

Natural selection drives the link between male immune function and reproductive potential

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Abstract: In the ground cricket *Allonemobius socius* (Scudder 1877), males provide females with a hemolymph-based nuptial gift. The size of the gift depends on when copulation is terminated, which can be controlled by either sex. Here we show that more immunocompetent males provide larger nuptial gifts, increasing their reproductive potential. To address if this pattern was the consequence of sexual selection for an honest signal (i.e., females assess mate immune quality through the hemolymph), we examined which sex controlled gift size. We found that the probability that males initiated the end of copulation increased when gift size was small (stereotypical of less immunocompetent males). Thus, early termination of copulation was the consequence of male behavior, suggesting that the association between immunocompetence and gift size was not due to sexual selection, but to natural selection for male vigor.

Résumé : Les mâles du grillon de Caroline, *Allonemobius socius* (Scudder, 1877), présentent aux femelles une offrande nuptiale à base d'hémolymphe. La taille de l'offrande dépend du moment de la fin de l'accouplement qui peut être contrôlée par l'un ou l'autre partenaire. Nous montrons que les mâles les plus immunocompétents fournissent des offrandes nuptiales plus importantes, ce qui augmente leur potentiel reproductif. Afin de déterminer si ce pattern est la conséquence de la sélection sexuelle d'un signal fiable (c.-à.-d. que les femelles évaluent la qualité immunitaire de leur partenaire par l'hémolymphe), nous avons observé quel partenaire contrôle la taille de l'offrande. La probabilité que le mâle décide du moment de la fin de l'accouplement augmente lorsque l'offrande est petite (signe stéréotypé d'un mâle moins immunocompétent). La fin hâtive de l'accouplement est donc due au comportement du mâle, ce qui laisse croire que l'association entre l'immunocompétence et la taille de l'offrande nuptiale ne s'explique pas par la sélection sexuelle, mais plutôt par la sélection naturelle de la puissance sexuelle mâle.

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Allonemobius socius (Scudder 1877) males are unique among crickets in that they possess a specialized spur on their hind tibia that females chew during copulation, providing a hemolymph-based nuptial gift (Fedorka and Mousseau 2002a). Previous work has shown that multiply mated males who provided a larger total gift (i.e., a longer total chewing duration over four matings) also exhibited a stronger antibacterial defense (antibacterial defense was measured 24 h post copulation; Fedorka et al. 2004). This is intriguing, and two mechanisms may account for this pattern. First, increased mating frequency may increase antibacterial enzyme levels. This seems reasonable considering that increased mating frequency is associated with a higher number of

chewing (wounding) events, which continually places the male's circulatory system at risk of infection. Second, males who provide larger gifts are in a better initial immune condition. The latter hypothesis may be driven by either sexual or natural selection. For instance, a female may choose to mate longer with a more immunocompetent male (sexual selection for an honest signal via the hemolymph), or males of higher initial quality can withstand a greater hemolymph loss (natural selection for male vigor). The mechanism underlying the relationship between immunity and gift size in our previous data, however, remains unresolved because immunity was recorded after all matings had occurred (Fedorka et al. 2004).

Here we investigate the hypothesis that larger nuptial gifts are provided by more immunocompetent males. To this end, we measured two immune parameters prior to mating, phenoloxidase activity (key component of invertebrate innate immunity) and lysozyme activity (measure of bacterial defense), and examined their association with several male reproductive phenotypes. Using a microsyringe (Hamilton Co., Reno, Nevada), we removed 1.5 μ L of hemolymph from between the 2nd and 3rd abdominal sternites of cold anesthetized (5 min on ice), virgin males 12 \pm 1 days post eclosion ($n = 65$). The hemolymph was dispensed into 20 μ L of phosphate-buffered saline (PBS) and frozen to induce cell

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Table 1. Correlation matrix of male reproductive behavior in *Allonemobius socius*.

	Courtship duration (min)	Gift size (min)	Spermatophore attachment duration (min)
Gift size (min)	-0.33 (0.0037)	—	—
Spermatophore attachment duration (min)	-0.03 (0.8133)	0.53 (0.0001)	—
Postcopula spermatophore attachment duration (min)	0.29 (0.0324)	-0.32 (0.0162)	0.61 (0.0001)

Note: Estimates are Pearson product-moment correlations followed by their associated *P* value in parentheses. *P* values in boldface type remain significant after a sequential Bonferroni correction.

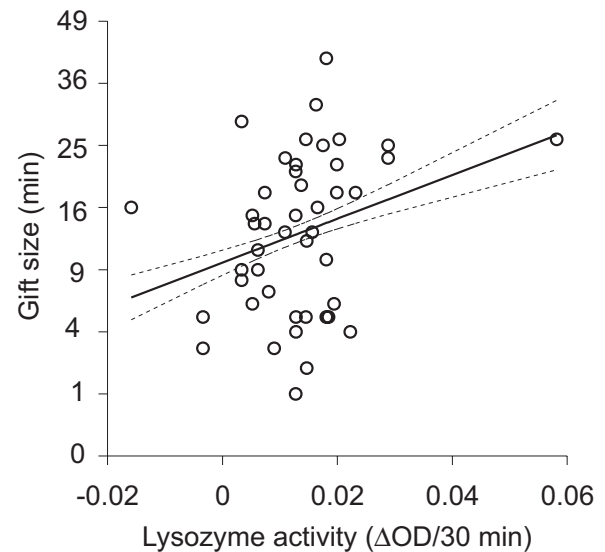
lysis. To estimate phenoloxidase activity, 90 μL of a 15 mmol/L of L-Dopa-buffered solution were added to 10 μL of the frozen hemolymph sample. Likewise, 90 μL of a *Micrococcus leutus* buffered solution (3 mg of freeze-dried *M. leutus*/L) were added to the remaining 10 μL of hemolymph to estimate lysozyme activity. The total change in optical density (OD) in both samples over 30 min served as our activity estimates, which were recorded at 490 nm using a microplate reader (model 550, Bio-Rad, Hercules, California; OD range 0.000 (transparent) to 3.500 (opaque)).

Twenty-four hours after removing the hemolymph, males were placed in a mating arena (75-mm Petri dish lined with filter paper) with a virgin female of similar age. In this system, males perform a courtship song and dance to entice females into copulation. If receptive, the female will mount the male in a brief “mock copulation”, causing the male to stop courting and form a spermatophore (approximately 20 min). Male courtship than renews, the couple unites, and the spermatophore is passed to the female. While adjoined, the male brings his hind tibia forward, allowing the female to “chew” on his spur until the pair separates (upwards of 30 min). Shortly thereafter, the female will remove and consume the spermatophore.

Considering the mating ritual, four reproductive behaviors were assayed: (1) courtship duration (measured as the time from initial courting to copulation), (2) gift size (measured as the duration of female spur chewing; Fedorka and Mousseau 2002a), (3) spermatophore attachment duration, and (4) postchewing spermatophore attachment duration (measured as the duration the female kept the spermatophore attached after uncoupling from the male). Body size estimates were obtained by measuring pronotum length to the closest 0.01 mm using a camera-mounted dissecting microscope and NIH Image[®] version 1.62 (National Institutes of Health 1999). Experimental crickets were third generation laboratory-reared individuals derived from gravid, wild-caught females from central South Carolina (for a description of stock maintenance see Fedorka et al. 2004). All data were analyzed using JMP[®] version 5.1 (SAS Institute Inc. 2003).

We found that the reproductive behaviors were not normally distributed (Shapiro–Wilks test, all $P < 0.01$) and were square root transformed to improve normality. Male body size was not associated with either immune parameter (pairwise Pearson’s correlation estimates, all $P > 0.24$), and was removed from further analysis. Males who experienced a brief courtship duration also provided larger nuptial gifts (Pearson’s correlation estimated, $r = -0.33$, $P < 0.005$; Table 1). In addition, females who received larger gifts kept the

Fig. 1. Male immune function predicts male reproductive potential in *Allonemobius socius*. Males who exhibited a greater lysozyme activity (antibacterial defense) prior to mating provided a larger nuptial gift during mating. This pattern is likely due to natural selection for male vigor and not due to sexual selection via female choice for honest signals. ΔOD is total change in optical density and broken lines are $\pm\text{SE}$.



spermatophore attached longer ($r = 0.53$, $P < 0.0001$; Table 1), allowing more sperm to transfer to the females’ reproductive tract. When the immune parameters were examined, we found that only gift size was significantly associated with lysozyme activity (multiple regression, $F_{[1,67]} = 8.14$, $P = 0.0058$; Fig. 1). All other reproductive behaviors were not associated with either immune parameter (multiple regression, all $P > 0.21$). These data suggest that males of superior immune quality court for a shorter period of time and provide a larger nuptial gift, which is positively associated with male reproductive fitness (Fedorka and Mousseau 2002b).

It remains unclear, however, whether this pattern was due to female choice for an honest signal (sexual selection) or to variance in male vigor (natural selection). To further investigate these mechanisms, we examined previously video-taped mating trials ($n = 105$; for details regarding these trials see Fedorka and Mousseau 2004) to determine which sex controlled the termination of copulation, and hence the size of the nuptial gift. Copulation control can be unambiguously assigned considering that the sexes are stationary during copulation and move-off in stereotypically different direc-

tions when copulation is complete. Thus, control was assigned to the first individual who moved away from their stationary partner. Female choice for an honest signal would be supported if females were shown to terminate copulation more often, or if shorter mating bouts (stereotypical of males with a lower lysozyme activity) were the consequence of female behavior. We found that males terminated mating significantly more often than females (66 of 105 trials; likelihood ratio test = 7.02, $P = 0.0081$). In addition, we found that the probability of male control increased with decreasing gift size (logistic regression, likelihood ratio test = 3.79, $P < 0.05$). Thus, the increased reproductive potential of immunocompetent males appears to be due to natural selection for male vigor and not to sexual selection via female choice for an honest signal.

These data suggest that males who invested more in immunity were able to invest more into reproduction. This phenomenon is similar to the “big car – big house” paradox in the evolution of life histories, where individuals of the highest quality incur a reduced trade-off cost compared with lower quality individuals (Reznick et al. 2000; Zuk and Johnsen 2000).

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