

# Immunity and the expression of a secondary sexual trait in a horned beetle

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Resource-based trade-offs are known to be important in determining investment in both sexual ornamentation and immunity in insects. Because of the strict resource limitation experienced during metamorphosis, we predict that if insects are trading off ornament size and immunity this should be most apparent immediately after eclosion and that the relationship between sexual ornament size and immunity should change with maturation feeding because of the changing patterns of resource availability during this process. We therefore present an investigation into the relationship between morphology, sex, and two measures of immune response (phenoloxidase [PO] activity and encapsulation ability) in the horned beetle *Euoniticellus intermedius*, immediately after eclosion and after maturation feeding. Both measures increased with maturation, with sex differences in PO activity becoming discernable after maturation feeding. PO activity was positively correlated with horn length in male beetles both on eclosion and after maturation feeding, and encapsulation ability was positively correlated with elytra length. We conclude that resource limitation in the pupating insect does have effects on immunity but that this resource limitation does not translate into a measurable trade-off between horn length and immunity. The correlation between horn length and PO activity may arise because both horn length and PO activity are correlated with the same elements of the animal's biology that allow some animals both to grow a long horn and to sustain a higher level of PO activity. *Key words:* immunity, insect, metamorphosis, sex differences, sexual selection, trade-offs. [*Behav Ecol* 17:466–472 (2006)]

Because sexually selected traits are costly to produce, their expression is believed to depend on an animal's overall phenotypic condition. This implies that only those animals that have obtained more resources than are necessary for continued survival and functioning are able to grow or maintain relatively large sexually selected traits (Tomkins et al., 2004). One facet of this "condition dependence" that is now supported by an increasing body of evidence is the relationship between immunity and sexual trait expression; in the arthropods, a number of recent publications have reported positive correlations between sexually selected characters and immune function, in species including scorpion flies (Kurtz and Sauer, 1999), damselflies (Rantala et al., 2000; Siva-Jothy, 2000), crickets (Rantala and Kortet, 2003; Ryder and Siva-Jothy, 2000; Simmons et al., 2005), beetles (Rantala et al., 2002, 2003), and wolf spiders (Ahtiainen et al., 2004).

These positive correlations are consistent with a model, whereby costly immune investment and expression of secondary sexual traits are both mediated by male quality, so that "high-quality" males obtain sufficient resources during development to allow them to invest in ornamentation or weaponry while maintaining an effective immune response. Such a mechanism is supported by a number of recent studies that have demonstrated trade-offs between immune reactivity and the expression of sexually selected traits. Injecting male crickets (*Gryllus campestris*) with an immune system elicitor leads to reduced calling rates (Jacot et al., 2004), and a second experiment demonstrated that if juvenile males of the same species have their immune system activated in the same way, then once they reach adulthood the harp, the modified part of the forewing used in calling, is smaller and less melanized (Jacot et al.,

2005). Finally, in the wolf spider *Hygrolycosa rubrofasciata*, elevated rates of drumming (the signal produced by the male) are associated with a decreased lytic activity of antibacterial peptides in the animal's hemolymph (Ahtiainen et al., 2005).

One important group of sexually selected traits is not represented in this set of examples; however, those that are formed from cuticular material during metamorphosis in holometabolous insects. These traits include some of the best known and most spectacular of the sexually selected traits found in the insects, including the horns of scarab and dynastid beetles, the enlarged mandibles of stag beetles, and the eyestalks of diopside flies (Andersson, 1994). The expression of these traits is determined during metamorphosis, a period during which the animal does not feed and so experiences strict resource limitation. This leads to clear trade-offs between the rapidly growing tissues that become adult body parts; in particular, dung beetles trade-off horn size and the size of nearby structures such as eyes or antennae (Emlen, 2001), and also show increased horn growth when a more distant structure (the imaginal disc-like structure that grows into the genitalia) is experimentally removed (Moczek and Nijhout, 2004). Further evidence of trade-offs during metamorphosis comes from the allometric relations of stag beetle mandibles, which show a greater decline in slope with body size in species with relatively large mandibles, as would be predicted if the ultimate size of these structures is limited by pupal resources (Knell et al., 2004). Unlike body parts such as mandibles or horns, which are sclerotized and therefore have their sizes determined at pupation, it is possible for the insect to continue to invest in components of the immune system after eclosion by using resources acquired by feeding as an adult, either during a period of maturation feeding before the animal becomes sexually mature or later on in its life, potentially uncoupling immune reactivity from expression of the sexually selected trait. Thus, if both immunity and sexually selected trait expression are limited by the availability of the same resources, we might expect any relationship between trait expression and immunity to change over time after eclosion if the insect has had an opportunity to

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Received 22 September 2005; revised 20 December 2005; accepted 17 January 2006.

feed and acquire resources that it cannot invest in its sexually selected trait.

Here, using the dung beetle *Euoniticellus intermedius* as a study animal, we report the relationship between two measures of the insect immune system, (encapsulation ability and phenoloxidase [PO] activity), two sexually selected traits (horn length and pronotum length), and a measure of overall body size (elytra length), both shortly after eclosion and after a period of maturation feeding. We also compare these measures of immunity between male and female beetles. Specifically, we aim to test the following hypotheses.

1. If immunity is resource limited, then freshly eclosed beetles should have lower immune reactivity than beetles that have undergone maturation feeding.
2. We predict that the condition dependence of both sexually selected trait expression and immunity should lead to positive correlations, with high-quality males being able to invest in both aspects of their biology.
3. If relationships between the sexually selected characters and immune function are determined by resource-based trade-offs, we predict that any correlation between horn or pronotum length and immune reactivity should be less clear in animals that have undergone maturation feeding.
4. Different sexes have different life-history priorities and so should invest differently in traits such as immune function (Rolff, 2002). Therefore, we predict that there should be sex differences in immune function.

## METHODS

*E. intermedius* is a small (roughly 1 cm long) dung beetle that is native to Southern Africa. *E. intermedius* feeds mostly on cow dung; female beetles burrow into the soil beneath the dung pat and drag dung into the burrow to create hollow balls (the "brood balls") into each of which they lay an egg. The eggs hatch into larvae after about 2 days, and adults will emerge after about a month (Blume, 1984; Pomfret JC, unpublished data). Male beetles guard and mate with females in these tunnels. Male *E. intermedius* carry a curved blunt horn on their heads, and the pronotum is also sexually dimorphic, being enlarged into a projecting hump in males (Blume, 1984). Unlike many other horned dung beetles (Emlen and Nijhout, 2000), *E. intermedius* males are not dimorphic with respect to horn size, and there is no evidence that small males pursue "sneak" strategies to obtain mates (Pomfret and Knell, in press). Both horn and pronotum length are important in determining the outcome of contests between males, but their relative importance changes depending on whether small or large males are competing. When contests are between small males both pronotum and horn size, along with body size determine victory, whereas when larger males fight, horn length is by far the most important determinant of contest outcome (Pomfret and Knell, in press). Adult *E. intermedius* engage in a period of maturation feeding after eclosion and do not become sexually mature for approximately 1 week (Pomfret JC, unpublished data).

All beetles used were taken from a large (>100 animals), outbred laboratory colony of *E. intermedius*, originally started using insects collected in South Africa and maintained at 28°C with a 12:12 h light:dark cycle. Adult beetles were kept in 1-l plastic beakers, with 500 ml of damp sand and 200 ml of cow dung and two males and two females to every beaker. Every week the contents of the pots were sieved and any brood balls were removed. These were separated and placed individually in moist sand in small plastic containers (50 ml). On emer-

gence from their brood balls, beetles were assigned randomly to two groups. The first group, the freshly emerged treatment, was kept in damp cotton wool for up to 24 h before being measured and sampled for immune assays. The second group, the maturation feeding treatment, was kept in an excess of dung for 7 days to allow them a period of maturation feeding. After this time, they were placed in damp cotton wool for 24 h to mimic the environment to which the freshly emerged beetles were exposed. Randomly selected male and female beetles from each treatment were either allocated to have hemolymph sampled for PO activity or for measurement of the encapsulation response. Sampling of males was continued for a longer period than for females in order to give a higher sample size for the more complex analysis required. Final sample sizes for PO measurement were as follows: freshly eclosed females, 32; 1-week-old females, 31; freshly eclosed males, 97; 1-week-old males, 92. Final sample sizes for the encapsulation assay were as follows: freshly eclosed females, 39; 1-week-old females, 33; freshly eclosed males, 90; 1-week-old males, 93. Elytra length (a linear measure of body size) and pronotum length of every beetle was measured to the nearest 0.1 mm using Vernier calipers. The heads of the male beetles were photographed with a Nikon Coolpix 950 digital camera under a binocular microscope, and the length of the horn was measured using NIH Image v. 1.62 software.

## Measurement of PO activity

PO is an important component of the insect immune response. This enzyme plays a key role in the synthesis of melanin and has been linked with resistance to a wide variety of insect parasites (Cotter and Wilson, 2002), including viruses (Reeson et al., 1998; Washburn et al., 1996), fungi (Bidochka and Hajek, 1998; Hung and Boucias, 1996), nematodes (Hagen et al., 1994), and parasitoids (Wilson et al., 2001), and more recently also to resistance to the bacterial pathogen *Bacillus thuringiensis* (Rahman et al., 2004).

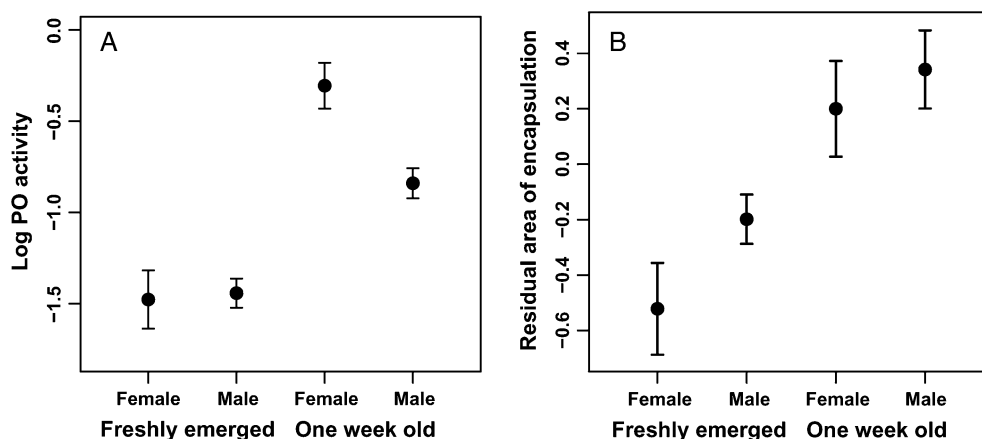
Beetles were swabbed with 70% ethanol and killed using ethyl acetate. They were then pinned and the elytra, wings, and abdominal membrane were removed. One microliter of hemolymph was removed from the body cavity using a pipette. This hemolymph was added immediately to 10 µl of ice-cold phosphate-buffered saline (pH 7.4) and frozen; this process disrupts the hemocytes allowing the release of cellular PO. After thawing, 100 µl of 5 mM L-Dopa in sterile water was added (Ashida and Söderhäll, 1984). The absorbance was measured at 492 nm, and the reaction was allowed to proceed at 28°C in a temperature-controlled spectrophotometer (Multiskan Ascent, Thermo Labsystems, Waltham, MA). It was not possible to carry out replicate assays because of the small amount of hemolymph that could be extracted from each beetle. This will increase the overall variance in the data but should not add any bias. The absorbance was then measured every minute for 20 min, giving two measures of PO activity: the maximum rate of increase of absorbance and the overall increase in absorbance over this period. Pilot studies showed that 20 min was enough for the reactions to enter and remain in the linear phase. Only the data on overall increase in absorbance are analyzed further here: the data for the maximum rate of increase of absorbance gave qualitatively the same results as those found by analysis of the data for the overall increase in absorbance, and because the latter are easier to analyze and interpret, we present these data only.

## Measurement of the cellular encapsulation response

Cellular encapsulation is the component of the insect's immune system that deals with larger parasites such as protists or

**Figure 1**

Summary of measures of immune reactivity. (A) Mean log PO activity for freshly emerged and 1-week-old beetles. (B) Area of encapsulation for freshly emerged and 1-week-old beetles. The data shown are residuals after the fitting of a generalized linear model with gamma errors and implant length as an explanatory variable. Error bars are 1 SE in all cases.



the eggs and larvae of parasitoids. During the process, the parasite is surrounded with several layers of hemocytes, which may or may not become melanized (Gillespie et al., 1997). Cellular encapsulation is a widely studied component of insect immunity because it can be assayed by challenging the insect with an artificial parasite (usually a piece of nylon) that is implanted into the animal's hemocoel, and then dissected out after a set period of time, and the extent of cellular encapsulation around the nylon measured (Cotter and Wilson, 2002; König and Schmid-Hempel, 1995; Ryder and Siva-Jothy, 2000, 2001; Schmid-Hempel R and Schmid-Hempel P, 1998; Siva-Jothy, 2000; Siva-Jothy et al., 1998; Wilson et al., 2003).

The beetles were swabbed with 70% ethanol, and a sterile needle was used to make a puncture in the beetles' cuticle on the ventral side of the abdomen. An approximately 2-mm long piece of surface-sterilized nylon monofilament (diam. 0.1 mm) was inserted through the puncture into the hemocoel. After 24 h, the beetle was killed using an anesthetic (ethyl acetate) and dissected under a microscope to extract the nylon monofilament. Encapsulation in these beetles was found to be largely cellular, with only a few implants displaying any significant melanization, and so only cellular encapsulation was measured. The nylon implant was placed in a drop of buffer on a microscope slide and squashed underneath a 0.1-mm thick coverslip to achieve an even thickness of cells surrounding the filament. The implant was photographed under a stereomicroscope, and the total area of the implant plus the encapsulation around it, along with the length of the nylon implant, were measured using NIH Image v1.62 image analysis software.

### Analysis

Two sets of models were fitted to both the PO activity and encapsulation data. Firstly, in order to investigate sex differences and the effects of maturation feeding on the two immune function estimates, models with two factors, age and sex, were fitted to the data sets for both males and females. Secondly, a model with one factor (age) and three continuous explanatory variables, namely, elytra length, horn length, and pronotum length were fitted to the data for male beetles only to investigate the relationships between the various morphological variables measured, maturation feeding and immune reactivity. PO activity was log transformed prior to analysis, which allowed the use of standard general linear models for these data. Generalized linear models with gamma errors and inverse link functions were used to analyze the encapsulation data, with implant length included as a covariate. All models were initially fitted with all terms up to two-way interactions included, and these full models were reduced by sequential

removal of explanatory variables to produce "minimal adequate models" that represent the most parsimonious descriptions of the relationship between the explanatory variables and the data. Variables to be removed were chosen on the basis of their statistical significance on a deletion test and also by inspecting the Akaike Information Criterion (AIC) scores of models with and without the variables in question (Crawley, 2002). All analyses were carried out using R v2.01 for Mac OS X.

## RESULTS

### Age and sex differences

Both PO activity and encapsulation increased after a week of maturation feeding, as shown in Figure 1. The PO activity data shows a distinct sex difference, with females showing higher activity than males, but only in those beetles that have undergone maturation feeding. This pattern is reflected in the significant age by sex interaction term (Table 1). By contrast, no sex difference was detectable in the encapsulation data, with age being the only term remaining in the minimal adequate model (Table 2).

### Morphological correlates of immune reactivity

Table 3 shows the fitted minimal adequate model to explain variability in PO activity in male beetles plus the results for other terms that were not retained. As expected from the previous analysis, the animal's age is an important predictor of PO activity, and the only other explanatory variable that remains in the model is horn length. Figure 2 shows the positive correlation between horn length and PO activity in both freshly emerged (Figure 2A) beetles and in those that had undergone maturation feeding (Figure 2B). If horn length is not included in the model, then elytra length emerges as a significant explanatory variable, suggesting that part of the

**Table 1**  
ANOVA table for the analysis of the relationship between log PO activity, sex, and age

Explanatory variable	df	SS	F	Probability
Age	1	2.56	4.25	.04
Sex	1	34.9	58.1	<.001
Age:sex	1	3.84	6.38	.01
Error	248	148		

Table 2

**Explanatory variables retained and removed (shown in italics) as the result of fitting a generalized linear model (gamma errors and an inverse link function) with age and sex as explanatory factors and implant length as a covariate to the data for area of encapsulation**

Explanatory variable	Coefficient	Standard error	Change in scaled deviance when removed	Probability ( $>\chi^2$ , 1 df)
Intercept	0.480	0.778		
Implant length	-0.067	0.006	102	<.001
Age	-0.036	0.008	19.98	<.001
Sex			2.64	.123
<i>Sex:age interaction</i>			0.54	.463

relationship between horn length and PO activity arises because of the correlation between horn length and body size, with large beetles having somewhat higher PO activity. Nonetheless, horn length remains a highly significant predictor of PO activity even when elytra length is included in the model ( $p = .001$ ), indicating that there is a component of the variability in PO activity that is predicted by horn size independent of body size.

Table 4 shows the results of fitting a minimal adequate model to the data on encapsulation area. In this case, elytra length and age are the two significant explanatory variables, with encapsulation ability increasing with both age and elytra length (Figure 3). A possible age by elytra length interaction was suggested by the apparent poor correlation between elytra length and encapsulation in freshly emerged beetles (Figure 3A), and a model with this interaction term has a slightly lower AIC than the one without it (480.9 versus 481.9), but this was not supported by a deletion test, which gave a non-significant probability of .111 for the interaction term. Thus, although there is some evidence for an interaction between age and elytra length, this is weakly supported in this data set. Two further comments are firstly that the pronotum length by horn length interaction was only marginally nonsignificant, but inclusion of this term was not supported by the AIC score for the model (484.7). Secondly, although examination of Figure 3B may suggest a curved relationship, including a qua-

Table 3

**Explanatory variables retained and removed (shown in italics) as the result of sequential removal of terms from a general linear model fitted to the data for PO activity in male beetles**

Explanatory variable	Coefficient	Standard error	F	Probability
Intercept	-2.429	0.228		
Age	0.604	0.107	32.10	<.001
Horn length	0.992	0.216	21.02	<.001
<i>Elytra length</i>			0.130	.718
<i>Pronotum length</i>			0.001	.991
<i>Age:pronotum length</i>			2.98	.086
<i>Elytra:pronotum length</i>			2.00	.159
<i>Pronotum:horn length</i>			2.90	.090
<i>Age:elytra length</i>			0.336	.563
<i>Elytra:horn length</i>			0.104	.748
<i>Age:horn length</i>			0.003	.954

The  $F$  values and probabilities are from partial  $F$  tests comparing the goodness of fit between models with and without the variable in question when less significant terms have already been removed (for details see Crawley, 2002).

dratic term in the model does not substantially improve the fit. Removal of the group of points with particularly high residuals that can be noted in this figure does not change the outcome of the analysis substantially.

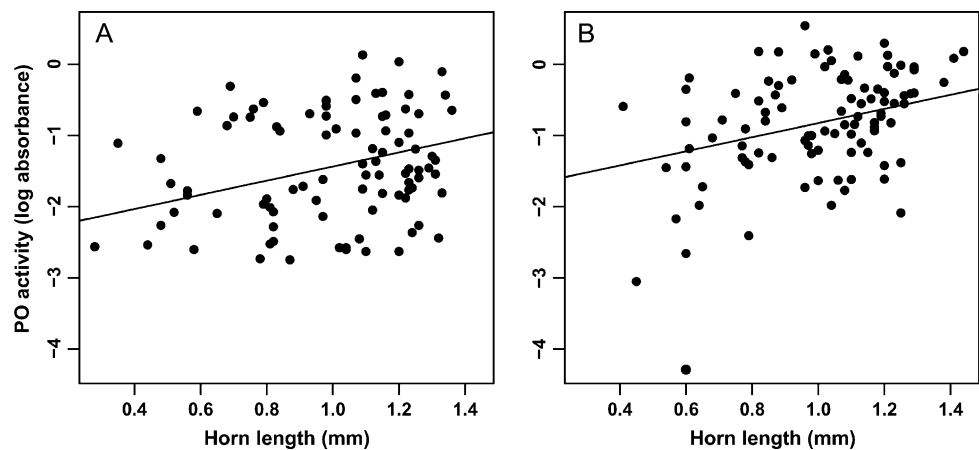
## DISCUSSION

Our results show a varied set of relationships emerging between our measures of immune reactivity, beetle age, sex, and (in males) morphology. These can be summarized as follows.

1. Both encapsulation ability and PO activity were higher in beetles that had undergone a week's maturation feeding than in freshly eclosed beetles.
2. PO activity was positively correlated with horn length in both freshly eclosed male beetles and in those that had undergone a week's maturation feeding.
3. Encapsulation ability was positively correlated with elytra length; larger beetles were better able to encapsulate a nylon implant. There was some suggestion in the data that this effect was related to maturation, but this was not well supported.
4. Sex differences were only observed in PO activity between male and female beetles that had undergone maturation feeding.

Thus, our prediction that freshly eclosed beetles should have reduced immune reactivity in comparison with 1-week-old animals is confirmed. In addition to the effect of increased resource availability in animals after maturation feeding, there are two further alternative explanations for this pattern. Firstly, freshly eclosed animals could be exposed to a lower risk of infection than those that are a week old and might not invest in immunity for this reason, but this is not likely to be correct; freshly eclosed animals will spend most of their time foraging for and consuming dung, which will expose them to much the same risks of infection as sexually mature animals, which will be more occupied with reproductive activity but in the same environment (dung pats) as the freshly eclosed animals. Secondly, the changes in our measures of immune investment could reflect physiological processes in the maturing beetle that are unrelated to immunity. Most importantly, the overall increase in PO activity might be related to cuticular melanization and sclerotization after eclosion. We sampled hemolymph, not cuticular PO activity, but cuticular PO is derived from proPO produced in the hemocytes (Ashida and Brey, 1995), and we note that Cotter and Wilson (2002) reported strong correlations between these measures in larvae of *Spodoptera littoralis*. Whether cuticular melanization should increase or decrease hemolymph PO activity is not clear: increased proPO production in the hemocytes could lead to a general increase in PO activity or alternatively increased rates of transport of proPO from the haemolymph to the cuticle





**Figure 2**  
Log PO activity plotted against horn length, with the predicted lines from the fitted minimal adequate model. (A) Freshly emerged beetles. The equation of the line is  $y = -2.43 + 0.99x$ . (B) One-week-old beetles. The equation of the line is  $y = -1.83 + 0.99x$ .

could lead to reduced activity in the hemolymph. Ascribing the overall change in PO activity with maturation to increased resource availability would therefore be premature, but the increase in encapsulation ability is less likely to arise from a link with processes such as melanization and is most likely to be a simple consequence of resource availability.

This pattern of PO activity increasing during maturation, but to a higher level in females than in males, corresponds to the pattern found in yellow dung flies, *Scathophaga stercoraria* (Schwarzenbach et al., 2005). Schwarzenbach et al. (2005) discuss the possible reasons why female insects might invest more in PO activity than males, including the need to melanize egg cuticle, a greater need to resist sexually transmitted diseases, greater exposure to pathogens because of their oviposition behavior, or different life-history priorities of the two sexes leading to more investment in reproductive effort and less in immunity in males (Rolff, 2002). Both our results and those of Schwarzenbach et al. (2005) contrast with two other studies of PO activity in insects. Armitage and Siva-Jothy (2005) report no effect of sex on PO activity in the mealworm beetle, *Tenebrio molitor*, 8 days after eclosion, and Rolff (2001) found that freshly emerged adult dragonflies had higher PO activity than mature adults. Dragonflies are hemimetabolous and so this latter discrepancy may be a result of the very different physiological processes and resource availability that

these insects will experience before emerging as adults: for example, it may be that the timing of cuticular melanization is different.

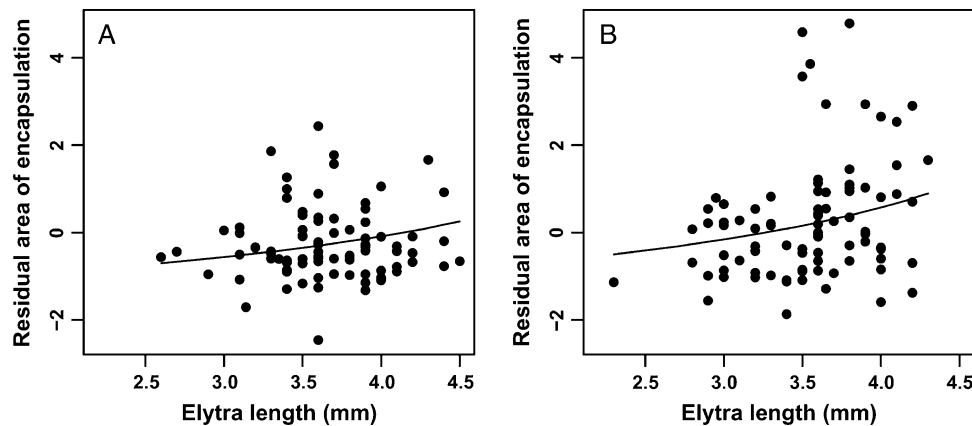
The greater ability of larger animals to encapsulate a nylon implant may simply reflect the greater total number of hemocytes that are likely to be available to larger animals simply by their possession of more hemolymph, given that encapsulation ability has been shown to be correlated with hemocyte count in a number of cases (Eslin and Prévost, 1996, 1998; Kraaijeveld et al., 2001; Wilson et al., 2003). Alternatively, it may be the case that larger animals are able to invest relatively more in those aspects of their biology that determine encapsulation ability. This result contrasts with the negative relationship reported between encapsulation rate and body size in the Mediterranean field cricket, *Gryllus bimaculatus* (Rantala and Roff, 2005). One possible explanation for this discrepancy is that Rantala and Roff measured the extent to which an encapsulated nylon filament is melanized, rather than the amount of encapsulation produced around it, as we have. Thus, the results of the two studies are not directly comparable, but we note that Cotter and Wilson (2002) found a positive correlation between area of encapsulation and melanization in *Spodoptera littoralis*. This implies that the discrepancy between these two studies reflects a real difference between the two organisms, which would be worthy of further investigation.

**Table 4**

**Explanatory variables retained and removed (shown in *italics*) as the result of sequential removal of terms from a generalized linear model with gamma errors and an inverse link function fitted to the data on area of encapsulation in male beetles**

Explanatory variable	Coefficient	Standard error	F	Probability
Intercept	0.586	0.050		
Implant length	-0.059	0.008	55.9	<.001
Age	-0.034	0.009	14.33	<.001
Elytra length	-0.040	0.012	11.90	<.001
<i>Horn length</i>			0.576	.449
<i>Pronotum length</i>			0.077	.782
<i>Pronotum:horn length</i>			3.787	.053
<i>Age:elytra length</i>			2.560	.111
<i>Elytra:pronotum length</i>			0.292	.590
<i>Elytra:horn length</i>			0.526	.469
<i>Age:horn length</i>			0.311	.577
<i>Age:pronotum length</i>			0.187	.666

The changes in scaled deviance and probabilities are calculated by comparing the goodness of fit between models with and without the variable in question, when less significant terms have already been removed (Crawley, 2002).



**Figure 3**

The relationship between elytra length and encapsulation ability. Residual area of encapsulation after the fitting of a generalized linear model—relating area of encapsulation to implant length with gamma errors—is shown on the y-axis and elytra length on the x-axis. The lines are the predicted values from a generalized linear model with gamma errors and with age and elytra length as explanatory variables fitted to these residuals: note that these lines are for illustrative purposes and do not truly represent the minimal adequate model given in Table 4. (A) Freshly emerged beetles. (B) One-week-old beetles.

Although we have found evidence for resource limitation of immune responses, our prediction that the relationship between sexually selected trait expression and immunity should change with maturation feeding is not supported by these data. In general, the biological mechanisms by which increased resource availability or better “condition” leads to a stronger immune response and more investment in sexual ornamentation are unclear. The fat body of the insect is important in synthesizing some components of the immune system, such as antimicrobial peptides (Hoffmann, 1995), and Koskimäki et al. (2004) reported that dominant male damselflies (*Calopteryx virgo*) had both a better encapsulation response and more fat reserves than subordinate males. This suggests a possible direct link between resource availability and some components of immune reactivity. Alternatively, a hormonally mediated interaction has been suggested (Rantala et al., 2003): injection of juvenile hormone into adult male mealworm beetles (*Tenebrio molitor*) simultaneously increases the attractiveness of their pheromones and decreases their immune reactivity. Given the nature of the insect immune system, it is of course possible that different mechanisms may mediate any relationship between secondary sexual characters and the expression of different components of the immune system.

In the case of *E. intermedius*, there are two plausible explanations for the consistency of the relationship between PO activity and horn length. Firstly, the positive correlation between horn length and PO activity might not be mediated by resource allocation trade-offs. It could be that both horn length and PO activity are, instead, correlated with some other aspect of the animal’s biology that enables some animals (those with long horns) to maintain higher PO activity than others, regardless of resource availability. Alternatively, it may be that the factors that lead to variability in resource availability in insects before and during metamorphosis continue to operate once the animal is an adult. If some beetles are less able to digest or assimilate their food both as a larva and as an adult, for example, they will have less resources during metamorphosis and therefore smaller horns. Once eclosed, they will acquire less resources during maturation feeding and may therefore continue to express lower PO activity than other beetles.

Positive correlations between sexually selected characters and immunity in arthropods have now been widely reported

(Ahtiainen et al., 2004; Kurtz and Sauer, 1999; Rantala and Kortet, 2003; Rantala et al., 2000; Ryder and Siva-Jothy, 2000; Simmons et al., 2005; Siva-Jothy, 2000), and our findings conform with recent findings relating success in intrasexual contests with a superior immune response (Koskimäki et al., 2004; Rantala and Kortet, 2004). This growing body of evidence is consistent with the immunocompetence handicap hypothesis (Folstad and Karter, 1992; Sheldon and Verhulst, 1996) and suggests that there may be a general connection between sexual selection and immunity in arthropods. It is important to be cautious about drawing such a conclusion, however. As discussed in (Adamo, 2004), the relationship between individual assays of immune function and disease resistance is complex. Furthermore, in several cases, only one component of the immune system has been found to correlate with the trait in question (e.g., Ahtiainen, 2004; Siva-Jothy, 2000, this study), and this, together with recent evidence that arthropods may trade-off components of their immune response against each other (Cotter et al., 2004; Rantala and Roff, 2005) indicates that drawing conclusions about overall “immunocompetence” is probably a dangerous oversimplification. Future studies of the relationship between sexually selected traits and immunity will need to concentrate more on the fitness consequences of variations in immune responses if we are to know what the true evolutionary significance of these relationships is.

We are grateful to Ken Wilson and to two anonymous referees for helpful comments on earlier drafts of the manuscript.

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