Sexual antagonism for testosterone maintains multiple mating behaviour

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Summary

1. The persistence of multiple mating remains one of the fundamental questions in evolutionary biology. In theory, multiple mating is predicted to improve female fitness cumulatively through direct and/or genetic benefits. However, intra-locus sexual conflicts may potentially constrain or even eliminate these benefits owing to the gender load imposed by sexually antagonistic selection.
2. Here, we tested whether sexually antagonistic selection can maintain the variance in multiple mating behaviour of bank voles (*Myodes glareolus*) by manipulating the hormone testosterone through artificial selection in the laboratory. Among mammals, testosterone is a sexually dimorphic fitness-related trait under selection and is known to affect mating behaviour. We conducted mating trials in which females derived from family-based selection of testosterone were sequentially paired with four males of different testosterone profiles.
3. We show that artificial selection for high testosterone increased the mating rate of males, but clearly decreased the number of partners that females mated with (and *vice versa*). Because multiple mating was beneficial for the reproductive success of both sexes, as evidenced by the positive Bateman gradients, the divergent evolutionary interests of testosterone between the sexes can maintain this polygynandrous mating system.
4. Our results highlight how mating rate is concordantly selected in both sexes; however, it is largely influenced by testosterone, which is under sexually antagonistic selection.
5. This study is the first one to emphasise the direct and indirect effects of the endocrine system not only on reproductive physiology and behaviour but also for the evolution of genetic mating strategies in mammals.

Key-words: bank vole, life history evolution, mating behaviour, mating system, sexual conflict, sexually antagonistic selection

Introduction

Sexual conflicts (Parker 1979) are considered an important force in the evolution of sexual dimorphism and co-evolutionary arms races between the sexes for optimal fitness strategies (Arnqvist & Rowe 2005). Intra-locus conflict, in which there is a conflict between the same set of genes in males and females, results from sexually antagonistic selection on sex-specific trait optima (Bonduriansky & Chenoweth 2009; van Doorn 2009). If the evolution of sexual dimorphism fails to resolve the antagonism, it manifests itself in a gender load, in which the realised fitness of either sex is appreciably less than the fitness optima for the trait(s) in question (Bedhomme & Chippindale 2007). Many traits associated with reproduction have been

identified as being sexually antagonistic, because of the divergent evolutionary interests between males and females (see Arnqvist & Rowe 2005 and references therein).

Previous studies have indicated that (inter-locus) sexual conflict over multiple mating is expected to be absent or minimal in species that are genetically monogamous, given the shared interest in common offspring, but could escalate with increasing promiscuity (Holland & Rice 1999; Hosken, Garner & Ward 2001; Stewart, Morrow & Rice 2005). Attempts at quantifying the level of promiscuity in avian and mammalian species indicate that true genetic monogamy is likely to be extremely rare (Kleiman 1977; Griffith, Owens & Thuman 2002). The mating rate is typically divergent between the sexes because it is predicted to have a higher variance and stronger association to fitness in males than females, which is expected to render males more mate-limited and females more selective, on average (Bateman 1948). However, more

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recent evidence from sex-role-reversed species and polyandrous mating systems has begun to challenge the application of these tenets (Ågren 1990; Jones *et al.* 2000). The widespread prevalence of polygamous mating systems raises an interesting possibility that the fitness benefits associated with multiple mating for one or both sexes outweigh the costs of the gender load imposed by the mating system.

Considerable research has focused on the benefits and costs of mating as well as mating with multiple partners (Daly 1978; Tregenza & Wedell 1998; Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Fedorka & Mousseau 2002; Gowaty *et al.* 2010). Whereas multiple mating in males is predicted to increase their reproductive success, females that mate multiply can also gain direct benefits (e.g. nuptial gifts, increased ovulation, improved fertilisation success, Fedorka & Mousseau 2002; the avoidance of physical harm/infanticide, Poikonen *et al.* 2008), as well as indirect genetic benefits (e.g. good genes, greater genetic diversity for their offspring and increased genetic compatibility, Jennions & Petrie 2000; Fedorka & Mousseau 2002). On the other hand, the co-evolution of male harm and female resistance may potentially help shape the mating rate in species where males exact appreciable harm on their mates during reproduction (Kokko 2005; Gay *et al.* 2011). Furthermore, if a female mates with multiple males, there is a possibility that a given mate's share of offspring paternity decreases or is eliminated altogether (Stockley 1997). Hence, there can be benefits as well as costs associated with multiple mating for both sexes.

Our study aims to examine the evolutionary costs and benefits behind female multiple mating and to determine whether sexual conflict over multiple mating behaviour increases with female promiscuity. We tested our aims by artificially selecting high- and low-testosterone bank voles (*Myodes glareolus*) in the laboratory (Mills *et al.* 2009). This hormone has been shown to affect sexual behaviour in a variety of mammals (Park & Rissman 2011). The reproductive success of male bank voles is affected by testosterone through its effects on mobility, mate searching, social status and number of mates (Mills *et al.* 2007a,b, 2009). The expression and effects of testosterone on mammalian females are much less known, but previous work in other mammals indicates that there may be a positive fitness correlation between the sexes in the selection of testosterone (Ketterson, Nolan & Sandell 2005 and references therein). Male bank voles with high-testosterone levels mate with multiple females; however, it is not known how-/whether this fitness advantage extends to genetically related females. Therefore, we also aimed to determine whether females related to males with high-testosterone levels also mate with multiple males.

Materials and methods

STUDY SPECIES

Bank voles (*Myodes glareolus*) operate in a non-resource-based mating system, where males do not provide any parental care for their offspring or resources for their mates. Breeding females are territorial, while the home ranges of males overlap several of these territories; hence, there are frequent opportunities for multiple mating by both sexes (Bondrup-Nielsen & Karlsson 1985; Koskela, Mappes & Ylönen 1997). Our laboratory population was kept at 22 °C on a 16:8 light/dark photoperiod and provided food and water *ad libitum* when not engaged in a trial. Individuals were kept in cages measuring $43 \times 26 \times 15$ cm that contained wood chips and autoclaved hay. The use of these animals adhered to ethical guidelines for animal research in Finland.

ARTIFICIAL SELECTION FOR TESTOSTERONE

One hundred male and 100 female bank voles were live-trapped from Konnevesi, Finland (62°37′N, 26°20′E). Seventy-five microlitres intra-orbital blood samples were taken from males (see Oksanen *et al.* 2003 for detailed procedure). The resulting plasma was analysed for testosterone using the radioimmunoassay technique (see methods in Mills *et al.* 2007b). Males were evaluated as low (<4 ng mL^{-1} plasma) and high (>7 ng mL^{-1} plasma) for plasma testosterone. They were placed in two testosterone groups, low and high, with their sisters. Matings were then carried out between non-relatives to create four testosterone groups: HH (high-testosterone sire and dam), HL (high-testosterone sire, low-testosterone dam), LH (low-testosterone sire, high-testosterone dam) and LL (low-testosterone sire and dam). Males and females were used until they produced offspring. Matings consisted of placing dams in a cage ($43 \times 26 \times 15$ cm) with an assigned sire for a period of 2 weeks, after which time the male was removed and female pregnancy was assessed. Approximately 1 week later, the female gave birth and first-generation litter sizes were measured. These first-generation males and females were categorised by their aforementioned testosterone group and used in the multiple mating experiment. Testosterone was measured from the adult males in this study using the method described above, and the male testosterone level was averaged for each testosterone group. These group averages for testosterone level were assigned to the females in this study that were concurrently bred to each group. While it is acknowledged that testosterone is sensitive to stress and environmental factors (e.g. Greiner *et al.* 2010; Van Hout *et al.* 2010), our study was carried out in the laboratory where environmental conditions and possible stressors were standardised among study individuals. Additionally, the repeatability of testosterone measures was assessed in a recent study on bank voles by measuring testosterone twice in a 2-week interval, which found this hormone measure to be consistent over time (repeatability = 0·637, $N = 56$ individuals, F ratio = 4·504; Schroderus *et al.* 2010).

MULTIPLE MATING EXPERIMENT

For the purposes of our experiment, we define multiple mating to refer to a female mating with several males during a single period of oestrus of *c.* 24 h (polyandry). First-generation mature males and females, one of each sex from each of the four testosterone groups (HH, HL, LH and LL), were assigned to a unique male (total of 33 male groups) and female group. We aimed to use an equal number of females from each of the four testosterone groups, but fewer successful matings between LL dams and sires occurred such that we created the following number of unique female groups: 26 HH, 31 HL, 23 LH and 15 LL. All females used in these multiple mating behavioural trials were assessed to be in (post-partum) oestrus, having given birth in the previous 24 h.
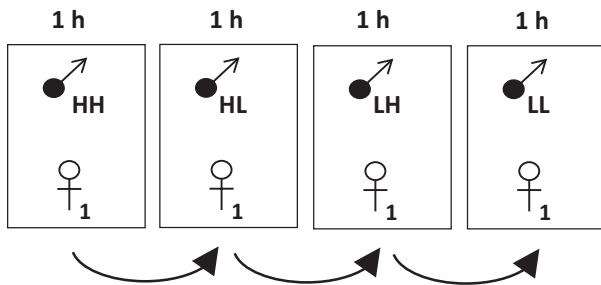
Fig. 1. Experimental design of mating trials. One female from one of the testosterone selection groups was individually paired with four males consecutively for 1 h each. The males originated from each of the four testosterone groups as well; however, the pairing of related individuals was avoided. The order of males was randomised across trials. Testosterone group designations: individual born to parents both from low-(LL) or high-(HH) testosterone families or else to parents where the sire (HL) or dam (LH) were from high-testosterone families.

Multiple mating trials were carried out such that a female was sequentially exposed to the four males from the male group, one at a time (and only once) in cages measuring $43 \times 26 \times 15$ cm. Each pairing lasted 1 h, for a total trial length of *c.* 4 h (Fig. 1). So, in every trial, each female was given the option to mate with four different males, each originating from the four different testosterone groups, during the 4 h trial. The order of males was randomised (e.g. HH males were not always first in the order), and their body masses did not differ between the treatment testosterone groups (ANOVA: $P > 0.05$). The female was never related to any of the males and was only exposed to a specific male group once. Mating success was recorded (whether a mating occurred) by observation of a mating and/or the presence of mating plug. A total of 227 trials were conducted, resulting in 188 trials in which the female mated with at least one male. The females ($N = 95$) achieved successful parturition in 165 of these trials. Mean values were taken for female and male fitness measures if they were used multiple times in mating trials (females and males were used an average of 2·36 and 6·68 times, respectively, whether or not the female became pregnant). If a female was used in multiple trials, an average of 27·4 days $\pm$ 1·37 SEM elapsed between these trials, which prevented any possible (unlikely) effects of sperm storage. The female's willingness to mate did not change significantly ($F_{6,156} = 0.315$, $P = 0.929$) if she was used multiple times; so, we dropped this variable from our models. A period of time elapsed between trials for males (12·1 days $\pm$ 0·61 SEM for males that mated, 11·1 days $\pm$ 0·48 SEM for males that did not mate) and the effect of time between trials for males generally did not significantly affect their copulatory behaviour [generalised linear mixed model (GLMM): $F_{40,623.09} = 1.20$, $P = 0.191$]. We define mating success to be the average number of partners (1–4 for females, 0–1 for males) a given individual was observed to mate with per trial, averaged for all of the trials an individual participated in. We define female reproductive success as the average litter size. Male reproductive success was the number of pups sired in a litter (as determined by genetic paternity analyses), averaged for all the trials it participated in.

PATERNITY ASSIGNMENT

A 2–3 mm portion of the terminal end of each newborn pup's tail was taken at birth for DNA paternity analysis and stored at −70 °C. Likewise, a 2–3 mm terminal ear clipping was obtained from each male that participated in the trials and stored at −70 °C. Genotyping

was assigned using seven microsatellites according to the procedure outlined in Mills *et al.* (2007b), Rikalainen *et al.* (2008), and Gockel *et al.* (1997). Likelihood-based analysis of paternity was conducted with CERVUS 3.0.3 software (Kalinowski, Taper & Marshall 2007). We used the following parameters: 10 000 cycles, 100% of candidate parents sampled, 100% of loci typed and a genotyping error rate of 1%. The 'one parent known' option was used to assign paternity. For each male group ($N = 4$ males), the candidate father with the highest LOD score (95% CI) was accepted as the sire. We genotyped all pups in a litter: there were a total of 928 pups analysed, of which only 14 pups could not be assigned paternity (1·5%).

STATISTICS

Selection differentials were calculated to test the effect of the respective mating successes on reproductive success (Fig. 2). We standardized the measures as follows: standardized male or female mating success = (mating success – mean mating success for all trials)/standard deviation; relative female reproductive success = litter size/population mean; and relative male reproductive success = average number of offspring sired per litter/population mean. The number of offspring sired per litter was used in the calculation of the male gradients to allow for a direct comparison of reproductive success between sexes.

Bateman gradients (β_{ss}) were determined by the least-square regression of reproductive success on mating success and were the slopes of the linear regressions. To assess the strength of conflict over multiple mating in this system, we calculated the absolute value of the difference in slopes between the linear male and female Bateman gradients:

$$\beta_{SC} = |\beta_M - \beta_F|$$

This formula assumes that a greater difference in the slopes between the sexes would correspond to a greater degree of sexual conflict over that particular trait. If there is no significant difference between the Bateman gradients between males and females, it can be said that their evolutionary interests for this particular trait coincide. This method was outlined clearly in a meta-analysis (Cox & Calsbeek 2009) that was used to measure sexually antagonistic selection. We used an ANCOVA to ascertain a statistically significant difference between slopes (Fig. 2).

A recent measure of SA selection proposed by Innocenti & Morrow (2010) was developed to distinguish between concordant ($I > 0$) and discordant ($I < 0$) selection, which was also calculated for this study using the formula:

$$I = \frac{\beta'_M \beta'_F}{\sqrt{\frac{\beta'^2_M + \beta'^2_F}{2}}}$$

where *I* is the joint index for the intensity of sex-specific selection and β_M and β_F are the respective male and female Bateman gradients.

In our analysis of male and female reproductive success in relation to female mating success (number of mating partners; Fig. 3), we used a generalised linear mixed model (GLMM) where 'sex', 'female mating success' and their interaction were fixed effects and the 'individual code' of the male or female vole was a random effect. An earlier version of the model included the male T-group as a fixed effect in the analysis of female reproductive success; however, this factor was not significant ($P > 0.05$) and therefore dropped from further analyses.

Male mating success was calculated as an average from multiple trials. This was performed to control for the first-male advantage in
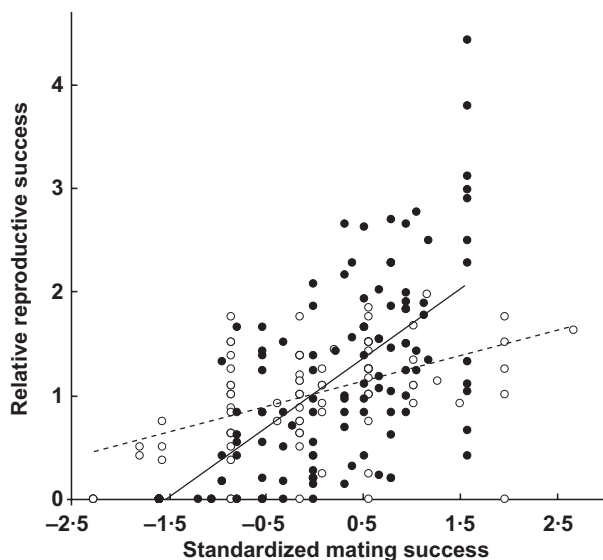
Fig. 2. Bateman gradients (linear regression slopes) for the evolutionary interests of males and females. Relative reproductive success (average litter size for females, average number of offspring sired for males) in relation to standardised mating success (number of mating partners) for female (white circle and dashed line) and male (solid circle and solid line) bank voles.
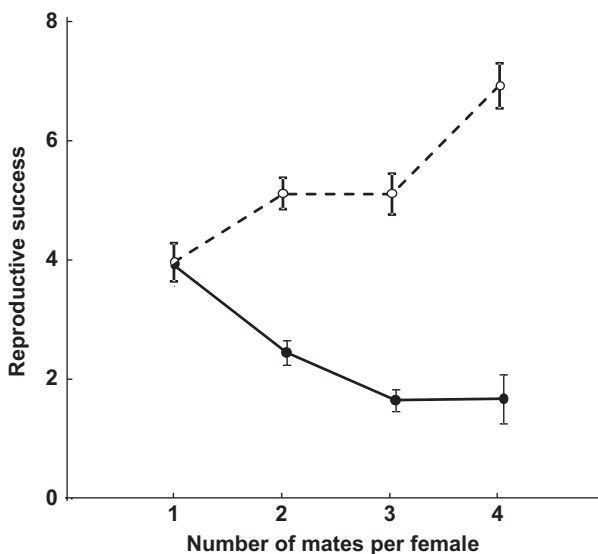
Fig. 3. Conflict over the female mating strategy of polyandry. Average male (solid circle and solid line) and female (open circle, dashed line) reproductive success ($\pm$ SEM) according to female mating success (number of mating partners) in trials.

these trials. A GLMM assessed male reproductive success across male positions (1–4) within a trial, where the 'proportion of pups sired in a litter' was the dependent variable, the 'male position' was a fixed factor and the unique 'male code' was the random factor.

A GLMM approach tested female mating success in relation to the testosterone levels of their selection groups ('female mating success' was the dependent variable, 'average testosterone level of males in the same testosterone group' was a covariate fixed factor and the unique 'female code' was the random factor; Fig. 4).
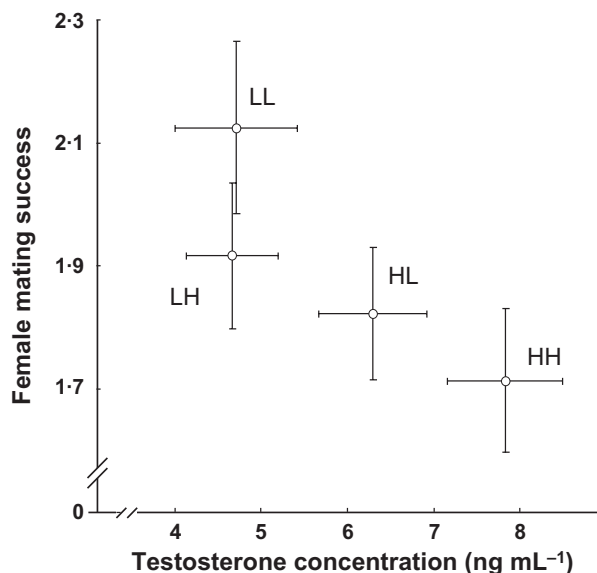
Fig. 4. Female mating behaviour associated with testosterone. Female mating success ($\pm$ SEM) in relation to male mean plasma testosterone concentration (ng mL^{-1} $\pm$ SEM) in testosterone selection groups. Mating success is the average number of partners (between 1 and 4) mated with during a trial. Testosterone group designations: individual born to parents both from low-(LL) or high-(HH) testosterone families or else to parents where the sire (HL) or dam (LH) was from high-testosterone families.

We tested differences in testosterone between first-generation male testosterone groups using a generalised linear model (GLM), and the *post-hoc* Tukey method for pairwise comparisons. GLM analyses were also performed in the comparisons of male reproductive and mating successes in relation to their testosterone levels, whereby the number of offspring sired and mates were the respective dependent variables, and the main effect of 'male testosterone group' and 'male testosterone level' nested within 'male testosterone group' were fixed factors in both analyses. Selection differentials were calculated to test the effect of male plasma testosterone concentration on reproductive success. We standardised the measures as follows: standardised testosterone concentration = (testosterone concentration − population mean testosterone concentration)/standard deviation; relative male reproductive success = average number of offspring sired per litter/population mean. In our calculations, we used these values to test for directional selection (linear) or stabilizing selection (quadratic curve). All statistical analyses were conducted using SPSS software v.16.0.1 (IBM SPSS, Armonk, New York, USA).

Results

SEXUAL SELECTION AND CONFLICT

The sex-specific Bateman gradients were both positive in direction (female regression: $y = 0.249x + 1.000$, $R^2 = 0.254$, $\beta_{SS} \pm SE$: 0.249 ± 0.044, $N = 95$, $t = 5.62$, $P < 0.001$; male regression: $y = 0.666x + 1.000$, $R^2 = 0.499$, $\beta_{SS} \pm SE$: 0.666 ± 0.058, $N = 136$, $t = 11.56$ $P < 0.001$, Fig. 2). The difference in slopes between Bateman gradients was significant, such that the male gradient increased significantly more than the female gradient

($\beta_{sc} = 0.417$; ANCOVA, sex * mating success: $F_{1,227} = 28.25$, $P < 0.001$). Furthermore, we also found that the quadratic slope between female mating success and reproductive success was significant ($y = -0.077x^2 + 0.243x + 1.076$, $R^2 = 0.254$, $N = 95$, $t = -2.68$, $P = 0.009$). This indicates that selection was positive, but weakened at higher female mating rates. Hence, while both females and males increased their respective reproductive successes when their mating success increased, males ultimately benefited more from multiple mating than females, likely causing disparity in mating rates. Additionally, we used the joint index to calculate the intensity of sex-specific selection (Innocenti & Morrow 2010) and found that $I = 0.330$. The positive nature of this measure indicates that the selection for multiple mating behaviour is concordant.

By mating with multiple males, females imposed fitness costs on their partners by reducing their share of paternity within a litter: as female mating success increased, male reproductive success decreased (GLMM: mating success $F_{3,481.35} = 6.96$, $P < 0.001$; sex $F_{1,363.83} = 41.59$, $P < 0.001$; mating success * sex $F_{3,481.35} = 27.28$, $P < 0.001$, Fig. 3). Of the 165 litters following these matings, 37% resulted in multiple male paternity (2 sires = 30.9% of litters; 3 sires = 6.06% of litters). There was a strong first-male advantage in this experiment (40.8% of pups sired by first male down to 13.0% by the fourth male; GLMM: $F_{3,867.23} = 16.65$, $P < 0.001$).

FITNESS EFFECTS OF TESTOSTERONE

Plasma testosterone concentration was significantly greater in the high-testosterone (HH) group of males compared to the low-testosterone (LL, LH) groups (GLM: $F_{3,132} = 5.692$, $P < 0.001$. Tukey pairwise: HH-HL $P = 0.301$; HH-LH $P = 0.002$; HH-LL $P = 0.006$). Furthermore, the testosterone level positively affected male mating success (number of partners: GLMM, $F_{4,121} = 8.45$, $P < 0.001$) and reproductive success (number of offspring; GLMM: $F_{4,121} = 3.28$, $P = 0.014$). The importance of testosterone for male fitness was further demonstrated by a significant selection gradient for higher testosterone values (directional selection: $y = 0.250x + 1.03$, $R^2 = 0.062$, $\beta \pm$ SE: 0.250 ± 0.087, $N = 128$, $t = 2.89$, $P = 0.005$). In contrast, the average mating success was higher for females selected for lower testosterone (number of partners: GLMM, $F_{1,186} = 4.39$, $P = 0.038$, Fig. 4). Furthermore, selection for lower testosterone seemed to increase female reproductive success; however, the result was marginally insignificant between groups (GLMM; $F_{1,34.89} = 3.19$, $P = 0.083$). Hence, contrary to males, selection for lower testosterone seemed to benefit female reproduction.

Discussion

We have found experimental evidence for sexual antagonism in mammals and how this phenomenon can maintain multiple mating behaviour in both sexes. Selection for high testosterone, which is associated with higher reproductive success in males, reduced female mating frequency. The conflict is realised because the mating rate of females positively correlates with their reproductive success: females that have more partners also have more offspring. However, males also suffer a cost in this mating system because this increase in reproductive success for females is coupled with a reduction in paternity for the males. The sexually antagonistic selection of testosterone in this mating system produces a differential effect on mating behaviour between the sexes, such that males from high-testosterone families and females from low-testosterone families mate the most. However, because both sexes gain more offspring by increasing their mating success, the sexually antagonistic selection of testosterone maintains the strategy of multiple mating. In this way, selection can favour either polyandrous females or polygynous males (Gowaty *et al.* 2010).

In this polygynandrous mating system, mating with multiple partners benefitted both sexes through the increases in their respective reproductive successes, as shown by the positive Bateman gradients (Fig. 2). The gradient was stronger for males than females, which indicates that as in several other systems, bank vole males are more limited for partners than females. This can result in stronger competition over females than males, which is in line with previous theory and research on this species (Mills *et al.* 2007b). It was interesting to discover a female's litter size increased with mating success (Figs 3 and 4), especially given the scarcity of evidence for this in mammals (Ågren 1990; Stockley 2003; Mills *et al.* 2007b). If the observed litter size benefit in our study can be attributed to ovulation stimulation, this may provide an interesting mechanism for females to regulate their reproductive effort and success in relation to their own physiological optima or potentially to extrinsic factors such as population density or the resources available to them. Alternatively, genetic incompatibility may be another explanation for a litter size benefit to multiple mating (Zeh & Zeh 1996). Recent work on the genetic incompatibility hypothesis has provided evidence for this in other species (Wilson *et al.* 1997; Tregenza & Wedell 1998; Stockley 2003).

Even though there was a positive Bateman gradient for both sexes, the slopes of the linear gradients were statistically different (Fig. 2). We interpret this difference in Bateman gradients to be a quantifiable measure of the evolutionary conflict over the mating strategy: a difference in the evolutionary interests between males and females. Additionally, the significant female quadratic regression indicates that the selection in females weakened at higher mating rates and may thus hint that the optimum mating rate in females was reached. While selection gradients have recently been advocated by Hunt *et al.* (2009), Cox & Calsbeek (2009) and Innocenti & Morrow (2010) to provide insight into the strength of sexual or sexually antagonistic selection, we propose that sex-specific selection gradients can also be used quite robustly to assess the presence and strength of sexual conflicts. However, caution is urged, and multiple measures are potentially needed, as our results indicate that divergent

evolutionary interests are possible even with positive selection in both sexes. Statistically assessing the difference between sex-specific selection gradients, as in the current study, may provide an additional convenient measure by which different systems, traits and species can be compared for sexual conflicts in a standardised way (Jones 2009).

Sexual conflict over mating behaviour increased with female promiscuity (Fig. 3). While female reproductive success increased with her mating rate, the average share of the litter paternity for her partner decreased. This finding is in line with current theory (Holland & Rice 1999; Hosken, Garner & Ward 2001; Stewart, Morrow & Rice 2005) and confirms that the level of promiscuity is associated with the strength of the sexual conflict over mating rate. Despite the conflict, it appears that the net fitness benefits obtained by both males and females in mating with multiple partners are greater than the costs imposed by the differential selection of testosterone on mating rate.

Males with higher testosterone and females with lower testosterone values mate the most (Fig. 4), and our earlier studies show that male bank voles with high testosterone also have a higher reproductive success (Mills *et al.* 2007b, 2009). Given that reproductive success increases with mating success in both sexes (Fig. 2), this could ultimately have a stabilizing effect on selection for testosterone, thus maintaining the genetic variation and gender load of this important reproductive trait. Alternatively, selection could favour the evolution of sex-specific mechanisms (e.g. sex-limited gene expression) to limit the negative effects of testosterone in females, although recent theory indicates that the costs of sexual antagonism are, at best, only partially resolved by such mechanisms (Connallon, Cox & Calsbeek 2010). The heritability of testosterone was estimated to be $h^2 = 0.32$ in a recent study of bank voles by Schroderus *et al.* (2010). It has previously been found in other species that family effects of testosterone selection are strong (e.g. King, Cline & Hubbard 2004), which emphasises testosterone's potential importance for fitness. Contrary to initial expectations of a positive fitness correlation between the sexes during selection, testosterone did not have a similar effect on females as it did on males when mating behaviour was examined (Ketterson, Nolan & Sandell 2005; Mills *et al.* 2009). There is evidence from other mammals that the negative changes in mating behaviour may be the result of *in utero* masculinization of females (Hernández–Tristán, Arevalo & Canals 1999; Lummaa, Pettay & Russell 2007). Females and males from the high-testosterone group (HH) would have been exposed to each other in the uterus, which is known to masculinise females. If this is the case here, then physiological processes specific to one sex, such as androgenization, may indirectly lead to sexual conflicts of traits (e.g. mating rate) mediated by other traits (e.g. testosterone).

Our experiment also revealed evidence of a first-male sperm precedence in mammals. This kind of advantage, which can also be obtained through dominance in male–male competition, assures that the first male will sire the greatest proportion of a litter and could even make costly processes such as mate guarding or care ultimately unnecessary for males. It may even potentially explain why males have not evolved mechanisms to prevent females from mating so frequently, given the costs to males of female multiple mating (Fig. 3). However, the appreciable proportion of multiple-sired litters in this study indicates that there are likely postcopulatory selection processes such as sperm competition or cryptic female choice operating in this species as well.

To conclude, our results reconcile the evolutionary and mechanistic causations of a sexual conflict by combining the inheritance of a sexually dimorphic reproductive trait (testosterone) with the mechanism (multiple mating) that maintains the conflict. There was a considerable benefit through increased reproductive success for both males and females to mate with multiple partners; yet, the reproductive interests between sexes diverge owing to reduced paternity success associated with polyandry, as well as the sexually antagonistic effect of the endocrine system on mating behaviour. To our knowledge, this is one of the first studies to experimentally assess the role of sexual antagonism on multiple mating behaviour in mammals, confirming work by Robinson *et al.* (2006) and Foerster *et al.* (2007) that show this family of vertebrates is experiencing evolutionary conflicts of interest between the sexes. Given the uncertain association between the genotype and phenotype, pleiotropic genes (as evidenced by negative genetic correlations; Schroderus *et al.* 2010), as well as the cascading effects of hormones and development, we urge caution when interpreting results that assume traits to be directly in conflict. It is possible these behavioural traits are actually mediated by other physiological traits under sexually antagonistic selection.

Acknowledgements

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