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Strategic sperm allocation under parasitic sex-ratio distortion

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Parasitic sex-ratio distorters are a major selective force in the evolution of host mating behaviour and mate choice. Here, we investigate sperm limitation in the amphipod *Gammarus duebeni* and the impact of the microsporidian sex-ratio distorter *Nosema granulosis* on sperm allocation strategies. We show that males become sperm limited after three consecutive matings and provide uninfected, high fecundity, females with more sperm than infected females. We show that sperm limitation leads to a decrease in female productivity. The outcome of sex-ratio distortion has been shown theoretically to be sensitive to the mating limits of males. Our results indicate that strategic sperm allocation under male rarity will have a greater impact on infected females and has the potential to regulate spread of parasitic feminisers in host populations.

Keywords: sexual selection; *Gammarus duebeni*; microsporidia; *Wolbachia*

1. INTRODUCTION

Maternally transmitted intracellular parasites increase their transmission efficiency by manipulating host reproduction in a number of ways, including distorting the primary sex-ratio through male killing or feminization (Bandi *et al.* 2001). Parasitic feminisers convert genetic males into phenotypic females, thereby increasing the relative frequency of the transmitting (female) sex. Feminization is induced by the bacterium *Wolbachia* (Bouchon *et al.* 1998) and by parasites from the eukaryotic phylum Microspora (Terry *et al.* 2004). Parasitic sex-ratio distorters have the potential to affect host population size and stability, and are a selective force in the evolution of mating behaviour (Charlat *et al.* 2003) and mate choice (Jiggins *et al.* 2000; Moreau *et al.* 2001) as they often result in female-biased populations (Dyson & Hurst 2004).

Males of many species are sperm limited and prudently allocate their limited supply at each mating to maximize their overall fertilization success (Gage 1998; Wedell *et al.* 2002). Frequently, males provide females of lower reproductive value with fewer sperm, or provide fewer sperm per mating when experiencing high female encounter rates. Modulation of ejaculate expenditure by males can at times directly result in sperm limitation for females (Wedell *et al.* 2002).

Because reproductive parasites can distort sex-ratio they may affect sexual selection, including sperm allocation by males. A recent study found that, under sperm depletion, *Armadillidium vulgare* females infected with feminizing *Wolbachia* have reduced fertility, most likely due to inefficient sperm usage (Rigaud & Moreau 2004). However, the impact of eukaryotic sex-ratio distorters on sperm allocation has not previously been studied. Here, we investigate sperm allocation strategies in the amphipod *Gammarus duebeni* under sex-ratio distortion by the feminizing microsporidium *Nosema granulosis* and show that males provide uninfected, high fecundity, females with more sperm than infected females.

2. MATERIAL AND METHODS

(a) *Animal collection and maintenance*

G. duebeni were collected from Budle Bay, Northumberland (55°40′N, 1°43′W), and maintained in brackish water (specific gravity 1005°) at 12 °C with rotted sycamore leaves and *Enteromorpha* spp (marine algae) for food and shelter.

(b) *Sperm numbers*

Animals were anaesthetized, weighed and the testes and seminal vesicles dissected. The testis was ruptured between the seminal vesicle and sperm duct, sperm were collected in 20 μl of distilled water, thoroughly mixed and the total volume of the fluid measured. For each male, three 10 μl sperm samples were placed on slides and dried. The numbers of sperm were counted under dark field phase microscopy at 100× magnification and the mean used to estimate the total number of sperm stored by the male (repeatability $F_{39,80}=35.75$, $p<0.0001$).

(c) *Parasite screening*

The infection status of females was determined by screening their eggs for the presence of microsporidia (Kelly *et al.* 2001*a*). Females were anaesthetized, the eggs flushed from the marsupium and stained with DAPI and examined under fluorescence microscope. In infected eggs, the diplokaryotic nuclei of microsporidia are visible in the perinuclear cytoplasm. This population of *G. duebeni* harbours two species of microsporidian sex-ratio distorter: *N. granulosis* and *Dictyocoelum duebenum*. We discriminated between the parasite species on the basis of size and excluded *D. duebenum* infected individuals. Uninfected females were used in these experiments unless otherwise stated.

(d) *Experimental design*

To measure sperm numbers and investigate the build up of males' sperm stores, 110 males were mated. Ten males were weighed, dissected and sperm numbers counted on the day that they had mated (week 0). The remaining males were allocated randomly to 10 groups of 10 solitary males. Sperm counts were taken at weekly intervals (10 males per week) for 10 weeks.

Sperm limitation was investigated by comparing the numbers of sperm that remained in the seminal vesicle of males that had mated with one, two or three females, consecutively. The impact of sperm limitation on productivity was investigated by measuring egg production and embryo viability in uninfected females. Thirty males were allowed to mate and then kept isolated for 10 weeks to build-up sperm stores. Males and females were allocated randomly to three treatment groups. In the first group, males were mated with one female and then dissected and the numbers of sperm remaining in the seminal vesicle estimated. In the other two groups, males were mated with either two or three females in succession and remaining sperm counted as above. The numbers of eggs laid by all females were counted. Embryo viability could not immediately be measured, as *G. duebeni* eggs will undergo three to four divisions even in the absence of fertilization. Therefore, the females were maintained for two weeks before the eggs were flushed from the brood pouch and the number of viable embryos was recorded.

To investigate ejaculate adjustment by males with respect to the parasite status of the female, 20 males were allowed to mate then kept in isolation for 10 weeks before being mated with either an uninfected or a *N. granulosis* infected female. After mating, the males were dissected and the numbers of sperm remaining in the seminal vesicle were recorded.

The data were analysed using GLIM (Numerical Algorithms, Oxford 1985). Sperm count data were square root transformed and analysed using ANCOVA. Egg numbers were analysed specifying a

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Poisson error structure (data corrected for overdispersion). Embryo viability data were analysed specifying a binomial error structure, taking the total number of eggs as the binomial denominator (data corrected for overdispersion). Overall productivity for each female was calculated as the number of eggs laid × the viability of the eggs.

3. RESULTS

The number of sperm stored in the seminal vesicle increased over the observation period ($F_{10,91}=5.6$, $p<0.01$). There was no effect of male weight on sperm numbers ($F_{1,91}=0.86$, $p>0.05$). However, males are sperm limited. The number of sperm was significantly affected by the number of times a male had mated ($F_{2,28}=6.30$, $p<0.01$; mean ± s.e. sperm remaining after 1 mating: $32.7\times10^3\pm6.9\times10^3$, 2 matings: $39.8\times10^3\pm4.5\times10^3$ and 3 matings: $14.3\times10^3\pm1.0\times10^3$) but unaffected by male weight ($F_{1,27}=0.18$, $p>0.05$). The difference in numbers of sperm remaining is due to the decrease in sperm numbers after three matings; (Tukey *post hoc* 3 versus 1 mating, $p<0.05$; 3 versus 2 matings, $p<0.05$).

Females suffer reduced productivity under sperm limitation. The number of eggs laid by a female differed between the treatment groups ($X_2^2=13.66$, $p<0.01$), indicating that the number of sperm received significantly affected egg production. The mean number of eggs produced by females from the different treatments was: male mated once 14.2 ± 0.8, twice 12.3 ± 1.4, three times 6.4 ± 1.7. Model simplification showed there was no difference in the number of eggs laid by females in the first two treatment groups; combining the treatments to a factor with two levels (males mated once or twice versus three times) caused no significant increase in the deviance of the data from the model ($X_1^2=0.72$, $p>0.05$). Surprisingly, egg number was not affected by female weight ($X_1^2=0.04$, $p>0.05$), and weight did not differ between infected and uninfected females ($F_{1,19}=2.45$, $p>0.05$). Embryo viability was also reduced by sperm limitation. All of the embryos from the first two experimental groups were viable. However, only $68.6\pm8.7\%$ of the eggs in the third group was viable. There was a significant difference in embryo viability between the three treatments ($X_2^2=42.3$, $p<0.01$). Simplification of the model to a factor with 2 levels (as described above) caused no significant increase in the deviance of the data from the model ($X_1^2=0.003$, $p>0.05$), indicating that egg viability was significantly reduced only when males had mated three times. Egg viability was unaffected by female size ($X_1^2=0.06$, $p>0.05$). As a consequence, the total productivity of each female was significantly affected by sperm limitation (figure 1, $F_{2,19}=5.54$, $p<0.05$) but unaffected by female weight ($F_{1,17}=0.63$, $p>0.05$). Simplification of the model to a factor with two levels (as described above) caused no significant increase in the deviance of the data from the model ($F_{1,18}=0.98$, $p>0.05$), indicating that female productivity was lower when males had mated three times.

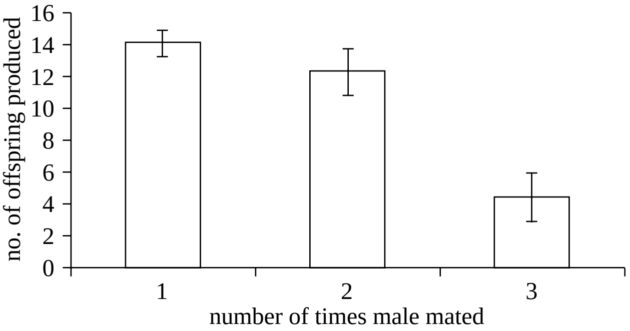

Figure 1. Number of offspring produced by females mated with a male who had mated once, twice or three times. Means ± s.e.

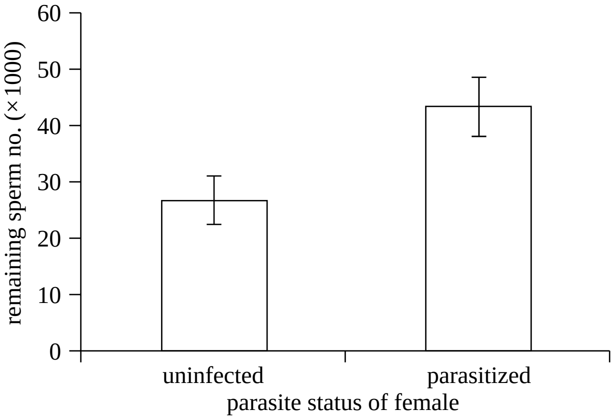

Figure 2. The number of sperm remaining after mating with infected and uninfected females. Means ± s.e.

Male *G. duebeni* adjust their ejaculate in response to the infection status of their mate. Males allocated more sperm to uninfected females than to parasitized mates resulting in more sperm remaining after copulation (figure 2, $F_{1,17}=6.44$, $p<0.05$). Sperm allocation was not affected by male weight ($F_{1,17}=0.423$, $p>0.05$).

4. DISCUSSION

Male *G. duebeni* are sperm limited and provide *N. granulosis*-infected females with fewer sperm. Selection should favour males that avoid mating with parasitized females. Although vertical transmission will select for low parasite virulence (Dunn *et al.* 2001), *N. granulosis* reduces female fecundity (Kelly *et al.* 2001*a*). In addition, as the parasite feminizes male hosts, males mating with uninfected females will be favoured, as these will produce more offspring of the rarer (male) sex (Fisher 1930). Males do not show any mating preference for uninfected females although, in the laboratory, males were found to invest a longer time guarding uninfected than infected females before copulation (Kelly *et al.* 2001*b*). The ejaculate adjustment observed in this study provides another mechanism by which males can adjust their reproductive investment and limit the costs of mating with parasitized females.

Our observations of sperm limitation are in accord with studies of the terrestrial crustacean *A. vulgare* in which multiple male mating resulted in sperm depletion (Rigaud & Moreau 2004), and where males transferred fewer sperm to females infected with feminizing *Wolbachia*. However, while sperm limitation causes reduced fecundity and fertility in (uninfected) *G. duebeni*, only *A. vulgare* infected with the feminizing *Wolbachia* suffered a reduction in fertility. This suggests sperm depletion does not directly reduce fertility in *A. vulgare* but that infected females have reduced ability to use sperm (Rigaud & Moreau 2004).

In contrast with previous studies (Terry *et al.* 1998; Kelly *et al.* 2001*b*), egg production was unaffected by female weight, suggesting that sperm limitation overrides any effect of female size on fecundity. There is no evidence that fecundity is influenced by any direct benefits provided by males at mating. Female *G. duebeni* appear to adaptively regulate egg production and size in response to environmental conditions. In southern populations, females produce small eggs in summer and larger eggs in winter, while in northern populations large eggs are produced throughout the year (Dunn & McCabe 1995). It is, therefore, possible females may be able to regulate egg laying also in direct response to available sperm supplies and reallocate resources towards increased egg production once sufficient sperm supplies are available. This possibility is currently being examined.

Feminizing parasites have the potential to increase or decrease host population size due to male rarity and may even lead to extinction if males become too rare (Hatcher *et al.* 1999). The predicted outcome of sex-ratio distortion has been shown to be particularly sensitive to the mating limits of males. In the field, the operational sex-ratio is surprisingly male-biased and fluctuates over the season (0.6–0.8; Kelly *et al.* 2001*a*). The decrease in female productivity observed here under sperm limitation could be important for host population size and stability. Moreover, the reduced mate guarding and transfer of lower sperm numbers to infected females mean that male rarity will have a greater impact on infected females and has the potential to regulate spread of the parasitic feminizer. It remains to be seen whether infected females are able to adjust their behaviour to reduce the costs of sperm limitation, by mating more frequently for example. In butterflies, male rarity under sex-ratio distortion by a male killing *Wolbachia* leads to sex-role reversal (Jiggins *et al.* 2000). Females form leks and solicit matings, with uninfected females having a higher mating success than infected females.

Parasitic sex-ratio distorters are common among arthropods and are likely to be a force affecting sexual selection in many species (Jiggins *et al.* 2000; Charlat *et al.* 2003; Rigaud & Moreau 2004; Terry *et al.* 2004). Here, we have shown that male *G. duebeni* provide *N. granulosis*-infected females with fewer sperm, which results in reduced offspring production. This mirrors the finding in *A. vulgare* infected with feminizing *Wolbachia*, indicating that ejaculate adjustment in relation to parasitic endosymbionts may be common as has been shown for other factors affecting male fertilization returns (Gage 1998; Wedell *et al.* 2002). We predict male ejaculate tailoring will have important consequences for the spread of the parasite as it undermines the transmission advantage, and therefore directly impacts on host population dynamics.

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