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Sexually dimorphic immune response in the harem polygynous Wellington tree weta *Hemideina crassidens*

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Abstract. Adult males are often less immunocompetent than females. One explanation for this is that intense sexual selection causes males to trade-off investment in immunity with traits that increase mating success. This hypothesis is tested in the Wellington tree weta (*Hemideina crassidens*), a large, sexually dimorphic orthopteran insect in which males possess enormous mandibular weaponry used during fights for access to female mates. Field-collected males have a significantly greater immune response (greater melanotic encapsulation) than females, suggesting that body condition, longevity or an allied trait is important to male fitness, or that females require materials for egg production that would otherwise be used to boost immunity. Although immunity is expected to trade-off against reproductive traits in both sexes, there is no significant relationship between immune response and weapon or testes size in males, nor fecundity in females.

Key words. Encapsulation, harem defence, immune response, melanization, sexual dimorphism, weaponry.

Introduction

Immune defence is a key life-history trait whose costs are traded-off against other fitness components in both vertebrates and invertebrates (Zuk & Stoehr, 2002). Interestingly, investment in immune defence often differs between the sexes, with males tending to be less immunocompetent than females (Schwarzenbach *et al.*, 2005). This sexual dimorphism is assumed to reflect how each sex maximizes fitness (Rolff, 2002; Zuk & Stoehr, 2002). Rolff (2002) argues that, because males gain greater fitness returns from a higher mating rate than do females, they might sacrifice investment in traits that increase longevity if, by so doing, they can elevate their mating success. A consequent prediction is that sexual dimorphism in immunocompetence will increase under more intense sexual selection because males will invest even fewer resources into immune defence (Rolff, 2002; Zuk & Stoehr, 2002). That is, for any given aspect of immune defence, males should mount a smaller immune response than females.

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There are two implicit assumptions that affect key predictions about the extent and direction of sexual dimorphism in immunity (Stoehr & Kokko, 2006). First, that increased survival (i.e. longevity) is more important to female than male fitness (Stoehr & Kokko, 2006). Longevity is, however, often a key fitness component for males (Clutton-Brock, 1988) and it is still unclear whether greater longevity selects for increased immunocompetence (van Boven & Weissing, 2004). Second, parasites often have nonlethal effects on traits, such as body condition, that have different effects on fitness in each sex. A recent theoretical model explicitly examines both assumptions (Stoehr & Kokko, 2006). If longevity is equally important to both sexes, the prediction that male immunity is negatively correlated with the strength of sexual selection still holds (unless parasites are particularly detrimental to male fitness). However, which sex invests more into immunity depends on how parasites impact condition and how condition affects reproductive success for each sex (Stoehr & Kokko, 2006). The values of these four parameters determine which sex invests more into immunity, and a shift from greater male to greater female immunocompetence can occur even as the intensity of sexual selection increases.

There are numerous predictions about phenotypic relationships between immunocompetence and the expression of sexually selected traits (Westneat & Birkhead, 1998; Adamo &

Spiteri, 2005; Lawniczak *et al.*, 2007). Indicator models propose that sexual signals convey information about male quality, which includes immunocompetence (Kokko *et al.*, 2006; Lawniczak *et al.*, 2007). It is often stated that only high-quality males can invest heavily into costly sexual signals without sacrificing investment into other essential traits, generating a positive correlation between sexual traits and key fitness components such as body condition or immunity. Life-history models show, however, that negative relationships will arise if the rewards of increased mating success compensate for reduced investment into other traits (Grafen, 1990; Kokko, 1997; Hunt *et al.*, 2004). Trade-offs between reproduction and immune function should also exist for females. For example, female insects might suffer fecundity costs of increased parasite resistance (Gwynn *et al.*, 2005) if egg production utilizes compounds required by the immune system (melanin, Li & Christensen, 1993).

The present study tests for a sex difference in immune defence in the harem-defending polygynous Wellington tree weta (*Hemideina crassidens*) (Orthoptera: Anostostomatidae), a huge (approximately 70 mm body length), flightless, nocturnal orthopteran insect endemic to New Zealand. *Hemideina crassidens* is strongly sexually dimorphic in head size (Kelly, 2005a) with males using their enormous mandibles as weapons during contests for cavities in trees (hereafter termed galleries) that are used as diurnal refugia by adult females (Kelly, 2006a). Females aggregate often in galleries (Kelly, 2006c) and mating occurs mainly in or near galleries (Field & Jarman, 2001). Males with larger weaponry defend typically bigger harems (Kelly, 2005a, 2008a).

Male Wellington tree weta are under strong sexual selection because of the opportunity for harem defence (Kelly, 2005a, 2008a). If optimal allocation to immune defence is lower generally for males in species with intense sexual selection, then male weta should be less immunocompetent than females if parasite intensity and reproductive condition-dependence are broadly similar for both sexes (Stoehr & Kokko, 2006). First, it is determined whether males that invest more in traits that increase mating success (i.e. weaponry) and fertilization success (i.e. testes size) show a reduced immunocompetence. Second, because there might be a fecundity cost of parasite resistance, or egg production might suppress immune function, the study also investigates whether more fecund females have lower immunocompetence.

Individual immunity is estimated by assaying encapsulation rate. Encapsulation is a cellular process in which specialized haemocytes aggregate and form compact capsules around invading, foreign (nonself) material (Beckage, 1998). As part of capsule formation, phenoloxidase converts tyrosine-based precursor molecules to melanin via a cascade of reactions (Siva-Jothy *et al.*, 2005; see also Beckage, 1998). The encapsulation rate is typically quantified as the degree of melanization of a novel, standardized antigen (e.g. nylon monofilament) inserted into the haemocoel for a set time; the darkness of the implant reflects the degree of melanization (Siva-Jothy *et al.*, 2005; Lawniczak *et al.*, 2007). Encapsulation does not assay the humoral arm of the immune system directly and, consequently, does not quantify antimicrobial protein activity. Because the present study is conducted in the field on a remote island, assays of enzyme activity (i.e. lysozyme) and haemocyte counts cannot be performed. Notwithstanding the apparent benefits of performing several different immune assays (Adamo, 2004), encapsulation (as well as melanization) provides a simple and reliable assay of an individual's immune system (Siva-Jothy *et al.*, 2001) because it requires the coordination of several cell-mediated and humoral immune components (Ratcliffe, 1993). Although some controversy exists with respect to the importance of melanization (Cerenius *et al.*, 2008), at least in *Drosophila* (Leclerc *et al.*, 2006) and mosquitoes (Schnitger *et al.*, 2007), melanization is correlated usually with other measures of immunity in Orthoptera, such as lytic activity, phenoloxidase activity and haemocyte load (Fedorka *et al.*, 2004; Rantala & Kortet, 2004; Rantala & Roff, 2005).

Materials and methods

Study site and animals

This study was conducted during April and May 2006 on Te Hoiere/Maud Island (41°02′S, 173°54′E), a 309-Ha scientific reserve in the Pelorus Sound, New Zealand. Adult tree weta were collected opportunistically at night by scanning vegetation. None of the experimental animals were missing limbs, parts of limbs or antennae. All animals were housed overnight in the laboratory in individual plastic containers (12×11.5 cm) that were fitted with perforated lids and fed apple, carrot and dry cat food *ad libitum*. They were kept in the dark and experienced natural temperatures (day: ~19 °C, night: ~12 °C).

Immune trait and assay

The morning after collection, a small-gauge, ethanol-sterilized needle was used to create a hole between the eighth and ninth abdominal sternites on the right side of cold-anesthetized tree weta (approximately 10 min in a −20 °C freezer). An 8.5-mm piece of monofilament (fishing line: 0.7 mm in diameter) was placed directly into the hole to simulate the invasion of a novel parasite. Pilot studies showed that, because tree weta are much larger than insects typically used in behavioural ecological studies of immune response, the monofilament sizes typically inserted into crickets (< 3 mm) were completely encapsulated after 24 h, revealing negligible variation among individuals. Therefore, 8.5-mm pieces of monofilament were used; these were cut under a dissecting microscope to ensure accurate and consistent size. After implantation, tree weta were returned to their container for 24 h in the dark, again under natural temperature conditions, but without food. The monofilament was knotted at one end so that it could be removed nondestructively after 24 h. In total, encapsulation data were collected from 62 males and 59 females.

After removal, the monofilament was immediately placed in 70% ethanol. Each monofilament was soaked in distilled water for 1 h before photographing it with a digital camera-mounted dissecting microscope equipped with a light-emitting diode ring light illuminator. Monofilaments were photographed over a single day and haphazardly with regard to sex and date of insertion. Image J software (http://rsb.info.nih.gov/ij/) was used to measure the degree of melanization as the mean grey scale darkness of the pixels (0 = white; 255 = black). The degree of darkness could be due to melanization and/or cellular encapsulation.

Body and weapon size measurements

After monofilament removal, individuals were killed and dissected. The hind legs from all individuals and the heads of males were detached using microscissors. Both femurs of both sexes and male heads were photographed using a digital camera (Nikon E8700, Nikon Corp., Sydney, Australia) mounted on a retort stand perpendicular to the stand's base. All traits were laid flat next to a ruler. Body size estimates were obtained by measuring femur length and male weapon size was estimated by measuring male head length (±0.01 mm). Male testes were also dissected and placed in 70% ethanol for approximately 1 month. All testes were oven-dried at the same time at 55 °C for 2 days and weighed (±0.1 mg) on a Mettler AE240 electronic balance (Mississauga, Ontario, Canada).

Female fecundity and reproductive investment

The sternal region of each female's abdomen was cut open using microscissors, all eggs were removed from the ovarioles and calyx and placed in 70% ethanol. Each female's egg load was photographed using the set-up described above. Egg size was measured as the longest axis of each fully mature egg (±0.01 mm), identified based on colour (Maskell, 1927). Total reproductive investment was estimated by multiplying the total number of eggs by mean egg length. Because female tree weta do not appear to oviposit frequently in the wild (i.e. egg laying bouts are typically separated by several days to weeks) (Kelly, 2008b), it was assumed that a female's egg load at dissection would provide a suitable estimate of her average daily fecundity.

Statistical analysis

Two measures (separated by >72 h) of mean grey scale, femur lengths, male head length and testes weight were made and then mean values used in subsequent analyses. A model II analysis of variance (ANOVA) to estimate measurement error (Yezerinac *et al.*, 1992) was used, which was very small for all traits [% measurement error = 0.22% ($n = 117$ individuals), 1.01% ($n = 116$), 0.91%, ($n = 117$), 0.10% ($n = 62$) and 0.43% ($n = 62$) for mean grey value, left femur, right femur, male head length and testes weight, respectively]. Prior to data analysis, all variables were confirmed to be normally distributed by visually examining quantile plots. Morphological traits were $\log_{10}$-transformed. Data are presented as the mean ± SE and 95% confidence intervals are given for correlation coefficients. Statistical analyses were performed using R, version 2.8.0 (R Development Core Team, 2008).

Results

Both weapon size ($r = 0.93$, $P < 0.0001$, $n = 353$; Kelly, 2005a) and testes size ($r = 0.30$, $P < 0.001$, $n = 139$; Kelly, 2008b) are positively related to body size. Therefore, given that many traits potentially influencing immunity in males and females covary with body size and that the females (femur length, 21.27 ± 0.22 mm) were significantly larger than the males (18.46 ± 0.20 mm) ($F_{[1,115]} = 90.49$, $P < 0.0001$), body size was statistically controlled in all subsequent analyses.

Sexually dimorphic melanization

Males (mean ± SE; 152.30 ± 1.39 mean grey value, $n = 62$) exhibited significantly greater melanization than females (147.61 ± 1.48 mean grey value, $n = 55$) (one-way ANOVA, $F_{1,115} = 5.35$, $P = 0.02$). A similar result was obtained when the analysis was controlled statistically for body size (male adjusted mean ± SE, 154.09 ± 1.54 mean grey value, $n = 62$; female adjusted mean ± SE, 144.41 ± 1.66 mean grey value, $n = 55$; analysis of covariance controlling for body size: sex: $F_{1,114} = 5.59$, $P < 0.0001$; sex × femur interaction: $F_{1,113} = 0.011$, $P = 0.915$).

Female fecundity, reproductive investment and encapsulation ability

Mean egg size (grand mean: 5.34 ± 0.013 mm, $n = 54$ females) differed significantly among females (model II ANOVA with female as random factor: $F_{53,462} = 36.85$, $P < 0.0001$) and was correlated positively with body size [$r = 0.42$ (0.171–0.618), $P = 0.002$, $n = 54$]. Egg number was correlated positively with female body size weakly [$r = 0.19$ (−0.033 to 0.395), $P = 0.082$, $n = 57$] and reproductive investment significantly [$r = 0.25$ (0.031–0.445), $P = 0.033$, $n = 54$] (tests are one-tailed because fecundity and reproductive investment were predicted to be related positively to body size). Larger females exhibited significantly greater melanic encapsulation than smaller females (Fig. 1 and Table 1). There was no relationship between melanic encapsulation and either reproductive investment, total fecundity or number of melanized (black) eggs (Table 1).

Male weapon size, reproductive investment and encapsulation ability

Larger males exhibited greater melanic encapsulation than smaller males (Fig. 1 and Table 1). Males with larger weaponry

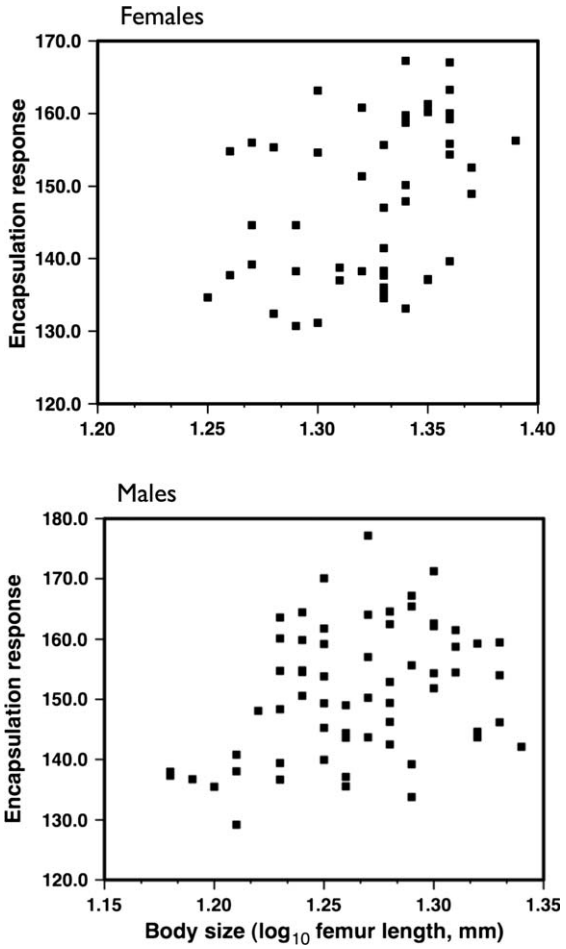

Fig. 1. Relationship between body size ($\log_{10}$ femur length, mm) and encapsulation response (mean grey value) for adult female (top panel, $n = 55$) and male (bottom panel, $n = 62$) tree weta.

exhibited significantly greater encapsulation; however, this relationship disappeared after controlling for body size (Table 1). There was no relationship between testes weight and encapsulation ability (Table 1).

Discussion

There is a significant difference between the sexes in encapsulation rate in Wellington tree weta. Surprisingly, however, males exhibit significantly greater encapsulation ability than females. The present study is unusual because few studies on invertebrates show that males have a greater immunocompetence than females (Fedorka *et al.*, 2004; Zuk *et al.*, 2004; McKean & Nunney, 2005; Stoehr, 2007). Typically, male arthropods elicit weaker immune responses than females, for at least one assay of immunocompetence (Schwarzenbach *et al.*, 2005) although some studies also report no sexual differences (Yourth *et al.*, 2002; Zuk *et al.*, 2004; Rantala & Roff, 2005; Pomfret & Knell, 2006). Robb *et al.* (2003) report a sex difference in melanotic encapsulation in *H. maori* but males did have higher haemocyte counts.

Notwithstanding the general utility of encapsulation to assay immunocompetence, particularly in Orthoptera (Fedorka *et al.*, 2004; Rantala & Kortet, 2004), multiple measures of immune function can only improve our knowledge in any given study (Adamo, 2004). Thus, because encapsulation and melanization may relate to some but not all pathogens (Kanost & Gorman, 2008), the present findings are unlikely to be universal with regard to the variety pathogens that a tree weta will encounter in the wild.

According to one hypothesis (Rolff, 2002), male tree weta should display weaker encapsulation responses than females if males trade-off immunity with the demands of intense sexual selection. As Stoehr & Kokko (2006) note, however, when parasites have a relatively greater impact on male than female condition and/or male sexually selected traits are more condition-dependent than female fecundity, then male investment in immunocompetence can exceed that of females, even in the face of the trade-off imposed by sexual selection.

How condition affects either male weapon development or female fecundity in tree weta is not known. Male tree weta develop large weaponry, carry these massive weapons considerable distances in search of female-occupied galleries (up to 12 m night^{-1}, Kelly, 2006b) and engage in intense male–male combat for mates (Kelly, 2006a). Females on the other hand do not develop weaponry, do not fight conspecifics and,

Table 1. Pearson correlation coefficients (r) between encapsulation response (mean grey value) and morphological, life history and reproductive traits for female and male tree weta.

Trait	r (95% CI)	n	Partial r (95% CI)	n
Females				
Body size	0.296 (0.030–0.576)*	55		
Reproductive investment	0.026 (–0.273 to 0.320)	44	0.119 (–0.184 to 0.402)	44
Total fecundity	0.093 (–0.196 to 0.353)	47	0.177 (–0.179 to 0.394)	47
No. black eggs	–0.02 (–0.305 to 0.268)	47	0.03 (–0.263 to 0.318)	47
Males				
Body size	0.34 (0.099–0.543)**	62		
Weapon length	–0.357 (0.118–0.557)***	62	–0.117 (–0.137 to 0.357)	62
Testes weight	0.053 (–0.199 to 0.299)	62	0.10 (–0.154 to 0.341)	62

*$P = 0.028$; **$P = 0.007$; ***$P = 0.004$.

CI, confidence interval. The partial r denotes the strength of correlation when statistically holding the body size ($\log_{10}$ femur length) constant.

under certain conditions, move about forest patches less than males (Kelly, 2006b). This implies that condition might be more important for male than female fitness, such that males should invest relatively more in defending against parasite-mediated damage to body condition by maintaining higher immunocompetence. It is also noteworthy that females suffer continuous demands for melanin and its precursors as a consequence of egg tanning and are thus potentially immunocompromised (Schwarzenbach *et al.*, 2005). Future studies should examine to what degree fitness is condition-dependent within each sex in tree weta.

Alternatively, males may exhibit greater melanization if selection favours greater wound response in this sex, possibly because they are more likely to be injured as a result of male–male combat over access to mates (Robb *et al.*, 2003). By contrast to this hypothesis, females are found to exhibit significantly more injuries in the wild (Kelly, 2006c) presumably because males use their mandibles to pull females out of tree cavities by their hind legs (Kelly, 2008).

The results obtained in the present study are consistent with the finding that adult female *H. crassidens* in the wild tend to have higher prevalence of infection by ectoparasitic mites (Kelly, 2005b). Despite the fact that both sexes live in the same habitat and so should experience the same exposure rates to ectoparasites (Kelly, 2005b), it appears that females experience greater parasitic infection. Perhaps this is because females are less immunocompetent than males. This scenario is in contrast to *H. maori*, which exhibits no sex difference in either ectoparasitic infection by mites or in the levels of melanotic encapsulation (Robb *et al.*, 2003, 2004). Whether higher parasite loads in *H. crassidens* are a cause or consequence of a compromised immune system remains inknown. Future studies should perform host resistance tests on tree weta to relate the observed variation in immunity with disease susceptibility (Adamo, 2004).

In neither sex are predictions about phenotypic correlations strongly supported. Males are expected to invest more in traits that increase mate acquisition (larger weaponry) or fertilization success (larger testes) and so should experience trade-offs with immunity. Instead, males with larger weaponry have significantly greater encapsulation rates, although this relationship is nonsignificant after controlling for body size (which is itself correlated highly with weapon size). These data hint at the possibility that, as in the damselfly *Calopteryx virgo* (Koskimäki *et al.*, 2004; but see also Siva-Jothy *et al.*, 1998), larger and more-dominant males might be in better condition and, as a consequence, have better immunity. In females, the lack of a negative relationship between reproductive investment and the degree of melanization could be a result of some females with fewer eggs having oviposited just before capture.

Larger-bodied tree weta in both sexes have a greater encapsulation response than smaller individuals. This pattern might simply reflect the greater total number of haemocytes available to larger animals because of their greater volume of haemolymph (assuming number haemocytes/volume haemolymph is independent of body size). This is a reasonable assumption given that encapsulation ability is often correlated positively with haemocyte count (Siva-Jothy *et al.*, 2005). Alternatively, perhaps larger animals can invest relatively more into traits that determine encapsulation ability. Based on the available data, there is no clear pattern among taxa for the relationship between encapsulation ability and body size in males. For example, Pomfret & Knell (2006) report that larger male *Euoniticellus intermdius* dung beetles show a stronger encapsulation response, whereas Rantala & Roff (2005) report a negative relationship with body size in the field cricket *Gryllus bimaculatus*.

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