RESEARCH ARTICLE

Bateman gradients reflect variation in sexual selection in a species with dynamic sex roles

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Abstract

Bateman gradients, the slope of the regression of reproductive success on mating success, are among the most commonly reported measures of sexual selection. They are particularly insightful in species with reversed sex roles, where females are expected to be under sexual selection. We measured Bateman gradients in replicate experimental populations of the spermatophore gift-giving bushcricket *Kawanaphila nartee* (Orthoptera: Tettigoniidae). In this species, the operational sex ratio (OSR) and thus the sex competing for mates varies depending on the availability of pollen food resources: under pollen-limited regimens females are more competitive, whereas under pollen-rich regimens males are more competitive. We maintained populations in enclosures with either limited or supplemented pollen and calculated Bateman gradients for males and females under both conditions. Bateman gradients were significantly positive in males, and the slope was steeper in pollen-supplemented populations where the OSR was more male-biased. Bateman gradients for females were shallow and nonsignificant regardless of pollen availability. Our results show that the strength of sexual selection on males can depend on environmental context. The lack of significant gradients among females may reflect experimental limitations on our ability to estimate Bateman gradients in female *K nartee*.

KEYWORDS
Bateman gradient, *Kawanaphila*, operational sex ratio, sexual selection, spermatophore

1 | INTRODUCTION

There is much debate over the measurement and therefore effect of sexual selection on animal mating systems, where sexual selection is defined as selection on traits used in competition for access to limited mates and/or gametes (Darwin, 1871; Shuker & Kvarnemo, 2021). Pioneering experimental work by Bateman (1948) produced a metric (the Bateman gradient) comprising the slope of the regression of reproductive success on mating success (Arnold & Duvall, 1994; Henshaw & Jones, 2019) that has become widely used

for quantifying the strength of sexual selection (Anthes et al., 2017). Populations or sexes with steep Bateman gradients are inferred to be under intense sexual selection because increased variation in reproductive success via mating success results in fewer relatively successful individuals and more relatively unsuccessful individuals because of competition for limited fertilization opportunities. Bateman's (1948) own results reported positive, essentially linear gradients for male *Drosophila melanogaster* and essentially flat gradients for females. These results helped reinforce the longstanding notion (originating from Darwin, 1871) that males, rather than

females, are the primary subjects of sexual selection, and that in terms of matings, males tend to be competitive, and females tend to be choosy (i.e. the sexes express Darwinian sex roles).

Several authors have challenged Bateman's findings (Gowaty & Hubbell, 2005; Hoquet, 2020; Klug et al., 2010; Tang-Martínez & Ryder, 2005). Theoretical work has suggested that the variation in mating success that Bateman found could equally have arisen from random mating (Sutherland, 1985), and reanalysis of the original data has failed to find support for Bateman's conclusions (Snyder & Gowaty, 2007; Tang-Martínez & Ryder, 2005). Furthermore, female size and fecundity are often confounded, making interpretation of female Bateman gradients difficult, and gradients themselves vary between years or breeding seasons, further complicating their interpretation (Anthes et al., 2017). Nevertheless, female Bateman gradients are often reported alongside those of males and tend to be shallower than male gradients, but still often positive and significant (Hare & Simmons, 2019; Henshaw et al., 2018; Janicke et al., 2016).

The operational sex ratio (OSR), the ratio of sexually receptive males to sexually receptive females (Emlen & Oring, 1977), is often used as an alternative predictor of sexual selection (Janicke & Morrow, 2018; Kvarnemo & Ahnesjö, 1996). All else being equal, an OSR heavily biased towards one sex (usually males) in a given population at a given time reflects increased competition among individuals of that sex (Emlen & Oring, 1977) because of the limited number of potential mates (Kokko et al., 2012). Jones et al. (2002) suggest that Bateman gradients are positively correlated with OSR, a suggestion that has been verified experimentally (Mills et al., 2007), and that the Bateman gradient is both more accurate and easier to measure.

However, Kokko et al. (2012) argue that the Bateman gradient and the OSR are in fact complementary measures of sexual selection. The measurement of one or both of these metrics could be biased or otherwise nonrepresentative, depending on the context. For example, using only the OSR, the benefits of multiple matings may be missed: the OSR can only be biased towards one sex, yet both sexes can nonetheless be mate-limited (Kokko et al., 2012), and variation in mate quality can lead to mate choice resulting in greater selection than is suggested by the OSR alone (Owens & Thompson, 1994; Parker, 1983). By contrast, in cases where one sex achieves maximum fitness after a single mating (as in the female *Drosophila* of Bateman's study), Bateman gradients may not capture sexual selection on that sex; in this case, competition may be reflected in the OSR but not the Bateman gradient (Kokko et al., 2012). Consequently, female Bateman gradients may only be steep at the origin, as the number of matings increases from zero to one, but male Bateman gradients may continue to increase linearly with each additional mate (Kokko et al., 2012). Regardless, some combination of Bateman gradient and OSR appear to be the best and most practicable available tools for assessing the strength of sexual selection among animals.

Animal species with reversals of Darwinian sex roles provide ideal test cases for sexual selection theory, as they are perceived as the exception that proves the rule (Fritzsche & Booksmythe, 2013; Williams, 1966). Measurements of the strength of sexual selection

in females of sex role reversed species, including Bateman gradients, often find equal or greater sexual selection in females than males (Hare & Simmons, 2019), and there is some evidence that varying OSR affects the strength of sexual selection (Jones et al., 2004; Mills et al., 2007; Wacker et al., 2013). However, these studies do not examine how well Bateman gradients and the OSR capture sexual selection acting on the sexes in different environmental contexts, in part because the behavioural sex roles of these species are not manipulable.

Kawanaphila nartee is a zaprochiline tettigoniid (Orthoptera) endemic to kwongan heath in coastal areas of Western Australia (Rentz, 1993). The species is pollinivorous and is reproductively active only for a brief period in spring (Rentz, 1993). Male *K. nartee* provide a nutritive spermatophore gift during copulation. Males require five or more days to synthesize a spermatophore, depending on the availability of pollen resources in the environment. The spermatophore invariably comprises some 20% of the donor's body mass, the majority of which is translated directly into gametic tissue by the female (Gwynne & Bailey, 1988; Simmons, 1990). When environmental sources of food are limited, sex roles are reversed (Gwynne & Simmons, 1990): female *K. nartee* scramble and physically grapple with one another for access to a limited supply of receptive males; meanwhile, males are discriminatory of potential mates, preferring heavier females, probably because heavier females are more fecund and also more likely to enter a mating refractory period (Simmons & Gwynne, 1993). When environmental sources of pollen are abundant spermatophores can be synthesized quickly so that the availability of males is high, sex roles conform to Darwinian convention: male *K. nartee* compete acoustically to attract females, and females are discriminatory of potential mates, preferring males with the lower-frequency calls that characterize greater body mass and presumably their larger nutrient gifts (Gwynne & Bailey, 1988).

Plasticity in the sex roles of male and female *K. nartee* occurs naturally and consistently in the field every spring as early flowers producing little pollen (primarily kangaroo paws, *Anigozanthos manglesii*) are replaced by late flowers producing a super-abundance of pollen (grasstrees, *Xanthorrhoea preissei*; Simmons & Bailey, 1990). Because the OSR in this species is influenced by the availability of pollen, it can be altered experimentally (Gwynne & Simmons, 1990). Additionally, female *K. nartee* have ears that are larger and more sensitive than those of males, and variation in ear size among females is correlated with variation in their mating success: females with larger ears arrive at calling males before their rivals (Bailey & Simmons, 1991; Gwynne & Bailey, 1999). Together, these features position *K. nartee* as an ideal candidate for exploring metrics of sexual selection in both sexes (Hare & Simmons, 2020).

We recently found that female ear size is under sexual selection in field-caught samples of *K. nartee*, but only in the earliest periods of the breeding season, when intrasexual competition among females is most intense (Hare & Simmons, 2021). We also found that that Bateman gradients did not differ from zero among females at five different sampling points across the breeding season, regardless

of prevailing sex role. Our findings suggest that females may not be under sexual selection for multiple mating, but rather compete for their first essential mating opportunity (Hare & Simmons, 2021). However, our findings were correlational by their nature, and we were unable to calculate Bateman gradients for males. Here, we report Bateman gradients for both male and female *K. nartee* while experimentally manipulating food availability, and thus varying the OSR.

When pollen is abundant, male *K. nartee* are able to synthesize spermatophores more rapidly, resulting in a greater potential reproductive rate for males than females, leading to a shift in the OSR so that it is more male-biased; when pollen is scarce, the male potential reproductive rate is lower than that of females, leading to a shift in the OSR in favour of females (Simmons, 1995). We predicted that Bateman gradients should increase in males as pollen availability increases and the OSR becomes increasingly male-biased. We made this prediction because females produce more eggs under conditions of pollen abundance so the value of each additional mating for males should be increased and also because females mate less frequently, presumably because they acquire sufficient nutrients for gametogenesis from consuming pollen, and do not require the additional nutrients of spermatophores (Gwynne & Simmons, 1990; Simmons & Bailey, 1990). Males share paternity when females mate with multiple males (Simmons, 1995) so that reduced polyandry under resource abundance is predicted to elevate the male Bateman gradient (Parker & Birkhead, 2013). By contrast, under conditions of pollen limitation females produce fewer eggs and mate more frequently, meaning that each mating achieved by a male provides access to fewer eggs for fertilization. Moreover, we predicted female Bateman gradients would be steeper under limited pollen availability, when male-derived resources are predicted to be of greater value in gametogenesis.

2 | METHODS

Animals to be used in our experimental populations were collected from an area of natural bushland that forms Kings Park, a botanical garden in metropolitan Perth (31.9708°S, 115.8282°E), in winter and spring 2018. Penultimate-instar juveniles were collected to ensure individuals were unmated at the commencement of the experiment. Juveniles were separated by sex and raised to adult eclosion in a temperature-controlled room kept at 17°C with a 12h:12h light: dark cycle. After all animals had eclosed to adulthood, they were marked on the pronotum using enamel paints for the purpose of individual identification.

2.1 | Experimental manipulations

In total, 120 females and 120 males were available for use in experimental manipulations and were separated into six enclosures (1×1×1 m) each with 20 males and 20 females, following the methods of Gwynne and Simmons (1990). Enclosures were placed over naturally occurring stands of kangaroo paws in Kings Park, in an area of 10×10 m. Enclosures were assigned randomly to one of two treatments, which have been shown previously to induce Darwinian and reversed sex roles, respectively, by supplementing or limiting the amount of pollen available (Gwynne & Simmons, 1990; Vincent & Gwynne, 2014).

Each pollen-supplemented enclosure received a 1-L flask containing a grasstree scape (approx. 40cm) painted with honey and dusted with bee-collected pollen. Pollen was purchased from a health food store and ground to a fine powder in an electric grinder prior to application. Pollen supplements were refreshed midway through the experimental period. A 1-L flask containing additional fresh-cut kangaroo paws was placed in each pollen-limited enclosure. Flasks in the pollen-limited enclosures were not refreshed during the experimental period. Behavioural observations were made nightly for 21 days, from full dark (approx. 7.45pm) until the cessation of mating activity, which occurs approximately 3 h after sunset (approx. 10pm; Simmons & Bailey, 1990). The number of calling males, the number and identities of individuals engaged in pre-mating couplings and the number and identities of females feeding on spermatophores were recorded twice per night for each enclosure. Spermatophore consumption takes at least 1.5 h (Simmons & Gwynne, 1991); thus two observations per night were sufficient to identify all females that had mated on any given night. In one focal enclosure per night, rejected mating attempts and grapples between females were also recorded via continuous observation (for a full description of behavioural interactions see Simmons & Bailey, 1990).

The experiment was conducted for 21 days to ensure sufficient time for the different pollen treatments to affect sex roles. After 21 days, 134 surviving individuals were recaptured from the enclosures and frozen: 35 females and 42 males from the pollen-supplemented enclosures, and 32 females and 25 males from the pollen-limited enclosures. Mortality rates were approximately at the level expected given the duration of the experiment: after 3 weeks, mortality rates become high, especially in treatments with high mating frequency (Simmons & Kvarnemo, 2006). A mixed-effects binomial GLM (with enclosure identity as a random factor) indicated no significant differences in mortality among sexes ($\chi^2_1 = 0$; $p = 0.999$) or treatments ($\chi^2_1 = 2.268$; $p = 0.132$). An apparent effect of the interaction between sex and treatment, where male mortality was higher in pollen-limited enclosures (58%) than in pollen-supplemented enclosures (30%), was not statistically significant ($\chi^2_1 = 3.559$; $p = 0.059$; pairwise post hoc comparison: z-ratio = 2.272; $p = 0.105$). Females were later defrosted and body mass and pronotum length were measured. Each female was then dissected, the number of eggs recorded, and the spermatheca placed in an Eppendorf tube containing 1 ml molecular-grade ethanol. Males were also defrosted, and body mass and pronotum length measured. Following dissection, the head and forelimbs of each male and each female was placed in an Eppendorf tube containing 1 ml molecular-grade ethanol.

2.2 | Estimates of mating success

We obtained two estimates of mating success: matings were observed directly during our observations, and a genomic assessment was conducted to ensure that no matings had been missed. During observations, a mating was recorded as successful when a male transferred a spermatophore to a female and the female began feeding on the spermatophylax (sperm are transferred to the female's spermatheca during the feeding period; Simmons & Gwynne, 1991). Genomic assessment involved searching for the sperm genotypes of each male within the spermatheca of each female. To achieve this, we used four microsatellite markers developed previously for use in this species (Hare & Simmons, 2021). These microsatellite loci are highly variable and together allow for the accurate detection of 74% of matings (Hare & Simmons, 2021). DNA from male tissue samples was extracted using EDNA HiSpEx Tissue Kits (Fisher Biotec) and DNA from sperm in the spermatheca was extracted according to the protocol developed by Simmons et al. (2007). The number of mates for each female was estimated by dividing by two the maximum number of microsatellite alleles detected in the spermatheca using any one primer. For each male, the number of mates was estimated by identifying microsatellite allele combinations unique to each male and screening for the presence of those allele combinations in the spermathecal contents of females.

Data from genomic analysis for both sexes were cross-checked against observational data. Observational data are relevant to estimating mating success because spermatophores are transferred in all matings. Females spend over 90 min consuming the spermatophore following copulation, so that virtually all matings by females in all cages were detectable. Indeed, our estimates of mean female mating success by observation (2.40 ± 0.18) and by genomic analysis (2.36 ± 0.25) did not differ (paired $t_{66} = 0.141$; $p = 0.889$). By contrast, males may complete their contribution to mating in under 30 min, making observations across multiple enclosures more difficult. Accordingly, our estimate of mean male mating success by observation (0.64 ± 0.12) was significantly lower than our estimate by genomic analysis (1.30 ± 0.14; paired $t_{66} = -3.601$; $p < 0.001$). Moreover, our estimate of male mating success by genomic analysis was itself an underestimate, because we divided the number of unique haploid genotypes by two under the assumption that all males were homozygous, when in reality many would be heterozygous. Therefore, to achieve the best possible measure of mating success, in cases of discrepancy between observed and genotyped estimates the higher number of mates was used in subsequent analysis. This number still constitutes an underestimate, especially for males; in theory male mating success should be identical to that of females. Nevertheless, although we consider our estimates to be conservative, they should provide a reliable proxy for relative mating success among males.

2.3 | Estimates of reproductive success

Egg number was used as a point estimate of reproductive success. There is evidence from various insects that lifetime egg production increases with increasing nutrition or mating opportunities (Chapman & Partridge, 1996; Davey, 1965; Muthukrishnan & Pandian, 1987); among continuously ovipositing taxa, as opposed to batch layers, variation in the rate of egg production will be reflected by variation in the number of eggs found in the ovary at any given time. This is true specifically for *K. nartee* which lays eggs continuously and where nutrient availability affects both the number of eggs carried at point sampling (Simmons, 1990; Simmons & Bailey, 1990) and the lifetime number of eggs actually laid (Simmons, 1992, 1994). Although our estimate of the number of developing eggs in the ovaries provides a reliable estimate of female fecundity at the point of sampling, it is unlikely to provide a reliable estimate of lifetime fitness. As such our estimated Bateman gradients can only inform us of relative differences between diet treatments and sexes, the aim of our study, and not the absolute strength of sexual selection acting on males and females.

Female reproductive success was thus estimated as the number of eggs (both developing and mature) in the ovaries, which is determined by pollen availability and male nutrient donations (Simmons, 1990, 1992, 1994; Simmons & Bailey, 1990). Male reproductive success was calculated by summing the number of those eggs likely to be fertilized by each male following random utilization of rival sperm by females (Simmons, 1995). Thus, for each male, the number of eggs counted from a given mate was divided by the number of males identified contributing sperm to that female, and an equal portion was attributed to the focal male. That male's reproductive success was calculated by summing the number of eggs he was likely to have fertilized across all females carrying his sperm. Observational data were used to identify multiple mating events between the same male–female pairs (3/156 matings), and estimates of male reproductive success were adjusted accordingly so that each male was apportioned a percentage of eggs corresponding to his total proportional contribution to each female's sperm stores.

There is no evidence that female *K. nartee* can remove spermatophores before insemination is complete. On the contrary, the spermatophylax (the nutritive component of the spermatophore) overlays and surrounds the ampulla (the sperm-filled component of the spermatophore), so females must thoroughly consume the spermatophylax over the course of approx. 80 min before reaching the ampulla by which time all sperm in the ampulla have been transferred to the female's spermatheca (Simmons, 1994; Simmons & Kvarnemo, 1997). Nor is there evidence of significant mating-order bias in fertilization success among competing males: the proportion of eggs fertilized by the second of two males to mate is 0.69 ± 0.10, and this proportional representation does not differ significantly from a fair raffle (Simmons, 1995). Together these findings justify an even allocation of fertilizations to all males contributing to a female's sperm store, an approach, that is conservative because equal distribution of fertilization among multiple males reduces the slope of the male Bateman gradient (Greenway et al., 2021). Note that the method described here, measuring patterns of sperm storage by females, arguably provides greater accuracy than methods used in previous studies in which prior mating status is assumed or otherwise uncontrolled.

TABLE 1 Behavioural observations and estimated OSRs in enclosed populations of *Kawanaphila nartee* with experimentally manipulated pollen availability. Mating frequency and fecundity calculated per individual across the experimental period; all other means (standard errors) calculated per night

Variable	Pollen-supplemented Mean (*SE*)	Pollen-limited Mean (*SE*)	χ^2	*p*
Operational sex ratio/night	6.79 (0.05)	4.25 (0.04)	9.276	0.002
Calling males/night	9.65 (0.05)	7.41 (0.05)	16.098	<0.001
Female mating frequency	2.83 (0.05)	4.06 (0.06)	7.998	0.005
Male mating frequency	1.33 (0.03)	2.50 (0.06)	8.970	0.003
Female reproductive success (eggs)	8.74 (0.07)	5.63 (0.08)	25.50	<0.001
Spermatophores/night	1.40 (0.03)	2.22 (0.03)	4.404	0.036
Receptive females rejected/night	0.19 (0.01)	0.51 (0.01)	5.063	0.024

2.4 | Statistical analysis

All analyses were conducted using R v3.6.1 (R Core Team, 2021). Random factors were included using the 'lme' function of the package *nlme* (Pinheiro et al., 2019). Where assumptions of normality were violated, robust variance–covariance matrices were estimated using the package *vcov* (Chirico, 2017), and these matrices were analysed using the function 'ANOVA' (Type II Wald chi-square tests) in the package CAR package (Fox & Weisberg, 2019). Post hoc comparisons, where necessary, were carried out using the function 'pairs' in the package EMMEANS (Lenth et al., 2021).

To estimate the OSR on each night in each of our experimental populations, we divided the number of receptive males (the number of males observed calling and/or to have transferred a spermatophore) by the number of receptive females (estimated as the highest number of either couplings observed or females with spermatophores by the end of the night). Males only call when they are ready to mate, so the number of males that called and/or mated corresponds exactly to the number of receptive males in a given cage on a given night. However, females were only detected as receptive when they were observed in amplexus or feeding on a spermatophore; females that unsuccessfully sought a mate could not be detected and were not included in the calculation of the OSR. Thus our estimates of the OSR were substantially male-biased. Nonetheless, they were calculated to ensure that our nutrient manipulation had a significant effect on the OSR, with our expectation being that pollen-supplemented populations should have a significantly greater male bias. One was added to each variable to prevent division by zero on nights where no matings or spermatophores were recorded. The OSR was compared between treatments and throughout the experimental period using a mixed-effects model of covariance with time, food treatment and the interaction between time and treatment included as independent variables. Enclosure identity was included as a random factor.

To calculate Bateman gradients, we used a mixed-effects model including reproductive success (number of eggs for females and estimated eggs fertilized for males) as the dependent variable. For the independent variables, we included mating success (number of
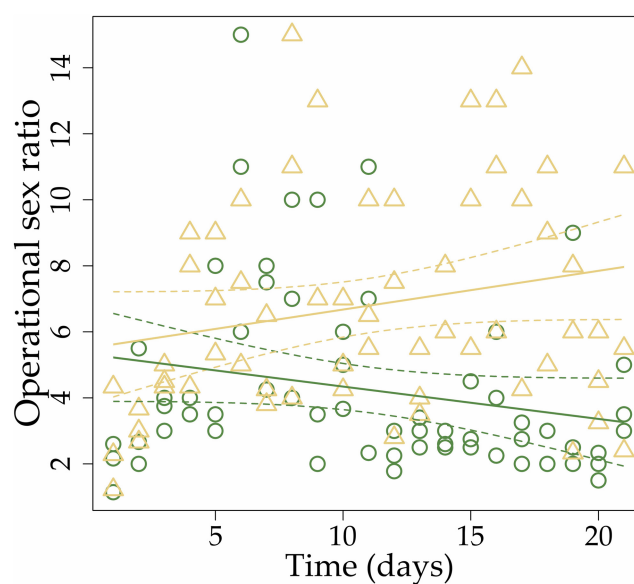
FIGURE 1 Variation in the operational sex ratio (OSR; with 95% confidence intervals, dashed lines) across the duration of an enclosure experiment (21 days) in which nutrient availability was manipulated via the supplementation or limitation of pollen. Green circular points and lines represent OSR data from pollen-limited enclosures; tan triangular points and lines represent OSR data from pollen-supplemented enclosures. OSR Calculated as the number of receptive males divided by the number of receptive females. Because not all receptive females could be observed, the estimated value of the OSR will be inherently male-biased.

mates), food treatment and sex in a three-way interaction; pronotum length; and, as a random intercept factor, enclosure identity. Pronotum length was included in order to account for problems associated with confounding body size and fecundity in females (Anthes et al., 2017; Henshaw et al., 2018). We present Bateman gradients based on absolute mating and reproductive success; however, this approach often leads to overestimation (Anthes et al., 2017) and does not necessarily allow for comparison between populations or species (Schielzeth, 2010). As such, we also present Bateman gradients calculated using *z*-standardized mating

success as the independent variable and mean-standardized reproductive success (i.e. an individual's reproductive success divided by the mean reproductive success) as the dependent variable (Collet et al., 2014; Schielzeth, 2010). Variables were standardized by sex and enclosure.

3 | RESULTS

3.1 | Operational sex ratios and mating behaviour

The degree of male bias in the OSR was greater in the pollen-supplemented compared with the pollen-limited enclosures (Table 1). There was no main effect of time on the OSR ($\chi_1^2 = 0.049$; $p = 0.824$), but there was a significant interaction between time and treatment ($\chi_1^2 = 6.169$; $p = 0.013$): the male bias increased in the pollen-supplemented treatment and decreased in the pollen-limited treatment as the experiment proceeded (Figure 1). Accordingly, there were increasingly more males calling per night in the pollen-supplemented enclosures than in the pollen-limited enclosures, but more spermatophores transferred in the pollen-limited enclosures, and males were more than twice as likely to reject females in the pollen-limited than in the pollen-supplemented enclosures (Table 1). Female and male mating frequencies were higher in the pollen-limited enclosures than in the pollen-supplemented enclosures, but female fecundity was 1.5 times higher in the pollen-supplemented enclosures than in the pollen-limited enclosures (Table 1). One female from the pollen-supplemented treatment and one female from the pollen-limited treatment did not mate at all during the experiment. The reproductive success of these females was recorded as zero regardless of how many eggs were found in the ovaries. By contrast, eight males from the pollen-supplemented treatment failed to mate during the experiment, whereas all males from the pollen-limited treatment achieved at least one mating. Thus, the manipulation of pollen availability in the enclosures had substantial effects on the OSR and reproductive behaviour of *K. nartee* within those enclosures.

3.2 | Absolute Bateman gradients

A model analysing data pooled from both sexes and all enclosures indicated a three-way interaction effect between mating success, food treatment and sex on our point estimates of reproductive success ($\chi_1^2 = 4.293$; $p = 0.038$). Subsequent analyses were broken down by sex. Among males, mating success and pollen availability significantly affected estimated reproductive success (Table 2). In both pollen-supplemented and pollen-limited populations, Bateman gradients were positive and significant (Table 2; Figure 2a). However, the Bateman gradient was significantly steeper in the pollen-supplemented treatment (Table 2). There was no effect of body size (pronotum length) on estimated male reproductive success (Table 2). Among females, estimated reproductive success was higher in pollen-supplemented enclosures than pollen-limited enclosures (Table 3). Bateman gradients were nonsignificant: there was no significant interaction between mating success and pollen availability ($\chi_1^2 = 0.202$; $p = 0.653$), and no significant effect of mating success on female reproductive success. The nonsignificant interaction between mating success and pollen availability was removed from the model (Figure 3a; Table 3). There was also no effect of pronotum length on female mating success (Table 3).

3.3 | Standardized Bateman gradients

A model analysing pooled standardized data from both sexes and all enclosures indicated a significant two-way interaction between mating success and sex on reproductive success ($\chi_1^2 = 39.048$; $p < 0.001$). Subsequent analyses were broken down by sex. Among males, standardized reproductive success significantly increased with standardized mating success across treatments (Figure 2b), but the interaction between mating success and treatment was nonsignificant (Table 4). There was no effect of body size (pronotum length) on standardized male reproductive success (Table 4). Among females, there was no effect of treatment, pronotum width, or mating success on reproductive success (Table 5; Figure 3b).

TABLE 2 Predictors of estimated reproductive success in male *Kawanaphila nartee* from enclosures in which the OSR was manipulated via the supplementation of pollen (three pollen-supplemented populations from which 42 animals were recovered, and three pollen-limited populations from which 25 animals were recovered)

Variable	Estimate (*SE*)	χ^2	Df	*p*
Mating success	2.223 (0.266)	82.993	1	<<0.001
Pollen availability	−0.875 (1.082)	12.972	1	<0.001
Pronotum length (mm)	0.489 (1.678)	0.085	1	0.771
Mating success: pollen availability		5.375	1	0.020
Residuals			58	
Bateman gradient (supplemented)	2.237 (0.310)	52.101	1	<<0.001
Bateman gradient (limited)	1.310 (0.152)	74.087	1	<<0.001

Note: A colon between variables indicates an interaction term. Bottom four rows are Bateman gradients calculated from models using data only from the treatments indicated (pollen-supplemented or pollen-limited).
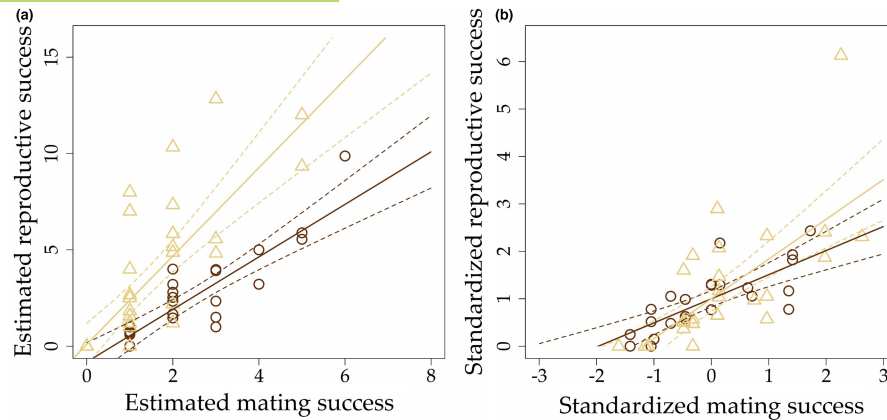
Abbreviation: *SE*, standard error.

FIGURE 2 Point estimates of Bateman gradients (with 95% confidence intervals, dashed lines) for male *Kawanaphila nartee* from enclosures in which the OSR was manipulated to be either more male-biased (three populations, from which 42 animals were recovered) or less male-biased (three populations, from which 25 animals were recovered) via the supplementation or limitation of pollen, respectively. Brown circular points and lines represent individuals from pollen-limited enclosures; tan triangular points and lines represent individuals from pollen-supplemented enclosures. Data estimated based on sperm genotyped from receiving females, assuming equal paternity share. Eight males the pollen-supplemented treatment had a mating success of zero. All other males, including all males in the pollen-limited treatment, achieved at least one mating. Points jittered to improve readability. (a): Bateman gradients calculated from absolute values. (b): Bateman gradients calculated using z-standardized mating success and mean-standardized reproductive success (Collet et al., 2014; Schielzeth, 2010).

TABLE 3 Predictors of estimated reproductive success in female *Kawanaphila nartee* from enclosures in which the OSR was manipulated via the supplementation of pollen (three pollen-supplemented populations from which 35 animals were recovered, and three pollen-limited populations from which 32 animals were recovered)

Variable	Estimate (*SE*)	χ^2	Df	*p*
Mating success	−0.215 (0.176)	1.488	1	0.223
Pollen availability	−3.377 (0.657)	26.415	1	<<0.001
Pronotum length (mm)	−0.463 (2.053)	0.051	1	0.822
Residuals			58	
Bateman gradient (supplemented)	0.131 (0.244)	0.290	1	0.590
Bateman gradient (limited)	0.298 (0.251)	1.409	1	0.235

Note: A colon between variables indicates an interaction term. Bottom four rows are Bateman gradients calculated from models using data only from the treatments indicated (pollen-supplemented or pollen-limited).

Abbreviation: *SE*, standard error.

4 | DISCUSSION

We predicted that Bateman gradients should vary in males according to variation in the operational sex ratio (OSR). In the absolute data from our experimentally manipulated populations, point estimates of Bateman gradients for males had significant and positive slopes, and the gradient was significantly steeper when the OSR was more male-biased (Table 2; Figure 2a). This finding is consistent with the majority of work published on Bateman gradients, in which male gradients are significant, linear, positive and interpreted as indicative of the strength of sexual selection (Janicke et al., 2016). Indeed, the resource-induced changes in the OSR were mirrored in the change in Bateman gradient: the OSR was 60% higher in pollen-supplemented populations than in pollen-limited populations, and the Bateman gradient was 66% higher (Tables 1 and 2). Reanalysis of the data using standardized values for mating success and reproductive success returned shallower

Bateman gradients, consistent with the view that Bateman gradients can be overestimated when using absolute values (Anthes et al., 2017).

Furthermore, the standardized Bateman gradients for males did not differ significantly from one another at the critical *p*-value of 0.05 (Table 4; Figure 2b). Interaction effects require greater statistical power to detect than main effects (Durand, 2013), and given that the standard errors around our standardized slope estimates did not indicate any overlap (Table 4; Figure 2b), we are cautious about rejecting the potential effect of pollen availability on male Bateman gradients. Nevertheless, a conservative interpretation of the data suggests that a shift in the OSR, and thus the behavioural sex roles, does not greatly affect the strength of sexual selection on male *K. nartee*: males are under consistent strong sexual selection for mating success, and a decrease in the OSR coupled with an increase in female intrasexual competition and male mate choice does not necessarily relax selection on males.
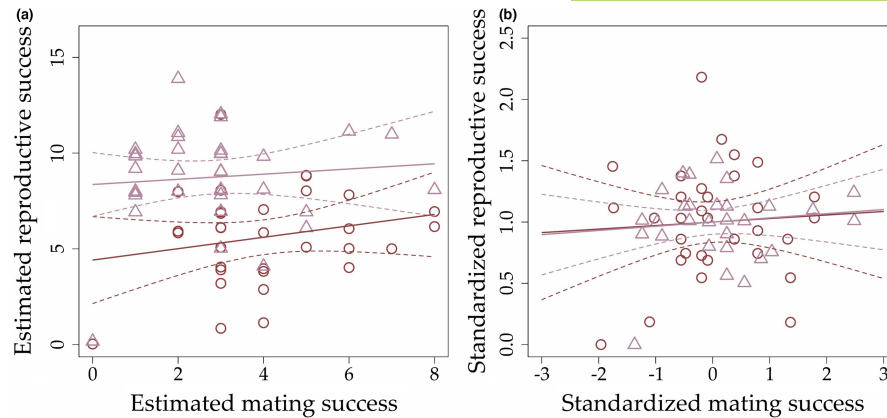
FIGURE 3 Point estimates of Bateman gradients (with 95% confidence intervals, dashed lines) in female *Kawanaphila nartee* from enclosures in which the OSR was manipulated to be either more male-biased (three populations, from which 35 animals were recovered) or less male-biased (three populations, from which 32 animals were recovered) via the supplementation or limitation of pollen, respectively. Dark red circular points and lines represent individuals from pollen-limited enclosures; mauve triangular points and lines represent individuals from pollen-supplemented enclosures. Data estimated based on sperm genotyped from receiving females, assuming equal paternity share. One female from the pollen-supplemented treatment and one female from the pollen-limited treatment had a mating success of zero. All other females achieved at least one mating. Points jittered to improve readability. (a): Bateman gradients calculated from absolute values. (b): Bateman gradients calculated using z-standardized mating success and mean-standardized reproductive success (Collet et al., 2014; Schielzeth, 2010).

TABLE 4 Predictors of mean-standardized reproductive success in male *Kawanaphila nartee* from enclosures in which the OSR was manipulated via the supplementation of pollen (three pollen-supplemented populations from which 42 animals were recovered, and three pollen-limited populations from which 25 animals were recovered)

Variable	Estimate (*SE*)	χ^2	Df	*p*
Mating success (z-standardized)	0.826 (0.116)	56.535	1	<<0.001
Pollen availability	0.004 (0.182)	<0.001	1	0.945
Pronotum length (mm)	0.398 (0.620)	0.412	1	0.521
Mating success: pollen availability		3.010	1	0.083
Residuals			58	
Bateman gradient (supplemented)	0.838 (0.135)	38.766[a]	1,40	<<0.001
Bateman gradient (limited)	0.508 (0.089)	32.654[a]	1,23	<<0.001

Note: A colon between variables indicates an interaction term. Bottom four rows are Bateman gradients calculated from models using data only from the treatments indicated (pollen-supplemented or pollen-limited). Mating success was z-standardized for this analysis (Collet et al., 2014; Schielzeth, 2010).

[a]*F*-ratio.

Abbreviation: *SE*, standard error.

For females, we predicted that Bateman gradients should also vary across our treatments, either by increasing access to sperm for fertilizations or by increasing access to nutrients used in gametogenesis. Although the Bateman gradient was positive in both treatments (Figure 3), these gradients did not differ significantly from zero, nor from each other, using either absolute or standardized values. Our point estimates of reproductive success are undoubtedly conservative and unlikely to reflect lifetime reproductive success. Females would have laid some number of eggs prior to our point sampling, and those that had a higher mating success may have laid more eggs prior to sampling. Thus it may be that our point estimates are inadequate to detect significance in the relatively shallow Bateman gradients in females compared with those in males.

Moreover, multiple mating impacts the quality of eggs laid by *K. nartee* as well as the quantity (Simmons, 1990). Many studies of

insects have revealed effects of multiple mating by females on hatching success, with a small but general effect size being supported (Slatyer et al., 2012). Such an effect would result in females with few matings having a lower reproductive success than egg counts suggest relative to those with many matings, making the Bateman gradient based on egg count shallower than the Bateman gradient based on hatched offspring. Male Bateman gradients were also likely conservative estimates for the same reasons, but also because we may not have recovered those males with the highest mating success: mating success of recaptured males was approximately half that of recaptured females (Table 1), suggesting that the most successful males died earlier than the less successful males, presumably due to increased investment in reproduction. The greater mortality of males in pollen-limited enclosures where matings were more frequent, though not statistically significant, also speaks to this

Variable	Estimate (SE)	χ^2	Df	p
Mating success (z-standardized)	0.031 (0.068)	0.378	1	0.539
Pollen availability	−0.002 (0.094)	<0.001	1	0.987
Pronotum length (mm)	−0.114 (0.312)	0.133	1	0.716
Mating success: pollen availability		<0.001	1	0.987
Residuals			58	
Bateman gradient (supplemented)	0.034 (0.051)	0.443[a]	1,33	0.506
Bateman gradient (limited)	0.029 (0.085)	1.114[a]	1,30	0.736

TABLE 5 Predictors of mean-standardized reproductive success in female *Kawanaphila nartee* from enclosures in which the OSR was manipulated via the supplementation of pollen (three pollen-supplemented populations from which 35 animals were recovered, and three pollen-limited populations from which 32 animals were recovered)

Note: A colon between variables indicates an interaction term. Bottom four rows are Bateman gradients calculated from models using data only from the treatments indicated (pollen-supplemented or pollen-limited). Mating success was *z*-standardized for this analysis (Collet et al., 2014; Schielzeth, 2010).

Abbreviation: *SE*, standard error.

[a]*F*-ratio.

possibility, but it remains speculative. In any case, the genetic estimates of mating success for females would include those males that had died before collection, but those males, and their presumably high variance in mating success, could not contribute to the male Bateman gradients. Despite these moderating factors, unambiguous and significant Bateman gradients were detected for males.

The lack of significance in female Bateman gradients is consistent with a previous field-based correlational study which likewise failed to find significant Bateman gradients in five temporally separated field samples, including the sex role reversed period of the breeding season (Hare & Simmons, 2021). It is possible that stochastic factors may have caused some females to acquire fewer resources within their treatment than others; possibly such females were more likely to mate multiply in an attempt to compensate for the lack of nutritional resources acquired by foraging, which would also flatten the gradient. However, overall our findings may speak to the lack of precision of our estimates of Bateman gradients and the general difficulty of measuring Bateman gradients in field-based assessments of female animals (Anthes et al., 2017), where slopes tend to be shallower and thus less easily detected than those of males.

Multivariate selection analysis indicates that female *K. nartee* are under sexual selection for increased ear size, evidently to maximize mate detection and thus mate acquisition during the early, sex role reversed portion of the breeding season (Hare & Simmons, 2021). To confirm variation in the relative strengths of Bateman gradients across males and females suggested by the point estimates reported here, future studies need to find ways in which lifetime male and female reproductive success can be measured so that accurate estimates of selection across both male and female lifespans can be obtained. These measurements will almost certainly require experimental designs based in the laboratory rather than the field.

Previous studies using the same experimental protocol as the present study found much larger shifts in OSR, from entirely female-biased in pollen-limited populations to entirely male-biased in pollen-supplemented populations (Gwynne & Simmons, 1990; Vincent & Gwynne, 2014). Indeed, in these studies OSR varied by one or even two orders of magnitude between experimental treatments.

However, the change in OSR in our experimental manipulation was less extreme. The difference between the OSR reported here and previously lies in our method of estimating OSR, which was inherently male-biased. Receptive females could only be counted towards the OSR when they were observed in amplexus or actually mated; females that unsuccessfully sought matings were not detected and thus were not included in the calculation. Nevertheless, enclosures with lower OSRs had higher mating rates, proportionally fewer males ready to mate at a given time, and higher incidence of males rejecting females than enclosures with higher OSRs. Thus, regardless of whether our overestimates of OSR fell below 1, significant relative differences in OSR between treatments constitute a strong indication of plasticity in the direction and strength of sexual selection. A stronger female bias in the OSR in *K. nartee* would almost certainly be associated with increased female intrasexual competition, especially early in the breeding season as females compete for their first (high-quality) mate (Hare & Simmons, 2021), and females have higher mating frequencies. It is possible then that had the OSR in our pollen-limited enclosures been skewed more strongly towards a female bias we might have seen significant and steeper female Bateman gradients (Janicke & Morrow, 2018).

In conclusion, our results show that in *K. nartee*, relative shifts in the OSR are not necessarily reflected in Bateman gradients. The prediction of sex role theory that the strength of selection on the sexes should be inverted during sex role reversal may warrant revisiting (Fritzsche & Booksmythe, 2013; Williams, 1966). It is clear that sex roles are not dichotomous, but rather exist along one or more spectra (Ah-King & Ahnesjö, 2013); in the case of *K. nartee*, sex role reversal among females may not necessarily entail sex role reversal among males, in the sense of exclusively competitive females and exclusively choosy males. We also caution against drawing conclusions regarding the operation of sexual selection based on one metric alone. We instead recommend that future studies approach sexual selection with multiple complementary tools and are encouraged to see that many existing studies already do so (Hafernik & Garrison, 1986; Jones et al., 2004; Kvarnemo et al., 2007; Mills et al., 2007; Pelissie et al., 2012; Simmons

et al., 2009). In the case of *K. nartee*, males and females are clearly under some degree of sexual selection that suggests but does not entirely conform to conventional concepts of sex roles. The complexity of behavioural sex roles is thus probably not reducible to a pair of opposing categories.

AUTHOR CONTRIBUTIONS

LWS conceived the study. LWS and RMH designed the experiment, collected field samples and analysed the data. RMH drafted the first version of the manuscript. LWS and RMH contributed to later versions of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.1zcrjdfvh.

PEER REVIEW

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