# Sex differences in immunity in two species of field crickets

**Marlene Zuk, Leigh W. Simmons, John T. Rotenberry, and Andrew M. Stoehr**

**Abstract:** Immune defense often differs between the sexes, with males often having a weaker response, at least among many vertebrates. We examined encapsulation ability, a cell-mediated immune response, in laboratory and field populations of two species of field crickets, *Teleogryllus oceanicus* (Le Guillou, 1841) and *Teleogryllus commodus* (Walker, 1869), which have different life histories. In the seasonally breeding *T. commodus*, males show a stronger encapsulation response than females in both the laboratory and the field, although the difference is more marked under field conditions. The aseasonal *T. oceanicus* showed no sex difference in encapsulation in either field or laboratory samples fed ad libitum, but when food was experimentally reduced, the same pattern of stronger male response emerged. It is possible that this pattern may result from selection on females to increase investment in reproduction when time and energy for breeding are limited, as is more likely for seasonal breeders or animals under food restriction.

**Résumé :** Au moins chez plusieurs vertébrés, les défenses immunitaires varient souvent en fonction du sexe et les mâles ont souvent des réponses plus faibles. Nous avons étudié la capacité d'encapsulation, une réponse immunitaire sous contrôle cellulaire, chez les populations sauvages et de laboratoire de deux espèces de grillons des champs, *Teleogryllus oceanicus* (Le Guillou, 1841) et *Teleogryllus commodus* (Walker, 1869), dont les cycles biologiques sont différents. Chez *T. commodus* à reproduction saisonnière, les mâles ont une réponse d'encapsulation plus importante que les femelles, tant en nature qu'en laboratoire, bien que la différence soit plus grande en nature. Chez *T. oceanicus* à reproduction non saisonnière, il n'y a pas de différences sexuelles dans l'encapsulation ni en nature, ni au laboratoire, lorsque les insectes sont nourris à volonté; cependant, lorsqu'il y a réduction expérimentale de la nourriture, la même réponse plus forte des mâles réapparaît. Il est possible que ce pattern soit dû aux forces de sélection sur les femelles pour accroître leur investissement dans la reproduction lorsque le temps et l'énergie sont limités, ce qui est plus probable chez les espèces à reproduction saisonnière ou chez les animaux privés de nourriture.

[Traduit par la Rédaction]

## Introduction

Immune defense is important for virtually all animals, and adaptations associated with disease resistance have attracted much recent attention from ecologists and evolutionary biologists (Sheldon and Verhulst 1996; Norris and Evans 2000; Rolff and Siva-Jothy 2003; Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003). Resistance has been shown to vary with numerous factors; for example, the ability of *Drosophila melanogaster* Meigen, 1830 to encapsulate parasitoid wasp eggs changes geographically, depending on the distribution of parasitoid species (Kraaijeveld and van Alphen 1995; Kraaijeveld and Godfray 1999). Immune response can also change with age (Rolff 2001; Doums et al. 2002), social situation (Klein 2000; Zuk and Johnsen 2000), season (Zuk and Johnsen 1998; Yourth et al. 2002), and nutritional status (Moret and Schmid-Hempel 2000; Siva-Jothy and Thompson 2002). Although earlier studies focused on vertebrates, it is becoming increasingly clear that invertebrates can also provide model systems for examining the evolution of immune defense (Siva-Jothy 2000; Zuk and Stoehr 2002; Rolff and Siva-Jothy 2003).

Like many other traits, immune defense is often different in males and females, with males, at least among vertebrates, frequently having a weaker response (Zuk 1990; Zuk and McKean 1996; Owens 2002). At a proximate level, this difference has generally been attributed to the immunosuppressive effects of testosterone (Alexander and Stimson 1988; Schuurs and Verheul 1990), which is lacking in invertebrates. On an ultimate level, however, differential selection on the sexes may have favored different investment levels in disease resistance (Zuk 1990; Rolff 2002; Zuk and McKean 1996; Moore and Wilson 2002). Males that take large risks in sexual competition because they may also accrue large gains might be expected to show more pronounced selection for investment in reproduction at the expense of disease resistance. Where male and female investment is relatively equal, the difference between the sexes in immune response/disease susceptibility may be slight (Zuk 1990; Zuk and McKean 1996). This disparity in how the sexes allocate resources could therefore result in sexual dimorphism in immune response even among taxa lacking testosterone. Efforts to examine the tendency for a sex difference in parasite levels or immune response in invertebrates have met with mixed results; Sheridan et al. (2000) found no evidence of a

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male bias in parasite levels of a variety of invertebrate hosts, whereas studies of crickets (species of *Gryllus* (Linnaeus, 1758)), scorpionflies (*Panorpa vulgaris* (Imhoff and Labram, 1836)), fairy shrimp (*Streptocephalus dichotomus* Baird, 1860), and the damselfly *Lestes viridis* (Vander Linden, 1825) showed a stronger immune response in females (Radhika et al. 1998; Kurtz et al. 2000; Adamo et al. 2001; Kurtz and Sauer 2001; Rolff 2001).

Regardless of whether vertebrates or invertebrates are examined, however, an increasing number of studies of sex differences in immune response or parasite infection also recognize that immune defense, like many other life-history traits, is highly context dependent (Zuk and Stoehr 2002; Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003). For example, encapsulation of mite feeding tubes by the damselfly *Lestes forcipatus* Rambur, 1842 was more vigorous later in the season (Yourth et al. 2002); also, older bumblebees (*Bombus terrestris* (Linnaeus, 1758) and *Bombus lucorum* (Linnaeus, 1761)) were less efficient at encapsulation than younger bumblebees (Doums et al. 2002). This variation in immune response under different social or environmental circumstances makes generalization about sex differences in immunity difficult, and suggests that it is important to study immune response in a variety of species with different life histories and under various conditions.

We were able to examine sex differences in encapsulation ability, one of the main mechanisms of cell-mediated immunity in arthropods, in two species of field crickets: *Teleogryllus oceanicus* (Le Guillou, 1841), which is a continuously breeding semi-tropical species that occurs throughout northern Australia and the Pacific Islands, and *Teleogryllus commodus* (Walker, 1869), which is a seasonal breeder found in southern Australia (Otte and Alexander 1983). As part of a long-term project examining call variation and sexual selection, we have established six laboratory populations of *T. oceanicus*, including three from the Hawaiian Islands where the cricket was introduced at least 130 years ago and where it is subject to an acoustically orienting parasitoid, the ormiine fly *Ormia ochracea* Bigot, 1889 (Zuk et al. 1993, 1995, 2001). We also compared male and female encapsulation ability in a field population of Hawaiian *T. oceanicus*, as well as in both laboratory and field populations of *T. commodus*. In the samples of *T. commodus* and in the field population of *T. oceanicus*, we also obtained the gonad and body masses of both males and females to assess the possible relative allocation of resources to immune defence versus reproduction. In the field-collected *T. commodus* sample, we noted the presence of gregarine parasites in the gut; these protozoa can have debilitating effects on cricket spermatophore replacement rate, developmental time, and mass gain, particularly when food is limited (Zuk 1987*a*, 1987*b*). Gregarines were absent from all other samples. Finally, we report the results of an experiment examining the effect of food deprivation on encapsulation ability of male and female *T. oceanicus*.

We addressed four questions. (1) Do the two species differ in encapsulation ability and do they both show a sex difference in encapsulation? Because *T. oceanicus* breeds throughout the year, males and females might have different patterns of energy allocation toward reproduction versus immunity than *T. commodus*, which has an abbreviated mating season in late summer and early autumn and which diapauses in the egg stage (Browning 1954). Female crickets devote a large portion of their tissues to eggs and associated structures, and when reproduction occurs in a relatively concentrated period, we expect them to face a more stringent trade-off than either conspecific males or crickets of either sex from a species in which breeding is more extended. (2) Are any sex differences in encapsulation seen in both laboratory and field populations and how are these related to relative investments in gonadal tissue? If resources are limiting in the field, animals may be more likely to show a cost of mounting an immune response; in *B. terrestris*, starved bumblebees had lower survivorship after an immune challenge, whereas bumblebees fed ad libitum lived as long as unchallenged controls (Moret and Schmid-Hempel 2000). Alternatively, if relaxed selection in the laboratory causes a reduction in costly immune machinery, laboratory populations might have poorer responses. Field-collected crickets might therefore display a sex difference in immune response not seen under laboratory conditions. (3) Are any sex differences in encapsulation ability exaggerated by food deprivation? By similar reasoning to that in question 2, food-deprived crickets may differ in their energy allocation to reproduction versus immune function. (4) Does encapsulation ability reflect infection with gregarine parasites and do the sexes differ in the prevalence of infection?

## Materials and methods

### Collection and maintenance of crickets

The three Australian *T. oceanicus* laboratory colonies came from crickets that were collected in 1996 from Mission Beach and Cairns in Queensland and Carnarvon in north Western Australia (details of sites in Zuk et al. 2001). The Hawaiian *T. oceanicus* laboratory colonies came from crickets that were collected in 1993, 1994, 1998, and 1999 from Hilo on the island of Hawai′i, the University of Hawai′i at Manoa on the island of O′ahu, and the island of Kaua′i. The *T. commodus* colony came from crickets collected during 2001 near Walpole, south Western Australia. For field samples, calling males were localized by ear and silent males and females were collected opportunistically from the grassy habitat preferred by both cricket species; all samples were collected during the breeding season. All crickets of both laboratory and field populations are macropterous.

Laboratory colonies were reared in humid incubators at 30 °C under a 12 h light : 12 h dark cycle (1900:0700). Between 20 and 30 adults were kept in plastic containers (approximately 15 cm × 27 cm × 38 cm) with egg cartons for cover and with Fluker's® cricket feed and water available ad libitum.

### Melanization assay of encapsulation response

As part of a cell-mediated immune response, arthropods marshal hemocytes and melanin to surround foreign particles such as parasitoid eggs or bacteria (Gupta 1991; Pathak 1993; Vass and Nappi 2001). This defense mechanism is commonly quantified by examining the degree to which inert implants such as nylon monofilaments or Latex® beads are covered by melanin (König and Schmid-Hempel 1995; Stolen et al. 1995; Rantala et al. 2000; Doums et al. 2002;

Rantala et al. 2003; Rantala and Kortet 2003). The darkness of the implant reflects the degree of melanization of the particle and is also correlated with hemocyte count and phenoloxidase (E.C. 1.14.18.1) activity, two other measures of immune function (Rantala et al. 2000, 2003). We assayed the encapsulation response by implanting a 3 mm long nylon monofilament (diameter 0.255 mm) in the body cavity of the cricket. The surface of the monofilament was roughened by rubbing it against sandpaper before use; this treatment enhanced the likelihood that hemocytes would stick to the implant. After implantation each cricket was housed separately in a 130-mL plastic vial with dry cat food and water ad libitum. The crickets were frozen 36 h after implantation.

The implant was dissected out from the body cavity and cleaned with 70% ethanol. It was then placed in a cavity slide, fixed with mounting medium (either Eukitt® or Permount®), and covered with a cover slip. To measure the degree of encapsulation, each implant was photographed with a digital camera attached to a microscope and the photograph analyzed using NIH Image software. The program provides a measurement of the mean grey-scale darkness of the pixels contained in a designated area, with 0 being completely white and 256 being completely dark. To control for variation in the color of the mounting medium, we first outlined the implant on the photograph in the screen; each outlined area of the implant in the photograph was then compared with an identical area in the same slide next to the implant, and the measurement used was the mean darkness of the implant minus the mean darkness of the control area.

### Morphological measures and gregarine assessment

When the implants were removed from the crickets, pronotum width was measured to the nearest 0.01 mm with digital calipers. For the *T. commodus* samples and the Hilo *T. oceanicus* field sample, wet body mass and gonad mass (eggs and ovarioles for females, testes and accessory glands for males) were taken to the nearest 0.0001 g with a Sartorius microbalance.

Gregarines are readily visible in the gut under a dissecting microscope (Zuk 1987*a*), and their presence or absence in the *T. commodus* field sample was noted at the time of dissection.

### Food-deprivation experiment

We randomly assigned male and female *T. oceanicus* from the Mission Beach population to one of two levels of dietary restriction. The level 1 diet was made by mixing 42 g of standard Fluker's cricket diet with 6 g of agar, 200 mL of distilled water, 2.5 mL of a solution containing methyl *p*-hydroxybenzoate in ethanol (to prevent fungal growth on the diet), and 0.125 g of aureomycin (to prevent bacterial growth). The diet was poured into containers while still warm, and after hardening, it was cut into small blocks of approximately 0.15 g. The level 2 diet was prepared in the same way, except that 21 g of cricket diet and 21 g of powdered cellulose were used in place of the 42 g of cricket diet in the level 1 diet. A random sample of 15 blocks from each diet did not differ significantly in mass (Student's *t* test, $t_{28}$ = 0.177, $p$ = 0.86). Although the diets differed from each other, both were nutritionally poor compared with the cricket chow used for colony maintenance as shown by the mass loss that crickets experienced on each diet (see Results).

Each cricket was placed in a separate transparent plastic 130-mL container with a perforated plastic lid. Each cricket was supplied with a small water-soaked sponge, a piece of egg carton for shelter, and a block of food (from the appropriate treatment group). Every other day we soaked the sponge with water if it was dry, replaced the food block, and, if necessary, changed the shelter and cleaned the container. We weighed each cricket on the first day of the experiment (day 0) and then again 5 days later (day 4). On the morning of day 14, the crickets were implanted with nylon implants as described above and 36 h later (the evening of day 15) were frozen.

Mortality was recorded over the course of the experiment. Values are means ± SE.

## Results

The two species differed in encapsulation scores, with *T. commodus* showing a stronger response in both males and females than *T. oceanicus* (Table 1, Fig. 1). Within the *T. oceanicus* populations, the Australian groups had a stronger response than the Hawaiian populations (Table 2, Fig. 1); no differences within Australia were apparent ($F_{[2,93]}$ = 1.42, $p$ = 0.25), but Hilo crickets had significantly lower encapsulation scores than the other two Hawaiian populations ($F_{[2,269]}$ = 15.51, $p < 0.001$; encapsulation scores for Hilo = 146 ± 2.83, Kaua′i = 167 ± 3.98, and O′ahu = 169 ± 3.47). *Teleogryllus commodus* also showed a sex difference in encapsulation ability, with males exhibiting a stronger response than females; however, no sex difference was apparent in *T. oceanicus* (Tables 1 and 2, Fig. 1). A comparison of laboratory and field samples revealed the same pattern of sex-biased immune response in *T. commodus* and no sex difference in *T. oceanicus* (Table 3, Fig. 2). In both species, encapsulation ability was lower in the field samples (Fig. 2). Note that the laboratory–field comparison for *T. oceanicus* only included crickets from Hilo, as this was the only population for which both laboratory and field samples were available.

The percentage of body mass devoted to gonadal tissue was not correlated with encapsulation ability for either sex in either species (*T. commodus*: females, $r$ = 0.08, $p$ = 0.46, $n$ = 91 and males, $r$ = 0.03, $p$ = 0.74, $n$ = 148; *T. oceanicus*: females, $r$ = –0.24, $p$ = 0.15, $n$ = 37 and males, $r$ = 0.08, $p$ = 0.58, $n$ = 47). Reproductive allocation, or gonad mass as a proportion of body mass, was greater in *T. commodus* females than in *T. oceanicus* females, whereas the proportion of gonad mass was slightly greater in *T. oceanicus* males than in *T. commodus* males as indicated by the significant interaction term from an ANOVA using sex and species as independent variables (Table 4, Fig. 3; percent gonad was arcsine square-root transformed in the analyses). Reproductive allocation did not differ in laboratory versus field-collected crickets (ANOVA, $F_{\text{sex} \times \text{field/laboratory status}}$ = 0.16, $p > 0.69$). Body size, whether measured as total wet mass or as pronotum width, was not correlated with encapsulation ability in any of the samples.

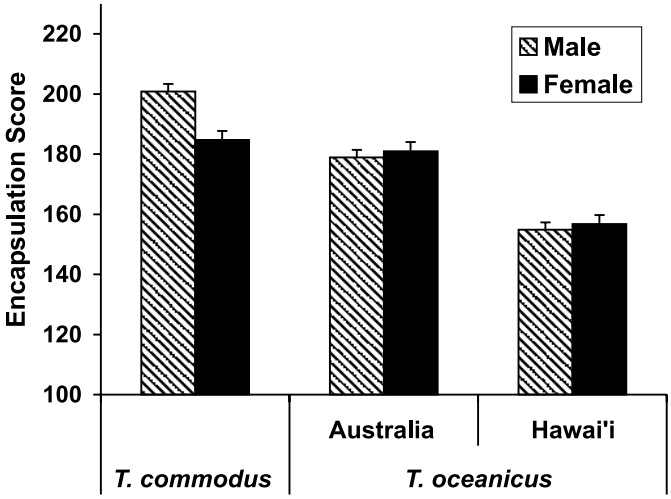
Gregarines were found more frequently in *T. commodus* males than in females, with 20 of 116 males (17.2%) and 2

**Table 1.** Results of the ANOVA for differences in encapsulation ability in male and female *Teleogryllus commodus* and *Teleogryllus oceanicus*.

| Source | df | Mean square | *F* | *P* |
|---|---|---|---|---|
| Model | 3 | 56 552 | 57.39 | <0.0001 |
| Species | 1 | 132 163 | 134.11 | <0.0001 |
| Sex | 1 | 7 221 | 7.33 | 0.0070 |
| Species × sex | 1 | 11 244 | 11.41 | 0.0008 |
| Error | 604 | 985 | | |

**Note:** Mean square and *F* values are from type III sums of squares.

**Fig. 1.** Encapsulation ability in *Teleogryllus commodus* ($n$ = 92 females and 148 males) and *Teleogryllus oceanicus* (scores from *T. oceanicus* are shown separately for populations from Australia (Cairns, Carnarvon, and Mission Beach pooled: $n$ = 45 females and 51 males) and Hawai'i (O'ahu, Kaua'i, and the Big Island pooled: $n$ = 130 females and 142 males)). Encapsulation is measured as the darkness score from 0 (completely white) to 256 (completely black) of a photograph of a nylon implant. See text for details.




of 61 females (3.3%) infested (Fisher's exact test, $p$ = 0.007). Encapsulation ability did not differ in groups with and without the parasites (201.41 ± 7.7 for infested males vs. 198.46 ± 2.41 for uninfested males; Student's $t$ test for unequal variances, $t_{114} = -0.36$, $p = 0.72$). Females were not compared because of the small sample size of infested crickets.

Both diets in the food-deprivation experiment caused the crickets to lose mass (0.054 ± 0.008 g ($n$ = 65) for the level 1 diet group and 0.068 ± 0.007 g ($n$ = 67) for the level 2 diet group), but the amounts lost did not differ significantly between treatments even when the sexes were separated (Student's $t$ test, $t_{130} = 1.31$, $p = 0.19$), so we pooled data from both treatments in subsequent analyses. Females lost significantly more mass than males (0.098 ± 0.011 vs. 0.041 ± 0.004 g, respectively; Student's $t$ test, $t_{130} = -4.66$, $p <$ 0.001). Although more males (47 out of 96) died during the course of the experiment than females (17 out of 50), this difference was not significant (Fisher's exact test, $p = 0.11$). Both diet and sex had significant effects on encapsulation rate when the data from the restricted diet groups were compared with Australian crickets fed ad libitum (Table 5). In contrast to other comparisons in *T. oceanicus*, males from the food-deprivation experiment had significantly higher encapsulation rates than females (204 ± 3.4 ($n$ = 50) vs. 185 ± 6.1 ($n$ = 31), respectively; Student's $t$ test for unequal variances, $t_{49} = -2.68$, $p = 0.01$).

**Table 2.** Results of the ANOVA for differences in encapsulation ability in male and female *T. oceanicus* from Hawai'i and Australia.

| Source | df | Mean square | *F* | *P* |
|---|---|---|---|---|
| Model | 1 | 14 761 | 20.76 | <0.0001 |
| Sex | 1 | 279 | 0.26 | 0.6075 |
| Population | 1 | 41 292 | 39.08 | <0.0001 |
| Sex × population | 1 | 0.42257 | 0 | 0.9841 |
| Error | 364 | 1 056 | | |

**Note:** Mean square and *F* values are from type III sums of squares.

## Discussion

Much of the literature on sex differences in immune response has focused on the proximate causes for the distinction, which has led to an emphasis on vertebrates (Alexander and Stimson 1988). However, these differences can be viewed in the same ultimate context as other differences between males and females, namely as the result of sexual selection and other forces acting on life history (Zuk 1990; Zuk and McKean 1996; Moore and Wilson 2002; Rolff 2002). We found that *T. commodus* and *T. oceanicus*, two very closely related species with many similarities in behaviour and morphology, nonetheless differed in the degree to which they exhibited a sex difference in encapsulation ability. However, unlike previous predictions (Zuk 1990) and several other studies of sex differences in immunity in invertebrates (Radhika et al. 1998; Adamo et al. 2001; Kurtz and Sauer 2001), we found a male-biased, rather than a female-biased, superiority in immune ability. What might account for this result?

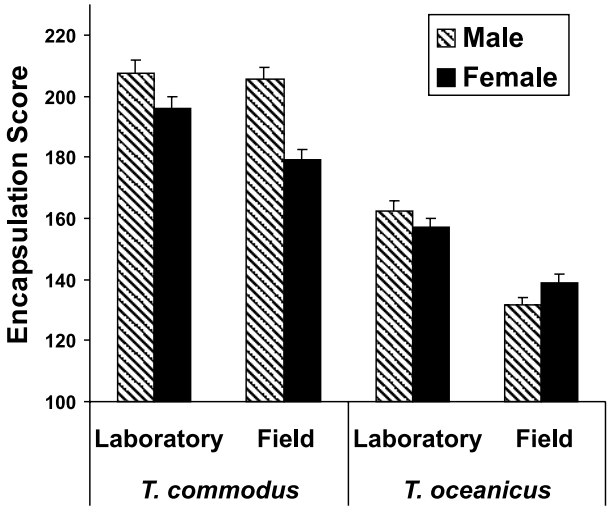
*Teleogryllus commodus* has one generation per year, with adults singing and mating from late summer to early autumn (March–April; Browning 1954), although nymphs are present throughout a large portion of the breeding period. In contrast, *T. oceanicus* breeds continuously, with approximately four generations per year (Otte and Alexander 1983; M. Zuk, personal observations). *Teleogryllus commodus* is therefore expected to concentrate its allocation of resources toward reproduction in a shorter time period than *T. oceanicus*, because crickets eclosing later in the season will be at a disadvantage unless they can reproduce relatively quickly. Selection should thus favor early reproduction with a demand for rapid marshalling of resources into reproductive activity as it does under any life-history regime with an increased chance for early mortality. This distinction should be particularly pronounced for females, which put a larger proportion of their body mass into reproductive tissue (Fig. 3). We suggest that the sex under the most constraints for reproductive effort, which may be female in *T. commodus*, might also be expected to show a decrease in investment in immunity.

**Table 3.** Results of the ANOVA for differences in encapsulation ability in male and female *T. commodus* and *T. oceanicus* from laboratory and field populations.

| Source | df | Mean square | *F* | *P* |
|---|---|---|---|---|
| *T. commodus* | | | | |
| Model | 3 | 7 538 | 11.03 | <0.0001 |
| Sex | 1 | 11 318 | 16.55 | <0.0001 |
| Laboratory/field | 1 | 7 474 | 10.93 | 0.0011 |
| Sex × laboratory/field | 1 | 780 | 1.14 | 0.2865 |
| Error | 236 | 161 358 | | |
| *T. oceanicus** | | | | |
| Model | 3 | 27 359 | 28.93 | <0.0001 |
| Sex | 1 | 618 | 0.65 | 0.4191 |
| Laboratory/field | 1 | 77 730 | 82.20 | <0.0001 |
| Sex × laboratory/field | 1 | 1 291 | 1.37 | 0.2434 |
| Error | 364 | 945 | | |

**Note:** Mean square and *F* values are from type III sums of squares.
*All *T. oceanicus* are from Hilo, Hawai'i.

**Fig. 2.** Encapsulation ability in *T. commodus* (laboratory: $n = 31$ females and 32 males; field: $n = 61$ females and 116 males) and *T. oceanicus* (laboratory: $n = 37$ females and 35 males; field: $n = 37$ females and 47 males).. Note that only crickets from Hilo on the Big Island of Hawai'i made up the *T. oceanicus* sample. Encapsulation is measured as the darkness score from 0 (completely white) to 256 (completely black) of a photograph of a nylon implant. See text for details.




The standard dogma of sexual selection is that males, because they are limited in reproductive success by the number of females they can inseminate, should be more risk-prone and concentrated in their reproductive effort than females, which are limited by the much less variable number of offspring they can produce and rear (Trivers 1972; Andersson 1994). For species in which females must sequester all of the resources necessary for egg production in a relatively short period, however, which includes many invertebrates, it may be that females rather than males have the "live hard, die young" strategy. This more general view of male and female reproductive strategies may explain why some studies have found sex differences in immune response in the predicted direction of female superiority and some have not. Adamo et al. (2001) found that female *Gryllus texensis*

**Table 4.** Results of the ANOVA for the percentage of body mass consisting of gonadal tissue in male and female *T. commodus* and *T. oceanicus*.

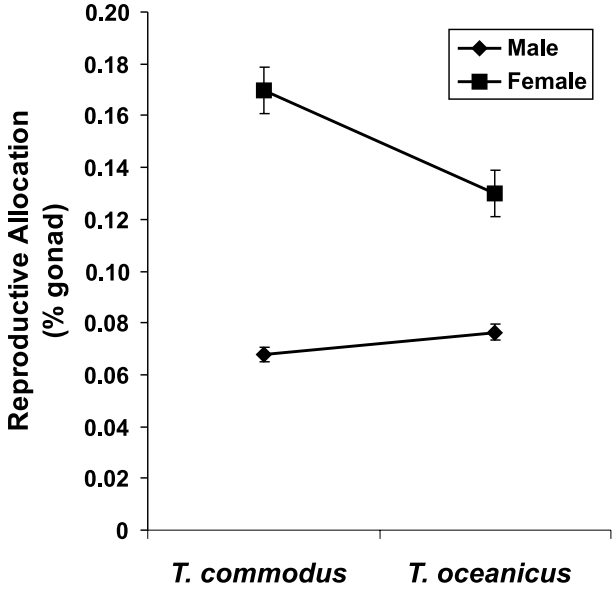
| Source | df | Mean square | *F* | *P* |
|---|---|---|---|---|
| Model | 3 | 0.4203 | 48.29 | <0.0001 |
| Species | 1 | 0.0050 | 0.58 | 0.45 |
| Sex | 1 | 0.7622 | 87.57 | <0.0001 |
| Species × sex | 1 | 0.0459 | 5.27 | 0.02 |
| Error | 325 | 0.0087 | | |

**Note:** Percentages were arcsine square-root transformed before analysis. Mean square and *F* values are from type III sums of squares.

(Cade and Otte, 2000), a species that breeds over an extended period, had higher prophenoloxidase (E.C. 1.14.18.1) activity (a component of the melanization response needed for encapsulation) and were less susceptible to bacterial infection than males, which is in contrast to the results reported here. Perhaps females invest relatively less in eggs and ovarian tissue in this species compared with the genus *Teleogryllus*, particularly *T. commodus*. It would be interesting to see if more "invertebrate-like" vertebrates, such as semelparous fish with very brief breeding periods, also show a male bias in the strength of the immune response. Poulin (1996) found that birds and mammals were more likely than the other vertebrates examined to have a male bias in parasite burdens, but variation in sampling techniques and other methodologies make interpretation of this taxonomic restriction difficult.

The tentative interpretation outlined above is supported by our comparison of laboratory and field populations; assuming that resources are more limiting in the field, we expect that allocation to immune defense might be decreased under these conditions, as indeed they were in both species. Interestingly, the difference between male and female encapsulation ability also became larger in the field sample of *T. commodus* compared with the laboratory sample, mainly driven by a large decrease in female immune response in the field sample compared with laboratory-reared crickets, rather than by an equivalent drop in the immune defense of both sexes. Females also appear to be more sensitive than

**Fig. 3.** Reproductive allocation, defined as the percentage of total wet mass consisting of gonads, in female and male *T. commodus* ($n$ = 91 females and 141 males) and *T. oceanicus* ($n$ = 37 females and 48 males). Note that field and laboratory samples were pooled and only crickets from Hilo on the Big Island of Hawai'i made up the *T. oceanicus* sample.




males to changes in resource availability in the genus *Drosophila* (Chippindale et al. 1997, 1998). It would be interesting to track changes in the sex difference in immune response over the course of the active season for *T. commodus*.

The results from the food-deprivation experiment similarly upheld the notion that females are more hard pressed to allocate sufficient resources to both immune defense and reproduction when resources are limiting; by stressing crickets nutritionally we uncovered a sex bias in the encapsulation ability of *T. oceanicus* that had not been evident in either the field or the laboratory populations. This sex bias in encapsulation ability was coupled with a sex bias in mass loss that was due to the diet manipulation. Again, this suggests that severe food restriction can change the apparent difference between the sexes in immune response and that immune defense, like many other life-history traits, is phenotypically plastic.

The Hawaiian populations of *T. oceanicus* had lower encapsulation abilities than the Australian populations. This finding may be related to the presence of an acoustically orienting parasitoid fly, *O. ochracea*, in the Hawaiian populations (Zuk et al. 1993, 2001). Although at first it might seem that selection pressure from a parasitoid would cause an increase and not a decrease in encapsulation ability, the biology of the fly as opposed to wasp parasitoids actually supports the lowered immune response in the affected populations. Unlike wasps, which inject inert eggs into the body cavity of their host, the flies deposit mobile planidium larvae on and around the host once they have localized it (Cade 1975; Adamo et al. 1995). The larvae then burrow inside the cricket and use the initial stages of the host encapsulation response to construct breathing tubes. An increased encapsulation response may thus be a disadvantage when dealing with the parasitoid, because it might enhance its larval growth. Alternatively, if the encapsulation response is costly, which seems likely given its reduction during resource restriction, but cannot effectively combat the parasitoid because of the advanced stage of development of larvae at infestation, selection may have favored decreased investment in cell-mediated immunity.

**Table 5.** Results of the ANOVA for differences in encapsulation ability in male and female *T. oceanicus* from Australia fed either a restricted diet or ad libitum.

| Source | df | Mean square | $F$ | $P$ |
|---|---|---|---|---|
| Model | 3 | 6322 | 8.47 | <0.0001 |
| Sex | 1 | 2903 | 3.89 | 0.05 |
| Diet | 1 | 8882 | 11.89 | 0.0007 |
| Sex × diet | 1 | 4535 | 6.07 | 0.0147 |
| Error | 173 | 746 | | |

**Note:** Mean square and $F$ values are from type III sums of squares.

Gregarine infestation in our sample of *T. commodus* was male-biased and not female-biased; furthermore, the presence of these parasites was unrelated to encapsulation ability. The male bias in gregarine infestation mirrors a similar finding in the related crickets *Gryllus veletis* (Alexander and Bigelow, 1960) from Michigan (Zuk 1987*a*) and *Gryllus bimaculatus* (de Geer, 1773) from southern Spain (Simmons and Zuk 1992), although a meta-analysis of invertebrate parasites from a variety of taxa revealed no difference between the sexes in parasite levels (Sheridan et al. 2000). In southern France, male *Calopteryx splendens* (Harris, 1782) with lighter, less homogenous wing pigmentation were less resistant to gregarines and showed a greater increase in prophenoloxidase activity following immune challenge with nylon implants but exhibited no difference in the degree of encapsulation of the implant (Siva-Jothy 2000). Encapsulation is mainly used to combat parasitoid eggs and similar objects in the body cavity (Gupta 1991); it may be that gregarines, which are protozoan gut parasites transmitted through fecal contamination of food or via grooming (Zuk 1987*b*), are not accessible to the cell-mediated immune system.

Although sex differences in immune response in many more species with seasonal and aseasonal reproduction would need to be compared to definitively test our idea, we think that this broader perspective on why sex differences in immune response occur may be helpful in stimulating further research. Maintenance of an effective defense against pathogens almost out of necessity involves trade-offs between immune response and other life-history traits (Zuk and Stoehr 2002). Viewing sex differences in immunity as evolved characteristics rather than incidental byproducts of physiology can help us to understand how those differences might result in patterns of male and female resistance among species with different mating systems, breeding phenology, or predation risk.

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