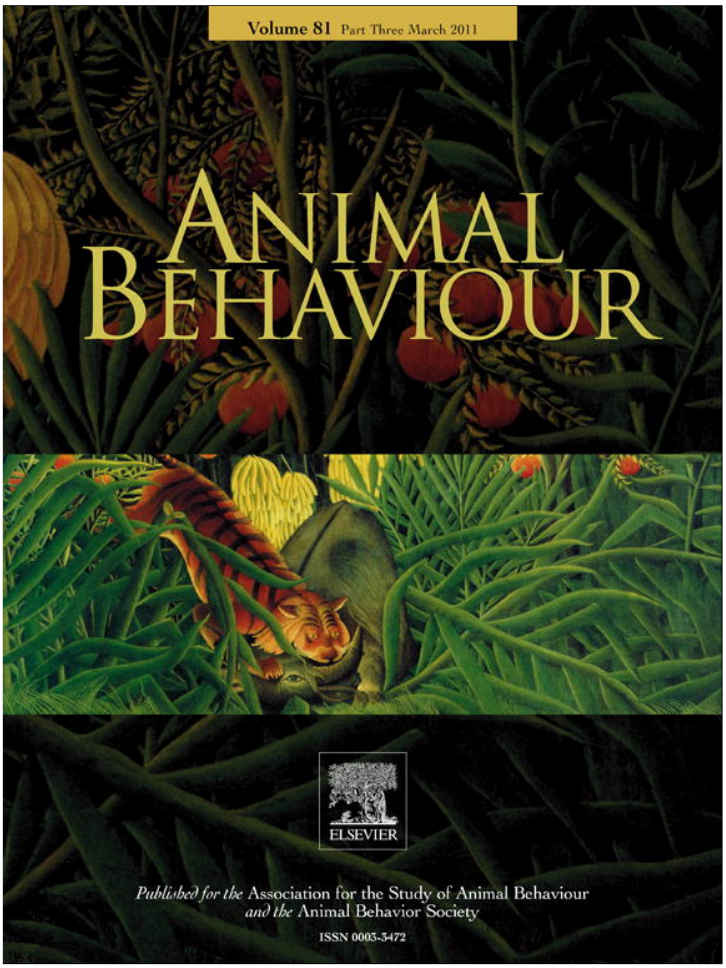

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Sex starved: do resource-limited males ensure fertilization success at the expense of precopulatory mating success?

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Theory predicts trade-offs between investment in various life history traits, and it is also now generally accepted that reproduction is costly for males. Males must therefore optimize resource allocation across several episodes of reproduction, and this includes investment in both pre- and postcopulatory fitness components. We investigated this in the sperm-polymorphic Indian meal moth, *Plodia interpunctella*. Resource-limited males were smaller, and had decreased precopulatory mating success, measured as lifetime number of matings. However, they transferred similar numbers of fertile sperm as males reared under high-quality larval conditions, and more nonfertile sperm. By mating less frequently, resource-limited males may allocate sufficient resources to the matings they achieve to ensure fertilization success under sperm competition.

In species in which females mate with multiple males, sexual selection occurs not only before copulation, but also after copulation in the form of sperm competition and/or cryptic female choice (Birkhead & Møller 1998). Theory predicts that sperm competition should favour increased allocation to sperm production (Parker 1998) and there is abundant evidence that this is the case (reviewed in Birkhead & Møller 1998). However, males possess finite resources from which various reproductive and somatic demands must be met and higher investment in one will decrease the resources available for investment in another (Roff 2002). While life history trade-offs have been reported in a wide range of animals, the majority of studies have focused on females (e.g. Kriegbaum 1997; Nussey et al. 2006; Rhen et al. 2006). Fewer studies have examined such trade-offs in males, possibly because traditional consensus, now largely invalidated (Wedell et al. 2002), held that males possess an almost unlimited supply of relatively cheap sperm (Bateman 1948). Recent evidence suggests that energetically expensive traits such as immunity are traded off against reproduction in males (reviewed in Lewis et al. 2008), and a number of studies have reported trade-offs between male reproduction and other traits related to life history such as longevity (e.g. Martin & Hosken 2004; Oliver & Cordero 2009) and predation avoidance (e.g. Nakayama & Miyatake 2010). Less attention has been paid to potential trade-offs between different episodes of reproduction, although it has been shown that in, for example, the horned beetle, *Onthophagus nigriventris* (Simmons & Emlen 2006), and the flour beetle, *Gnatocerus cornutus* (Yamane et al. 2010), there are trade-offs between the development of weaponry fundamental to precopulatory mating success and testes size which predicts postcopulatory fertilization success. Similarly in coho salmon, *Oncorhynchus kisutch*, males that are more likely to experience sperm competition exhibit less intense spawning coloration, a trait linked to precopulatory mating success, but have higher sperm velocities than males with darker spawning coloration, suggesting a trade-off between investment in sexual coloration and sperm quality (Pitcher et al. 2009).

As in nearly all lepidopterans, Indian meal moth, *Plodia interpunctella*, males produce two types of sperm, normal fertile or 'eupyrene' sperm, and large numbers of anucleated nonfertile or 'apyrene' sperm (Meves 1902). The function of the latter is still contentious, but they are thought to play a role in sperm competition, by filling the female's sperm storage organ and thereby delaying female remating (Cook & Wedell 1999). The reproductive

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success of a male, both precopulatory and postcopulatory, is related to the numbers of both types of sperm transferred during copulation (Z. Lewis & N. Wedell, unpublished data). Males that transfer large numbers of nonfertile sperm have higher precopulatory reproductive success, measured as lifetime number of matings, while the transfer of large numbers of fertile sperm is associated with higher postcopulatory reproductive success, measured as fertilization success under sperm competition. Trade-offs are typically seen when resources are limited and, hence, individuals are under increased pressure to allocate their resources optimally (e.g. Ferkau & Fischer 2006; Fricke et al. 2008). *Plodia interpunctella* do not feed as adults, and males must therefore derive all the resources necessary for reproduction through larval feeding. As a result, this is an ideal species for examining life history trade-offs as there is no confounding factor of adult feeding. In addition, males reared on a low-quality diet at high densities transfer fewer of both types of sperm at mating (Gage & Cook 1994). Here we examined whether there is a trade-off between male precopulatory mating success and postcopulatory sperm competitive ability in *P. interpunctella*. We manipulated the larval rearing environment by varying food quality and density, following Gage & Cook (1994), and subsequently examined adult male sperm transfer, precopulatory reproductive success (lifetime number of matings) and postcopulatory reproductive success (fertilization success under sperm competition when in either the first or second male role). We also measured adult longevity and body size, to ascertain whether either pre- or postcopulatory reproductive success is traded off against somatic life history traits in resource-limited males; unlike in many insects (cf. Bangham et al. 2002), male body size is not subject to sexual selection in this species (Cook et al. 1997; although see Ingleby et al. 2010). As sperm are fundamental to reproductive success in this species, we predicted that males reared in a low-quality, high-density environment would concentrate their limited resources to optimize one of these aspects of reproduction at the expense of another.

METHODS

Rearing of Experimental Stock

A stock population of *P. interpunctella* was cultured using individuals from a strain that has been maintained at the Fuji Flavour Company, Tokyo, Japan for approximately 10 years (R. Sasaki, personal communication). Larvae were reared on a diet of bran midlings, honey, glycerol and yeast (ratio 10:1:1:1), at a temperature of $28 \pm 3\,^{\circ}C$ with a 16:8 h light:dark regime (Gage & Cook 1994). Effects of larval overcrowding were removed by providing larvae with excess food: 50 eggs per 200 ml of food. In addition to the wild-type strain, we used a golden mutant marker of *P. interpunctella*. These had appeared spontaneously in a wild-type culture and had been bred to form a pure strain. As the wild type has a greater sperm competitive ability than the golden mutant (Z. Lewis & N. Wedell, unpublished data), we used only the former to compare this ability between males. The golden mutant was reared under conditions identical to the experimental stock.

Experimental Treatments

Eggs of known laying date were collected from the stock population and randomly assigned to one of two experimental treatments: a high-quality larval environment and a low-quality larval environment (Gage & Cook 1994). The high-quality environment was identical to that of the stock population: a diet consisting of bran, honey, glycerol and yeast at a ratio of 10:1:1:1, with a population density of 50 eggs per 200 ml of food. Offspring reared in the low-quality environment were given food medium that contained no yeast, an important protein component of the moth's laboratory diet, and were stocked at higher densities (250 eggs per 200 ml of food) than offspring from the high-quality larval environment. Thus larvae developing under poor environmental conditions suffered both poor nutrition and intense competition for resources. Ten containers of each treatment were established, and experimental individuals were selected randomly from across all containers.

Mating Experiments

To collect virgin moths, fifth-instar larvae were separated according to sex. By this stage of the life cycle, larvae have ceased to feed and males can be distinguished from females as their testes become visible through the body wall (Ingleby et al. 2010). Subsequent matings took place during the 8 h dark phase of the light cycle.

On the day of eclosion, sperm transfer ability was estimated for all focal males; 200 males from each of the two treatments were paired individually in 30 ml vials with a virgin stock female also on her day of eclosion. Following copulation, each female was removed and killed immediately to prevent sperm migrating from the spermatophore; subsequently the fertile and nonfertile sperm transferred by each male were counted following a standard protocol (Lewis & Wedell 2007).

We then measured the sperm competitive ability of each male, which incorporates both his ability to defend his sperm against the subsequent ejaculates of rival males and his ability to compete for fertilizations offensively against previously inseminated sperm (Parker 1984). The former is generally measured as P_1, the proportion of eggs sired by the focal male when mating in the first male role, while the latter is measured as P_2, the proportion of eggs fertilized by the second of two males (Boorman & Parker 1976). In the current study, for half the males from each treatment, sperm competitive ability was measured as P_1, and for the other half as P_2. To measure P_1 and P_2, focal males were mated with golden females; as the golden mutation is recessive and exhibits typical Mendelian inheritance (Beeman & Schmidt 1982; Beeman 1983), by allowing a wild-type male and a golden male to compete for the fertilization of a golden female's eggs, we could accurately assign paternity to the two males by counting the wild-type and golden offspring that subsequently emerged. Despite potential reproductive differences between wild-type and golden females, the use of two types of female is unlikely to have affected our results, as all focal males across our treatments were treated in the same manner, and mated with stock females throughout the experiment except on their second mating. To measure P_1 the focal male was mated with a virgin golden female. On the following day (the third day of the mating experiments) this golden female was then mated with a golden male that had previously mated with a random virgin female. We never estimated sperm competitive ability using virgin males, as *P. interpunctella* males produce significantly more sperm on their first mating than on subsequent matings (approximately 20% more fertile sperm and 50% more nonfertile sperm; Gage & Cook 1994), and females are less likely to remate if provided with large quantities of sperm, which would cause practical difficulties for this experiment. Furthermore, in the wild, males are more likely to be nonvirgin than virgin; hence by allowing nonvirgin males to compete with each other, we obtained a more appropriate measure of sperm competitive ability. To measure P_2 the focal males were mated with a golden female that had previously mated with a golden male, also on his second mating. Thus, when measuring both P_1 and P_2 we allowed the ejaculates of two nonvirgin males, one golden mutant male and one wild-type male, to compete.

When the female had mated with both males, she was isolated in a 10 ml vial and allowed to oviposit for the remainder of her life. Her eggs were then transferred to standard rearing conditions as above. Fertilization success of the focal wild-type male was calculated by determining the proportion of wild-type offspring relative to golden offspring, following their emergence as adults.

Having estimated the sperm competitive abilities of our focal males on their second mating, for the remainder of the experiment we presented males with a virgin stock female, each day, until the males died. Each day we noted whether the males mated, thereby obtaining a measure of lifetime mating success; we subsequently confirmed whether each of these matings had been successful by dissecting the females and checking for the presence of a spermatophore or sperm packet. Upon death, male longevity was recorded, and body size estimated by measuring the length of the forewing (Ingleby et al. 2010).

Statistical Analyses

Analyses were conducted using SPSS version 15 (SPSS Inc., Chicago, IL, U.S.A.) and R version 2.8.1 (Ihaka & Gentleman 1996). The raw data for all variables were tested for normality, and, where appropriate, nonparametric tests used. Male body size was analysed using a *t* test with larval treatment as the explanatory variable. Male adult longevity was analysed using a Mann–Whitney *U* test. Lifetime number of matings was initially analysed using a general linear model (GLM) with number of nonfertile sperm as a covariate, as nonfertile sperm number has been shown to predict mating number (Z. Lewis & N. Wedell, unpublished data). Nonfertile sperm was removed from the final model as it was nonsignificant ($F = 1.84$, $P > 0.1$; overall $F = 1.84$, $P > 0.1$). Both fertile and nonfertile sperm numbers were analysed using GLMs with female body size and male body size as covariates, also removed in the final models (dependent variable: fertile sperm number: female body size: $F = 2.81$, $P = 0.1$; male body size: $F = 0.07$, $P > 0.9$; overall: $F = 0.04$, $P > 0.8$; dependent variable: nonfertile sperm number: female body size: $F = 1.49$, $P > 0.2$; male body size: $F = 1.06$, $P > 0.3$; overall: $F = 5.97$, $P = 0.02$). The proportions of offspring sired by males under sperm competition were analysed using two general linear mixed models (GLMMs), one for P_1 and one for P_2, with a binomial error structure and a logit link function. Male body size, female body size and total number of offspring produced by each female were initially included as covariates, but were removed from the final models as they were nonsignificant (dependent variable: P_1: female body size: $F = 0.14$, $P > 0.7$; male body size: $F = 2.11$, $P > 0.1$; total offspring: $F = 0.002$, $P > 0.9$; overall: $F = 0.14$, $P > 0.7$; dependent variable: P_2: female body size: $F = 0.01$, $P > 0.9$; male body size: $F = 0.50$, $P > 0.4$; total offspring: $F = 0.88$, $P > 0.3$; overall: $F = 0.01$, $P > 0.3$). Sample sizes are smaller for sperm competition data, as not all females would mate twice. All values given are means ± SE.

RESULTS

Adult males that had been reared in the low-quality (L) environment were smaller than those reared in the high-quality (H) environment (L: 4.56 ± 0.03 mm; H: 4.74 ± 0.04 mm; $t_{167.8} = 3.637$, $N = 224$, $P < 0.001$; Fig. 1a). There was no effect of treatment on the number of fertile sperm ejaculated by males on their first mating (L: 1062.60 ± 74.78; H: 1018.78 ± 89.81; $F_1 = 0.15$, $N = 224$, $P > 0.7$). However, males reared in the low-quality environment ejaculated more nonfertile sperm on their first mating (L: 30 831.30 ± 1864.88; H: 22 178.90 ± 2280.09; $F_1 = 7.89$, $N = 221$, $P = 0.005$; Fig. 1b).

In terms of reproductive success, males from the low-quality environment suffered in terms of lifetime mating number, mating less frequently over the course of their life span than males from the high-quality environment (L: 4.17 ± 0.11; H: 4.69 ± 0.17; $F_1 = 5.21$, $N = 231$, $P = 0.023$; Fig. 1c). However, there was no difference between males from the treatments in terms of sperm competitive ability; males from both sired a similar proportion of

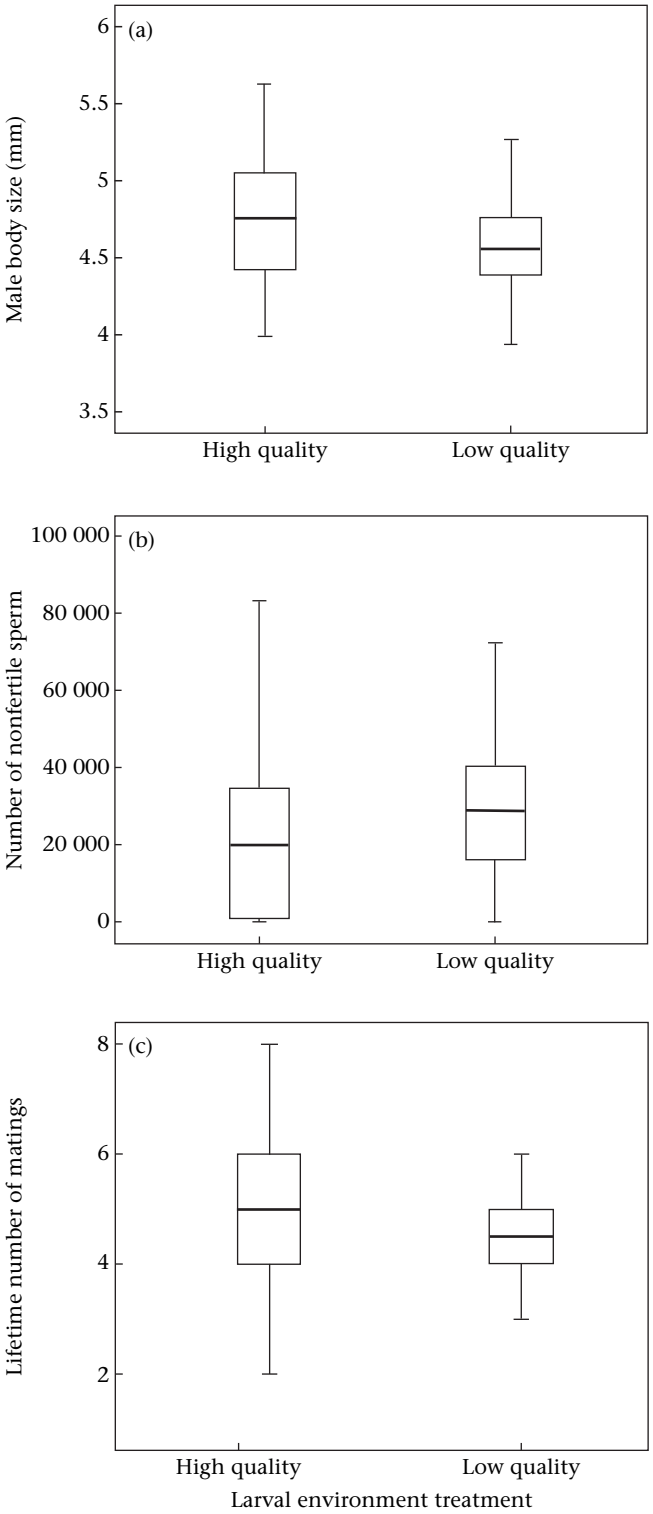

Figure 1. (a) Male body size, (b) number of nonfertile sperm transferred on the male's first mating, and (c) number of matings in a lifetime in relation to larval environment, which was either low or high quality. Box plots show the median, 25th and 75th percentiles, and minimum and maximum values.

offspring when competing against a mutant marker male, whether in the first male role (L: 0.61 ± 0.16; H: 0.69 ± 0.15; $F_1 = 0.018$, $N = 31$, $P > 0.8$) or the second male role (L: 0.47 ± 0.10; H: 0.58 ± 0.11; $F_1 = 0.009$, $N = 37$, $P > 0.8$). We found no difference in adult longevity across treatments (L: 7.47 ± 0.12 days; H: 7.81 ± 0.16 days; $U = 5404.50$, $N_1 = 93$, $N_2 = 132$, $P > 0.1$).

DISCUSSION

Our study shows that in *P. interpunctella*, the quality of the male larval environment affects male reproductive success. Resource-limited males achieved fewer matings over the course of their life span than males from a high-quality larval environment. However, resource-limited males ejaculated similar numbers of fertile sperm as males reared in a high-quality environment, and more nonfertile sperm. In addition, we found no effect of larval environment on male sperm competitive ability; males from both treatments sired a similar proportion of offspring when competing against a golden mutant marker male, whether in the first male role or the second male role. Resource-limited males were also smaller. Our results suggest that when reared under poor conditions males mate less frequently, but can allocate sufficient resources to the matings they achieve to ensure fertilization success under sperm competition.

The fact that resource-limited males mated less frequently but achieved similar fertilization success as males reared in a high-quality environment could have arisen because mating was more costly than sperm production. However, resource-limited males transferred more nonfertile sperm than males from the high-quality environment. As discussed in the Introduction, nonfertile sperm may delay remating in females. Perhaps by diverting resources from the soma (resource-limited males were also smaller) and mating, *P. interpunctella* males reared in a low-quality larval environment ejaculate similar numbers of fertile sperm as males that are not resource limited, thereby ensuring fertilization success under sperm competition for the matings they do achieve. In addition, by ejaculating more nonfertile sperm, they perhaps protect this investment by delaying female receptivity to rival males, as seen in the green-veined white butterfly, *Pieris napi* (Cook & Wedell 1999). It may be that nonfertile sperm are less costly to produce than fertile sperm, and are therefore easier to increase in numbers when resources are limited. Alternatively, resource-limited males may have ejaculated more nonfertile sperm because our low-quality environmental treatment incorporated high rearing densities. In both *P. interpunctella* and the closely related almond moth, *Cadra cautella*, males reared at high densities are known to transfer more sperm. In *P. interpunctella*, Gage (1995) has shown that males reared at high densities transfer more of both fertile and nonfertile sperm, in contrast to the current study where males from the treatment that incorporated high densities transferred more nonfertile sperm alone. This difference is likely to be because, in our study, this treatment also incorporated nutritional limitation. In *C. cautella*, males reared under high densities transferred more nonfertile sperm (McNamara et al. 2010), as found in the current study. The authors suggested that males could be responding to cues that reflect larval population structure, and thus future level of mating competition. At high densities males may anticipate a higher risk of sperm competition on emergence, and therefore invest in a strategy, transferring more nonfertile sperm to females, that will maximize their mating success under sperm competition.

Our experimental design makes it difficult to disentangle the relative effects of larval density versus nutritional limitation. However, our aim was to examine the effects of a poor-quality larval environment as a whole; we thus followed Gage & Cook (1994), whose treatments were shown to result in resource limitation. The fact that in the current study males from the poor-quality treatment were significantly smaller suggests that they were indeed resource limited. However, despite using the same larval treatments, Gage & Cook (1994) reported a significant decrease in the numbers of both fertile and nonfertile sperm ejaculated by resource-limited males. This is in contrast to our results where resource-limited males transferred similar numbers of fertile sperm as males reared in a high-quality environment and more nonfertile sperm. This may be because of differences between the two populations of *P. interpunctella* used and their respective evolutionary histories and/or rearing histories. For example, if the population used in the current study had a history of relatively higher levels of female remating, males may have historically been subject to stronger selection pressures to maximize their reproductive success under sperm competition, thus leading to the difference in results between the two studies.

There was no effect of larval rearing treatment on adult longevity. This is perhaps not surprising, as selection is expected to act on lifetime number of matings, rather than longevity per se. The amount of time a male lives after his final mating is irrelevant to his success. Mating number is expected to be under significant selection, as the more mates a male has the higher his potential fitness is. Therefore it may be reasonable that a male can produce more nonfertile sperm by sacrificing his lifetime number of matings rather than adult longevity.

In conclusion, we have shown that *P. interpunctella* males reared in resource-limited larval conditions were smaller and mated less frequently over the course of their life span than males reared in high-quality larval conditions. However, resource-limited males allocated sufficient resources to the matings they did achieve to ensure fertilization success under sperm competition. Our results suggest trade-offs between somatic development and reproduction, and possibly between pre- and postcopulatory reproductive success in this species.

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