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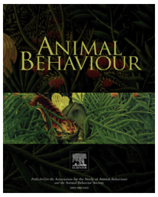

Operational sex ratio and density predict the potential for sexual selection in the broad-horned beetle

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Sexual selection can act on all aspects of the phenotype and the opportunity for selection (I_s) sets its maximal strength. Popular approaches to alter I_s include the manipulation of the operational sex ratio (OSR) and/or density, with an increase in I_s predicted with a male-biased OSR and at higher density. However, debate continues regarding the utility of I_s to measure meaningful changes in the strength of selection, as changes in I_s with OSR and density may only reflect stochastic processes. Here we tested whether the manipulation of OSR and density alters I_s in the broad-horned flour beetle, *Gnatocerus cornutus,* a species where males are under intense sexual selection and the targets of selection are known. We also recorded the average number of fights and mating behaviour of individuals in our competitive arenas. We found significant main effects of OSR and density on I_s, with the opportunity for selection being highest in male-biased high-density treatments. There were also significant effects of OSR and density on the average number of matings, whereas only density influenced the average number of fights. These results suggest that manipulation of OSR and density influence the opportunity for sexual selection in *G. cornutus* and our observations of fighting and mating behaviour provide a proximate mechanism for the change in I_s.

Sexually reproducing animals compete to fertilize the gametes of the opposite sex and this leads to variation in reproductive success that is known to favour extreme sexually selected ornaments and behaviours (Andersson, 1994; Crowley et al., 1991; Darwin, 1859; Eshel, 1979). Competition for mates is critically dependent on the relative rate of production of gametes in males and females and, therefore, the availability of sexually receptive mates (Clutton-Brock & Parker, 1992; Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 1996; Wade & Arnold, 1980), as well as aspects of the mating system that are often set by ecological factors (Emlen & Oring, 1977; Shuster & Wade, 2003). Typically, the sex that invests least in gametes or parental care should have greater variation in reproductive success, compete most strongly for mates and be under strong sexual selection (Emlen & Oring, 1977; Janicke, Häderer, Lajeunesse, & Anthes, 2016; Kvarnemo & Ahnesjö, 1996; Shuster & Wade, 2003; Weir, Grant, & Hutchings, 2011). To quantify how strong sexual selection may be, behavioural and evolutionary biologists have commonly quantified the opportunity for sexual selection (I_s), which measures the upper limits of sexual selection (Croshaw, 2010; Jennions, Kokko, & Klug, 2012; Klug, Heuschele, Jennions, & Kokko, 2010; Krakauer, Webster, Duval, Jones, & Shuster, 2011).

Theoretically, the opportunity for sexual selection should covary with the operational sex ratio (OSR, the ratio of sexually receptive males to sexually receptive females: Emlen & Oring, 1977; but see Jennions et al., 2012; Klug et al., 2010). In principle, OSR bias is expected to change the degree of competition and mate monopolization and therefore increase the strength of sexual selection on the sex that competes for mates (Emlen & Oring, 1977; Klug et al., 2010; Kvarnemo & Ahnesjö, 1996; Shuster & Wade, 2003; Wade & Arnold, 1980). For instance, populations with a male-biased OSR should show greater male–male competition and greater expression of sexually selected traits, or the opposite in sex-role-reversed systems, and some empirical studies confirm this (Aronsen, Berglund, Mobley, Ratikainen, & Rosenqvist, 2013; reviewed in ; Janicke & Morrow, 2018; Kvarnemo & Ahnesjö, 1996; Mills, Grapputo, Koskela, & Mappes, 2007; Wacker et al., 2013; Weir et al., 2011; but see ; de Jong, Wacker, Amundsen, & Forsgren, 2009; Fitze & Le Galliard, 2008; Hayes, Callander, Booksmythe, & Jennions, 2016; Head, Lindholm, & Brooks, 2008). As a result, manipulation of the OSR is frequently used in experimental

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evolution studies to relax or elevate the intensity of intrasexual competition and sexual selection. In general, the evolved response of male behavioural and physiological traits is consistent with the expectation that male-biased OSR increases the opportunity for sexual selection (I_S) compared to female-biased lines (reviewed in Edward, Fricke, & Chapman, 2010; Michalczyk et al., 2011). However, data from some experimental evolution studies show that population divergence is not always predictable (e.g. Chechi, Syed, & Prasad, 2017; Linklater, Wertheim, Wigby, & Chapman, 2007; Reuter et al., 2007). For example, in theory males should invest more in testes and ejaculate traits when the risk of sperm competition is high (Simmons, 2001). Yet, in *Drosophila melanogaster* that were derived from experimental evolution male-biased lines (3:1 male:female), males did not invest more in their first mating (Linklater et al., 2007) and they did not differ in either testis or accessory gland size (Chechi et al., 2017). Furthermore, in extremely female-biased lines (10:1 female:male) where there is no risk of sperm competition, evolved males had larger testes than males from less biased lines (4:1 female:male and 1:1 female:male) which may be a response to sperm depletion (Reuter et al., 2007).

Variation in density is also predicted to influence the strength of sexual selection by altering the intensity of competition for mates and the availability of mating partners for a choosing individual (Eshel, 1979). Theoretically, an increase in population density should increase the contact rate of competitors and potential mating partners (Emlen & Oring, 1977; Knell, 2009; Kokko & Rankin, 2006). This may increase intrasexual competition and mate success. Alternatively, intense intrasexual competition and mating may trade off if competition reduces the total amount of time available (Emlen & Oring, 1977; Knell, 2009; Kokko & Rankin, 2006). There is evidence that greater interference from other males and male–male competition to monopolize females favour more pronounced weaponry at high density in arthropods (e.g. in the pseudoscorpion *Dinocheirus arizonensis*: Zeh, 1987; in dung beetles, Onthophagini sp.: Pomfret & Knell, 2008; and in the European earwig, *Forficula auricularia*: Tomkins & Brown, 2004). However, an effect of density has not been found in two species of fish (guppies, *Poecilia reticulata*: Head et al., 2008; two-spotted gobies, *Gobiusculus flavescens*: Wacker et al., 2013) and the intensity of mate monopolization has been found to break down at high density as the defence of resources (i.e. territory or mates) can become increasingly difficult (Kokko & Rankin, 2006). For instance, soldier beetles, *Chauliognathus pennsylvanicus* (McLain, 1982), fungus beetles, *Bolitotherus cornutus* (Conner, 1989), guppies (Jirotkul, 1999) and bitterling, *Rhodeus sericeus* (Reichard, Jurajda, & Smith, 2004) all appear unable to maintain mate monopolization at high density. Density has also been used to manipulate I_S in experimentally evolving populations of the Indian meal moth, *Plodia interpunctella* (Gage, 1995) and the dung fly *Sepsis cynipsea* (Martin & Hosken, 2003). In each of these systems, morphological and behavioural responses of males and females to density show that high density increases the competitiveness of the social environment. In dung flies, the effects of sexual conflict on experimentally evolving lines was tested in replicate high- and low-density lines. High density increased the frequency of encounters, female resistance to mating and persistent mating attempts by males, as predicted in systems that evolve via sexual conflict (Martin & Hosken, 2003). In meal moths, males reared at high density invested more resources in traits related to sperm production, as predicted when mate encounter rate and sperm competition risk is high (Gage, 1995).

Despite the extensive use of OSR and density to manipulate I_S, the legitimacy of I_S to measure the potential for sexual selection has been debated for several decades (Croshaw, 2010; Downhower et al., 1987; Fairbairn & Wilby, 2001; Jennions et al., 2012; Klug et al., 2010; Krakauer et al., 2011; Sutherland, 1985). One of the limitations of I_S is that it does not account for stochastic variation in mating success (number of mates) or reproductive success (number of progeny) that is uncorrelated with the competitor's phenotype (Croshaw, 2010; Klug et al., 2010; Sutherland, 1985). For instance, if OSR becomes more male-biased and the addition of more competitors in the system decreases male monopolization of mates, variance in mating or reproductive success may only reflect chance. Consequently, changes in the measured opportunity for selection may not reflect real changes in the strength of selection (Croshaw, 2010; Klug et al., 2010; Sutherland, 1985). However, Klug et al. (2010) demonstrated that there is at least one condition where OSR, density and I_S are correlated with selection, which is illustrated by the example of a population of three males and three females becoming a population of four males and three females. In this theoretical scenario, an increase in density and a shift in OSR from unbiased to male-biased are associated with an increase in I_S if a single male with the most highly developed sexually selected traits completely monopolizes all mates and has high mating success but most of the other males remain unmated (Klug et al., 2010). Whether this theoretical outcome occurs in real-life scenarios and whether a change in I_S is driven by random or nonrandom processes requires knowledge of the biological mechanisms involved (Klug et al., 2010). Therefore, we used an experimental design that is similar to the one used by Klug et al. (2010; and outlined above) and evaluated whether I_S responds to a change in OSR and density in the broad-horned flour beetle, *Gnatocerus cornutus*, and whether that effect can be explained by male monopolization behaviour.

Male *G. cornutus* have mandibles that they use during fights to guard resources and monopolize females (Harano, Okada, Nakayama, Miyatake, & Hosken, 2010; Okada, Katsuki, Sharma, House, & Hosken, 2014; Okada, Miyanoshita, & Miyatake, 2006; Okada & Miyatake, 2009; Yamane, Okada, Nakayama, & Miyatake, 2010). During fights, males interlock their mandibles, shove, bite and even lift their opponent (Okada et al., 2006) and the male with the most developed mandibles is usually the winner in combat (Lane, Dickinson, Tregenza, & House, n.d.). Following a fight, the loser retreats and avoids aggressive interactions, with the losing experience persisting for up to 4 days (Okada & Miyatake, 2009). Males also exhibit same-sex mounting behaviour which appears to function as a form of passive aggression (Lane, Haughan, Evans, Tregenza, & House, 2016). Furthermore, males that lose fights or are mounted during same-sex behaviour are less likely to court females and have lower mating success (Lane, Dickinson, Tregenza, & House, 2016).

Since dominant male *G. cornutus* have strategies to dominate competitors and monopolize mates (Harano et al., 2010; Okada et al., 2006, 2014; Okada & Miyatake, 2009; Yamane et al., 2010) this seems an ideal system to evaluate the predicted relationship between OSR, density and I_S (Klug et al., 2010, Case 3). In this study, males in our male-biased high-density treatment had greater contact with competitors and were predicted to fight more to establish dominance so that a single male monopolizes all females. The average number of multiple matings in this treatment was predicted to be low as male–male aggression prevents competitors from mating or may reduce the time available for mating. The overall effect was predicted to decrease average mate success and increase the variance in mating success in this treatment, so that I_S would be highest in this treatment compared to all others.

METHODS

Study System

Stock populations of *G. cornutus* were derived from the Japanese National Food Research Institute (NFRI) where they were established more than 50 years ago (see Okada et al., 2006). In our laboratory, mixed-sex subpopulations (50 female and 50 male per subpopulation, $N = 9$) were maintained in pots (Thermoscientific Nalgene 500 ml, 120 mm outside diameter) that contained wholemeal wheat flour (Doves Farm Foods, Hungerford, U.K.) enriched with 5% brewer's yeast (ACROS Organics, Loughborough, U.K.). Rearing temperatures, humidity and photoperiod were set at 27 °C, 60% humidity and a 14:10 h light:dark regime (Okada et al., 2006). Every 4 weeks, final-instar larvae were removed from the subpopulation pots and placed in a large tray so that larvae from different subpopulations mixed randomly. A random subset of these larvae were placed into the individual wells of 24-well plates ($N = 38$) as pupation is hindered at moderate to high larval density (Tsuda & Yoshida, 1985). Pupation occurs in about 1 week and pupae eclose soon after (i.e. about 4–5 days). These beetles were randomly selected to serve as the parents of the next generation.

For this experiment, 144 final-instar larvae were collected daily from the subpopulations and placed in 24-well plates. These larvae were checked daily and eclosed beetles were placed into individual cells of unisex 24-well plates and provided with about 250 mg of wholemeal wheat flour per well. This protocol provided a supply of experimental, virgin beetles that were used during our study when they were 11–15 days of age.

Experimental Design

We manipulated OSR and density in competitive arenas (petri dishes; 70 mm in diameter and 20 mm high) as follows. Our low-density (L) treatment consisted of four individuals and our high-density (H) treatment consisted of eight individuals. Within each density treatment, we manipulated the sex ratio to be 1:1 female:male (equal, E) or 1:3 female:male (male-biased, MB; Table 1). This created four treatments established in a factorial design: L-E, L-MB, H-E and H-MB (Table 1).

On the morning of the behaviour trials, all beetles were marked on the pronotum with gel pen (Pentel Hybrid Gel Grip DX Metallic, Lane et al., 2016; green, blue, red, pink, bronze or gold) for easy identification. Males are easily distinguished from females, so the sexes were occasionally marked with the same colour but within a sex the colour marking was unique so that the identity of each male, his opponent(s) and the novel mates per male were known. Marked beetles were returned to an individual cell of a 24-well plate to recover until the afternoon. Pilot studies in the laboratory indicate that beetles resume normal fighting and mating about 2 h after application of the gel (Lane, Haughan et al., 2016). Therefore, the competitive arenas were observed during late photophase for 2 h. Our study was unbalanced as some replicates exhibited no behaviour and were removed from the analysis (L-E, $N = 37$; L-MB, $N = 36$; H-E, $N = 36$; H-MB, $N = 39$).

Behavioural Observations

The competitive arenas were lined with filter paper for traction and females were introduced into their respective competitive arenas followed by the males. During the next 2 h, fights were observed and the identity of the aggressor was recorded. The male that first pushed, bit or held another beetle with his mandibles was classified as the ‘aggressor’. We also observed mating and recorded the identity of each mating partner. Thus, at the completion of each observation period we had a tally of the number of fights (i.e. fights with the same or different males) that were initiated by the male aggressor, a tally of the number of mating partners per male and the total number of matings per male (i.e. mating with the same or different females).

Table 1
The treatment and numbers of females and males in each competitive arena

Density	OSR skew	
	Even	Male-biased
High	4♀:4♂	2♀:6♂
Low	2♀:2♂	1♀:3♂

Estimating the Opportunity for Sexual Selection

We used the above behavioural data to quantify whether one male had a mating partner monopoly (100% monopoly) or whether mating partners were shared between two males (50% shared mating success), three males (33.33%) or four males (25%: there were never more than four males acquiring a mating partner(s)) in each replicate. We also calculated the average number of fights and matings in each replicate of a treatment. Next, we calculated the average number of mates (i.e. mating success, M) as:

$$M = \left(\sum k_i\, m_i\right) \Big/ \sum m_i \qquad [1]$$

where k is the potential number of female mates in the ith class and m is the number of males in the ith class (Shuster & Wade, 2003). The number of ‘classes’ is limited by the number of females. For instance, if there are a total of six females there can be seven mating classes: the first mating class includes zero female mates as some males do not mate at all, the next class is ‘1’ (i.e. one mate partner) and so on. For example, in an H-E replicate, if one male mates with three females and all other males remain unmated, $M = ((0 \times 3) + (1 \times 0) + (2 \times 0) + (3 \times 1) + (4 \times 0))/4$.

We then calculated the variance in mating success (V_M):

$$V_M = \left[\left(\sum k_i^2 m_i\right) \Big/ \sum m_i\right] - \left[\left(\sum k_i m_i\right) \Big/ \sum m_i\right]^2 \qquad [2]$$

Finally, we calculated the opportunity for sexual selection (I_s):

$$I_s = V_M \big/ M^2 \qquad [3]$$

where V_M is the variance in mating success (estimated in Eq. (2)) and M is average mating success estimated in Eq. (1) (Wade, 1979; Wade & Arnold, 1980).

Statistical Analyses

We conducted four separate generalized mixed models using Bayesian inferences implemented by the MCMCGLMM package (v.3.5.1; Hadfield, 2010) and Lattice package (v.3.5.1; Deepayan, 2008) to test for an effect of OSR and density on monopolization, I_s, average number of fights and matings (version 3.5.1, R Core Team, 2018). We included OSR, density and the interaction as fixed effects and Petri dish identity as a random effect with a level for every observation. We ran chains for 30 000 iterations with a burn-in of 100 and a thinning interval of 50. In each model, we used a relatively uninformative prior ($\nu = 0.02$ for both the fixed and random effects), as we had very little a priori information about the expected parameter estimates.

RESULTS

Monopolization

There was a main effect of density ($P_{MCMC} = 0.013$) and OSR ($P_{MCMC} = 0.013$) for male monopolization but the interaction was not significant ($P_{MCMC} = 0.368$). We found that males were more likely to monopolize female mating partners in our low-density and male-biased OSR treatments (Table 2). Average mate monopolization was 51% in the high-density equal treatment (H-E), 70% in the low-density equal treatment (L-E), 69% in the high-density male-biased treatment (H-MB) and 79% in the low-density male-biased treatment (L-MB). A limitation of this approach is that in some replicates a male had a complete monopoly but only a single mating partner. In other cases, a male had a complete monopoly but several mating partners but our estimation of monopolization did not account for this. Therefore, we investigated this pattern further with our estimate of I_s which integrates not only whether a male had a monopoly but also the number of mating partners.

The Opportunity for Sexual Selection

There was a main effect of OSR ($P_{MCMC} < 0.002$), density ($P_{MCMC} < 0.002$) and a significant interaction between OSR and density ($P_{MCMC} = 0.017$). I_s was likely to be lower in our low-density treatments and higher in our male-biased OSR treatments (Fig. 1a). The density $*$ OSR interaction is shown in Fig. 1a, where a relatively large reduction in I_s was found in our low-density male-biased treatment.

Fighting and Mating Behaviour

There was a main effect of density ($P_{MCMC} = 0.013$) for the average number of fights but the main effect of OSR ($P_{MCMC} = 0.575$) and the interaction between these main effects was not significant ($P_{MCMC} = 0.712$). The average number of fights was likely to be lower in the low-density treatments (Fig. 1b).

There was an effect of OSR ($P_{MCMC} < 0.002$), density ($P_{MCMC} < 0.002$) and an interaction between OSR and density ($P_{MCMC} = 0.033$) for the average number of matings. The average number of matings was likely to be higher for low-density treatments and lower for male-biased OSR treatments. The density $*$ OSR interaction is shown in Fig. 1c, where a relatively large reduction in the average number of matings was found in our low-density male-biased treatment (Fig. 1c).

DISCUSSION

The OSR has been used extensively in field and laboratory studies to predict which sex will compete more strongly for mates (Emlen & Oring, 1977; Katsuki, Harano, Miyatake, Okada, & Hosken, 2012). Population density may also influence individual mating strategies (Kokko & Rankin, 2006) and, therefore, the opportunity for sexual selection (I_s). However, the legitimacy of I_s continues to be debated as the metric does not account for stochastic events that may bias estimates of I_s and be uncorrelated with changes in selection (Croshaw, 2010; Klug et al., 2010; Moura & Peixoto, 2013; Sutherland, 1985). Nevertheless, in systems where the degree of mate monopolization is high, it has been argued that estimates of I_s should reflect meaningful changes in selection (Klug et al., 2010; but see Krakauer et al., 2011). In the flour beetle *G. cornutus* we found that the manipulation of both OSR and density influenced the degree of male monopolization of mating partners. We found that a monopoly occurred more often in male-biased treatments (around 70–80% of the time). However, in contrast to our prediction, monopolization was more likely in the low-density treatments. This pattern suggests that monopolization is more likely when there are fewer females to monopolize (i.e. one or two) although we cannot know for sure. When we considered not only the degree of monopolization but also the number of mating partners we showed that the combined effect of OSR and density increased the opportunity for sexual selection, with I_s being highest in our male-biased OSR and high-density treatment. In our male-biased treatment, I_s was expected to increase because of greater competition for a few females, lower mean mating success and greater variance in mating success, although we cannot completely rule out the influence of stochastic events (Klug et al., 2010). The results of previous studies (Harano et al., 2010; Okada et al., 2006, 2014; Okada & Miyatake, 2010; Yamane et al., 2010) suggest that changes in I_s are likely to be associated with male sexually selected morphological and behavioural traits that contribute to the degree to which mate monopolization occurs (Lane, Dickinson et al., 2016; Lane, Haughan et al., 2016; Okada et al., 2006; Okada & Miyatake, 2010). In this study, we observed that one or two males were often more aggressive and tended to dominate aggressive interactions and potential mates, particularly in male-biased OSR treatments. Therefore, we propose that the differences in I_s that we found in our treatments reflect ‘real’ changes in selection, not just random processes. However, mating success is not the only contributor to reproductive success and, therefore, more work is required to show that male monopolization during mating success persists when postcopulatory components of sexual selection are incorporated into estimates of I_s (Fitze & Le Galliard, 2008; Henshaw, Kahn, & Fritzsche, 2016; Krakauer, 2011).

The increase in I_s with male-biased OSR and high density that we documented is consistent with empirical studies in seahorses, *Hippocampus subelongatus* (OSR: Kvarnemo, Moore, & Jones, 2007), dung beetles (OSR and density: Pomfret & Knell, 2008) and milkweed longhorn beetles, *Tetraopes tetrophthalmus* (density: McLain & Boromisa, 1987). However, studies in guppies (OSR and density: Head et al., 2008), two-spotted gobies (OSR and density: de Jong et al., 2009) and common lizards, *Zootoca vivipara* (Fitze & Le Galliard, 2008) failed to show similar effects on I_s. In these mating systems, other factors such as sex-specific mortality (which is associated with the cost of mate searching and breeding), encounter rates and variation in mate quality are likely to influence choosiness and sex roles (Kokko & Johnstone, 2002; Kokko & Monaghan, 2001). In contrast to these systems, male *G. cornutus* with large mandibles are the most competitive and are more likely to mate under competitive conditions, although they are not necessarily preferred by females (Harano et al., 2010; Okada et al., 2014; Yamane et al., 2010). Although females do not prefer competitive males, the direct benefits of mating (i.e. lifetime reproductive success) to an attractive male versus a competitive

Table 2

The mean of the posterior values (with 95% credible interval) of the main effect of low-density male-biased OSR and low density $*$ male-biased OSR for each response variable; monopolization, I_s, average numbers of fights and average number of matings

	Low density	Male-biased OSR	Low density*male-biased OSR
Monopolization	**17.662 (4.453 to 30.194)***	**16.884 (4.751 to 27.848)***	−7.484 (−25.010 to 9.361)
I_s	**−1.038 (−1.509 to −0.488)****	**1.853 (1.267 to 2.364)****	**−0.862 (−1.609 to −0.131)***
Average number of fights	**−0.986 (−1.791 to −0.246)****	0.221 (−0.701 to 0.916)	−0.196 (−1.174 to 1.009)
Average number of matings	**2.013 (1.267 to 2.637)****	**−1.099 (−1.734 to −0.408)****	**−1.0382 (−1.9807 to −0.1448)***

Significant values are in bold.

$^{*}P < 0.01$; $^{**}P < 0.001$.

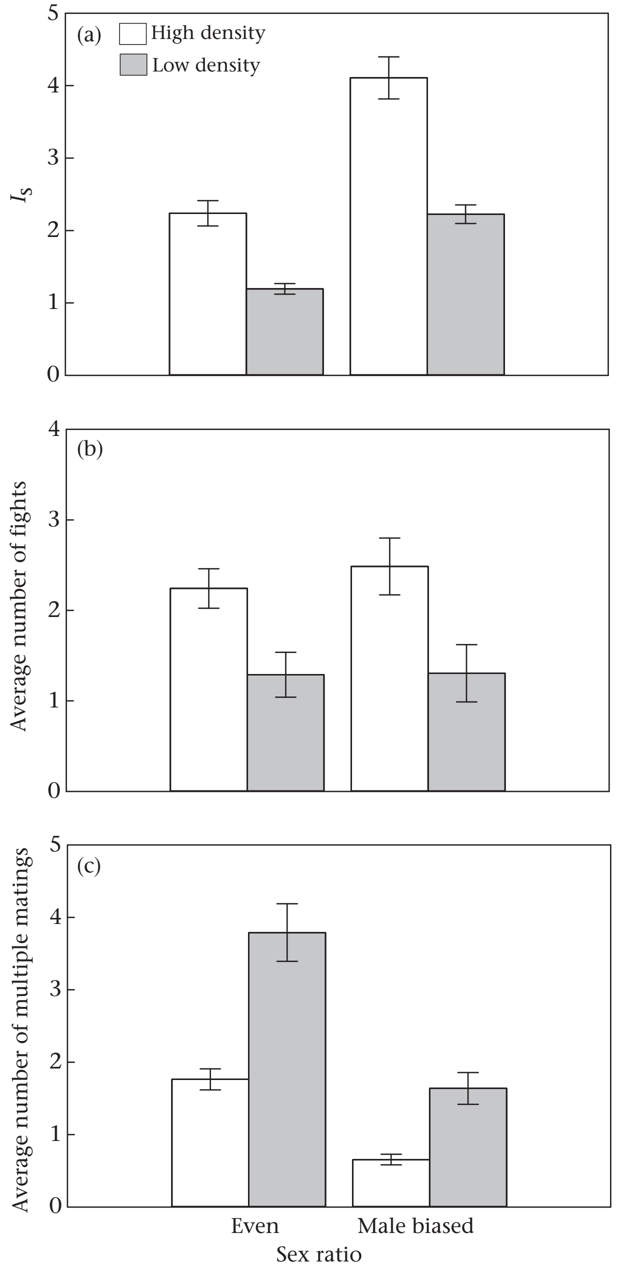

Figure 1. Mean (SE) effect of OSR (even, male-biased) and density (high, low) on (a) I_s, (b) the number of fights and (c) the number of multiple matings.

male are probably equivalent (Okada et al., 2014; also see Katsuki Harano, Miyatake, Okada, & Hosken, 2012). The cost of reproduction is low and multiple mating improves fecundity and lifetime reproductive success (Okada et al., 2015); this may lower female choosiness in male-biased populations and decrease the intensity of sexual selection in females. While we are unable to test this idea directly with our data, it does seem likely that male–male competition is of greater importance for sexual selection than the direct cost of mate choice (Okada et al., 2014) and reproduction (Okada et al., 2015) in the mating system of *G. cornutus*.

A possible explanation for why intrasexual competition increases with a male-biased OSR and increased density is that encounters with other males are more probable and, therefore, an act of aggression is a likely outcome. Increased intrasexual competition with male-biased OSR and at higher density is often seen (Pomfret & Knell, 2008; Tomkins & Brown, 2004; Zeh, 1987; but see ; Conner, 1989; Jirotkul, 1999; McLain, 1982; Reichard et al., 2004). However, the effect that this has on male mating success is more varied. In some mating systems, an increase in male density can result in an increase in male–male competition, greater male insistence to mate and an increase in mean mating success (Arnqvist, 1992; cited in Laurer, Sih, & Krupa, 1996). For example, in the water strider, *Aquarius remiges*, an increase in male density produced a decrease in female resistance and an increase in mean male mating success (Laurer et al., 1996). Conventionally, however, it is predicted that a male-biased OSR and/or higher density should be coupled with stronger male–male competition and decreased mating success due to time constraints or because males have less energy to invest in courtship (Andersson, 1994; Weir et al., 2011). This prediction is consistent with our results, which show that males always fought but especially when the density was high and this was associated with a decline in the number of matings, particularly in male-biased populations. However, we were unable to determine whether the decreased mating we observed was due to time constraints or lower encounter rates with females (or both). Importantly, we know that there are short-term fitness consequences of intrasexual competition in *G. cornutus*. Winning males are more likely to monopolize females as losing males disperse from a fight site and are less likely to engage in fights with other competitors (Okada & Miyatake, 2010). Furthermore, loser males are less likely to court a female and therefore have lower mating success (Lane, Haughan et al., 2016).

Researchers have used I_s extensively to predict the maximum potential for premating sexual selection to occur (Shuster & Wade, 2003; reviewed: Klug et al., 2010; Krakauer et al., 2011). Although our results support the view that OSR and density influence I_s, several authors have cautioned that I_s may correlate poorly with the strength of selection targeting individual traits associated with mating success in many mating systems (Henshaw et al., 2016; Klug et al., 2010). For example, Henshaw et al. (2016) showed that the accuracy of I_s to predict short-term linear selection differentials on mating traits was weak compared to six other proxies tested. However, I_s was shown to always be higher for males ($r_2 = 0.70$) than females ($r_2 = 0.08$), although this sex difference was highly dependent on the type of mating system being examined (Henshaw et al., 2016). Specifically, I_s accurately tracks the realized strength of sexual selection in systems with promiscuous mating, where male–male competition is strong and females exclusively invest in offspring, as characterized in species such as red deer, *Cervus elaphus*, and American red squirrels, *Tamiasciurus hudsonicus* (Henshaw et al., 2016). The mating system of *G. cornutus* appears to be similar to the red deer and American red squirrel mating systems where the accuracy of I_s in predicting selection on premating traits is good (Henshaw et al., 2016). *Gnatocerus cornutus* is highly promiscuous, males are competitive (although they do not control a harem), sperm competition risk is high and only females invest in offspring. It is, therefore, likely that I_s will reliably predict the potential for premating sexual selection in this species. Furthermore, we already know that there is strong sexual selection acting on some individual male traits (i.e. mandible size, body size and cuticular hydrocarbons; Lane, Dickinson et al., 2016) during contests between two males. However, it is currently unknown how these previous estimates of selection on individual traits contribute to the variation in I_s we observe in the different sociosexual environments (OSR and density). Ideally, we would establish replicate experimental evolution populations of *G. cornutus* that manipulate OSR and density to quantify the evolved response of individual sexually selected traits when I_s is elevated or relaxed.

In conclusion, OSR and density manipulations seem to alter the opportunity for selection in flour beetles in the manner expected.

Nevertheless, cautions about the general utility of these manipulations remain (e.g. Jennions et al., 2012).

Author contributions

C.M.H. conceived the study and C.M.H., D.H. and J.H. designed it. C.M.H. and J.R. reared the beetles and conducted the behavioural assays. C.M.H. and J.R. analysed the data. C.M.H. wrote the manuscript and all authors commented on drafts of it.

Declaration of interest

We have no competing interests.

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