Evolution, 58(11), 2004, pp. 2478–2485

IMMUNE SUPPRESSION AND THE COST OF REPRODUCTION IN THE GROUND CRICKET, *ALLONEMOBIUS SOCIUS*

KENNETH M. FEDORKA,[1,2] MARLENE ZUK,[1] AND TIMOTHY A. MOUSSEAU[3]
[1]*Department of Biology, University of California Riverside, Riverside, California 92521*
[3]*Department of Biology, University of South Carolina, Columbia, South Carolina 29208*

Abstract.—One of the most common life history trade-offs in animals is the reduction in survivorship with increasing reproductive effort. Despite the prevalence of this pattern, its underlying physiological mechanisms are not well understood. Here we test the hypothesis that immune suppression mediates this phenotypic trade-off by manipulating reproductive effort and measuring immune function and mortality rates in the striped ground cricket, *Allonemobius socius*. Because *A. socius* males provide females with a hemolymph-based nuptial gift during copulation, and many structural components of immunity reside in the hemolymph, we also predicted that sexual selection may differentially affect how disease resistance evolves in males and females. We found that an increased mating effort resulted in a reduced immune ability, coupled with an increased rate in age-specific mortality for both sexes. Thus, immune suppression appears to be a link between reproductive effort and cost in this system. In addition, males and females appeared to differentially invest in several aspects of immunity prior to mating, with males exhibiting a higher concentration of circulating hemocytes and a superior bacterial defense capability. This pattern may be the result of previously established positive selection on gift size due to its affect on female fecundity. In short, female choice for larger gifts may lead to a sexually dimorphic immune ability.

Key words.—Hemolymph, immunocompetence, life-history trade-off, sexual dimorphism, sexual selection, survivorship.

Received June 25, 2004. Accepted August 11, 2004.

Selection acts to maximize fitness by favoring those individuals (genotypes) who produce the largest number of successful offspring (Reznick et al. 2000). However, an increase in reproductive effort can create a shortfall in resources that are necessary for other important life-history traits such as somatic maintenance, growth, or survival (Roff 1992). This cost of reproduction need not be limited to an internal trade-off (physiological costs), but may be realized through other exogenous forces such as an increased exposure to predators or disease (ecological costs). From a logical standpoint, reproductive costs make sense; otherwise evolution would give rise to organisms that exhibit the best of all possible phenotypes, that is, organisms with an unbounded growth rate coupled with an unlimited reproductive output (Reznick et al. 2000).

In particular, the survival costs associated with reproduction have been empirically well established across a diverse array of organisms including plants, insects, fish, and birds (e.g., Tatar and Promislow 1997; Hutchings et al. 1999; Hautekeete et al. 2001; Wheelwright et al. 2003). For instance, in the western gull, *Larus occidentalis*, males and females who exhibited an earlier age at first reproduction, or females that attempted to breed more frequently, had a reduced probability of survival (Pyle et al. 1997). Although survival trade-offs of this nature are common, the physiological mechanisms underlying this relationship are not well understood. Those studies that have addressed the underlying physiology have generally focused on differences in metabolic rates and energy consumption with regard to reproductive status (reviewed in Zera and Harshman 2001). However, the question often remains which energetically costly physiological system serves as the link between reproductive effort and reproductive cost.

One possible candidate that has received recent attention is the immune system (Sheldon and Verhulst 1996). For instance, previous work has shown that mating effort induces immune suppression in both the damselfly, *Matrona basilaris japonica* (Siva-Jothy et al. 1998) and the fruit fly *Drosophila melanogaster* (McKean and Nunney 2001). However, few studies have linked a reduction in immunocompetence due to mating directly with a reduction in survivorship (but see Nordling et al. 1998). The reason for the negative relationship between mating effort and immunity is likely due to the competitive allocation of resources (Sheldon and Verhulst 1996), where shared components necessary for reproduction are diverted from immune defense during periods of sexual activity. However, recent work in the mealworm beetle, *Tenebrio molitor*, suggests that immune suppression may also be due to physiological antagonism between hormones associated with increased sexual activity and key immune components (Rolff and Siva-Jothy 2002).

Considering that the sexes possess different optimal reproductive phenotypes (Rice 1996), we might also expect the trade-off pattern between reproduction and immunity to be sexually dimorphic (Kurtz et al. 2000). In other words, because males are expected to invest more in current reproduction than females (Trivers 1972), fewer resources may be available for immune defense. Consequently, males would suffer higher rates of parasitic infection coupled with a shorter life span (Zuk 1990). This pattern of infection and longevity is well established in vertebrates and is usually attributed to the detrimental side effects of testosterone on immunity (Zuk and McKean 1996; Moore and Wilson 2002). However, due to the divergent selection pressures on males and females, one may expect this pattern to exist in other sexually reproducing organisms, regardless of hormonal in-

[2] Present address: Department of Genetics, University of Georgia, Athens, Georgia 30602; E-mail: fedorka@uga.edu.

teractions. Previous investigations into sex-specific parasite loads in testosterone-free systems (i.e., arthropods), however, have provided inconsistent results (Sheridan et al. 2000; Zuk et al. 2004).

Here, we investigated the cost of reproduction as mediated by immune response in the striped ground cricket *Allonemobius socius*. Among crickets, *Allonemobius* are unique because males possess a specialized tibial spur that the females chew during copulation, providing a hemolymph-based nuptial gift (Fedorka and Mousseau 2002a). Previous studies suggest that the size of the gift is positively related to male body size and to female reproductive success, creating direct selection on gift size due to a female mating bias for large males (Fedorka and Mousseau 2002b). Because many of the structural components of immunity reside in the hemolymph (Gupta 1991), this system offers a unique opportunity to examine how sexual selection influences the evolution of disease resistance. Due to both the cuticular wounding and hemolymph loss associated with spur feeding, we predicted that males would incur an exaggerated reproductive cost by engaging in prolonged or repetitive mating bouts. In addition, the oral transfer of defensive components between individuals is not uncommon (e.g., Eisner et al. 1997). Therefore, females may gain an immune advantage through increased courtship feeding if hemolymph-bound immune components are in some way transferable. Thus, how the sexes invest in immunity may be dimorphic, with males exhibiting a higher initial investment to compensate for their future loss.

In this study, we addressed two main hypotheses concerning the cost of reproduction and immunity: (1) increased reproductive effort reduces immune response, which leads to a reduction in survivorship, and (2) the trade-off patterns among reproduction, immunity, and survivorship are sex specific.

Methods

Immune Traits

Although not as complex as the vertebrate immune system, invertebrate immunity is multifaceted and exhibits a number of both generalized and specific defensive components (Pathak 1993). The cell-mediated response serves in an indiscriminate capacity, healing wounds, phagocytizing foreign material, and encapsulating macroparasites. In contrast, the humoral response regulates pathogenic challenges by producing a number of inducible, relatively specific antimicrobial peptides (e.g., cecropin, lysozyme, drosomycin, and lectin). However, unlike the vertebrate humoral system, there is little evidence that invertebrates possess an immunological memory (but see Kurtz and Franz 2003).

To obtain a robust description of an individual's cell-mediated and humoral immunity, four immune measures were obtained including hemocyte load, phenoloxidase activity, lytic activity, and encapsulation ability. (1) Hemocyte load refers to the concentration of circulating hemocytes (blood cells) in the hemolymph, which previous studies have shown to be positively associated with parasitic resistance (Eslin and Prevost 1998; Kurtz et al. 2000; Fellows and Godfray 2000; Kraaijeveld et al. 2001). (2) Phenoloxidase activity provided a measure of the hemolymph-bound enzyme, prophenoloxidase, which is also a key component of invertebrate innate immunity (Söderhäll and Cerenius 1998; Siva-Jothy et al. 2001; Rolf and Siva-Jothy 2002). (3) Lytic activity provided a measure of another hemolymph-bound enzyme, lysozyme, which provides a general defense against bacterial insults (Rantala and Kortet 2003). (4) Encapsulation ability provided a measure of the degree to which a macroscopic invading body (e.g., parasitoid egg) is engulfed by hemocytes and melanized, thereby killing the invader. Encapsulation requires the coordination of several cell-mediated and humoral immune components (Ratcliff 1993) and therefore provides a simple and reliable assay as to the direct effectiveness of an individual's immune system (Siva-Jothy et al. 2001).

Mating and Immune Assay

Experimental crickets were second generation laboratory-reared individuals derived from gravid, wild-caught females from central South Carolina. All crickets were maintained in $10 \times 10 \times 8$ cm plastic cages containing ground cat food, a carrot slice, dampened cheesecloth (water source and oviposition material), and strips of brown paper towel for cover. Every two days the food, carrot and paper towel were replaced. At this time, newly eclosed adults were separated and caged as sex-specific cohorts. All cages were kept in a constant environment at 28.5° C and a 12:12 (L:D) photoperiod provided by a Precision incubator (model 818; Precision Scientific, Chicago, IL). The age of laboratory reared experimental crickets was 12 ± 1 days posteclosion.

In this system, mating begins with a male performing a courtship song and display that culminates with the male orienting his abdomen toward a stationary female. If receptive, the female will briefly mount the male in a ''mock copulation'' lasting only a few seconds. The male will then cease courting and begin to form a spermatophore. When it is complete, he will renew his courtship behavior, again enticing the female to mount. At this time, the couple will adjoin abdomens as the male adheres the spermatophore to the female's seminal receptacle. The male will then bring his hind tibia forward allowing the female to chew on his spur until the pair separate (upwards of 30 min), transferring a substantial amount of hemolymph to his mate during this period (2.5% of his body mass on average; for details, see Fedorka and Mousseau 2002a).

To address the hypothesis that immune suppression serves as an underlying physiological link between reproductive effort and survivorship, we created three treatments that varied in mating frequency. Treatment A consisted of virgins (n = 60 males and females each), treatment B consisted of singly mated individuals (n = 60 males and females each), and treatment C consisted of multiply mated individuals (n = 46 males and females each). For all treatments individuals were randomly chosen from the stock population. Individuals from treatment B were mated 12 ± 1 days postadult eclosion. Treatment C individuals began mating one week after adult eclosion, and were provided a different partner once per day over five days (each individual acquired four matings on average). Males and females were rotated within this treatment so that mating partners had the same level of mating experience (i.e., 0, 1, 2, and 3 prior matings).

All matings were conducted in a mating arena constructed of a 75 mm petri dish lined with filter paper. The duration (in minutes) that the female chewed on the tibial spur was recorded for each mating trial. This provided an estimate of the nuptial gift size, with longer chewing duration resulting in a larger gift (Fedorka and Mousseau 2002a). All individuals were 12 ± 1 days old upon completion of their respective treatments, whereupon they were placed in a 50 mm petri dish along with a carrot slice and dampened cotton ball and maintained as above. Individuals were then examined every two days to estimate the rate of age-specific mortality, which was calculated as $-\ln(p_x)$, where p_x is the probability of survivorship for individuals entering age class x.

At 13 ± 1 days of age, we assayed all individuals for their immune function. For treatments B and C, this occurred 24 hours after their last mating. Crickets were cold anesthetized on ice for five minutes and 2.5 μl of hemolymph were removed between the 2nd and 3rd abdominal sternites using a microsyringe fitted with a 31-gauge, 0.5-inch needle (Hamilton Co., Reno, NV). Immediately following, we placed a 2 mm piece of monofilament directly into the hole created by the needle to simulate the invasion of a novel parasite. The monofilament strand was a piece of standard fishing line (0.2 mm diameter), roughened on sandpaper to better facilitate hemocyte adhesion, and knotted at one end. The knot allowed us to nondestructively remove the monofilament after six hours to assess the degree of encapsulation. This defense mechanism is commonly quantified by calculating the amount of melanin covering inert implants such as nylon monofilaments or Latex beads (Rantala et al. 2000; Rantala and Kortet 2003; Doums et al. 2002; Stolen et al. 1995; König and Schmid-Hempel 1995), with the darkness of the implant reflecting the degree of melanization.

From the syringe, the hemolymph was separated into two samples by dispensing 1.5 μl into a microcentrifuge tube containing 20 μl of phosphate buffered saline (PBS), and dispensing the remaining hemolymph into a microcentrifuge tube containing 9 μl of anticoagulant (39 mg NaOH + 85 mg NaCl + 37 mg EDTA + 79 mg citric acid + 1 L distilled water). Once separated, the PBS-bound hemolymph was frozen and maintained for several weeks at approximately −17°C, while the anticoagulant-bound hemolymph was immediately dispelled onto a hemocytometer and the number of hemocytes counted per milliliter.

Once all crickets had been processed in the above manner, the PBS-bound hemolymph was removed from the freezer and further separated into two 10 μl samples. To estimate the amount of prophenoloxidase in the hemolymph, 90 μl of a 15 mM L-Dopa buffered solution were added to the first sample. To estimate the amount of lysozyme in the hemolymph, 90 μl of a *Micrococcus leutus* buffered solution (3 mg of freeze-dried *M. leutus* per liter) were added to the second sample. These methods were modified from Rantala and Kortet (2003, 2004). The total change in optical density, OD, in both samples over 30 min served as our estimates of phenoloxidase and lytic activity, which was recorded at 490 nm using a microplate reader (model 550; Bio-Rad, Hercules, CA; OD ranges from 0.000 (transparent)–3.500 (opaque)).

Encapsulation ability was measured as the degree to which the monofilament was melanized after six hours, which was quantified using a camera-mounted dissecting microscope and National Institutes of Health (NIH) image software available at: http://rsb.info.nih.gov/nih-image. The degree of melanization was measured as the mean grey scale darkness of the pixels, with 0 being completely white and 255 being completely dark. Again using NIH image software, all crickets had their pronotum length measured as an estimate of body size. All analyses were performed using SAS (1999) version 8.12.

Results

Body size was not correlated to any immune parameters for either sex (all $P > 0.18$) and was therefore not included in subsequent immune analyses. However, several of the immune parameters were correlated with one another. For instance, in treatment A (mating control group) hemocyte load was found to be positively correlated with encapsulation ability ($r = 0.33$, $P < 0.01$) and with lytic activity ($r = 0.33$, $P < 0.01$). In addition, lytic activity was negatively associated with phenoloxidase activity, although this relationship was not significant ($r = -0.18$, $P = 0.09$). Thus, the concentration of circulating hemocytes appears to provide some insight into an individual's immune ability.

When the sexes were considered separately, immune function appeared to be sexually dimorphic, with males exhibiting a 34% greater hemocyte load (mean ± SE for males and females, respectively: 13.9 ± 1.2 vs. 10.4 ± 1.7; Wilcoxon two-sample test, $Z = -3.10$, $P < 0.002$), a 36% greater lytic activity (0.015 ± 0.002 vs. 0.011 + 0.001; $Z = -1.91$, $P = 0.056$), and 45% lower phenoloxidase activity (0.012 ± 0.004 vs. 0.022 ± 0.003; $Z = 4.05$, $P < 0.0001$) when compared to females. When the correlation between immune parameters were reexamined within each sex, female hemocyte load remained positively correlated with both lytic activity ($r = 0.37$, $P < 0.02$) and encapsulation ability ($r = 0.48$, $P < 0.01$) and male hemocyte load was positively associated with lytic activity ($r = 0.25$, $P < 0.08$).

When longevity was examined across treatments, we found a clear cost to reproduction in both sexes. As mating effort increased, males and females exhibited a shorter life span (Fig. 1; one-way ANOVA: $F_{2,286} = 10.43$: $P < 0.0001$; no significant sex effect or a sex by treatment interaction: $F_{2,287} = 0.60$, $P < 0.44$ and $F_{2,287} = 1.179$, $P < 0.17$, respectively), with virgins living approximately 40% longer than multiply mated individuals. Likewise, we found that mating effort dramatically increased the intrinsic baseline mortality rate (Fig. 2, intercepts) in both males and females (ANCOVA: $F_{2,72} = 8.52$, $P < 0.001$ and $F_{2,68} = 26.85$, $P < 0.0001$, respectively). Age-specific mortality rates (Fig. 2, slopes) within each sex did not differ across the treatments (age by treatment interaction: $F_{2,72} = 0.12$, $P < 0.89$ and $F_{2,68} = 0.08$, $P < 0.93$, for males and females, respectively). Interestingly, singly mated males exhibited a lower baseline mortality rate than their virgin counterparts (ANCOVA: $F_{1,54} = 7.85$, $P < 0.01$), whereas singly mated and unmated females showed no observable difference (ANCOVA: $F_{1,54} = 0.0$, $P < 0.96$). The male pattern in treatment B could be due to an up regulation of male immune parameters due to the small challenge posed by the single cuticular wounding event from

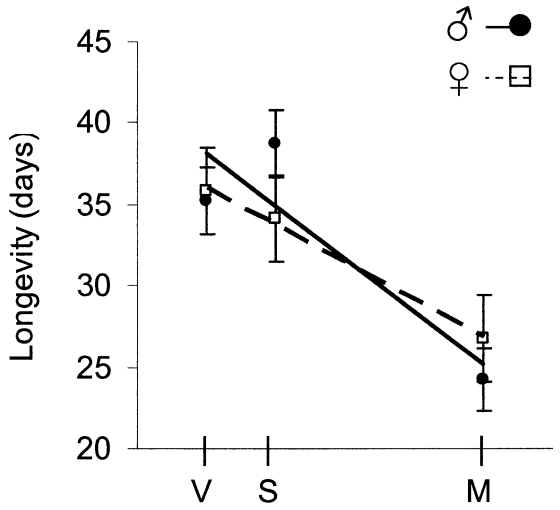

FIG. 1. Trade-off between mating effort and longevity (mean day ± SE) in *Allonemobius socius*. As mating effort increased from no mating (virgin, V) to single (S) and multiple (M) matings, both males (filled circle) and females (square) exhibited a reduced longevity. The multiply mated treatment consisted of four matings on average.

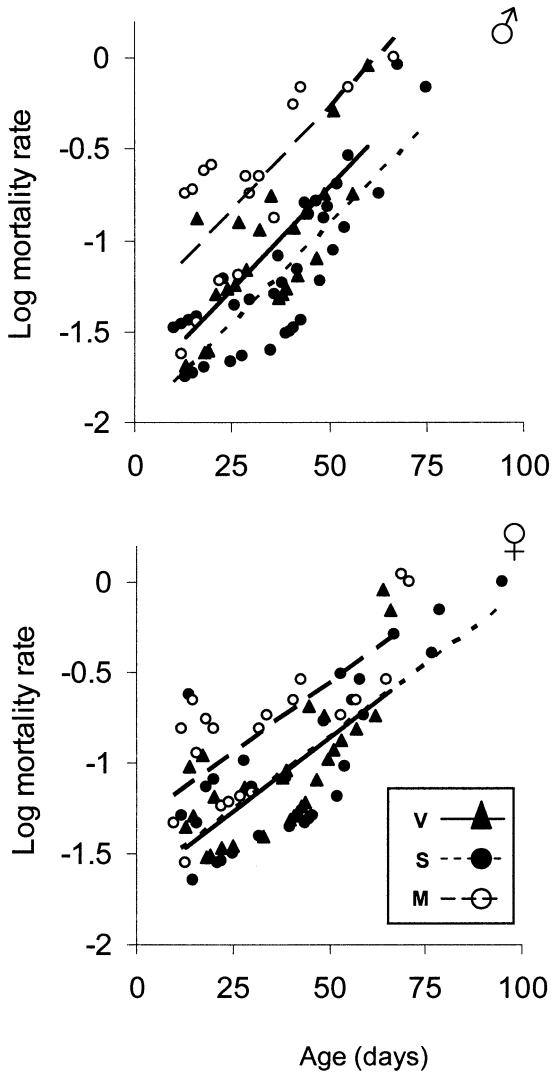

FIG. 2. Male and female mortality rates. As mating effort increased (V, virgin; S, singly mated; M, multiply mated), intrinsic baseline mortality rates (intercepts) increased for both sexes. However, age-specific mortality rates (slopes) within each sex did not differ between the treatments. When the sexes are directly compared, multiply mated males exhibited a significantly higher mortality rate than multiply mated females, suggesting that males incur a greater reproductive cost.

spur chewing. This up regulation may have cleared any standing infections that by themselves were not enough to induce an immune response, leading to longer lived singly mated males.

When the sexes are directly compared they showed no significant difference in their intrinsic baseline mortality rates (Fig. 2; ANCOVA: $F_{1,48} = 0.82$, $P < 0.37$; $F_{1,60} = 2.69$, $P < 0.11$; $F_{1,32} = 2.20$ $P < 0.15$ for treatments A–C, respectively). However, multiply mated males did exhibit a significantly higher age-specific mortality rate than multiply mated females (ANCOVA interaction: $F_{1,48} = 1.57$, $P < 0.22$; $F_{1,60} = 0.60$, $P < 0.44$; $F_{1,32} = 3.96$, $P = 0.055$; treatments A–C, respectively). Furthermore, males consistently exhibited a qualitatively higher age-specific mortality rate than females for all treatments (average male slope ± SE > average female slope ± SE: $b = 0.23 \pm 0.006 > b = 0.15 \pm 0.006$, respectively; $t_4 = -8.69$, $P < 0.001$). Thus, males appear to incur a greater fitness cost in terms of higher rates of intrinsic mortality due to mating.

As mating effort increased, individuals also exhibited a reduction in several aspects of immunity. Both males and females exhibited a reduction in hemocyte load (Fig. 3; MANOVA: $F_{2, 202} = 9.03$, $P = 0.0002$), lytic activity (MANOVA: $F_{2, 202} = 5.81$, $P < 0.01$) and encapsulation ability (MANOVA: $F_{2, 202} = 14.57$, $P < 0.0001$). The above MANOVA model included the significantly correlated immune response variables (i.e., hemocyte load, lytic activity, and encapsulation) along with the dependant variables of mating treatment and sex. In no case was there a significant interaction between sex and treatment (all $P > 0.18$), suggesting that the rate of decrease in immune function was similar between the sexes. However, males exhibited a qualitatively steeper slope for these immune parameters than did females (average male slope ± SE vs. females average slope ± SE: -0.34 ± 0.03 vs. $-.020 \pm 0.06$, respectively; $t_4 = -2.11$, $P = 0.05$), suggesting that immunosuppression may still be greater in males, although further investigation is needed to determine if this is true.

In contrast, females exhibited an increase in phenoloxidase activity as mating effort increased (Kruskal Wallis: $\chi^2 = 9.40$, $P < 0.01$; males exhibited no significant change in phenoloxidase: $F_{2,159} = 0.98$, $P < 0.38$). One possible mechanism underlying the female result was that they obtained a limited resource from the nuptial gift that allowed for the increase in phenoloxidase activity. Unfortunately, we found no association between gift size and female phenoloxidase activity after controlling for the number of matings ($r = 0.08$, $P < 0.42$). However, we did find a negative association between gift size and a female's ability to encapsulate (Spearman rank: $r = -0.29$, $P < 0.05$), implying that the gift may reduce female immunity. Unexpectedly, we found a positive asso-

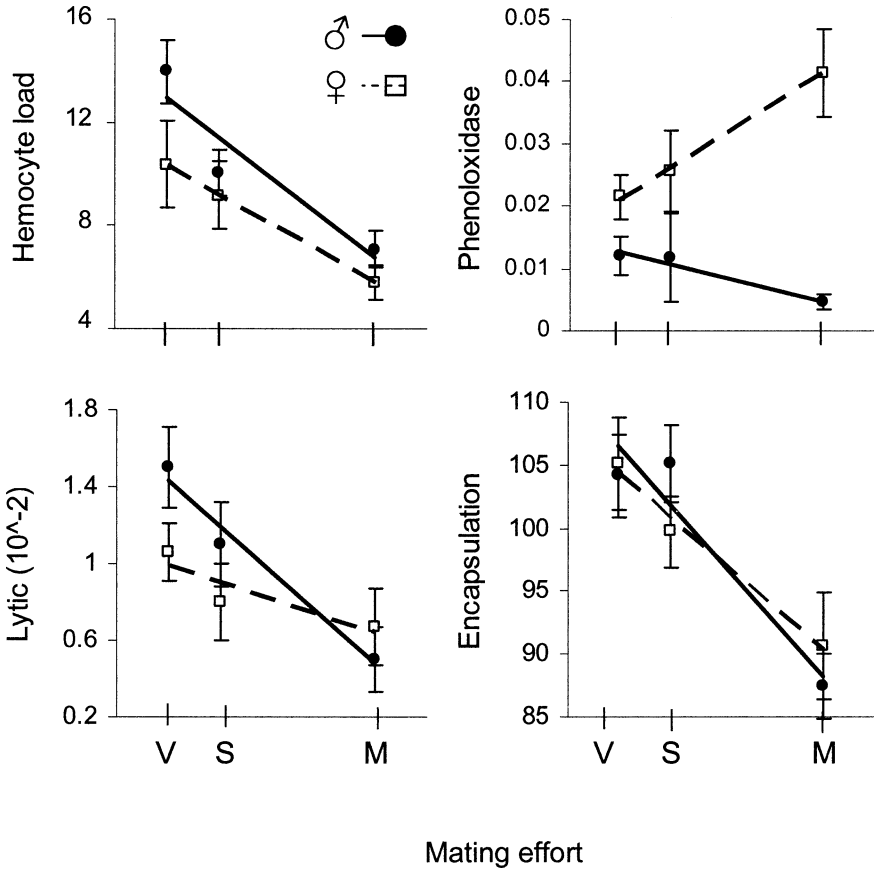

FIG. 3. Trade-off between mating effort and immunity (mean + SE). Both males and females exhibited significant reductions in hemocyte load, lytic activity, and encapsulation. There was no significant overall effect of treatment on phenoloxidase activity. However, there was a significant sex difference. When the sexes are examined separately, female phenoloxidase activity increased with mating effort, whereas males showed no significant change. See the text for more detail regarding the units of measure.

ciation between the size of the gift and male lytic activity (Spearman rank: $r = 0.29$, $P < 0.01$; controlled for number of matings) and encapsulation ability (Spearman rank: $r = 0.22$, $P < 0.053$; controlled for number of matings), suggesting that males who provided larger gifts had stronger immune responses.

Some aspects of immune defense were also directly related to longevity. For instance, individuals who exhibited a higher hemocyte load ($r = 0.14$, $P < 0.05$) and encapsulation ability ($r = 0.18$, $P < 0.01$) lived longer. Considering the above relationships, these data strongly suggest that mating effort decreases immunocompetence, which in turn decreases expected lifespan. Thus, immunosuppression appears to be a physiological mechanism mediating the expression of reproductive costs.

DISCUSSION

Increased reproductive effort has a considerable effect on immunity and survivorship in the ground cricket, *A. socius*. As mating frequency increased, hemocyte load, lytic activity, and encapsulation ability all decreased. Furthermore, the decrease in immune defense was directly correlated to the decrease in survivorship. These data imply that immune suppression mediates the expression of reproductive survival costs in this system. Considering that the fundamental properties of immune defense and reproduction are evolutionarily conserved, it is probable that the phenotypic trade-off described here is prevalent across a diverse array of taxa.

Although the competitive allocation of resources appears to be the most parsimonious explanation for the trade-off between reproduction and immunity, several other contributing mechanisms may exist. In males, the cuticular wounding and hemolymph loss incurred during nuptial feeding may increase the risk of pathogenic infection, as well as severely impede an effective immune response. In females, male substances may be received during copulation that negatively affect her immune capacity. For instance, in *Drosophila*, male accessory gland proteins (ACPs) transferred along with sperm have been shown to reduce female longevity (Lung et al. 2002). This pattern may be the result of the ACP's manipulation of female reproduction (Heifetz et al. 2000; Chen et al. 1988), an unintended toxic side effect or a direct interference with female immunological pathways (Barratt and Pockley 1998). Although the effects of ACPs have only been well characterized in a few species, similar effects may be widespread considering the potential ubiquity of sexual conflict.

Prior to mating, we found that males exhibited a greater hemocyte load, lytic activity, and lower phenoloxidase activity than females, suggesting that initial investment in im-

munity is sexually dimorphic. This divergent pattern is in accordance with previous work that showed that the size of the hemolymph gift was under strong, positive selection due to its direct benefits on female fecundity (Fedorka and Mousseau 2002b). Consequently, males may invest heavily in the number of circulating hemocytes to offset the immune costs of a larger hemolymph donation, making them initially more immunocompetent with regard to bacterial insults (lytic activity and hemocyte load were positively correlated) and less immunocompetent with regard to phenoloxidase activity (lytic and phenoloxidase activity were negatively associated).

Sex differences in immune function are common, and may be due largely to basic differences in sex-specific reproductive agendas (Zuk and McKean 1996). For instance, vertebrate males often experience a lower immunocompetence (e.g., Klein and Nelson 1997), higher rates of parasitic infection (Moore and Wilson 2002) and mortality (e.g., Promislow 1992). Invertebrate males show similar patterns (Radhika et al. 1998; Kurtz et al. 2000; Krutz and Sauer 2001). For instance, in the cricket, *Gryllus texensis*, males exhibit a significant decrease in immunity as they become sexually active when compared to juveniles, females, and early adulthood males (Adamo et al. 2001). However, due to the complex nature of life history trade-offs, differences in immune defense may be difficult to predict (Moret and Schmid-Hempel 2000; Doums et al. 2002; Zuk and Stoehr 2002). For instance, in the seasonally breeding cricket, *Teleogryllus commodus*, males show a stronger encapsulation response than females, where as the aseasonal *T. oceanicus* show no sexual difference (Zuk et al. 2004). This pattern appears to be the result of *T. commodus* females investing proportionally more in gonad weight compared to *T. oceanicus* females and males as well as *T. commodus* males (in that order). In other words, due to the ephemeral breeding season, female *T. commodus* may over invest in reproduction, which may lead to fewer resources available for immune defense. Thus, a weaker male immunity is not the universal rule.

In addition to the initial dimorphic immune investment found here, immunological response to mating also appears to be sexually dimorphic. Because many of the structural components of immunity reside in the hemolymph (Gupta 1991), we expected that males would incur a larger cost once mating began. Indeed, multiply mated males exhibited a greater mortality rate than multiply mated females (treatment C). In addition, for all treatments males exhibited a greater decline than females in all of the immune components measured, although these differences were not significant. The lack of significance found here may be due to our experimental design. In the field, males are sexually active for several weeks (Fedorka and Mousseau 2002b) and may be presented with more mating opportunities than allowed for in our experiment. If the function described in Figure 1 accurately approximates the true relationship between lifespan and mating, and if minor extrapolations are appropriate, than we would predict a 50% difference in life span between the sexes after only eight matings. Such a large difference in mortality rates between the sexes can play an important role in how senescence evolves in this system.

Considering the unique nature of the nuptial gift, we predicted that gift size might positively covary with female immunity, and negatively covary with male immunity. Surprisingly, we found the opposite pattern in that gift size was negatively associated with female encapsulation and positively associated with male lytic activity and encapsulation ability. This is an intriguing observation and several explanations exist that may account for the male pattern. First, males may actively up-regulate the level of lysozyme in their hemolymph in response to increased spur chewing. This seems reasonable because an increase in nuptial feeding frequency is associated with an increased number of wounding events, continually exposing the circulatory system to potential bacterial infection. Second, males in better initial condition, as indicated by their immune profile, may be better able to withstand longer chewing durations, which ultimately increases the amount of their sperm that is transferred to the female. This phenomenon is similar to the ''car-house'' paradox, in which individuals of higher initial quality can afford to pay a higher price without penalty (Reznick et al. 2000). For example, in red jungle fowl (*Gallus gallus*) roosters, males with more robust immune responses became dominant after being placed with a strange male, and their immunity actually became stronger (Zuk and Johnsen 2000). Third, a female may be able to assess male immune quality through courtship feeding and adjust her reproductive behavior accordingly. In other words, females may choose to mate longer with males of ''good genes'' (e.g., Ryder and Siva-Jothy 2000). These hypotheses are currently being investigated.

Previous studies in other systems including humans have provided empirical evidence regarding the immunosuppressive effects of mating (Barratte and Pockley 1998; McKean and Nunney 2001; Rolff and Siva-Jothy 2002). However, few have examined the long-term survival consequences of mating-induced immune suppression as presented here (but see Nordling et al. 1998). It is conceivable that the true relationship between immunity and survival in our study is correlative and not causal, and a more direct test would involve the experimental manipulation of immunity. Furthermore, the extent to which the phenotypic trade-off between mating and immunity is evolutionarily relevant will depend on its underlying genetic architecture (Reznick 1985). To this end, we are determining the degree to which this trade-off is genetically controlled.

In summary, our data suggest that an increased reproductive effort has a dramatic negative effect on immunocompetence in both sexes. We have also shown that the reduction in immunity is associated with a reduction in survivorship, providing a potential physiological mechanism that underlies the survival cost of reproduction. Last, it appears that sexual selection has greatly influenced the dimorphic evolution of disease resistance in this system.

Acknowledgments

We thank V. Tsan for assisting with cricket maintenance, A. Stoehr for assisting with data collection, M. Rantala for assisting with the immunity protocols, and W. Winterhalter for providing the cricket stocks. This work was supported by a National Science Foundation Postdoctoral Fellowship to KMF, a grant from the UCR Academic Senate to MZ and by a National Science Foundation grant to MZ and TAM.

Literature Cited

Adamo, S. A., M. Jensen, and M. Younger. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly G. integer): trade-offs between immunity and reproduction. Anim. Behav. 62:417–425.

Barratt, C. L. R., and A. G. Pockley. 1998. Sperm survival in the female reproductive tract: presence of immunosuppression or absence of recognition? Mol. Hum. Reprod. 4:309–313.

Chen, P. S., E. Stummzollinger, T. Aigaki, J. Balmer, M. Bienz, and P. Bohlen. 1988. A male accessory-gland peptide that regulates reproductive behavior of female *Drosophila melanogaster*. Cell 54:291–298.

Doums, C., Y. Moret, E. Benelli, and P. Schmid-Hempel. 2002. Senescence of immune defense in *Bombus* workers. Ecol. Entomol. 27:138–144.

Eisner, T, M. A. Goetz, D. E. Hill, S. R. Smedley, and J. Meinwald. 1997. Firefly ''femmes fatales'' acquire defensive steroids (lucibufagins) from their firefly prey. Proc. Natl. Acad. Sci. 94: 9723–9728.

Eslin, P., and G. Prevost. 1998. Hemocyte load and immune resistance to *Asobara tabida* are correlated in species of *Drosophila melanogaster* subgroup. J. Insect Physiol. 44:807–816.

Fedorka, K. M., and T. A. Mousseau. 2002a. Tibial spur feeding in ground crickets: larger males contribute larger gifts (Orthoptera: Gryllidae). Fla. Entomol. 85:317–323.

Fedorka, K. M., and T. A. Mousseau. 2002b. Nuptial gifts and the evolution of male body size. Evolution 56:590–596.

Fellows, M. D. E., and H. C. J. Godray. 2000. The evolutionary ecology of resistance to parasitoids by *Drosophila*. Heredity 84: 1–8.

Gupta, A. P., ed. 1991. Immunology of insects and arthropods. CRC Press, Boca Ratton, FL.

Hautekeete, N. C., Y. Piquot, and H. Van Dijk. 2001. Investment in survival and reproduction along a semelparity-iteroparity gradient in the Beta species complex. J. Evol. Biol. 14:795–804.

Heifetz, Y., O. Lung, E. A. Frongillo, and M. F. Wolfner. 2000. The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. Curr. Biol. 10:99–102.

Hutchings, J. A., A. Pickle, and C. R. McGregor-Shaw. 1999. Influence of sex, body size, and reproduction on overwinter lipid depletion in brook trout. J. Fish Biol. 55:1020–1028.

Klein, S. L., and R. J. Nelson. 1997. Sex differences in immunocompetence differ between two *Peromyscus* species. Am. J. Physiol. 273:R655–660.

König, C., and P. Schmid-Hempel. 1995. Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terristris* L. Proc. R. Soc. Lond. Biol. Sci. 260:225–227.

Kraaijeveld, A. R., E. C. Limentani, and H. C. J. Godray. 2001. Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Proc. R. Soc. Lond. B 268:259–261.

Kurtz, J., and K. P. Sauer. 2001. Gender differences in phenoloxidase activity of *Panorpa vulgaris* hemocytes. J. Invertebr. Pathol. 78:53–55.

Kurtz, J., A. Wiesner, P. Götz, and K. P. Sauer. 2000. Gender differences and individual variation in the immune system of the scorpionfly *Panorpa vulgaris* (Insecta: Mecoptera. Dev. Comp. Immunol. 24:1–12.

Kurtz, J., and K. Franz. 2003. Evidence fro memory in invertebrate immunity. Nature 425:37–38.

Lung, O., U. Tram, C. M. Finnerty, M. A. Eipper-Mains, J. M. Kalb, and M. F. Wolfner. 2002. The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. Genetics 160:211–224.

McKean, K. A., and L. Nunney. 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 98:7904–7909.

Moore, S. L., and K. Wilson. 2002. Parasites as viability cost of sexual selection in natural populations of mammals. Science 297: 2015–2018.

Moret, Y., and P. Schmid-Hempel. 2000. Survivial for immunity: the price of immune system activation for bumblebee workers. Science 290:1166–1167.

Nordling, D., M. Andersson, S. Zohari, and L. Gustafsson. 1998. Reproductive effort reduces specific immune response and parasitic resistance. Proc. R. Soc. Lond. B. 265:1291–1298.

Pathak, J. P. N., Ed. 1993. Insect immunity. Kluwer, Dordrecht, The Netherlands.

Promislow, D. E. L. 1992. Costs of sexual selection in natural populations of mammals. Proc. R. Soc. Lond. B 247:203–210.

Pyle, P., N. Nur, W. J. Sydeman, and S. D. Emslie. 1997. Cost of reproduction and the evolution of deferred breeding in the western gull. Behav. Ecol. 8:140–147.

Radhika, M., A. K. Abdul Nazar, N. Munuswamy, and K. Nellaippan. 1998. Sex-linked differences in phenoloxidase in the fairy shrimp *Streptocephalus dichotomous* Baird and their possible role (Crustacea: Anostraca). Hydrobiologia 377:161–164.

Rantala, M. J., J. Koskimaki, J. Taskinen, K. Tynkkynen, and J. Suhonen. 2000. Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens* L. Proc. R. Soc. Lond. Biol. Sci. 267:2453–2457.

Rantala, M. J., and R. Kortet. 2003. Courtship song and immune function in the field cricket *Gryllus bimaculatus*. Biol. J. Linn. Soc. 79:503–510.

———. 2004. Male dominance and immunocompetence in a field cricket. Behav. Ecol. 15:187–191.

Ratcliff, N. A. 1993. Cellular defense responses of insects: unresolved problems. *In* N. E. Beckage, S. N. Thompson, and B. A. Federici eds. Parasites and pathogens of insects. Academic Press, London.

Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44:257–267.

Reznick, D., L. Nunney, and A. Tessier. 2000. Big houses, big cars, superfleas, and the cost of reproduction. Trends Ecol. Evol. 15: 421–425.

Rice, W. R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 381:232–234.

Roff, D. A. 1992. The evolution of life histories. Chapman and Hall. New York.

Rolff, J., and M. T. Siva-Jothy. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. Proc. Acad. Sci. 99:9916–9918.

Ryder, J. J., and M. T. Siva-Jothy. 2000. Male calling song provides a reliable signal of immune function in a cricket. Proc. R. Soc. Lond. B 267:1171–1175.

SAS Institute. 1999. Version 8. SAS Institute, Cary, NC.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11:317–321.

Sheridan, L. A. D., R. Poulin, D. F. Ward, and M. Zuk. 2000. Sex differences in parasitic infections among arthropod hosts: is there a male-bias? Oikos 88:327–334.

Siva-Jothy, M. T., Y. Tsubaki, and R. E. Hooper. 1998. Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. Physiol. Entomol. 23:274–277.

Siva-Jothy, M. T., Y. Tsubaki, R. E. Hooper, and S. J. Plaistow. 2001. Investment in immune function under chronic and acute immune challenge in an insect. Physiol. Entomol. 26:1–5.

Söderhäll, K., and L. Cerenius. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunology 10:23–28.

Stolen, J. S., T. C. Fletcher, S. A. Smith, J. T. Zelikoff, S. L. Kaattari, R. S. Anderson, K. Söderhäll, and B. A. Weeks-Perrin. eds. 1995. Techniques in fish immunology. 4. Immunology and pathology of aquatic invertebrates. SOS Publications, NJ.

Tatar, M., and D. E. L. Promislow. 1997. Fitness costs of female reproduction. Evolution 51:1323–1326.

Trivers, R. L. 1972. Parental investment and sexual selection. *In* B. Campbell, ed. Sexual selection and the descent of man. Aldine Press, Chicago, IL.

Wheelwright, N. T., K. A. Tice, and C. R. Freeman-Gallant. 2003. Postfledging parental care in Savannah sparrows: sex, size and survival. Anim. Behav. 65:435–443.

Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 32:95–126.
Zuk, M. 1990. Reproductive strategies and sex differences in disease susceptibility: an evolutionary viewpoint. Parasitol. Today 6:231–233.
Zuk, M., and T. S. Johnsen. 2000. Social environment and immunity in male red jungle fowl. Behav. Ecol. 11:146–153.
Zuk, M., and K. A. McKean. 1996. Sex differences in parasite infections: patterns and processes. Int. J. Parasitol. 26: 1009–1024.
Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. Am. Nat. 160:S9–S22.
Zuk, M., L. W. Simmons, J. T. Rotenberry, and A. M. Stoehr. 2004. Sex differences in immunity in two species of field crickets. Can. J. Zool. 82:627–634.

Corresponding Editor: T. Tregenza