Original Article

Exploring the effects of extreme polyandry on estimates of sexual selection and reproductive success

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Multiple mating by females can dramatically alter selection on males by creating indirect interactions between rivals via sperm competition. Exactly how this behavior alters the relationship between male mating and fertilization success depends on multiple factors: re-mating frequency, sperm usage patterns, and mating assortment (the extent to which the most promiscuous individuals mate with each other). Here, we explore the role these elements play in determining sexual selection in a highly polygyandrous species, the squash bug *Anasa tristis*. Using replicated semi-natural enclosures, in which individuals were able to freely interact for a 2-week period, we tracked matings between individuals and subsequent female offspring production. Multiple mating was extremely common, resulting in very high levels of sperm competition intensity. However, network analysis revealed that the most promiscuous males mated with less polyandrous females, and therefore experienced lower levels of sperm competition. As a result, estimated male reproductive success increased with mating success, but this relationship varied according to the mode of sperm utilization with which it was calculated. Furthermore, females with more mating partners produced more offspring, suggesting they also benefit from mating multiply. Our findings highlight that polyandry has numerous and complex effects on sexual selection which may only be exposed when examined under naturalistic conditions.

Key words: **Bateman gradients, mating systems, polyandry, sexual networks, sperm competition.**

INTRODUCTION

Polyandry, or multiple mating by females, is now recognized to be the rule rather than the exception in many species (Kokko and Mappes 2013; Pizzari and Wedell 2013). In previous decades, male mating success was assumed to translate directly into offspring production: the more mates, the more offspring sired (Bateman 1948). However, female polyandry fundamentally challenges this assumption because it can initiate postcopulatory sexual selection (Parker 1970; Kvarnemo and Simmons 2013; Parker and Birkhead 2013; Shuster et al. 2013). After mating, sperm from multiple males may directly compete to fertilize a limited number of eggs, and this sperm competition can occur concurrently with the operation of cryptic female choice (Wade and Arnold 1980; Eberhard 1996; Simmons 2001, 2005). Therefore, multiple mating by females has the potential to de-couple male mating success and paternity and erode both precopulatory and resulting total sexual selection. The extent to which polyandry influences sexual selection depends on multiple factors, primary of which are 1) the frequency

and variance of female multiple mating across the population, 2) whether males and females re-mate randomly, and 3) how females use the sperm they receive from their various partners.

Considerable variation in male re-mating rates has been documented across a range of species. In extreme cases, a few successful males may mate with tens of females while the least successful males fail to secure any copulations over the course of a single breeding season (e.g., elephant seals, Le Boeuf 1974). Such observations of intense intrasexual competition for reproductive opportunities and resulting mating skew indicate that far stronger sexual selection forces act on males than females (Bateman 1948; Arnold and Duvall 1994; Jones et al. 2002; Collet et al. 2014). However, recent molecular paternity assignment advances have revealed that individual female mating rates are also far from uniform, both within and across populations (Taylor et al. 2014). Some females mate with multiple partners, others mate with a single partner, and some females fail to mate at all (Rhainds 2010; Kokko and Mappes 2013; Taylor et al. 2014, Greenway et al. 2015). This variance may reflect differences in mate encounter rates, choosiness, and the cost–benefit trade-offs of mating multiply for females. In some species, mating can provide direct benefits, and so females can accrue reproductive success with each additional copulation (Evans and

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Magurran 2000; Gowaty et al. 2010). However, in other species, the costs associated with mating may reduce reproductive output if females continue to re-mate with new partners (Maklakov et al. 2005). Under both conditions, a female's mating history can considerably influence the reproductive payoff a male achieves from mating with her. Multiple mating can alter not only the extent of sperm competition but also the number of offspring the female will subsequently produce. For any population, the interaction between individual male and female re-mating rates (i.e., who is re-mating with whom) is central to understanding the effects of polyandry on the strength of precopulatory sexual selection and total selection.

Considering variation in re-mating rates in both sexes, non-random re-matings can dramatically influence the strength of sexual selection. Under positive assortment, more polygynous males mate with more polyandrous females, and less polygynous males mate with less polyandrous females (McDonald and Pizzari 2016, 2017). This scenario effectively levels the sexual selection playing field for males, eroding variance in their reproductive success and weakening the relationship between male mating success and fertilization success (Collet et al. 2012, Figure 1b). Positive assortment contrasts with negative mating assortment, where the least polygynous males mate with the most polyandrous females. Under this negative mating assortment scenario, males with the lowest mating success face the highest levels of sperm competition, steepening the association between mating success and reproductive success (e.g., Morimoto et al. 2019, Figure 1c). Thus, the strength and direction of mating assortativity is likely to determine the extent to which mating systems and resulting episodes of sexual selection depart from traditional assumptions and is therefore crucial to take into account.

In addition to assortativity, male fertilization success depends to a large extent on patterns of female sperm usage. In many species, polyandrous females store ejaculates from multiple males simultaneously in a specialized sperm storage organ prior to fertilization (Eberhard 1996). In some species, this process confers the first or last male to mate with a fertilization advantage, and in other species, sperm from multiple males mixes inside storage organs, turning fertilization into a fair raffle in which paternity is proportional to the amount of sperm a male contributes (Birkhead and Hunter 1990; Neff and Wahl 2004; Shuster et al. 2013). Thus, depending on sperm usage, the order in which males mate with females can have a large bearing, or no bearing at all, on their ability to convert mating success into paternity (Wade and Arnold 1980; Pischedda and Rice 2012; Pélissié et al. 2014). Under last male precedence, a female's mating history is of relatively little consequence to a male's paternity share, provided he is the last to inseminate her prior to oviposition or egg release. In the case of a fair raffle, or shared paternity, males are likely to receive diminishing fitness returns from mating with increasingly polyandrous females regardless of whether they mated first or last (Wedell 2007). As a result, sperm usage patterns likely mediate the impacts of polyandry and mating assortment on the relationship between mating and fertilization success.

It is necessary to track freely interacting males and females under naturalistic conditions if we wish to meaningfully evaluate the contribution of each of these polyandry-associated components to the action of sexual selection: variance in female mating success, mating assortment, and sperm usage by females (e.g., Collet et al. 2012, 2014). Until recently, the reproductive success of each sex has been largely estimated in isolation, which has obscured potential feedback between male and female multiple mating rates and their respective resulting fitness consequences (Alonzo and Pizzari 2013; Bocedi and Reid 2016). This is the case when measuring assortativity; the patterns shown in Figure 1b and c assume females do not vary in fecundity. In reality, female reproductive output can vary considerably due to both intrinsic and extrinsic factors.
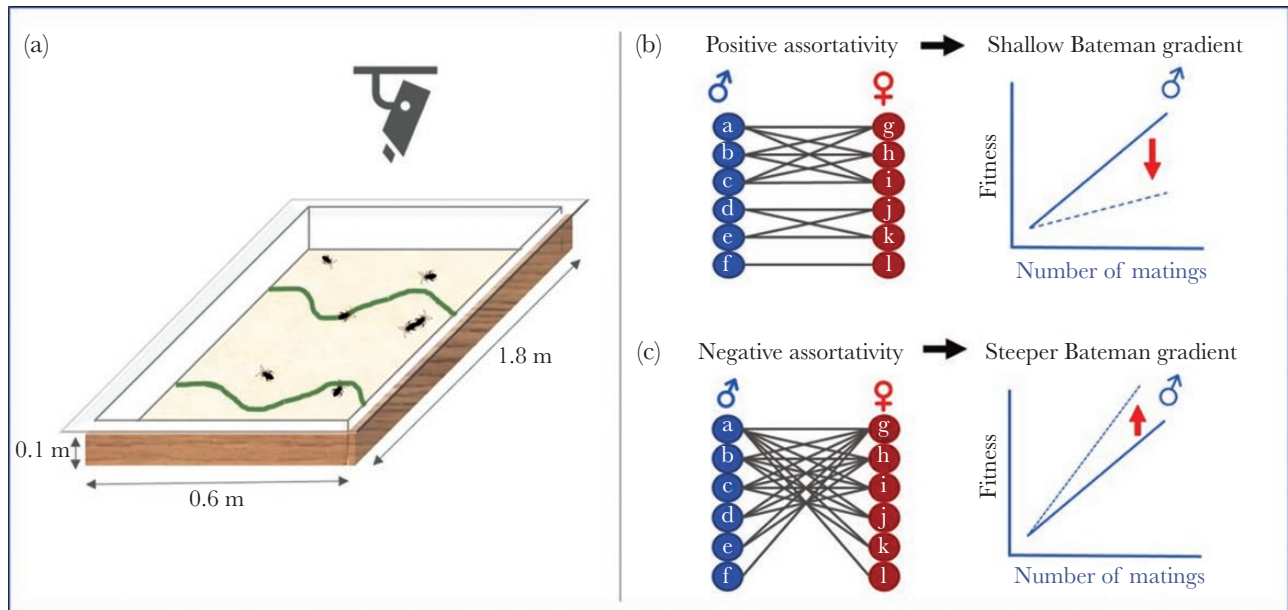

Figure 1
(a) Schematic illustrating the dimensions of the experimental arena set-up, constructed of polywall and covered with a transparent plexiglass sheet with a Gigapan-mounted camera positioned overhead taking panoramic images at hourly intervals. (b) Positive assortative mating between the most promiscuous males (blue) and females (red) is predicted to uncouple male mating success and reproductive fitness, resulting in a shallow or flat Bateman gradient. (c) On the other hand, negative assortative mating between the most promiscuous males (blue) and the least promiscuous females (red) is predicted to strengthen the relationship between male mating success and reproductive fitness, potentially resulting in a steeper positive Bateman gradient.

Males may preferentially mate with more fecund females, exposing themselves to a higher risk of sperm competition but gaining access to larger numbers of ova to fertilize. The situation is complicated even further if females receive direct benefits from mating multiply and their fecundity increases with each partner they mate with (Eady et al. 2000; Kamimura 2003; Noble et al. 2013). In this study we used a network-based approach to capture the inherent nonindependence of reproductive success of the individuals within a mating population (McDonald and Pizzari 2016). We then incorporated the estimated fitness consequences of both direct mating interactions between males and females and indirect interactions between rival males via projected sperm competition risk. This approach enabled us to gain a comprehensive and ecologically relevant understanding of the various impacts of polyandry on sexual selection.

Using arenas in which individuals could freely interact, we examined the roles of mating partner number and assortativity, and their consequences under different sperm precedence scenarios, for male and female reproductive success in the squash bug *Anasa tristis* De Geer (Hemiptera: Coreidae). *Anasa tristis* is found throughout the Americas and typically feeds on cucurbits; adults live for several weeks in the field and are often observed at high densities when food resources are abundant (Beard 1940). Unlike other coreid species, *A. tristis* males do not possess sexually selected weaponry, and there is no evidence for precopulatory contest behavior. Instead, postcopulatory competition is likely to dominate, as males and females have been observed mating multiple times and with multiple individuals (J.H., unpublished data). Copulations are initiated by males, which attempt to mate by climbing on the backs of females and extending their genitalia. Females are larger than males and can reject male mating attempts by vigorously shaking them off and moving away. Once in copula, matings are prolonged and can last for upwards of 8 h. However, females can acquire sufficient sperm from a 30-min mating to fertilize the eggs she lays for at least 4 weeks, and mating duration does not appear to impact female reproductive success (Sears et al. 2020). In this study, we (1) compared variance in mating success of male and female *A. tristis* in small replicated experimental populations under seminatural conditions. We (2) then examined the extent to which mating interactions between males and females were assortative, asking whether the most promiscuous males mated more frequently with the most or least polyandrous females in the populations. Finally, we (3) quantified the consequences of variance in mating success for female reproductive output, and estimated the roles that mating success, mating assortment, and sperm usage played in determining male reproductive output and sexual selection.

METHODS

Insect collection and rearing

We collected experimental *A. tristis* (Hemiptera: Coreidae) as late stage juveniles from eleven different community gardens in Alachua Co., FL, in June 2013. We reared these juveniles communally in mesh insect cages in a shaded greenhouse with seasonal variation in temperature and photoperiod, and provisioned them with potted host plants of both *Curcubita pepo* and *C. moschata*, as well as with cut fruit and wet cotton in petri dishes. Cages of juveniles were checked for adults every 48 h to ensure that experimental adults were unmated. Newly eclosed adults were removed from juvenile cages and housed in single-sex mesh cages. Adults were maintained on the same diet and in the same greenhouse as juveniles. To

ensure that experimental individuals were reproductively mature, bugs were only entered into trials once they reached 14–28 days post final molt, during which period females typically have a clutch of eggs present in their reproductive tract (Beard 1940). To track mating interactions in enclosures containing multiple individuals, we individually marked each adult on the pronotum with a water-based paintpen (Elmer's Painters Opaque Paint Markers) and gave each bug a unique black ink numeric ID (Sharpie Permanent Ultra Fineliner).

Experimental design and scan sampling protocol

Behavioral trials were set up in eight replicated seminatural enclosures within a climate-controlled greenhouse during July and August 2013. Each enclosure contained 10 male and 10 female *A. tristis* individuals: sex ratio and density were matched to those commonly observed in field populations (J.H., unpublished data). Enclosures were 0.6 × 1.8 m in area, ~ 0.1 m deep, constructed of wooden frames lined with thin, flexible polywall, and covered with large panels of plexiglass (Figure 1). To encourage insects to remain on the bottom of each enclosure, the polywall lining was painted with Fluon™. Insects were provisioned with long, vining stems of large potted cucurbits (*C. pepo* and *C. moschata*) threaded into enclosures through small holes (~1.3 cm in diameter) drilled in the enclosure sides and anchored in 3–4 cm of sand that covered the bases of the enclosures.

We documented the mating interactions of individuals 12 times per day (i.e., once an hour between 8 am and 7 pm) over a 14-day period, using high-resolution photographs taken by cameras (Canon Powershot G15) housed in robotic camera mounts (Gigapan Epic Pro) suspended over each of the enclosures. This resulted in 168 observation points at which each individual's mating status and partner identity were documented. As matings are prolonged in *A. tristis*, lasting on average ≥ 2 h (Sears et al. 2020), we anticipated this sampling frequency should capture the vast majority of daytime mating interactions. We cannot eliminate the possibility that matings occurred at night, but mating and other reproductive behavioral activity (e.g., oviposition) is correlated with temperature and typically peaks during the heat of the day (Beard 1940). While consecutive mating observations between the same individuals likely reflect one continuous copulation, we also observed instances of females mating with different males an hour apart. In a previous study, pairs separated for around 0.9 ± 1.6 h between matings, and inter-mating intervals ranged between 2 min and 9.65 h (Sears et al. 2020). As a result, it is possible that consecutive mating observations may also represent shorter repeated copulations. Because the sampling technique precluded our ability to distinguish between these two scenarios, we focused predominantly on the number of different mating partners per individual rather than mating frequency in our analysis. Due to their positioning in the arena, some insects were either not visible or had obscured ID numbers in some panoramas, but we were able to successfully identify both partners in 70.25% of matings captured. In instances in which one of the two partners could be identified, in 607 cases only the female could be identified, and in 454 instances only the male in the pairing was identifiable.

To quantify female reproductive output, we separated females into individual clear plastic containers at the end of the 2-week behavioral trial, and we maintained them for an additional 4 weeks in Florida Reach-In environmental chambers (Walker et al. 1993) at 26.0 ± 0.1 °C on a photoperiod of 16:8 (L:D). This month-long oviposition time period approximates their natural reproductive

lifespan (Bonjour et al. 1993). We collected all the eggs they produced during this period and incubated them in petri dishes under the same conditions for an additional ≥ 2 weeks before visually scoring them for hatching success under a dissecting microscope. Eggs of *A. tristis* typically hatch in 7–10 days under laboratory conditions (Beard 1940). We then measured the pronotal width of insects to the nearest 0.1 mm using digital calipers (Mitutoyo Digimatic) to account for any association between body size and mating success.

Data analysis

We first visualized variance in male and female mating success and calculated mating network connectance within each enclosure using the R package *bipartite* (Dormann et al. 2008). We quantified levels of precopulatory sexual selection on body size in both sexes using linear mixed models (LMMs, model: number of mates~ body size) with enclosure ID included as a random effect. We then assessed the level of sperm competition between males using a metric of sperm competition intensity (SCI), the harmonic mean number of other mates a male's partners have, following McDonald and Pizzari (2016). An SCI value of 1 indicates that a male's partners are monandrous, whereas higher values of SCI indicate increased levels of sperm competition. To quantify mate assortment, we calculated the sperm competition intensity correlation (the SCIC) for each replicate trial (the relationship between SCI and male mating success). Positive SCIC values suggest positive assortment, indicating that the most promiscuous males and females are mating with each other, whereas negative SCIC values indicate the opposite. To assess whether SCIC values differed from chance, we randomly shuffled male and female mating combinations per trial while holding partner number per individual constant and then calculated SCIC as above. We repeated this randomization 1000 times per trial to generate a null distribution of SCIC values and then compared the observed SCIC value to this distribution to determine if observed levels of assortativity were significantly different from those expected by chance. Finally, we examined whether variation in observed SCIC values across replicates was associated with replicate average female polyandry levels using simple linear models.

Sperm usage scenarios

In the absence of paternity data, we estimated male reproductive success under two of the most likely sperm usage scenarios, last male precedence—in which the last male to mate with a female fathered 100% of a her offspring, and under a fair raffle scenario—in which paternity was divided between the males who mated with a female, weighted by the number of times they were observed in copula with her, and rounded up to an integer value. Both high last male precedence (>90%) and sperm mixing have been documented in related species (Sillén-Tullberg 1981; Balfour et al. 2020). While in reality, sperm usage likely falls somewhere between these two scenarios, we wanted to assess the scale of impact that estimating paternity under these contrasting conditions would have on predicted male reproductive output. We next estimated Bateman gradients using simple linear models constructed using male reproductive success estimated under the two sperm use conditions. To enable comparison with other studies, we then calculated standardized gradients, by repeating the same analysis after standardizing reproductive success to have a mean of 1 and mating success to have a mean of 0 and an SD of 1, following Lande and Arnold (1983). To obtain the significance of fixed effects and overall fit

of models, we used generalized linear mixed models (glmer, lme4 package (Bates et al., 2015)) with a Poisson distribution and log-link; male or female reproductive success was the dependent variable and replicate trial was included as a random effect. We ran the models with and without male SCI as a covariate to compare its role in explaining variance in estimated reproductive success under the two sperm utilization scenarios. To do so, we calculated AICs and marginal R^2 values using the *MuMIn* package for full and reduced models under both conditions following Johnson (2014). We then calculated effect sizes and significance values for mating success and SCI from these GLMMs. All statistical analyses were conducted in R version 3.5.0 (R Core Team 2018). Analyses reported in this article can be reproduced using the data provided by Greenway et al. (2021) via Dryad (https://doi.org/10.5061/dryad.k98sf7m6b).

RESULTS

Quantifying mating rates and precopulatory sexual selection

Both males and females re-mated at very high frequencies over the course of the two-week experimental period. On average across all replicates, both males and females had 5.84 observed mating partners, but females had a nonsignificantly larger variance around this mean partner number with several individuals remaining unmated (Levene's test, W = 3.52, P-value = 0.063, Figure 2). At the enclosure level, mating network connectivity values (the percentage of all possible mating combinations which were realized) ranged between 41% and 68%. We did not detect a relationship between body size and mating success for either sex: larger individuals were no more likely to gain more mating partners than their smaller counterparts (GLMM LRT, males: $\chi^2 = 0.1498$, df = 1, P = 0.699, females: $\chi^2 = 0.009$, df = 1, P = 0.924, Supplementary Figure S2).

Males were observed mating at an average of 47.49 timepoints over the 14-day trial, with individual mating frequencies ranging from 0 to 81. However, rather than investing their mating efforts evenly across their mating partners, observed male mating effort was significantly skewed toward one or two of the females. On average, males were observed 2.33 times more often in copula with their most frequent (or "primary") mate than expected by chance (one-sample t-test, t = 17.96, df = 79, P < 0.0001). On average, experimental females were observed mating at 64.5 timepoints over the course of 14 days, and individual mating frequency varied considerably from 0 through 110 observations (out of a total possible 168 observations). Mating activity remained fairly constant over the 14-day period; between 222 and 347 mating observations were documented per day across the eight replicates, and we recorded almost identical mating activity on Day 1 versus Day 14 of the experiment (225 vs. 221).

Quantifying levels of sperm competition

High levels of polyandry likely created intense sperm competition. In many of the arena replicates, almost all males interacted indirectly with each other via shared females. Sperm competition intensity (SCI) was consequently high, but importantly, it decreased with increasing mating success (Figure 3, LMM, $\chi^2 = 17.571$, df = 1, P < 0.001). Sperm competition intensity correlations (SCICs) varied across replicate arenas but were consistently negative (mean −0.199, ranging between −0.054 and −0.571), meaning that the degree of a male's polygamy tended to scale negatively with the degree of polyandry exhibited by
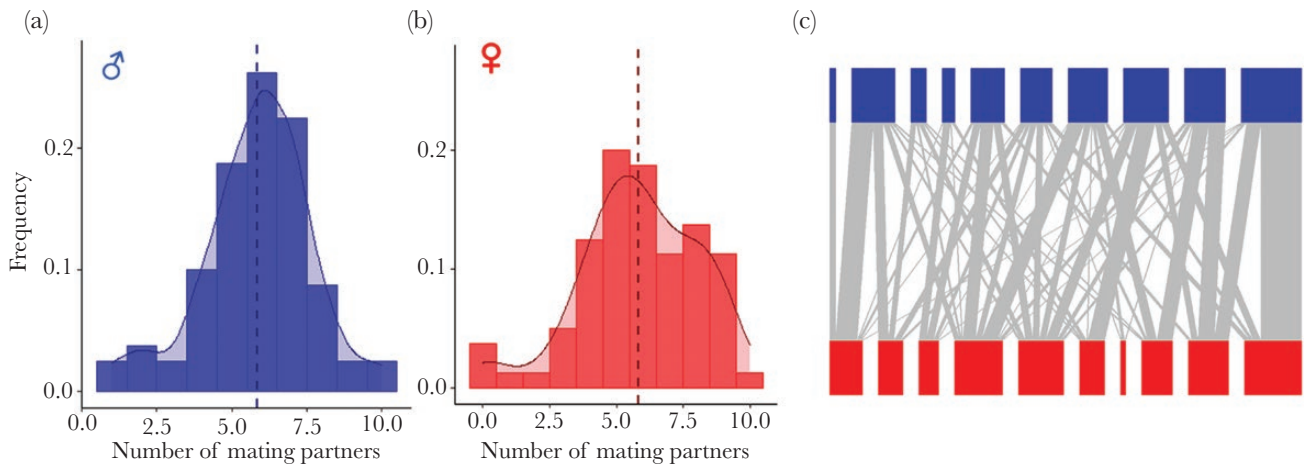
Figure 2
(a) Males and (b) females demonstrate high levels of multiple mating and considerable variance in mating success, sharing a mean of 5.84 mating partners each out of a possible 10 available during the 2-week trial period. This multiple mating led to high mating network connectance as exemplified through bipartite visualization of one arena replicate (c) in which matings between individual males (blue blocks) and females (red blocks) are represented as gray lines (see Supplementary Figure S1 for bipartite visualizations of all replicates).
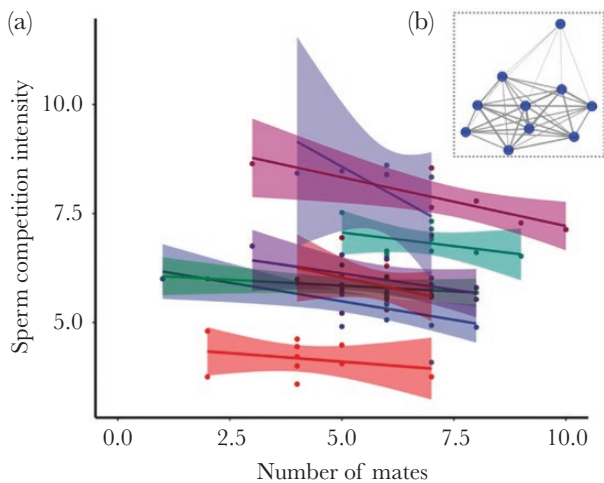

Figure 3
Relationship between mating success and sperm competition risk. (a) Overall, enclosures with higher levels of mating success had higher sperm competition intensity. However, within each of the eight replicates, males with the highest mating success experienced relatively lower levels of sperm competition, indicating negative mating assortativity between males and females. (b) An example network of intense indirect sperm competition interactions (gray lines) between males (blue nodes) in one replicate enclosure.

his mates. At the replicate population level, he extent of negative assortment tended to strengthen with mean levels of polyandry (Figure 4a), although this relationship was not statistically significant. This negative assortativity between individual male and female re-mating rates did not differ from that generated by chance (Randomization tests, $P > 0.05$ across all replicates, Supplementary Figure S3) and likely emerges as a property of the mating network rather than constituting a male or female preference (McDonald and Pizzari 2017).

Estimating variation in reproductive success

The relationship between individual male mating success and estimated offspring production (i.e., the estimated Bateman gradient)

was positive under both fair raffle (FR) and last male precedence (LMP) sperm usage scenarios (Table 1). This positive correlation is somewhat unsurprising given the estimation method used. However, LMP generated a significantly steeper estimated Bateman gradient than FR, as well as greater variance and skew in male reproductive success, with 33.8% of males siring no offspring in this situation (Mating partner: sperm usage scenario interaction, LRT, $X_2 = 6.199$, $P = 0.013$, Table 1, Figure 5). While incorporating a male's sperm competition index (SCI) alongside his mating success increased model fit under both FR and LMP scenarios, this effect was considerably more marked under LMP, in which it explained around 11% of variance in estimated offspring production (Table 1). Intriguingly, number of mates was also positively associated with reproductive success for females, explaining 25% of variance in offspring production (Table 1, Bateman gradient = 5.8, standardized $\beta = 0.19$). We then reran the analysis excluding females that were never observed mating during the experiment. While this reduced the Bateman gradient, a positive association between mating success and offspring production persisted (Bateman gradient = 3.21, standardized $\beta = 0.10$, Table 1).

Across replicate arenas, as with SCIC, we found considerable variation in estimated Bateman gradients under both sperm usage scenarios. Under FR, the association between replicate-level male mating success and estimated reproductive success ranged from highly positive (standardized $\beta = 0.54$) in some replicates through to slightly negative in one case. Under this scenario, the estimated Bateman gradient became qualitatively shallower as group level polyandry increases (LM, $F_{1,6} = 1.512$, $P = 0.26$), whereas under LMP there was no apparent relationship between the two (Figure 4).

DISCUSSION

As expected, we found that both male and female *A. tristis* re-mate at a high frequency under seminatural free-living conditions. Yet, the level of multiple mating events was even higher than expected—on average, 58% of all potential mating combinations were realized across replicate enclosures. As a result, we uncovered not only a dense mating network of direct interactions
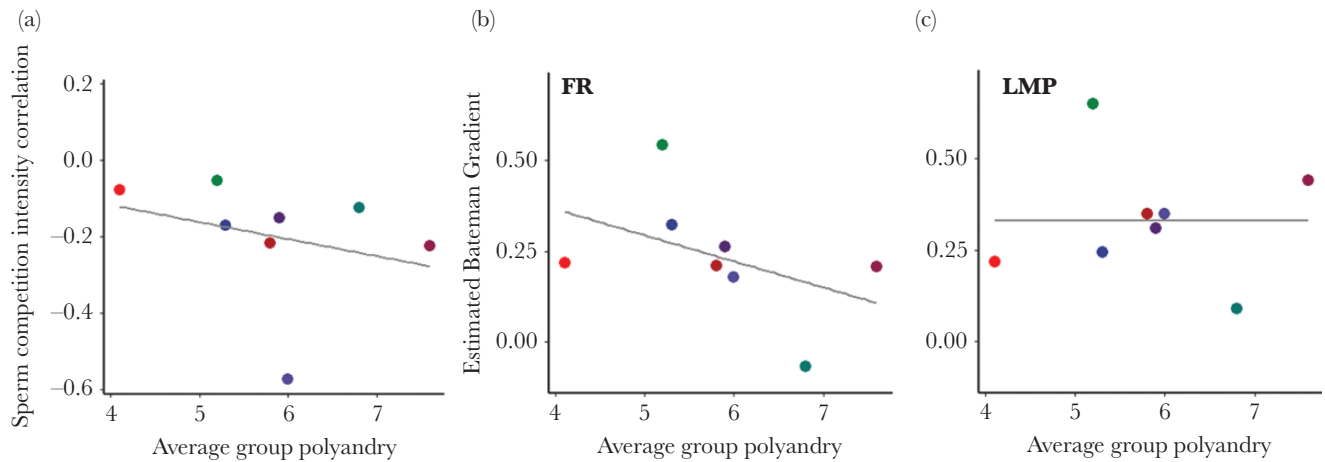
Figure 4

Relationship between average group levels of polyandry and across-trial variance in (a) sperm competition intensity correlations (b) estimated male Bateman Gradients under a fair raffle and (c) estimated male Bateman Gradients under last male precedence. Colors of each replicate group correspond with those in Figure 3a.

Table 1

Estimated male and actual female Bateman gradients. Gradients were obtained with a linear model and standardized following Arnold (1994). We obtained AICs, marginal R^2 values, estimates and P-values for terms using a generalized-mixed model fitted with a Poisson distribution (see Methods)

Sex	Sperm usage	Gradient	Standardized Gradient	SCI included in model?	AIC	Marginal R^2	GLMM Estimate		Z-score	P value
Male	Fair Raffle (FR)	7.35	0.22	No	1155.2	0.41	Mating success	0.17	16.30	<0.0001
				Yes	1114.6	0.43	Mating success	0.15	14.16	<0.0001
							SCI	−0.14	−6.73	<0.0001
	Last Male Precedence (LMP)	9.94	0.30	No	5151.4	0.18	Mating success	0.25	22.66	<0.0001
				Yes	4690.8	0.29	Mating success	0.18	15.43	<0.0001
							SCI	−0.42	−22.40	<0.0001
Female	All	5.15	0.19			0.25	Mating success	0.11	13.42	<0.0001
	Excluding unmated	3.21	0.10			0.06	Mating success	0.05	5.972	<0.0001

between males and females, but also indirect interactions between rival males, in the form of likely sperm competition within the reproductive tracts of shared mating partners. Taking a network-based approach revealed the presence of negative assortativity: the most polygynous males faced relatively lower levels of sperm competition than their less successful counterparts. This negative assortativity likely contributed to the maintenance of a positive relationship between male mating success and estimated reproductive success, even in the face of high rates of polyandry. The most successful males appeared to effectively "out-mate" their rivals and achieved higher estimated paternity rates under both sperm usage scenarios we explored.

Patterns of polyandry observed in this study challenge the traditional assumption that males exhibit greater variance in mating success than females (Kokko et al. 2012; Collet et al. 2014). Male and female mating rates in these closed experimental populations were intrinsically intertwined. High rates of female multiple mating generated an approximately normal distribution of male *A. tristis* mating success and equivalent variance in male and female mating rates. We found no evidence for precopulatory selection on body size in either sex, although we cannot rule out selection on unmeasured traits. Intriguingly, the only individuals that were never

observed mating during the observation period were females, rather than males as one would typically assume (but see Rhainds (2010)). While the scan sampling technique used restricted our ability to identify all mating pairs, females that were never observed mating also produced no offspring, providing some confidence that they indeed remained unmated over the course of the study. More broadly, this result also suggests sampling observations are likely representative of actual mating patterns. Furthermore, given that we were unable to attribute identities to all observed mating pairs, our estimates of mating frequency and sperm competition are, if anything, conservative and represent the lower bound of possible polyandry.

We found evidence for negative mating assortment; more polygynous males mated with on average less polyandrous females. This negative association indicates that polyandry has the potential to strengthen postcopulatory sexual selection in this species rather than relax it (McDonald et al. 2013; McDonald and Pizzari 2016). Importantly, while this negative relationship between male and female re-mating rates can arise through variation in male attractiveness and female choosiness, it can also emerge as a property of small mating networks, as was the case in our study (McDonald and Pizzari 2016). As the mating network of potential male-female mating combinations approaches saturation (as seen in *A. tristis*), the
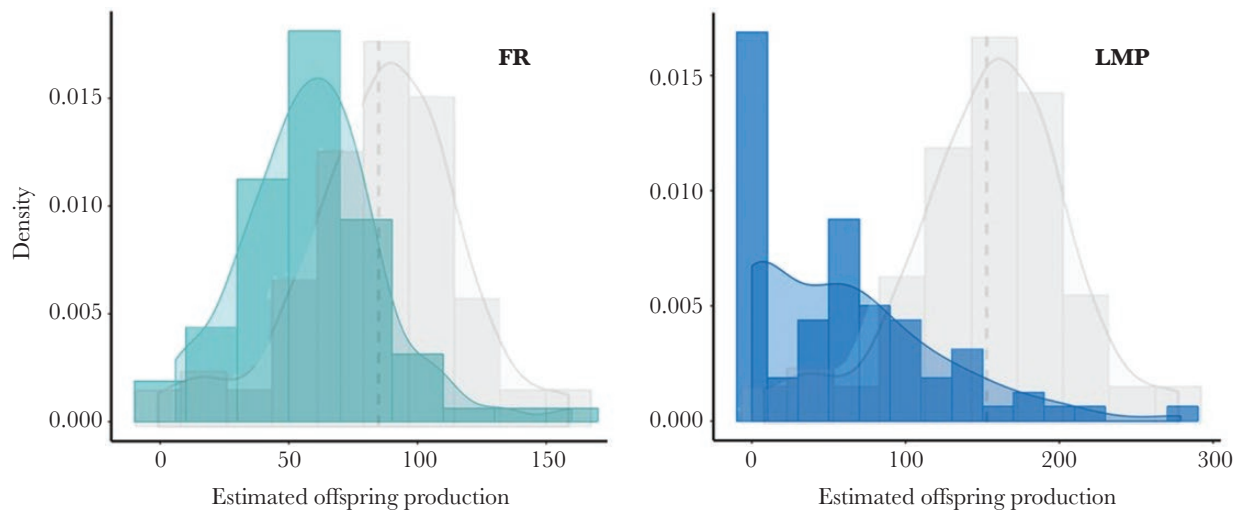
Figure 5
Variance in estimated male offspring production under Fair Raffle (left) and Last Male Precedence (right) sperm usage scenarios. Pale gray bars illustrate the shape of the observed distribution of male mating success for visualization purposes only.

likelihood of negative Sperm Competition Intensity Correlations increases, resulting in higher levels of sperm competition for less successful males (Morimoto et al. 2019). The fact that such an effect can arise by chance without active mate choice highlights the importance of considering local population structuring and movement. Wild *A. tristis* are found at locally high densities and have patchy distributions restricted by their host plants (Palumbo et al. 1991; Bonjour et al. 1993), and such mating network saturation is likely under natural conditions. Furthermore, despite the very high levels of multiple mating and apparent absence of precopulatory sexual selection observed, males did not distribute their mating efforts evenly across each of their mates. Instead, they skewed their mating efforts toward one or two females. This concentration of effort may constitute a mate guarding strategy in the face of sperm competition, as the more time a male spends in copula with a female, the less opportunity rivals have to inseminate her before she oviposits (Alcock 1994; Burdfield-Steel and Shuker 2014). This is likely to be an efficient postcopulatory competitive strategy in *A. tristis* as females tend to lay egg clutches every 1–2 days (Beard 1940).

By estimating paternity under two potential sperm usage scenarios, either paternity proportional to time spent mating (a shared raffle) or last male precedence, we found that the association between mating success, sperm competition intensity and predicted offspring production changed considerably. In particular, last male precedence increased the variance in estimated reproductive success by relegating 33.8% of males into becoming offspring-less (Figure 5). Under this scenario, we demonstrated that SCI plays a large role, explaining 11% of variance in predicted offspring production. On the other hand, under a fair raffle, the effects of SCI were less pronounced and variance in male reproductive success was roughly equal to male mating success plus additional variance introduced by differences in female fecundity, recapitulating Wade and Arnold's (1980) theoretical model. The extent to which these mating duration and order effects are considered postcopulatory components of sexual selection has been a topic of some debate, considering they are generally mediated by properties of the mating itself rather than sperm competitive ability or active cryptic female choice mechanisms (Rose et al. 2013; Pélissié et al.

2014; Evans and Garcia-Gonzalez 2016). However, the effects of these mating characteristics often largely outweigh any variation in sperm competition ability, for example, highlighting the importance of examining mating patterns of freely interacting individuals under naturally realistic encounter rates (Pélissié et al. 2014; Evans and Garcia-Gonzalez 2016; Marie-Orleach et al. 2016). Given that sperm usage patterns vary across individuals and populations, relatively small changes in these patterns can have potentially large knock-on effects on sexual selection and mating system evolution in polyandrous systems, as they ultimately determine variance in male reproductive success (Wade and Arnold 1980; Lewis and Austad 1990; Herberstein et al. 2011; Collet et al. 2014). Future paternity analysis is required to conclusively parse apart the contribution of sperm usage dynamics, sperm competitive traits, and cryptic female choice to realized paternity.

Interestingly, *A. tristis* females appear to benefit from multiple mating, with partner number explaining 25% of variation in female offspring production. Several potential mechanisms underly this positive association. Given the frequency of mating in this system, males may rapidly become sperm depleted and thereby constrain female reproductive output, as is the case in the related coreid *N. femorata* (Greenway et al. 2020). Indeed, female sperm limitation is a widespread phenomenon (Wedell et al. 2002) and has been posited as a driver of increased levels of polyandry as females continue re-mating to ensure access to adequate sperm stores to fertilize their eggs (Friesen et al. 2014; Bocedi and Reid 2016; Sutter et al. 2019). Exploring the role of sperm limitation further will be crucial to fully understanding the pay-offs of mating with multiple partners for both sexes. Mating multiply may also increase reproductive output via increased genetic compatibility or receipt of non-sperm seminal products (Zeh and Zeh 1997; Barbosa et al. 2012; Hopkins et al. 2017). An alternative explanation for the observed positive female Bateman gradient is that males directed more matings toward more fecund females. If this were the case, we might expect that larger females would produce more offspring (as is typical in insects (Honěk 1993)) and would also have higher mating success. However, no such association has been found (Supplementary Figure S2, Hamel et al. 2015, 2018). Larger females neither mated with more partners nor produced more offspring. As a result, from

these results alone we cannot determine the direction of causality between female fecundity and mating success (Collet et al. 2014). What is clear, however, is that male–female multiple mating dynamics and reproductive success are both complex and almost inextricably linked in this species.

In this study, we bridge the gap between laboratory and field studies examining the influence of polyandry on sexual selection by enabling individuals to freely interact over a prolonged time frame (Rodríguez-Muñoz et al. 2010; Fisher et al. 2016). This arena-based approach necessitates some loss of fine-scale behavioral resolution (e.g., mating duration information) accessible through laboratory studies. However, it provides invaluable gains in insight into the complex interplay between male and female mating rates and pre- and postcopulatory elements of sexual selection which are inaccessible under more controlled conditions. While high levels of polyandry have typically been assumed to de-couple mating and fertilization success, we found that strong positive Bateman gradients can persist, in the presence of considerable variance in female mating success and assortative mating patterns. Furthermore, under such high levels of sperm competition, sperm usage and sperm precedence dynamics play a key role in determining the distribution of male reproductive success and the subsequent strength of selection. Taken together, our findings add to the increasing weight of evidence that the capacity of polyandry to mediate the strength and direction of sexual selection in nature is both profound and nuanced.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Greenway et al. (2021).

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