total body length, mouth to end of ovipositor, in mm) was also included as a scaled covariate in the model. We analysed the distance between forewings in a LM that was structured the same as the leg asymmetry LM. We also analysed female body size in a LM with mode of origin as the fixed effect and day of ecdysis as the scaled covariate.

2.3 | Effect of reproductive origin on mating interactions and resistance behaviours

We recorded sexual interactions under red light using tablet devices with built-in video cameras (Samsung Galaxy Tab A 6). To test whether females of sexual and parthenogenetic origin differed in the number of mating attempts they experienced, we counted the number of times that males attempted to use their vomer to grip onto the ventral surface of the female ovipositor and analysed this response in a generalised linear mixed-effects model (GLMM) with a Poisson error structure and log link function. Female reproductive origin was the fixed effect, with day of ecdysis, body size and female age at pairing as scaled covariates. Female identity was included as an observation-level random effect (OLRE) to correct for overdispersion.

From the video footage, we calculated the proportion of mating attempts that elicited different resistance behaviours previously documented in this species—playing dead (thanatosis), abdomen curling, kicking and walking away (Burke & Bonduriansky, 2019)—and summed these proportions together to give a composite score of resistance for each female. We then analysed this score in a LM with a Gaussian error structure and identity link function. Separate analyses of each resistance behaviour are reported in Appendix S1. We also assessed mating success using a GLMM with a binomial error structure and logit link function. The response variable was the number of females that successfully mated during the pairing period treated as a success–failure binomial proportion. The rest of the model was structured the same as the GLMM for mating attempts.

2.4 | Effect of reproductive origin on the performance of mated versus virgin females

Females in the mated treatment that did not mate during the 3-day pairing period were kept with their male partner until mating occurred. Copulation was determined by the presence of a conspicuous spermatophore (Clark, 1974). All focal females were then separated from males and housed in individual enclosures thereafter. We allowed females to lay eggs for 28 days to estimate fecundity and subsampled 20 of these eggs from each female (or all eggs if females laid fewer than 20) to assess hatching rate. These eggs were kept in damp coco-peat at 27°C, 60% relative humidity and 13:11 hr day–night cycle. Newly emerged hatchlings were counted twice a week and killed by freezing. We stopped checking for hatchlings after three consecutive months of no emergence. We later sexed the hatchlings based on abdomen morphology (Figure S1) using a Leica MS5 stereo microscope. Some hatchlings (152/940 = 16%) could not be sexed because their abdomens had deteriorated after death.

We assessed the effect of female origin (sexual vs. parthenogenetic) and mating status (mated vs. virgin) on four measures of reproductive performance: latency to oviposit, egg output, hatching rate and total offspring (estimated as the product of egg output—including cases of zero egg output—and hatching rate). For oviposition latency, we analysed the time in days from final ecdysis to first oviposition (which was log-transformed to meet parametric assumptions) using a linear mixed-effects model (LMM) with a Gaussian error structure and identity link function. Egg output was analysed using a GLMM with a Poisson error structure and log link function; hatching rate was analysed as a binomial presence–absence proportion in a GLMM with a binomial error structure and logit link function; and expected total number of offspring was analysed using a LMM with a Gaussian error structure and identity link function. The interacting fixed effects in each of these models were female reproductive origin and mating status. We also included day of adult ecdysis and body size as covariates. Male identity was included as a random effect. GLMMs for egg output and hatching success also included female identity as an additional OLRE to correct for overdispersion.

To assess whether mated females of sexual versus parthenogenetic origin produced a similar proportion of sons to daughters, we analysed male and female hatchling counts as a binomial proportion using a GLMM with a binomial error structure and logit link function. Reproductive origin was the fixed effect, with day of adult ecdysis and body size included as covariates, and male identity included as a random effect.

2.5 | Statistical analyses

All the aforementioned mixed models were fitted with random intercepts by maximum likelihood using the lmer and glmer functions in the lme4 package (Bates et al., 2015) in R version 3.4.4 (R Core Team, 2017). Likelihood ratio tests (LRTs) were used to determine the significance of model effects, using the lrtest function in the lmtest package (Zeileis & Hothorn, 2002). The significance of two-way interactions and covariates was assessed by comparing the full model to a model in which each interaction or covariate was independently removed. The significance of main effects was only assessed if higher level interactions involving those main effects were nonsignificant. This was done by independently removing each fixed effect from a reduced model in which the interaction effect was already removed. We report model coefficients and standard errors for each factor, as well as chi-square statistics and p-values from LRTs, for each model comparison. For significant interaction effects, we performed post-hoc pairwise Bonferroni contrasts on the predicted marginal means of treatment combinations, with Kenward–Roger approximations where degrees of freedom were estimable, using the lsmeans R package (Lenth, 2016). The unit of replication in all analyses was the focal female. Summary statistics are reported in Table 1.

3 | RESULTS

3.1 | Morphology

Reproductive origin affected female body size. Sexually produced females were, on average, 4% longer at maturity (coef$\pm SE = 0.58 \pm 0.17$; $\chi_1^2 = 11.24$, $p < 0.001$; Figure 2A; Table 1) than their asexually produced counterparts. But there was no seasonality effect on body size (coef$\pm SE = 0.02 \pm 0.09$; $\chi_1^2 = 0.08$, $p = 0.78$). Reproductive origin also affected levels of deformity in wings, but not fluctuating asymmetry in legs. Distance between forewings was, on average, 249% larger in parthenogenetically produced females (coef$\pm SE = -3.12 \pm 0.48$; $\chi_1^2 = 45.34$, $p < 0.001$; Figure 2C; Table 1), suggesting that wing development was more unstable in these females. Inter-wing distance was not correlated with female body size (coef$\pm SE = -0.35 \pm 0.21$; $\chi_1^2 = 2.85, p = 0.09$). Wing development appeared to be influenced by seasonality, since females that moulted later in the experimental period had larger inter-forewing distances (coef$\pm SE = 0.51 \pm 0.22$; $\chi_1^2 = 5.49$, $p = 0.02$). By contrast, leg asymmetry did not differ between females of parthenogenetic and sexual origin (coef$\pm SE = -13.49 \pm 20.40$; $\chi_1^2 = 0.45$, $p = 0.50$; Figure 2B; Table 1). Leg-length asymmetry was not correlated with body size (coef$\pm SE = -1.16 \pm 9.89$; $\chi_1^2 = 0.01$, $p = 0.90$) or seasonality (coef$\pm SE = 8.95 \pm 10.41$; $\chi_1^2 = 0.76$, $p = 0.38$).

3.2 | Mating and resistance

Reproductive origin affected mating behaviour, with females of sexual origin experiencing 18% more mating attempts than females of parthenogenetic origin (coef$\pm SE = 0.59 \pm 0.28$; $\chi_1^2 = 4.41$, $p = 0.04$; Figure 3A; Table 1). This suggests that parthenogenetically produced females were either less attractive to males than sexually produced females, or better able to repel males. The frequency of mating
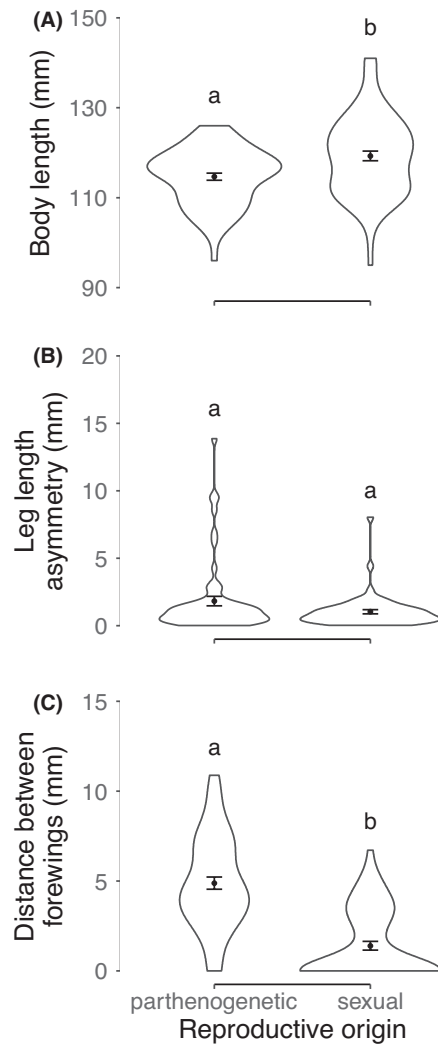
FIGURE 2 Violin plots of morphological differences between females of sexual and parthenogenetic origin showing density distributions and means $\pm SE$s of body length (A), leg length asymmetry (B) and inter-forewing distance (C). Areas within each violin shape are proportional to the sample size. Dissimilar symbols above plots indicate significant differences according to likelihood ratio test
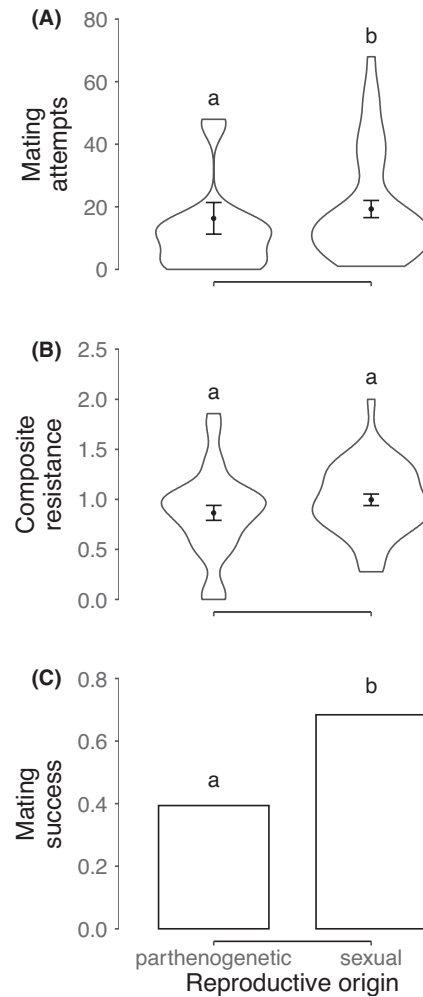

FIGURE 3 Plots show differences in mating attempts (A), resistance (B) and mating success (C) between females of sexual and parthenogenetic origin. Violin plots show density distributions and means $\pm SE$s. Areas within each violin shape are proportional to the sample size. Dissimilar symbols above plots indicate significant differences according to likelihood ratio test

attempts did not covary with body size (coef $\pm SE$ = 0.16$\pm$0.14; χ^2_1 = 1.26, p = 0.26), seasonality (coef $\pm SE$ = 0.17$\pm$0.18; χ^2_1 = 0.84, p = 0.36) or female age at pairing (coef $\pm SE$ = 0.15$\pm$0.18; χ^2_1 = 0.73, p = 0.39). Despite receiving more mating attempts, sexually produced females resisted mating attempts to a similar degree as parthenogenetically produced females (coef $\pm SE$ = 0.05$\pm$0.09; χ^2_1 = 0.34, p = 0.56; Figure 3B; Table 1). We found greater resistance in larger (coef $\pm SE$ = 0.10$\pm$0.05; χ^2_1 = 5.355, p = 0.02) and younger females (coef $\pm SE$ = −0.13$\pm$0.06; χ^2_1 = 4.78, p = 0.03), but resistance was not dependent on seasonality (coef $\pm SE$ = 0.05$\pm$0.06; χ^2_1 = 0.66, p = 0.42). Abdomen curling was by far the most common behavioural manifestation of female resistance, with females of sexual and asexual origin, on average, deploying this behaviour in 73% and 59% of mating attempts, respectively (see Appendix S1 for results of behavioural analyses, and Table 1 for average proportions

of mating attempts resisted using different behaviours). All females in the mating treatment group mated only once. Mating success was significantly affected by reproductive origin. While a majority (68%) of sexually produced females mated during the 3-day pairing period, fewer than half (39%) of parthenogenetically produced females mated during the same period (coef $\pm SE$ = 1.16$\pm$0.53; χ^2_1 = 5.01, p = 0.03; Figure 3C; Table 1). Mating success did not covary with seasonality (coef $\pm SE$ = −0.52$\pm$0.39; χ^2_1 = 1.99, p = 0.16), body size (coef $\pm SE$ = −0.0007$\pm$0.26; χ^2_1 = 0.00, p = 0.998) or female age (coef $\pm SE$ = 0.30$\pm$0.36; χ^2_1 = 0.69, p = 0.41).

3.3 | Reproductive performance

Reproductive origin and mating status both affected oviposition latency. Females of sexual origin oviposited 7.5 days sooner on average
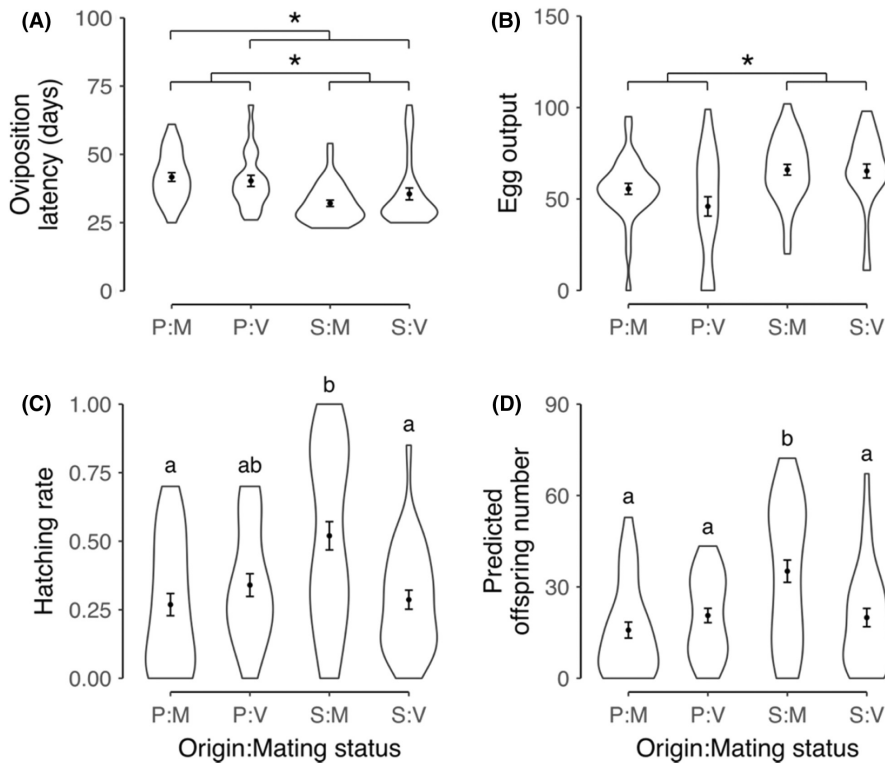
FIGURE 4 Violin plots showing the density distributions and means $\pm$ SEs for reproductive performance of mated (M) and virgin (V) females of sexual (S) and parthenogenetic (P) origin. Areas within each violin shape are proportional to the sample size. Combinations of mode of origin and mating status are shown on the x-axis. Asterisks in plots A and B indicate significant treatment effects according to likelihood ratio test. Dissimilar letters in plots C and D indicate significant pairwise comparisons according to Bonferroni post-hoc tests. Note that the response variable in D is hatching rate×egg output

than females of parthenogenetic origin (coef $\pm$ SE = -0.15 ± 0.04; $\chi_1^2 = 8.83$, $p = 0.003$; Figure 4A; Table 1), and mated females oviposited 1.2 days sooner than virgin females (coef $\pm$ SE = 0.03 ± 0.04; $\chi_1^2 = 4.15$, $p = 0.04$; Figure 4A; Table 1). There was no interaction between origin and mating status (coef $\pm$ SE = 0.15 ± 0.07; $\chi_1^2 = 0.62$, $p = 0.43$; Figure 4A). Smaller females took longer to start ovipositing (coef $\pm$ SE = -0.11 ± 0.02; $\chi_1^2 = 20.82$, $p < 0.001$), but there was no effect of day of ecdysis on oviposition latency (coef $\pm$ SE = 0.03 ± 0.02; $\chi_1^2 = 3.46$, $p = 0.06$).

Mating status had a significant effect on egg output: virgin females produced, on average, 10% fewer eggs than mated females (coef $\pm$ SE = -0.23 ± 0.11; $\chi_1^2 = 4.49$, $p = 0.03$; Figure 4B; Table 1). However, egg output was not affected by reproductive origin (coef $\pm$ SE = 0.18 ± 0.11; $\chi_1^2 = 2.73$, $p = 0.10$; Figure 4B) or the interaction between mating status and reproductive origin (coef $\pm$ SE = 0.32 ± 0.21; $\chi_1^2 = 2.24$, $p = 0.13$; Figure 4B). Larger females produced significantly more eggs (coef $\pm$ SE = 0.29 ± 0.06; $\chi_1^2 = 25.22$, $p < 0.001$), whereas females that reached adulthood later produced fewer eggs due to a seasonality effect (coef $\pm$ SE = -0.11 ± 0.05; $\chi_1^2 = 4.27$, $p = 0.04$). Of females of parthenogenetic origin, 22% of the virgin group and 3% of the mated group produced no eggs at all, whereas all females of sexual origin produced eggs (see sample sizes in Table 1).

While mated females were more fecund, the hatching success of eggs depended on the interacting effects of mode of origin and mating status (coef $\pm$ SE = -1.82 ± 0.52; $\chi_1^2 = 11.69$, $p < 0.001$; Figure 4C). Mating resulted in elevated hatching only in sexually produced females: mated females of sexual origin produced eggs with a higher hatching rate than eggs of mated females of parthenogenetic origin (coef $\pm$ SE = 1.44 ± 0.36, $Z = 4.04$, $p < 0.001$; Figure 4C; Table 1)

and eggs of virgin females of sexual origin (coef $\pm$ SE = 1.24 ± 0.35, $Z = 3.53$, $p = 0.003$; Figure 4C). All other pairwise comparisons between treatment combinations were nonsignificant ($0.53 \leq Z \leq 2.28$, $0.14 \leq p \leq 1.00$; Figure 4C). Hatching success did not covary with day of ecdysis (coef $\pm$ SE = -0.08 ± 0.13, $\chi_1^2 = 0.38$, $p = 0.54$) or body size (coef $\pm$ SE = 0.10 ± 0.14, $\chi_1^2 = 0.56$, $p = 0.46$). Hatching rates ranged, on average, from 0.27 to 0.52 (see Table 1), which is consistent with hatching rates of eggs laid by wild-caught females (~0.3 to ~0.4; Alavi, 2016: chapter 5). Of the cohort of females of sexual origin that laid eggs, 8% from the mated group and 3% from the virgin group produced eggs that all failed to hatch (see sample sizes in Table 1). Of the parthenogenetically produced females that produced eggs, 4% of the virgin group and 22% of the mated group produced eggs that all failed to hatch (see sample sizes in Table 1).

Virgin females produced 100% daughters, except for one virgin female of sexual origin who produced a single son parthenogenetically. Among mated females, offspring sex ratio (proportion of offspring that were male) was female biased (mean $\pm$ SE = 0.21 ± 0.03). Females of sexual origin produced 25% more male offspring, on average, than females of parthenogenetic origin (sexual: 0.23 ± 0.04; parthenogenetic: 0.19 ± 0.05; Table 1), but this difference in sex ratio was not significant (coef $\pm$ SE = 0.55 ± 0.57, $\chi_1^2 = 0.94$, $p = 0.33$). Female body size (coef $\pm$ SE = -0.17 ± 0.29, $\chi_1^2 = 0.34$, $p = 0.56$) and day of ecdysis (coef $\pm$ SE = 0.21 ± 0.29, $\chi_1^2 = 0.56$, $p = 0.46$) had no effect on offspring sex ratio.

Estimated reproductive output (i.e. hatching rate×egg output) also depended on the interaction between mode of origin and mating status (coef $\pm$ SE = -20.86 ± 6.30; $\chi_1^2 = 16.09$, $p < 0.001$; Figure 4D). Mating resulted in more expected offspring only in sexually produced females: mated females of sexual origin produced

significantly more offspring than virgin females of sexual origin (coef $\pm SE = 15.68 \pm 4.32$, $t_{109.1} = 3.63$, $p = 0.003$; Figure 4D; Table 1) and more offspring than mated females of parthenogenetic origin (coef $\pm SE = 17.11 \pm 4.30$, $t_{118} = 3.98$, $p < 0.001$; Figure 4D; Table 1). All other pairwise comparisons between treatment combinations were nonsignificant ($0.31 \leq t \leq 2.56$, $0.07 \leq p \leq 1.00$; Figure 4D; Table 1). Notably, among females of parthenogenetic origin, mated females did not achieve higher reproductive output than virgin females (coef $\pm SE = 5.18 \pm 4.73$, $t_{112} = 1.09$, $p = 1.00$; Figure 4D). Indeed, estimated mean reproductive output of parthenogenetically produced females was 30% higher in the virgin treatment group than in the mated treatment group, albeit not significantly so (Figure 4D; Table 1). These findings suggest that benefits of mating are conditional on female reproductive origin. Larger females produced a greater number of expected offspring (coef $\pm SE = 3.57 \pm 1.68$; $\chi_1^2 = 7.36$, $p = 0.01$) and later-maturing females produced fewer expected offspring (coef $\pm SE = -2.48 \pm 1.59$; $\chi_1^2 = 5.19$, $p = 0.02$).

4 | DISCUSSION

Relative to females of sexual origin, females of parthenogenetic origin were smaller and more likely to exhibit deformities in their vestigial wings but were no less symmetrical in their legs. This suggests that parthenogenetically produced females have a reduced capacity to buffer some developmental processes but that important functional traits may be well canalised against such perturbations. While most sexually produced females mated when paired with males, fewer than half of parthenogenetically produced females mated during the 72-hr no-choice pairing period. Furthermore, mated females and females of sexual origin both oviposited sooner, suggesting, respectively, a stimulatory effect of males and delayed development of parthenogenetically produced females. Mated females had higher fecundity than virgin females, but reproductive origin had no effect on egg output. Mated females of both sexual and parthenogenetic origin produced offspring with female-biased sex ratios, suggesting that some parthenogenesis took place even after mating. However, the advantage of mating did not carry through consistently to offspring production: mating was clearly advantageous for females of sexual origin, resulting in 76% more offspring relative to females of sexual origin that did not mate, but parthenogenetically produced females gained no net fecundity benefit from mating. Indeed, the estimated reproductive output of parthenogenetically produced females was 30% higher (but not significantly so) when they did not mate. Taken together, our results suggest that automictic parthenogenesis in *E. tiaratum* has some negative effects on female phenotype, but mating provides no fitness benefit to parthenogenetically produced females.

Our finding that adult females of parthenogenetic origin were smaller than their sexually produced counterparts and exhibited signs of developmental instability in their wings is consistent with costs of high homozygosity. The extremely low heterozygosity generated by terminal fusion automixis in *E. tiaratum* (Alavi et al., 2018), and the consequent expression of recessive load could be responsible for

the morphological patterns we observed, as has been suggested for similar developmental aberrations in other automictic taxa (Andersen et al., 2006; Schuett et al., 1997). Deviations from the normal phenotype due to reduced heterozygosity (as a result of inbreeding, automixis or other processes) could signify a general breakdown in developmental buffering (Lerner, 1954; Palmer & Strobeck, 1986; Vøllestad et al., 1999) and could negatively affect fitness-related traits (Leary et al., 1985). However, in our experiment, parthenogenetically produced females exhibited relatively low fluctuating asymmetry in a functional trait (legs), despite substantial abnormalities in a non-functional trait (vestigial wings), suggesting that past selection may have acted to mitigate effects of elevated homozygosity on parthenogenetic development (Crespi & Vanderkist, 1997).

In a facultatively parthenogenetic species, it follows from Hamilton's rule (Hamilton, 1964; Howard, 1979) that a comprehensive estimate of female reproductive performance requires weighting each offspring by the expected number of copies of maternal alleles in the offspring genome. All else being equal, this value is expected to be approximately twice as high for parthenogenetically produced offspring (which carry only maternal alleles) as for sexually produced offspring (which carry both maternal and paternal alleles). Maternal fitness is also considered to reflect offspring performance (Howard, 1979; Hunt et al., 2004), although the exact relationship between parental fitness and offspring performance is complex (see Fitzpatrick & Wade, 2022; Wolf & Wade, 2001). Given their smaller body size and higher rates of deformities, it is possible that parthenogenetically produced *E. tiaratum* offspring would have reduced rates of survival or reproduction in the wild, resulting in fewer grand-offspring. Daughters and sons could contribute unequally to maternal reproductive performance as well. From a mother's perspective, sons are expected to have lower genetic value than daughters on average because daughters can be produced either sexually or parthenogenetically, whereas sons are nearly always produced sexually. However, sons could have high value when multiple mates are available (as might occur in female-biased populations; Kawatsu, 2015), unless female resistance substantially reduces the probability of successful mating and fertilisation. These factors are very difficult to estimate in *E. tiaratum*. We therefore quantified the reproductive performance of focal females as the total number of offspring (i.e. estimated hatchlings produced), without taking into account offspring sex or development from fertilised/unfertilised eggs. Estimating maternal fitness would also require data on how mating versus not mating affects female survival in the wild.

Despite the theoretical advantages of asexual reproduction (Maynard Smith, 1978; Williams, 1975), virgin females did not outperform mated females in any of the components of fitness that we measured—a finding consistent with previous studies on *E. tiaratum* (Alavi, 2016; Burke et al., 2015) and other facultatively parthenogenetic species (Archetti, 2004; Burke & Bonduriansky, 2018a; Corley & Moore, 1999; Corley et al., 1999; Engelstadter, 2008; Kobayashi & Miyaguni, 2016; Kramer & Templeton, 2001; Lamb & Willey, 1979; Levitis et al., 2017). Yet, we also found that the benefits of mating were mostly confined to sexually produced females. For females of sexual

origin, mating resulted in faster oviposition onset, higher hatching success and a greater expected number of offspring. By contrast, for females of parthenogenetic origin, mating slightly reduced oviposition latency and increased egg output but had negative effects on hatching success and offspring production. Moreover, completely unviable eggs were laid by more than 22% of parthenogenetically produced females in the mated treatment group but only 4% of parthenogenetically produced females in the virgin treatment group. These findings of reduced performance and viability after mating are contrary to predictions based on the effects of sexual versus parthenogenetic reproduction on offspring heterozygosity. Mating should allow homozygous females of automictic origin to restore heterozygosity in their offspring through outcrossing, thereby enhancing offspring viability. The fact that effects of mating were not universally beneficial suggests that mating in this species is more costly for parthenogenetically produced females than for sexually produced females. Such costs could reflect three non-mutually exclusive processes. First, males in our experiment may have allocated less sperm to parthenogenetically produced females, resulting in lower rates of fertilisation and therefore lower reproductive performance. Indeed, we found that males attempted to mate less frequently with parthenogenetically produced females. However, plastic discrimination by virgin males exposed to females only briefly in a no-choice setting is unlikely (see also Dougherty & Shuker, 2015). Second, development via parthenogenesis could affect the morphology and/or function of adult females' sperm-storage organs or other structures involved in fertilisation. Such perturbations could lead to elevated rates of fertilisation failure among mated females of parthenogenetic origin, resulting in a greater proportion of parthenogenetically than sexually produced offspring. This seems possible as *E. tiaratum* females are known to produce some offspring parthenogenetically following the receipt of sperm (Alavi, 2016: chapter 5), and we found that mated females of parthenogenetic origin tended to produce a higher proportion of daughters (perhaps reflecting a higher rate of parthenogenetic reproduction) than did mated females of sexual origin, although the difference in offspring sex ratios was far from statistical significance. Previous studies have reported sexual trait decay in obligately asexual lineages descended from sexual ancestors (Kraaijeveld et al., 2016; Schwander et al., 2013; van der Kooi & Schwander, 2014), and loss of heterozygosity associated with automictic parthenogenesis is known to affect trait development and functionality (Card et al., 2021). Whether female reproductive organs are negatively impacted following a single generation of parthenogenesis remains unclear. However, a reduced rate of fertilisation cannot explain the high rate of complete hatching failure that we observed in parthenogenetically produced females that mated. Third, it is possible that parthenogenetically produced females are optimised for parthenogenetic reproduction through a maternal effect, and eggs primed for parthenogenetic development may become unviable when fertilised or exposed to seminal products. Such unviability would be analogous to effects of polyspermy on developmental failure in obligately sexual organisms (Rothschild, 1954). This kind of fertilisation interference could potentially explain the high incidence of total egg unviability in females of parthenogenetic origin that mated and received sperm.

Mated females produced female-biased offspring sex ratios, and the extent of this female bias was substantial in mated females of both sexual and parthenogenetic origin. Although we did not determine whether offspring were produced from fertilised or unfertilised eggs, the overproduction of daughters by mated females suggests that many of these females reproduced parthenogenetically even after receiving sperm. Evidence of post-mating parthenogenesis in *E. tiaratum* has previously only been found among parthenogenetically produced females (Alavi, 2016: chapter 5), but true rates of parthenogenesis in that study may have been underestimated due to low sampling. There are several possible reasons for post-mating parthenogenesis in our focal females. First, females may require larger stores of sperm to achieve high rates of fertilisation than a single mating can provide. Indeed, in many insects, a single mating is not sufficient to maximise female fitness (Arnqvist & Nilsson, 2000), in part because multiple matings may be required to avoid sperm limitation (Mark & Iv, 1999; Rondeau & Sainte-Marie, 2001; Warner et al., 1995). Second, fertilisation rates could be reduced by incompatibility. In *Timema* stick insects, genetic incompatibility results in reduced reproductive success, favouring polyandry (Arbuthnott et al., 2015). Genetic incompatibility could have reduced the rates of successful fertilisation and thereby contributed to the production of female-biased broods by our mated females, and rates of genetic incompatibility might have been high in our genetically diverse laboratory stock. Third, the timing of insemination may have influenced the proportion of offspring that developed sexually versus parthenogenetically. For example, females that mate at a younger age may be less effective at storing or utilising sperm (see Schnakenberg et al., 2012), whereas mature eggs ready to be oviposited may escape fertilisation even after insemination has occurred (see Richerson et al., 1976). Fourth, if producing sons is overly costly, facultatively parthenogenetic females may be selected to skew the sex ratio of their offspring by reducing fertilisation rates (Kawatsu, 2013).

If sexually produced females benefit from mating but parthenogenetically produced females do not, then sexual conflict theory predicts that parthenogenetically produced females will be selected to mate at a lower rate than sexually produced females, or avoid mating entirely (Burke & Bonduriansky, 2017; Kawatsu, 2013). In support of this prediction, we found that most females of parthenogenetic origin did not mate, despite being paired with a male for 3 days in a small container where escape was impossible. Although delayed oviposition by parthenogenetically produced females suggests that they might have been less likely to mate because they were less reproductively mature, we found no effect of female age on mating success. Furthermore, females of parthenogenetic origin did not have higher composite scores for resistance behaviours, although larger and younger females were more reluctant to mate. Previous work has shown that *E. tiaratum* females can alter their chemical signals to hide from or repel males (Burke et al., 2015), and such chemical deterrents may have been used by parthenogenetically produced females to ward off males and avoid mating. It is also possible that males perceived parthenogenetically

produced females as unattractive and so attempted to mate with them less frequently, as has been documented in a cyclically sexual rotifer (Gómez & Serra, 1996), or transferred less sperm when mating did occur. Male discrimination of potential mates is widespread in animals (Bonduriansky, 2001; Wedell et al., 2002), and larger female insects tend to be more attractive because of their capacity to produce more eggs (Bonduriansky, 2001; Honěk, 1993), although smaller females are preferred in some species (Myers et al., 2015; Nakano et al., 2019; Wearing-Wilde, 1996). Indeed, we found that sexually produced females were larger than parthenogenetically produced females, and that larger females were significantly more fecund. However, virgin male insects are typically eager to mate (e.g. Michaud et al., 2013; Ortigosa & Rowe, 2003) and unlikely to reject females in a no-choice pairing (e.g. Dougherty & Shuker, 2015; although see Nandy et al., 2012; Wedell et al., 2002). Female chemical resistance rather than male preference may therefore be more likely to explain the lower mating rate of parthenogenetically produced females, but further experiments will be required to identify the exact mechanism.

Our laboratory population was created by crossing individuals sourced from different locations and may therefore have been more heterozygous than is typical of natural populations of this species. However, high heterozygosity is unlikely to have qualitatively altered our conclusions. Although little is known about natural populations of *E. tiaratum*, males' ability to disperse by flying is likely to maintain moderate heterozygosity in natural mixed-sex populations of this species (see Alavi et al., 2018). In a less outbred population, the positive fitness effect of mating could be reduced because sexually produced offspring would be less heterozygous, but sexual reproduction would still be expected to confer benefits for females. Likewise, parthenogenetic reproduction could have fewer negative effects on fitness because of prior purging of deleterious alleles, but parthenogenesis would still result in reduced heterozygosity. Genetic incompatibility could have lowered females' parthenogenetic performance if our outbred population possessed a greater diversity of genotypes less suited to parthenogenetic reproduction, but there is no reason to believe that female reproductive origin and mating would interact in a qualitatively different way in such a population compared to a less outbred population. Thus, it is unlikely that the interactions between mating status and female origin that we observed are artefacts of atypically high heterozygosity in our study population.

Origin-dependent differences in mating rate and reproductive performance could have implications for distributions of sex and parthenogenesis in natural populations. Although the demography of wild populations of *E. tiaratum* is poorly known, many other species of facultatively asexual animals exhibit geographical variation in sex ratio and reproductive mode: some populations have a balanced sex ratio while others show strong female bias or consist entirely of females (Buckley et al., 2009; Burns et al., 2018; Cermak & Hasenpusch, 2000; Horne & Martens, 1998; Kramer & Templeton, 2001; Law & Crespi, 2002; Morgan-Richards et al., 2010; Wegner, 1955). Models of facultatively parthenogenetic metapopulations suggest that sexual conflict could generate and maintain geographical variation in sex ratio and reproductive mode: effective

female resistance to mating coupled with high productivity can generate female-biased populations that resist invasion by males over many generations (Burke & Bonduriansky, 2018b), whereas males can persist only if they are sufficiently coercive (Burke & Bonduriansky, 2018a; Kawatsu, 2013). Our findings here provide insights into how these dynamics might proceed in real organisms. Parthenogenetic reproduction can occur in phasmids when a female originating from a mixed-sex population disperses as an egg, juvenile or virgin adult to a new location or host plant where males are absent and produces an all-female brood. Our results suggest that female-biased or all-female populations could persist for multiple generations because parthenogenetically produced daughters gain no benefit (or even pay a net cost) from mating and can avoid mating even when encountering males. Whether such effects occur in natural populations remains an open question.

In many facultatively parthenogenetic taxa, sexual reproduction appears to confer immediate benefits to females, such as increased fecundity and offspring fitness (Corley & Moore, 1999; Lamb & Willey, 1979; Levitis et al., 2017), potentially resulting from direct stimulatory effects of mating on female fecundity (Burke & Bonduriansky, 2018a; Neiman, 2004, 2006; West-Eberhard, 2003) and/or advantages of high heterozygosity for offspring (Chapman et al., 2009; Taylor et al., 2010). Our study confirms the beneficial effects of mating for female reproductive output on average, but also shows that these benefits can be contingent on female reproductive origin. Our results suggest that selection on whether or not to mate could hinge on the state of individual females—not just on ecological conditions, as is often reported (e.g. Griffiths & Bonser, 2013; Grishkan et al., 2003; King et al., 2009; Koch et al., 2009; Mau et al., 2015)—and that sexual conflict over reproductive mode could therefore be state dependent. However, the nature and context dependence of sexual interactions in facultative organisms—especially female resistance behaviours—still remains relatively unexplored (although see Burke et al., 2015; Schwander et al., 2013).

Our results suggest that the mode of reproduction by which females are produced can affect the relative costs and benefits of mating and therefore alter selection on female behaviour in *E. tiaratum*. Sexually produced females appear to be strongly selected to continue reproducing sexually, but parthenogenetically produced females may be selected to mate less frequently or avoid mating altogether. While the fitness benefits of sex that we observed could help to explain why sexual populations are rarely invaded by obligately asexual variants, the fact that females of asexual origin gained no benefits from mating and were less likely to mate could also help to explain how all-female populations of facultative parthenogens might withstand invasion by males from neighbouring mixed-sex populations. Facultatively parthenogenetic phasmids frequently undergo transitions to obligate parthenogenesis (Bradler & Buckley, 2018), and the establishment of all-female populations could be the first step in this process.

AUTHORS' CONTRIBUTIONS

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REFERENCES

Alavi, Y. (2016). *The evolutionary significance of parthenogenesis and sexual reproduction in the Australian spiny leaf insect*, Extatosoma tiaratum (PhD thesis). University of Melbourne.

Alavi, Y., van Rooyen, A., Elgar, M. A., Jones, T. M., & Weeks, A. R. (2018). Novel microsatellite markers suggest the mechanism of parthenogenesis in *Extatosoma tiaratum* is automixis with terminal fusion. *Insect Science*, *25*(1), 24–32. https://doi.org/10.1111/1744-7917.12373

Andersen, D. H., Pertoldi, C., Loeschcke, V., & Scali, V. (2006). Developmental instability, hybridization and heterozygosity in stick insects of the genus *Bacillus* (Insecta; Phasmatodea) with different modes of reproduction. *Biological Journal of the Linnean Society*, *87*(2), 249–259. https://doi.org/10.1111/j.1095-8312.2006.00572.x

Arbuthnott, D., Crespi, B. J., & Schwander, T. (2015). Female stick insects mate multiply to find compatible mates. *The American Naturalist*, *186*(4), 519–530. https://doi.org/10.1086/682675

Archetti, M. (2004). Recombination and loss of complementation: A more than two-fold cost for parthenogenesis. *Journal of Evolutionary Biology*, *17*(5), 1084–1097. https://doi.org/10.1111/j.1420-9101.2004.00745.x

Arnqvist, G., & Nilsson, T. (2000). The evolution of polyandry: Multiple mating and female fitness in insects. *Animal Behaviour*, *60*(2), 145–164. https://doi.org/10.1006/anbe.2000.1446

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bedford, G. O. (1978). Biology and ecology of the Phasmatodea. *Annual Review of Entomology*, *23*(33), 125–149. https://doi.org/10.1146/annurev.en.23.010178.001013

Bonduriansky, R. (2001). The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews*, *76*(3), 305–339. https://doi.org/10.1017/S1464793101005693

Bradler, S., & Buckley, T. R. (2018). Biodiversity of Phasmatodea. In R. G. Foottit & P. H. Adler (Eds.), *Insect biodiversity: Science and society* (Vol. *2*, 1st ed., pp. 281–313). Wiley-Blackwell). https://doi.org/10.1002/9781118945582.ch11

Brock, P. D. (2001). Studies on the Australasian stick-insect genus *Extatosoma* gray (Phasmida: Phasmatidae: Tropoderinae: Extatosomatini). *Journal of Orthoptera Research*, *10*(2), 303–313. https://doi.org/10.1665/1082-6467(2001)010[0303:SOTASI]2.0.CO;2

Brock, P. D., & Hasenpusch, J. (2007). Studies on the Australian stick insects (Phasmida), including a checklist of species and bibliography. *Zootaxa*, *1570*, 1–84.

Brock, P. D., Lee, M., Morgan-Richards, M., & Trewick, S. A. (2012). Missing stickman found: The first male of the parthnogenetic New Zealand phasmid genus Acanthoxyla Urarov, 1944 discovered in the United Kingdom. *Atropos*, *60*, 16–23.

Buckley, T. R., Marske, K. A., & Attanayake, D. (2009). Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: The New Zealand stick insect *Argosarchus horridus* (white). *Molecular Ecology*, *18*(22), 4650–4663. https://doi.org/10.1111/j.1365-294X.2009.04396.x

Burke, N. W., & Bonduriansky, R. (2022). Data for: Sexually but not parthenogenetically produced females benefit from mating in a stick insect. *Dryad*. https://doi.org/10.5061/dryad.63xsj3v4t

Burke, N. W., & Bonduriansky, R. (2017). Sexual conflict, facultative asexuality, and the true paradox of sex. *Trends in Ecology and Evolution*, *32*(9), 646–652. https://doi.org/10.1016/j.tree.2017.06.002

Burke, N. W., & Bonduriansky, R. (2018a). The fitness effects of delayed switching to sex in a facultatively asexual insect. *Ecology and Evolution*, *8*(5), 2698–2711. https://doi.org/10.1002/ece3.3895

Burke, N. W., & Bonduriansky, R. (2018b). The geography of sex: Sexual conflict, environmental gradients and local loss of sex in facultatively parthenogenetic animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1757), 20170422. https://doi.org/10.1098/rstb.2017.0422

Burke, N. W., & Bonduriansky, R. (2019). Exposure to juvenile males during development suppresses female capacity for parthenogenesis in a stick insect. *Animal Behaviour*, *154*, 85–94. https://doi.org/10.1016/j.anbehav.2019.06.016

Burke, N. W., Crean, A. J., & Bonduriansky, R. (2015). The role of sexual conflict in the evolution of facultative parthenogenesis: A study on the spiny leaf stick insect. *Animal Behaviour*, *101*(3), 117–127.

Burns, M., Hedin, M., & Tsurusaki, N. (2018). Population genomics and geographical parthenogenesis in Japanese harvestmen (Opiliones, Sclerosomatidae, Leiobunum). *Ecology and Evolution*, *8*, 36–52. https://doi.org/10.1002/ece3.3605

Card, D. C., Vonk, F. J., Smalbrugge, S., Casewell, N. R., Wüster, W., Castoe, T. A., Schuett, G. W., & Booth, W. (2021). Genome-wide data implicate terminal fusion automixis in king cobra facultative parthenogenesis. *Scientific Reports*, *11*(1), 1–9. https://doi.org/10.1038/s41598-021-86373-1

Carlberg, U. (1983). Copulation in *Extatosoma tiaratum* (MacLeay) (Insecta: Phasmida). *Zoologischer Anzeiger*, *210*, 340–356.

Cermak, M., & Hasenpusch, J. W. (2000). Distribution, biology and conservation status of the peppermint stick insect, *Megacrania batesii* (Kirby) (Phasmatodea: Phasmatidae), in Queensland. *Memoirs of the Queensland Museum: Nature*, *46*(1), 101–106.

Chapman, J. R., Nakagawa, S., Coltman, D. W., Slate, J., & Sheldon, B. C. (2009). A quantitative review of heterozygosity-fitness correlations in animal populations. *Molecular Ecology*, *18*(13), 2746–2765. https://doi.org/10.1111/j.1365-294X.2009.04247.x

Charlesworth, D., Morgan, M. T., & Charlesworth, B. (1990). Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution*, *44*(6), 1469–1489. https://doi.org/10.2307/2409330

Charlesworth, D., Morgan, M. T., & Charlesworth, B. (1993). Mutation accumulation in finite outbreeding and inbreeding populations. *Genetical Research*, *61*(01), 39–56. https://doi.org/10.1017/S0016672300031086

Clark, J. T. (1974). A conspicuous spermatophore in the phasmid *Extatosoma tiaratum*. *Entomologist's Monthly Magazine*, *110*, 81–82.

Corley, L. S., Blankenship, J. R., Moore, A. J., & Moore, P. J. (1999). Developmental constraints on the mode of reproduction in the facultatively parthenogenetic cockroach *Nauphoeta cinerea*.

Evolution and Development, 1(2), 90–99. https://doi.org/10.1046/j.1525-142x.1999.99001.x

Corley, L. S., & Moore, A. J. (1999). Fitness of alternative modes of reproduction: Developmental constraints and the evolutionary maintenance of sex. *Proceedings of the Royal Society B: Biological Sciences, 266*(1418), 471–476. https://doi.org/10.1098/rspb.1999.0661

Crespi, B. J., & Vanderkist, B. A. (1997). Fluctuating asymmetry in vestigial and functional traits of a haplodiploid insect. *Heredity, 79*(6), 624–630. https://doi.org/10.1038/hdy.1997.208

Crnokrak, P., & Barrett, S. C. H. (2002). Perspective: Purging the genetic load: A review of the experimental evidence. *Evolution, 56*(2347), 2347–2358.

Dougherty, L. R., & Shuker, D. M. (2015). The effect of experimental design on the measurement of mate choice: A meta-analysis. *Behavioral Ecology, 26*(2), 311–319. https://doi.org/10.1093/beheco/aru125

Eliceiri, K., Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Engelstadter, J. (2008). Constraints on the evolution of asexual reproduction. *BioEssays, 30*(11–12), 1138–1150. https://doi.org/10.1002/Bies.20833

Engelstaedter, J. (2017). Asexual but not clonal: Evolutionary processes in populations with automictic reproduction. *Genetics, 206*(June), 993–1009. https://doi.org/10.1101/081547

Fitzpatrick, C. L., & Wade, M. J. (2022). When is offspring viability fitness a measure of paternal fitness and when is it not? *Journal of Heredity, 113*(1), 48–53. https://doi.org/10.1093/jhered/esab055

Gerber, N., & Kokko, H. (2016). Sexual conflict and the evolution of asexuality at low population densities. *Proceedings of the Royal Society B: Biological Sciences, 283*(1841), 20161280.

Gómez, A., & Serra, M. (1996). Mate choice in male *Brachionus plicatilis* rotifers. *Functional Ecology, 10*, 681–687.

Goudie, F., Allsopp, M. H., Beekman, M., Oxley, P. R., Lim, J., & Oldroyd, B. P. (2012). Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). *Evolution, 66*(6), 1897–1906. https://doi.org/10.1111/j.1558-5646.2011.01543.x

Griffiths, J. G., & Bonser, S. P. (2013). Is sex advantageous in adverse environments? A test of the abandon-ship hypothesis. *The American Naturalist, 182*(6), 718–725. https://doi.org/10.1086/673476

Grishkan, I., Korol, A. B., Nevo, E., & Wasser, S. P. (2003). Ecological stress and sex evolution in soil microfungi. *Proceedings of the Royal Society B: Biological Sciences, 270*(1510), 13–18. https://doi.org/10.1098/rspb.2002.2194

Gummer, D. L., & Brigham, R. M. (1995). Does fluctuating asymmetry reflect the importance of traits in little brown bats (*Myotis lucifugus*)? *Canadian Journal of Zoology, 73*(5), 990–992. https://doi.org/10.1139/z95-116

Hamilton, W. D. (1964). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology, 7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Hedrick, P. W. (1994). Purging inbreeding depression and the probability of extinction: Full-sib mating. *Heredity, 73*(January), 363–372.

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology and Evolution, 31*(12), 940–952. https://doi.org/10.1016/j.tree.2016.09.005

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: A general relationship. *Oikos, 66*(3), 483–492. https://doi.org/10.2307/3544943

Horne, D. J., & Martens, K. (1998). Geographical parthenogenesis in European non-marine ostracods: Post-glacial invasion or Holocene stability? *Hydrobiologia, 391*, 1–7. https://doi.org/10.1023/A:1003508210166

Howard, R. D. (1979). Estimating reproductive success in natural populations. *The American Naturalist, 114*(2), 221–231.

Hunt, J., Bussière, L. F., Jennions, M. D., & Brooks, R. (2004). What is genetic quality? *Trends in Ecology and Evolution, 19*(6), 329–333. https://doi.org/10.1016/j.tree.2004.03.035

Kawatsu, K. (2013). Sexual conflict over the maintenance of sex: Effects of sexually antagonistic coevolution for reproductive isolation of parthenogenesis. *PLoS One, 8*(2), e58141. ARTN e58141 DOI 10.1371/journal.pone.0058141

Kawatsu, K. (2015). Breaking the parthenogenesis fertilization barrier: Direct and indirect selection pressures promote male fertilization of parthenogenetic females. *Evolutionary Ecology, 29*(1), 49–61. https://doi.org/10.1007/s10682-014-9749-0

King, K. C., Delph, L. F., Jokela, J., & Lively, C. M. (2009). The geographic mosaic of sex and the red queen. *Current Biology, 19*(17), 1438–1441. https://doi.org/10.1016/j.cub.2009.06.062

Kobayashi, K., & Miyaguni, Y. (2016). Facultative parthenogenesis in the Ryukyu drywood termite *Neotermes koshunensis*. *Scientific Reports, 6*(March), 30712. https://doi.org/10.1038/srep30712

Koch, U., Von Elert, E., & Straile, D. (2009). Food quality triggers the reproductive mode in the cyclical parthenogen Daphnia (Cladocera). *Oecologia, 159*(2), 317–324. https://doi.org/10.1007/s00442-008-1216-6

Komsta, L., & Novomestky, F. (2015). *Moments: Moments, cumulants, skewness, kurtosis and related tests*. R Package Version 0.14. https://cran.r-project.org/package=moments

Kraaijeveld, K., Anvar, S. Y., Frank, J., Schmitz, A., Bast, J., Wilbrandt, J., Petersen, M., Ziesmann, T., Niehuis, O., de Knijff, P., den Dunnen, J., & Ellers, J. (2016). Decay of sexual trait genes in an asexual parasitoidwasp. *Genome Biology and Evolution, 8*(12), 3685–3695. https://doi.org/10.1093/gbe/evw273

Kramer, M. G., & Templeton, A. R. (2001). Life-history changes that accompany the transition from sexual to parthenogenetic reproduction in *Drosophila mercatorum*. *Evolution, 55*(4), 748–761. https://doi.org/10.1554/0014-3820(2001)055[0748:LHCTAT]2.0.CO;2

Lamb, R. Y., & Willey, R. B. (1979). Are parthenogenetic and related bisexual insects equal in fertility? *Evolution, 33*(2), 774–775. https://doi.org/10.2307/2407801

Law, J. H., & Crespi, B. J. (2002). The evolution of geographic parthenogenesis in Timema walking-sticks. *Molecular Ecology, 11*(8), 1471–1489. https://doi.org/10.1046/j.1365-294X.2002.01547.x

Leary, R. F., Allendorf, F. W., Knudsen, K. L., & Thorgaard, G. H. (1985). Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. *Heredity, 54*(2), 219–225. https://doi.org/10.1038/hdy.1985.29

Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software, 69*(1), 1–33.

Lerner, I. M. (1954). *Genetic homeostasis*. Wiley.

Leung, B., Forbes, M. R., & Houle, D. (2000). Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. *American Naturalist, 155*(1), 101–115. https://doi.org/10.1086/303298

Levitis, D. A., Zimmerman, K., & Pringle, A. (2017). Is meiosis a fundamental cause of inviability among sexual and asexual plants and animals? *Proceedings of the Royal Society B: Biological Sciences, 284*(1860), 20170939. https://doi.org/10.1098/rspb.2017.0939

Lynch, M., Bürger, R., Butcher, D., & Gabriel, W. (1990). The mutational meltdown in asexual populations. *The Journal of Heredity, 84*(5), 339–344.

Mark, A. B. M., & Iv, J. B. (1999). Sperm ecnomy and limitation in spiny lobsters. *Behavioral Ecology and Sociobiology, 46*, 14–24.

Mau, M., Lovell, J. T., Corral, J. M., Kiefer, C., Koch, M. A., Aliyu, O. M., & Sharbel, T. F. (2015). Hybrid apomicts trapped in the ecological niches of their sexual ancestors. *Proceedings of the National Academy of Sciences of the United States of America, 112*(18), E2357–E2365. https://doi.org/10.1073/pnas.1423447112

Maynard Smith, J. (1978). *The evolution of sex*. Cambridge University Press.

Michaud, J. P., Bista, M., Mishra, G., & Singh, O. (2013). Sexual activity diminishes male virility in two *Coccinella* species: Consequences for female fertility and progeny development. *Bulletin of Entomological Research, 103*(5), 570–577. https://doi.org/10.1017/S0007485313000199

Morgan-Richards, M., Langton-Meyers, S., & Trewick, S. A. (2019). Loss and gain of sexual reproduction in the same stick insect. *Molecular Ecology, 28*, 3939–3941.

Morgan-Richards, M., Trewick, S. A., & Stringer, I. A. N. (2010). Geographic parthenogenesis and the common tea-tree stick insect of New Zealand. *Molecular Ecology*, *19*(6), 1227–1238. https://doi.org/10.1111/j.1365-294X.2010.04542.x

Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *1*(732), 2–9. https://doi.org/10.1016/0027-5107(64)90047-8

Myers, S. S., Buckley, T. R., & Holwell, G. I. (2015). Mate detection and seasonal variation in stick insect mating behaviour (Phamatodea: *Clitarchus hookeri*). *Behaviour*, *152*(10), 1325–1348. https://doi.org/10.1163/1568539x-00003281

Nakano, M., Morgan-richards, M., Liliana, A., & Mccormick, C. (2019). *Parthenogenetic females of the stick insect* Clitarchus hookeri *maintain sexual traits.* https://doi.org/10.3390/insects10070202

Nandy, B., Joshi, A., Ali, Z. S., Sen, S., & Prasad, N. G. (2012). Degree of adaptive male mate choice is positively correlated with female quality variance. *Scientific Reports*, *2*, 1–8. https://doi.org/10.1038/srep00447

Neiman, M. (2004). Physiological dependence on copulation in parthenogenetic females can reduce the cost of sex. *Animal Behaviour*, *67*(5), 811–822. https://doi.org/10.1016/j.anbehav.2003.05.014

Neiman, M. (2006). Embryo production in a parthenogenetic snail (*Potamopyrgus antipodarum*) is negatively affected by the presence of other parthenogenetic females. *Invertebrate Biology*, *125*(1), 45–50. https://doi.org/10.1111/j.1744-7410.2006.00038.x

Nougué, O., Rode, N. O., Jabbour-zahab, R., Ségard, A., Chevin, L. M., Haag, C. R., & Lenormand, T. (2015). Automixis in artemia: Solving a century-old controversy. *Journal of Evolutionary Biology*, *28*, 2337–2348. https://doi.org/10.1111/jeb.12757

Ortigosa, A., & Rowe, L. (2003). The role of mating history and male size in determining mating behaviours and sexual conflict in a water strider. *Animal Behaviour*, *65*, 851–858. https://doi.org/10.1006/anbe.2003.2112

Palmer, A., & Strobeck, C. (1986). Fluctuating asymmetry: Measurement, analysis, patterns. *Annual Review of Ecology and Systematics*, *17*, 391–421.

Pearcy, M., Hardy, O., & Aron, S. (2006). Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity*, *96*(5), 377–382. https://doi.org/10.1038/sj.hdy.6800813

Pertoldi, C., Scali, V., & Loeschcke, V. (2001). Developmental instability in sexually reproducing and parthenogenetic populations of *Bacillus rossius rossius* and *Bacillus rossius redtenbacheri*. *Evolutionary Ecology Research*, *3*, 449–463.

Pijnacker, L. P. (1969). Automictic parthenogenesis in the stick insect *Bacillus rossius* Rossi (Cheleutoptera, phasmidae). *Genetica*, *40*(1), 393–399. https://doi.org/10.1007/BF01787364

R Core Team. (2017). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing. https://www.r-project.org/

Richerson, J. V., Cameron, E. A., & Brown, E. A. (1976). Sexual activity of the gypsy moth. *The American Midland Naturalist*, *95*(2), 299–312.

Rondeau, A., & Sainte-Marie, B. (2001). Variable mate-guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. *Biological Bulletin*, *201*(2), 204–217. https://doi.org/10.2307/1543335

Rothschild, L. (1954). Polyspermy. *The Quarterly Review of Biology*, *29*(4), 332–342.

Schnakenberg, S. L., Siegal, M. L., & Bloch Qazi, M. C. (2012). Oh, the places they'll go: Female sperm storage and sperm precedence in *Drosophila melanogaster*. *Spermatogenesis*, *2*(3), 224–235. https://doi.org/10.4161/spmg.21655

Schuett, G. W., Fernandez, P. J., Gergits, W. F., Casna, N. J., Chiszar, D., Smith, H. M., Mitton, J. B., Mackessy, S. P., Odum, R. A., & Demlong, M. J. (1997). Production of offspring in the absence of males: Evidence for facultative parthenogenesis in bisexual snakes. *Herpetological Natural History*, *5*(1), 1–10.

Schwander, T., Crespi, B. J., Gries, R., & Gries, G. (2013). Neutral and selection-driven decay of sexual traits in asexual stick insects. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1764), 20130823. https://doi.org/10.1098/rspb.2013.0823

Schwander, T., Vuilleumier, S., Dubman, J., & Crespi, B. J. (2010). Positive feedback in the transition from sexual reproduction to parthenogenesis. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1686), 1435–1442. https://doi.org/10.1098/rspb.2009.2113

Stalker, H. D. (1956). On the evolution of parthenogenesis in Lonchoptera (Diptera). *Evolution*, *10*(4), 345–359. https://doi.org/10.2307/2406996

Stenberg, P., & Saura, A. (2009). Cytology of asexual animals. In I. Schön, K. Martens, & P. Van Dijk (Eds.), *Lost sex: The evolutionary biology of parthenogenesis* (pp. 63–74). Springer.

Suomalainen, E., Saura, A., & Lokki, J. (1987). *Cytology and evolution in parthenogenesis*. CRC Press.

Taylor, S. S., Sardell, R. J., Reid, J. M., Bucher, T., Taylor, N. G., Arcese, P., & Keller, L. F. (2010). Inbreeding coefficient and heterozygosity-fitness correlations in unhatched and hatched song sparrow nestmates. *Molecular Ecology*, *19*(20), 4454–4461. https://doi.org/10.1111/j.1365-294X.2010.04824.x

van der Kooi, C. J., & Schwander, T. (2014). On the fate of sexual traits under asexuality. *Biological Reviews*, *89*(4), 805–819. https://doi.org/10.1111/brv.12078

Vøllestad, L. A., Hindar, K., & Møller, A. P. (1999). A meta-analysis of fluctuating asymmetry in relation to heterozygosity. *Heredity*, *83*(2), 206–218. https://doi.org/10.1046/j.1365-2540.1999.00555.x

Warner, R. R., Shapiro, D. Y., Marcanato, A., & Petersen, C. W. (1995). Sexual conflict: Males with highest mating success convey the lowest fertilization benefits to females. *Proceedings of the Royal Society B: Biological Sciences*, *262*(1364), 135–139. https://doi.org/10.1098/rspb.1995.0187

Wearing-Wilde, J. (1996). Mate choice and competition in the barklouse *Lepinotus patruelis* (Psocoptera: Trogiidae): The effect of diet quality and sex ratio. *Journal of Insect Behavior*, *9*(4), 599–612. https://doi.org/10.1007/BF02213883

Wedell, N., Gage, M. J. G., & Parker, G. A. (2002). Sperm competition, male prudence, and sperm-limited females. *Trends in Ecology & Evolution*, *17*, 313–320. https://doi.org/10.1007/978-0-387-28039-4_3

Wegner, A. M. R. (1955). Biological notes on *Megacrania wegneri* Willemse and *M. alpheus* Westwood (Orthoptera, Phasmidae). *Treubia*, *23*, 47–52.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Williams, G. C. (1975). *Sex and evolution*. Princeton University Press.

Wolf, J. B., & Wade, M. J. (2001). On the assignment of fitness to parents and offspring: Whose fitness is it and when does it matter? *Journal of Evolutionary Biology*, *14*(2), 347–356. https://doi.org/10.1046/j.1420-9101.2001.00277.x

Zeileis, A., & Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News*, *2*, 7–10.

Zeyl, C., Mizesko, M., & de Visser, J. A. (2001). Mutational meltdown in laboratory yeast populations. *Evolution*, *55*(5), 909–917. https://doi.org/10.1111/j.0014-3820.2001.tb00608.x

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