Animal Behaviour 86 (2013) 977–985

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Evaluating the costs of a sexually selected weapon: big horns at a small price

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ARTICLE INFO

Article history:
Received 27 June 2013
Initial acceptance 7 August 2013
Final acceptance 24 August 2013
Available online 26 September 2013
MS. number: A13-00543R

Keywords:
cost
horn
male–male competition
rhinoceros beetle
sexual selection
Trypoxylus dichotomus

A major assumption of sexual selection theory is that ornaments and weapons are costly. Such costs should maintain the reliability of ornaments and weapons as indicators of male quality, and therefore explain why choosy females and rival males pay attention to these traits. However, honest signalling may not depend on costs if the penalty for cheating is sufficiently high, a situation that is likely to be true for most weapons because they are frequently tested during combat. We examined and summarized the costs of producing and carrying giant horns in the rhinoceros beetle, *Trypoxylus dichotomus*. Remarkably, we found no evidence for fitness costs. Previously we found that horns do not impair flight performance, and here we found that horns did not stunt the growth of other body structures or weaken the beetles' immune response. Finally, and most importantly, horns did not reduce male survival in the field. Collectively, our results provide strong evidence that the exaggerated horns of *T. dichotomus* are surprisingly inexpensive. We discuss why weapons may be inherently less costly than ornaments, and suggest that the lack of fitness costs offers a simple, yet unexpected, explanation for why rhinoceros beetle horns are both elaborate and diverse.

Sexual selection is a powerful evolutionary force that has given rise to many of nature's largest, most colourful, and most flamboyant traits (Darwin 1871; Andersson 1994). In particular, female choice has led to the evolution of bright, conspicuous ornaments that make males attractive to potential mates, and male–male competition has driven the evolution of big, bulky weapons that aid in direct male–male combat. Understanding the evolution and maintenance of these sexually selected traits remains an important challenge for evolutionary ecologists.

Theoretical studies of sexual selection suggest that ornaments and weapons are most likely to evolve when they are honest indicators of male condition (Pomiankowski 1987; Grafen 1990; Iwasa et al. 1991; Iwasa & Pomiankowski 1994); that is, when ornaments and courtship displays reliably signal a male's overall genetic quality, and weapons and aggressive displays accurately reflect a male's fighting ability and likelihood to attack. However, if males in poor condition exaggerate the size of their ornaments or weapons, they may attract females that would otherwise mate with higher-quality males, or successfully intimidate larger rivals that would otherwise win fights. Thus, if ornaments and weapons do not honestly reflect male genetic quality or fighting ability, choosy females and rival males would not benefit from attending to the sexual signals, and signalling should ultimately break down (Maynard Smith & Harper 2003; Searcy & Nowicki 2005). It is therefore critical to identify the mechanisms that maintain the reliability of ornaments and weapons as indicators of male condition in order to understand the stability of these signalling systems.

The prevailing explanation for honest signalling is that cheating is prohibitively costly (Zahavi 1975; Zahavi & Zahavi 1997). Specifically, if the marginal costs of signalling are greater for individuals of low quality than they are for individuals of high quality, then it will not be cost-effective for these males to cheat, and the size or intensity of a male's signals will reliably reflect his quality or condition (Zahavi 1975; Andersson 1982; Kodric-Brown & Brown 1984; Nur & Hasson 1984; Grafen 1990; Iwasa et al. 1991; Getty 1998; but, for discussions on the difficulties in defining and measuring individual quality and condition, see Wilson & Nussey 2010; Lailvaux & Kasumovic 2011). Thus, a central tenet of sexual selection theory is that ornaments and weapons are costly, and that costs explain why choosy females and rival males pay attention to these traits (Andersson 1994; Kotiaho 2001; Searcy & Nowicki 2005).

Others, however, have argued that sexual signals need not be costly to be honest (Enquist 1985; Hurd 1995; Maynard Smith & Harper 2003; Számadó 2011). Theoretical models indicate that as long as the penalty for cheating is sufficiently high, no costs are required to ensure reliability at the signalling equilibrium (Hurd 1995; Lachmann et al. 2001; Számadó 2011). This situation may

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http://dx.doi.org/10.1016/j.anbehav.2013.08.017

be uncommon for ornaments because choosy females cannot easily verify whether a male's ornament reliably reflects his genetic quality, and they cannot easily punish cheaters should they occur. By contrast, the penalty for bearing dishonest weapons is likely to be substantial. Because weapons are frequently tested in combat, small males that exaggerate their fighting ability by producing disproportionately large weapons are easily detected, and these cheaters are likely to be attacked and severely punished (Rohwer 1977; Møller 1987; Berglund et al. 1996; Lachmann et al. 2001). As a result, signal costs may be necessary to ensure the reliability of male ornaments, but low-cost signalling may be favoured in the context of male–male competition. Weapons may therefore be inherently less costly to produce and maintain than ornaments. This hypothesis is, however, difficult to test because the costs of producing and bearing weapons in many species are still largely unknown. Indeed, most sexual selection studies have focused (almost exclusively) on the evolution of male ornaments via female mate choice (Lande 1981; Kirkpatrick 1982; Iwasa et al. 1991; Kirkpatrick & Ryan 1991; Pomiankowski et al. 1991; Kokko et al. 2006), and the mechanisms driving the evolution and maintenance of male weapons remain poorly understood (Emlen 2008).

Here, we describe a series of experiments designed to measure the costs of producing and carrying an elaborate sexually selected weapon: the horns of the giant Asian rhinoceros beetle, *Trypoxylus dichotomus*. Large males have a long, forked head horn and a short thoracic horn; small males have a short head horn and a tiny thoracic horn; and females are hornless. In the largest males, horns can reach two-thirds the length of the rest of the body (Fig. 1). Males use their horns to fight for access to females, and those with the longest horns are more likely to win fights and gain mating opportunities (Siva-Jothy 1987; Karino et al. 2005; Hongo 2007).

We predicted that the horns of *T. dichotomus* males would incur four primary costs. First, the long, forked head horn may impair flight performance. Second, because horns develop at the end of the larval period after the larva has stopped feeding, resources allocated to the development of a horn come at the expense of allocation to other structures. As a result, horns may stunt the growth of other body parts. Third, allocation to horns may also deplete resources that could be devoted to adult immune activity, thereby weakening the male immune response. And finally, horns may make males more conspicuous to predators or parasitoids, so that they directly reduce male survival.

Contrary to our expectations, we recently found that horns do not significantly increase the force required to fly (McCullough & Tobalske 2013), and they have no effect on biologically relevant measures of flight performance in the field (McCullough et al. 2012). Nevertheless, horns are long extensions of the exoskeleton that are likely to be expensive to produce. Indeed, there is now strong evidence that horns trade off with other body parts in many species of dung beetles (Nijhout & Emlen 1998; Emlen 2001; Moczek & Nijhout 2004; Simmons & Emlen 2006; Parzer & Moczek 2008), and recent studies in insects and other arthropods have documented trade-offs between immune function and the expression of sexually selected ornaments and displays (Jacot et al. 2004, 2005; Ahtianen et al. 2005). However, surprisingly little is known about morphological trade-offs in rhinoceros beetles (Kawano 1995), and trade-offs between immune activity and the expression of male weapons have only begun to be explored (Pomfret & Knell 2006; Rantala et al. 2007; Demuth et al. 2012).

Figure 1. Large male *Trypoxylus dichotomus* showing the long, branched head horn and sharp thoracic horn.

Here, we tested for trade-offs in resource allocation between horns and four morphological traits (wings, eyes, forelegs, and genitalia) and three measures of immune response (encapsulation response, phenoloxidase activity, and haemocyte density). Additionally, we tested whether these potential trade-offs have appreciable fitness consequences by directly measuring male survival in the field. Collectively, this study and our other recent work (McCullough et al. 2012; McCullough & Tobalske 2013) provide a comprehensive examination of the costs of producing and carrying an exaggerated weapon.

METHODS

Beetles used in the morphological and immune analyses were purchased as final-instar larvae from a commercial insect distributor and reared to adulthood in the laboratory. Only males were used in the morphological and immune analyses. Males and females not only differ in the presence and absence of horns, but also in their reproductive investments. As a result, they are likely to differ in their allometric relationships and optimal immune responses due to different life-history constraints (Stearns 1992; Rolff 2002). All beetles were approximately 10 days old (emergence from underground pupal chambers = day 0) at the time of the experiments. Beetles used in the survival analyses were from a wild population in central Taiwan.

Morphological Traits

Beetles were placed individually in airtight containers and euthanized by freezing prior to measurements. Body mass was used as a proxy for overall body size. Beetles were weighed to the nearest 0.001 g with an analytical balance. Horn length, eye diameter, foretibia length and aedeagus (genitalia) length were measured to the nearest 0.01 mm with dial callipers. Wing length was measured using imaging software (ImageJ v.1.46, National Institutes of Health, Bethesda, MD; http://rsbweb.nih.gov/nih-image/) from digital photographs of flattened wings.

Horns are disproportionately long in large males and disproportionately short in small males. We therefore tested for morphological trade-offs by comparing the relationships between horn length and the four morphological traits after controlling for the possible confounding variation in body size. Specifically, we fitted general linear models (GLMs) for wing, eye, tibia and aedeagus size with horn length and body mass as explanatory variables. The interaction between horn length and body mass was not a significant explanatory variable in any of the models and therefore was not retained in the final analyses.

If horns stunt the growth of other body parts, then horn length should be negatively correlated with the four other body parts, after accounting for variation in body size. We therefore expected a negative partial effect of horn length in the GLMs predicting the size of wings, eyes, tibiae and aedeagi.

Immune Responses

Insects defend themselves against infections through a combination of cellular and humoral immune responses (Gillespie et al. 1997). We investigated both responses by measuring the beetles' encapsulation response, haemocyte density and phenoloxidase (PO) activity. Cellular encapsulation is the main line of defence against multicellular pathogens, such as nematodes, fungi and parasitoids (Gillespie et al. 1997). The process occurs when a pathogen is surrounded by layers of haemocytes (blood cells) and eventually killed by asphyxiation (Salt 1970) or the accumulation of cytotoxic compounds (Nappi et al. 1995). Encapsulation responses can be assayed by implanting an artificial parasite (e.g. a small piece of nylon) into the haemocoel to mimic an infection, and then measuring the degree of encapsulation around the implant (König & Schmid-Hempel 1995; Ryder & Siva-Jothy 2000; Rantala et al. 2000). An insect's haemocyte density may also indicate its cellular immune activity, as the ability to mount a successful encapsulation response depends, at least in part, on the number of haemocytes circulating in the haemolymph (Eslin & Prévost 1996, 1998). PO is a key enzyme in the synthesis of melanin pigment and a major component of the humoral immune response. It is activated by the recognition of foreign particles (Cerenius & Söderhäll 2004) and has been linked with resistance to a wide variety of pathogens, including fungi, nematodes, parasitoids, viruses and bacteria (Ourth & Renis 1993; Reeson et al. 1998; Wilson et al. 2001; Cerenius & Söderhäll 2004; Cotter et al. 2004).

Although the sizes of morphological traits are determined during metamorphosis, beetles may continue to invest resources in their immune system by feeding as adults (Pomfret & Knell 2006), recouping losses incurred during metamorphosis and obscuring evidence of putative trade-offs with horn growth. We tested this hypothesis by comparing the immune responses of beetles that were or were not allowed to feed after eclosion.

Upon emergence from their pupal chambers, males were randomly assigned to two treatment groups. Beetles in the 'fed' group were provided apple slices ad libitum for 10 days prior to immune assays, while beetles in the 'unfed' group were provided no food. Beetles in both treatment groups were kept in individual glass containers filled with mulch that was moistened regularly. We used body mass as a proxy for overall body size in the analyses. Body mass was slightly higher for beetles in the fed group (mean $\pm$ SD $= 5.35 \pm 1.55$ g; $N = 44$) compared to the unfed group (5.03 ± 1.39 g; $N = 45$), but body mass did not differ significantly between the two treatments (Welch's *t* test: $t_{85.56} = 1.03$, $P = 0.31$). We weighed beetles to the nearest 0.001 g with an analytical balance and measured horn length to the nearest 0.01 mm with dial callipers. Both morphological measurements were log transformed before analyses.

To measure encapsulation response, we anaesthetized beetles by placing them in the freezer for 10 min and then inserted two sterile pieces of nylon monofilament (3 mm long) into the body cavity through punctures into the left and right sides of the abdomen. We allowed the beetles' immune systems to react to the implants for 24 h and then dissected the implants from the body cavity. We photographed the implants under a stereomicroscope and analysed the grey value of reflected light using imaging software (ImageJ v1.46). We compared the grey value of each implant to the grey value of a control, unimplanted piece of nylon monofilament and used the difference between the two grey values as a measure of encapsulation rate (Rantala et al. 2007). Higher values indicate darker (i.e. more melanized) implants, and thus a stronger encapsulation response.

We were unable to extract measurable quantities of haemolymph from the body cavity, so we used a 'flush' method (Chino et al. 1987) to collect haemolymph for the PO activity assays and haemocyte counts. Before removing the implants, which is a destructive procedure, we slowly injected phosphate-buffered saline (pH 7.4) into the beetle's abdominal cavity. The volume of this injection was equivalent to half of the beetle's body mass. We then severed one of the mesathoracic legs and collected the diluted haemolymph from the wound. A 10 μl sample of buffered haemolymph was mixed with 20 μl of anticoagulant (Cotter et al. 2004) and reserved for the haemocyte counts. The remaining haemolymph was frozen immediately to disrupt the haemocyte membranes and release cellular PO for the PO assays.

We measured PO activity spectrophotometrically using dopamine as a substrate following the basic methods described in Cotter & Wilson (2002). We added 90 μl of 10 mM dopamine to 90 μl of the buffered haemolymph and incubated duplicate samples in a temperature-controlled plate reader (Multiskan Ascent) at 25 °C. The absorbance was measured at 492 nm every minute for 15 min. Preliminary results (E. L. McCullough, unpublished data) indicated that the reaction was in the linear phase during this time period. PO activity was expressed as the average rate of the reaction over the 15 min.

A sample of 12 μl of the haemolymph–anticoagulant mixture was pipetted onto each side of a Neubauer haemocytometer. Haemocytes were counted in four nonadjacent squares, and haemocyte density was expressed as the number of cells per millilitre of buffered haemolymph for each beetle.

We tested for immune trade-offs by examining the relationship between male horn length and the three measures of immune response, after controlling for the possible confounding variation in body size. Specifically, we fitted GLMs for encapsulation rate, PO activity and haemocyte density with horn length, body mass and treatment as explanatory variables. Models initially included all second-order interactions, and then were simplified by sequentially removing nonsignificant terms on the basis of deletion tests (Crawley 2007). We also compared models on the basis of their goodness of fit using the corrected Akaike's Information Criterion (AICc), with smaller AICc scores indicating better fit. No interaction terms remained in the final models. PO activity and haemocyte densities were log transformed prior to analyses to meet the assumptions of standard general linear models. Encapsulation rates were normally distributed, so we did not perform any data transformation.

If horn development limits the resources available to the males' immune system, then horn length should be negatively correlated with the three immune responses after accounting for variation in body size. We therefore expected a negative partial effect of horn length in the GLMs predicting encapsulation rate, PO activity and haemocyte density. Furthermore, if beetles invest additional resources towards their immune system by feeding as adults, we expected stronger immune responses for beetles that were allowed to feed following eclosion. We therefore expected a negative partial effect of treatment (unfed versus fed) in the GLMs for the three immune measurements.

Survival Analyses

We conducted a mark–recapture study to investigate the effects of horn length and body size on beetle survival. Beetles were monitored over the course of the breeding season (June–August) on the National Chi Nan University campus in central Taiwan. The campus grounds contain many ash (*Fraxinus* spp.) trees, which is the exclusive host plant of *T. dichotomus* in Taiwan. All beetles found in the study site were collected from their natural sap sites, and individually marked with quick-drying paints. Horn length was measured to the nearest 0.01 mm with dial callipers, and mass was measured to the nearest 0.1 g with a spring scale. Beetles were returned to their original feeding trees after being marked and measured.

We searched for beetles at their feeding trees every 4 h between 2000 and 0400 hours when the beetles are most active. Because the number of recaptures for females was very low, we only tested for differences in survival among males. Survival probabilities were estimated using the live recaptures model in Program MARK (White & Burnham 1999) and assuming constant survival and recapture (i.e. no heterogeneity with day or age). We examined the effect of horn length and body mass on male survival by including these variables in the models as individual covariates. To determine the significance of horn length and body mass in explaining the variability in male survival, we assessed the confidence intervals of the parameter estimates for horn length and body mass, and used likelihood ratio tests to contrast models with and without these variables included.

RESULTS

Morphological Traits

We found no evidence for resource allocation trade-offs for any of the measured morphological traits (Table 1, Fig. 2). Male body size was significantly positively correlated with the size of wings, eyes and foretibiae, but was not correlated with aedeagus size (Table 1). After controlling for the possible confounding variation in body size, horn length was not correlated with eye size, but it was significantly positively correlated with wing, foretibia and aedeagus size (Table 1). These results indicate that males with relatively long horns for their body size also had relatively long wings, foretibiae and aedeagi.

Immune Responses

We found no evidence for resource allocation trade-offs for encapsulation rate, PO activity or haemocyte density (Tables 2, 3, Fig. 3). Horn length was not significantly correlated with either PO activity ($R^2 = 0.002$, $F_{1,87} = 0.16$, $P = 0.69$) or haemocyte density ($R^2 = 0.03$, $F_{1,76} = 2.12$, $P = 0.15$). Body mass and treatment also were not significant predictors of either PO activity or haemocyte density; model simplification indicated that the minimum adequate models predicting both PO activity and haemocyte density were the null models (Table 2). In contrast, both horn length and treatment were significant predictors of encapsulation rate (Table 2). There was a significant negative correlation between horn length and encapsulation rate ($R^2 = 0.09$, $F_{1,87} = 9.06$, $P < 0.01$), which indicates that males with long horns had lower encapsulation responses. However, when controlling for variation in body size (by retaining mass as an explanatory variable in the model), the partial effect of horn length was not statistically significant (Table 3). Additionally, the partial effect of treatment was significantly positive for encapsulation rate (Table 3), which is the opposite trend of what we would expect if males faced resource limitations for encapsulation rate.

Table 1

Results from GLMs examining the effects of body size and horn size on male morphological characters

Explanatory variable	Coefficient	SE	t_1	P
Wings				
Intercept	1.38	0.02		
Log(mass)	0.14	0.02	6.30	<0.001
Log(HL)	0.16	0.03	5.89	<0.001
Eyes				
Intercept	0.36	0.02		
Log(mass)	0.22	0.02	10.46	<0.001
Log(HL)	0.04	0.02	1.62	0.11
Foretibiae				
Intercept	0.79	0.02		
Log(mass)	0.11	0.02	5.76	<0.001
Log(HL)	0.18	0.02	8.46	<0.001
Aedeagus				
Intercept	0.71	0.03		
Log(mass)	0.01	0.03	0.18	0.86
Log(HL)	0.16	0.03	4.52	<0.001

HL: horn length.

Survival Analyses

The total period analysed consisted of 58 sampling nights, 186 marked individuals and 450 total recaptures. Neither horn length nor body mass were significant factors influencing male survival; the 95% confidence intervals for both parameters included zero (body mass: $\beta = 0.006$, CI $= -0.06$, 0.08; horn length: $\beta = -0.008$, CI $= -0.04$, 0.02). Furthermore, there was no evidence that the addition of body mass ($\chi^2_1 = 0.03$, $P = 0.87$) or horn length ($\chi^2_1 = 0.29$, $P = 0.59$) as a covariate explained significantly more of the variability in male survival compared to the null model. Male survival was high over the course of the breeding season ($\Phi = 0.90$, SE $= 0.01$).

DISCUSSION

Sexually selected traits can decrease fitness in a number of different ways, and their overall evolutionary cost may be a combination of several types of costs. As a result, studies that examine just one potential cost may underestimate some important fitness consequences of sexual trait exaggeration (Kotiaho 2001). We measured four of the most relevant costs associated with carrying and producing an elaborate weapon. Remarkably, we found no evidence for any of these costs. First, recent work has shown that *T. dichotomus* horns do not impair locomotion. Horns do not significantly increase the force required to fly (McCullough & Tobalske 2013) and have no effect on biologically relevant measures of flight performance (Hongo 2010; McCullough et al. 2012). Second, horns do not stunt the growth of other body structures. In fact, relative wing, tibia and aedeagus sizes were greater in males with relatively large horns. Third, horns did not weaken the beetles' immunocompetence. We found no correlations between relative horn size and the males' immune response, and the acquisition of additional resources through adult feeding had no effect on these relationships. Fourth, and most importantly, horns did not reduce male survival. Neither body size nor horn length was a significant predictor of male survival, and male survivorship was high throughout the breeding season. Collectively, these results suggest that the elaborate horns of *T. dichotomus* males are surprisingly inexpensive.

No Resource-based Trade-offs

Contrary to our expectation for morphological trade-offs, we found positive correlations among males between relative horn size and relative wing, tibia and aedeagus size. These results indicate that males that invest heavily in horn growth also invest heavily in the development of wings, legs, and genitalia. Correlated changes in morphological traits that are not the primary target of sexual selection are a common way by which males compensate for the costs of bearing ornaments and weapons (Oufiero & Garland 2007; Husak & Swallow 2011). For example, male birds with elongated tails (Evans & Thomas 1992; Andersson & Andersson

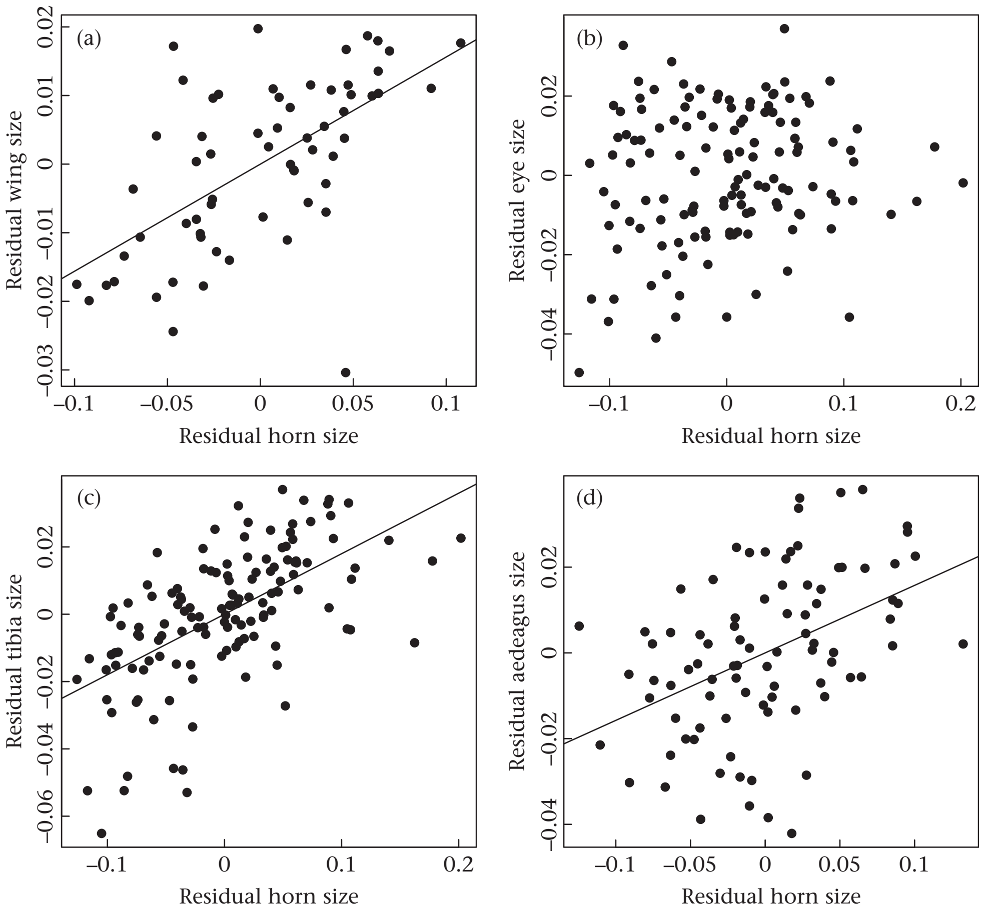

Figure 2. Partial regression plots showing the relationship between horn length and (a) wing length, (b) eye diameter, (c) tibia length and (d) aedeagus length after controlling for variation in body mass. Lines represent significant partial correlations.

Table 2
Explanatory variables retained and removed (shown in italics) for the minimal adequate models predicting male immune activity

Explanatory variable	Coefficient	SE	*F*	*df*	*P*
Encapsulation rate					
Intercept	57.17	16.82			
Treatment	11.60	3.02	11.44	1,87	<0.01
Log(HL)	−46.75	13.30	12.36	1,86	<0.001
Log(mass)			*0.47*	*1,85*	*0.50*
Log(mass):log(HL)			*1.99*	*1,84*	*0.16*
Log(HL):treatment			*0.05*	*1,83*	*0.82*
Log(mass):treatment			*0.34*	*1,82*	*0.56*
PO activity					
Intercept	−1.85	0.03			
Treatment			*3.66*	*1,87*	*0.06*
Log(HL)			*0.31*	*1,86*	*0.58*
Log(mass)			*2.13*	*1,85*	*0.15*
Log(mass):treatment			*3.91*	*1,84*	*0.05*
Log(HL):treatment			*2.10*	*1,83*	*0.15*
Log(mass):log(HL)			*0.61*	*1,82*	*0.44*
Haemocyte density					
Intercept	6.35	0.03			
Treatment			*2.07*	*1,76*	*0.15*
Log(mass)			*1.87*	*1,75*	*0.18*
Log(mass):treatment			*3.09*	*1,74*	*0.08*
Log(HL)			*0.09*	*1,73*	*0.77*
Log(mass):log(HL)			*0.55*	*1,72*	*0.46*
Log(HL):treatment			*0.07*	*1,71*	*0.79*

F statistics and *P* values are from partial *F* tests comparing the goodness of fit between models with and without the explanatory variable in question when less significant terms have been removed. HL: horn length. Food treatment is coded as fed = 0, unfed = 1.

1994; Balmford et al. 1994; Møller et al. 1995) and male stalk-eyed flies with exaggerated eyespans (Swallow et al. 2000; Husak et al. 2011) have relatively larger wings than female conspecifics, presumably to compensate for the costs of flying with ornaments. Although the horns of *T. dichotomus* incur negligible flight costs in present-day beetles (McCullough & Tobalske 2013), horns may have imposed substantial flight costs in the past and thereby driven the positive correlation between relative horn and relative wing size to overcome these costs (McCullough et al. 2012).

Similarly, male weapons may be developmentally linked with other traits that help males use their weapons more effectively during combat (Tomkins et al. 2005; Okada & Miyatake 2009). In numerous insect taxa, including dung beetles, flour beetles and earwigs, positive partial correlations have been found between exaggerated weapons and other body parts (e.g. legs and prothoraces) that are likely to influence fighting success (Tomkins et al. 2005; Okada & Miyatake 2009). In *T. dichotomus*, males use their

Table 3
Results from GLMs examining the effects of body size, horn length and food treatment on male encapsulation response

Explanatory variable	Coefficient	SE	t_1	*P*
Intercept	45.91	23.56		
Log(mass)	−17.13	25.01	−0.69	0.50
Log(HL)	−28.12	30.30	−0.93	0.36
Treatment	10.87	3.20	3.39	0.001

HL: horn length. Food treatment is coded as fed = 0, unfed = 1.

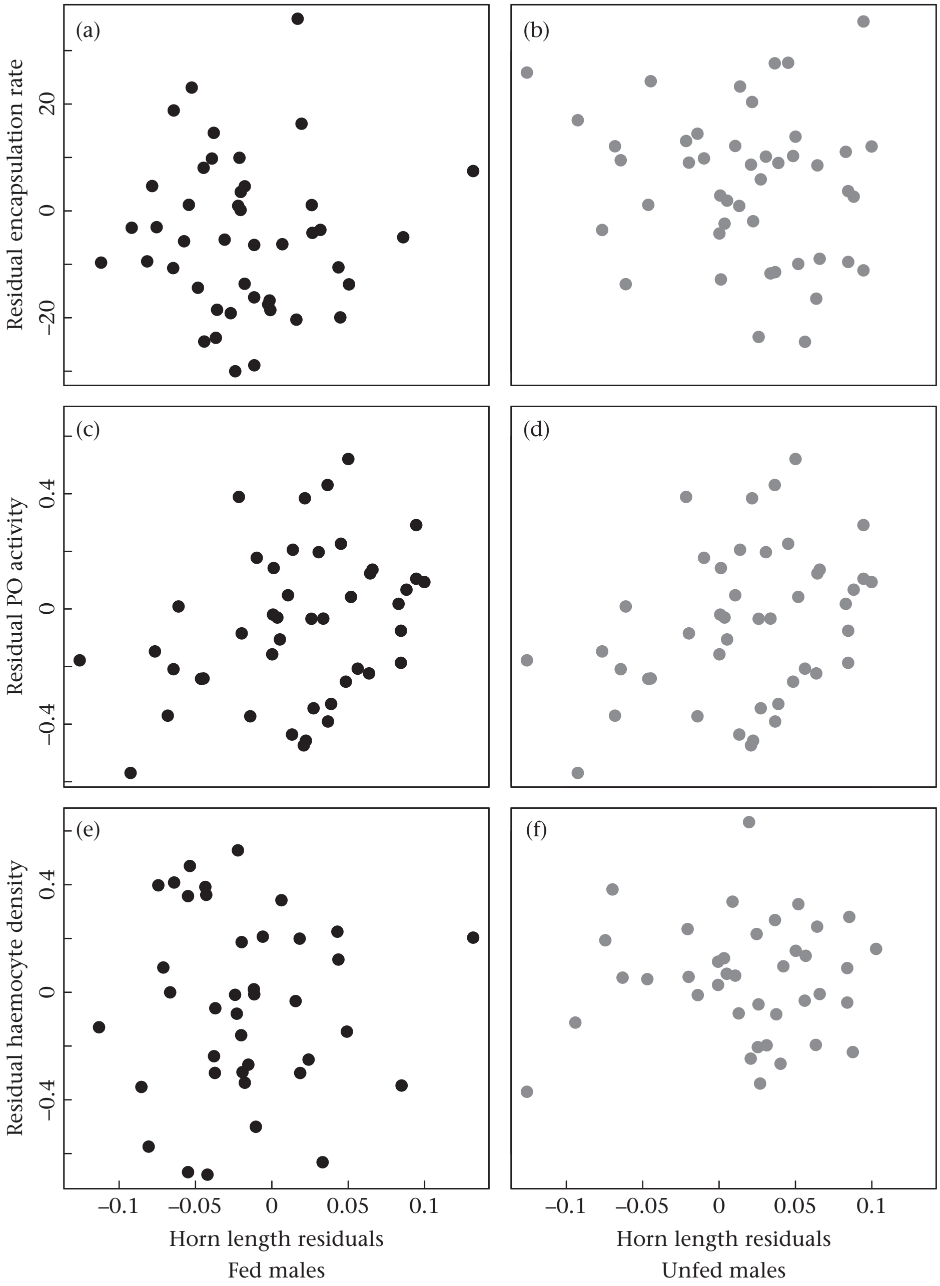

Figure 3. Partial regression plots showing the relationship between horn length and (a, b) encapsulation rate, (c, d) phenoloxidase (PO) activity and (e, f) haemocyte density after controlling for variation in body mass. Left panels (black points) show partial correlations for fed males; right panels (grey points) show partial correlations for unfed males.

horns as pitchforks to pry their opponents off the trunks and branches of trees (Siva-Jothy 1987; Hongo 2003). Long forelegs may help males raise themselves above the substrate, thereby gaining leverage for lifting opponents up and off the contested sites (Eberhard 1977). As a result, long foretibiae may improve a male's ability to dislodge his opponents, and selection on horns may drive correlated responses on tibia size.

The positive correlation between relative horn and aedeagus size is intriguing because male genitalia are typically considered to be under stabilizing selection for an intermediate, standard size that fits the average genitalia size of females (Eberhard et al. 1998). Our results are generally consistent with this 'one size fits all' hypothesis (Eberhard et al. 1998) because body mass was not a significant predictor of aedeagus length, and aedeagus length exhibited negative static allometry (standardized major axis slope = 0.20 for the log–log relationship between aedeagus length and body mass). There is no evidence for size-assortative mating in *T. dichotomus* (Siva-Jothy 1987; E. L. McCullough, unpublished data), and no study has examined the effect of variation in male genitalia morphology on mating or fertilization success. It is therefore unknown whether the positive correlation between relative horn and aedeagus size is adaptively significant. In water striders, males with long genitalia are better able to overcome female reluctance to mate (Preziosi & Fairbairn 1996; Sih et al. 2002), and similar processes may occur in *T. dichotomus* if there is intersexual conflict over mating decisions. Little is known about the strength of cryptic female choice and sperm competition in *T. dichotomus* or in other rhinoceros beetles, and future work will be necessary to determine how these selective pressures influence the shape and size of male genitalia.

We found no relationships between relative horn size and the three measures of immune response. Thus, in contrast to the morphological characters, males that invested heavily in horns did not also invest heavily in immune activity. Selection on morphological traits that help male beetles use their horns may be stronger than selection on male immune response due to the direct effect of horns on improving a male's reproductive success (Siva-Jothy 1987; Karino et al. 2005; Hongo 2007). As a result, males may invest preferentially in horns and other morphological characters before investing in immune activity. Whether males allocate larval resources among various fitness components in a hierarchical manner, and how these allocation decisions affect overall fitness, remain to be tested.

Despite the accumulating evidence for resource allocation trade-offs in beetles and other insects (Kawano 1995, 1997; Nijhout & Emlen 1998; Stevens et al. 1999; Emlen 2001; Moczek & Nijhout 2004; Parzer & Moczek 2008), these trade-offs are not universal (Simmons & Emlen 2006). In some cases, the evidence for trade-offs is weak, and in others, trade-offs are nonexistent (C. E. Allen & D. J. Emlen, unpublished data). Negative correlations between relative horn and relative wing size have been detected in species of *Chalcosoma* and *Dynastes* (in which horns can exceed the length of the body), so trade-offs appear to be important in at least a few rhinoceros beetle species with exceptionally large horns (Kawano 1995). We suspect that the variation in the strength of the trade-offs depends on individual variation in the ability to acquire resources (van Noordwijk & de Jong 1986). Indeed, trade-offs are most evident under poor or stressful conditions (Messina & Fry 2003; Sgrò & Hoffmann 2004; Boggs 2009), so populations or species that experience relatively benign conditions may be able to invest in all fitness-enhancing traits without constraints.

We note that the beetles used in our study may have experienced relatively benign developmental conditions, which would limit our ability to detect trade-offs. We found no differences in the relationships between horn length and body size between our laboratory-reared beetles, the beetles collected on the campus grounds for the survival analyses and the beetles from a rural population that was monitored briefly as a side project. We doubt that the conditions experienced by our experimental beetles were sufficient to mask any costs of carrying and producing horns, and therefore expect that the patterns observed here are representative of those found in natural beetle populations. However, future studies that experimentally manipulate larval diet are still needed to clarify how variation in resource acquisition affects the strength of resource allocation trade-offs.

No Survival Costs

We cannot rule out the possibility that horns incur significant, but as-yet-unidentified, costs. However, for these costs to be evolutionarily important, they must cause a reduction in individual fitness. We conducted a mark–recapture study to measure the effects of horn on male survival, and we found no evidence for survival costs. Although we were unable to measure female survival in this study due to very low recovery rates for females, our results are consistent with previous observations that males do not suffer higher predation costs. In *T. dichotomus*, hornless females actually suffer higher predation than males (Hongo & Kaneda 2009). Note, however, that both our study and that of Hongo & Kaneda spanned only a single breeding season. Survival estimates may differ from population to population, and from year to year. Future studies are required to assess whether survival costs vary among populations and seasons due to fluctuations in ecological and environmental conditions.

Costs and Sexual Selection Theory

Our findings contradict one of the most basic assumptions of sexual selection theory: that exaggerated ornaments and weapons are expensive to produce and carry. Yet we are not the first to find that sexually selected traits do not necessarily incur substantial costs (Kotiaho 2001; Husak & Swallow 2011). For example, male stalk-eyed flies with exaggerated eyespans exhibit higher survival than females (Worthington & Swallow 2010), and the large claws of male fiddler crabs confer a survival advantage against avian predators (Bildstein et al. 1989). More importantly, even when ornaments and weapons do incur measurable costs, several authors have questioned whether they are costly in a way that keeps them honest (Kotiaho 2001; Searcy & Nowicki 2005). Empirical support that sexual traits are differentially costly to individuals in poor condition, as required by the handicap principle, is equivocal at best (Kotiaho 2001; Cotton et al. 2004).

Although the handicap principle is the dominant explanation for honest signalling, several authors have argued that honesty does not require signal costs (Hurd 1995; Lachmann et al. 2001; Maynard Smith & Harper 2003; Számadó 2011). In fact, the realized cost of a signal can be zero if the cost of cheating is sufficiently high (Hurd 1995; Lachmann et al. 2001; Számadó 2011). This condition is probably true for most weapons: small males are likely to incur particularly high costs from fighting large males, and because weapons are routinely tested in fights, cheaters (i.e. males that exaggerate their fighting potential by producing oversized weapons) should be easily detected. As a result, selection on males to constantly 'call the bluff' on rivals will prevent males from producing weapons that do not accurately reflect their ability to fight (West-Eberhard 1983), and honest, yet low-cost, signals may be common. Importantly, it may be the high potential price paid by a weak male if it decided to fight a strong male that keeps weapons honest, rather than the actual, realized cost of producing or carrying the weapon itself (Lachmann et al. 2001).

Evolution and Diversification of Horns

Sexually selected traits are predicted to become increasingly exaggerated until survival costs outweigh the reproductive benefits of further trait elaboration (Fisher 1930; Lande 1980). This is unlikely to be the case for *T. dichotomus* because horns are not associated with any measurable fitness costs. Thus, what prevents males from developing even longer horns? One possibility is that maximum horn size is set by physical or mechanical limits. If oversized weapons are structurally weaker, or are more likely to break or perform poorly in combat, selection for males that build strong, functional weapons may prevent runaway horn growth (Alexander 1981). *Trypoxylus dichotomus* males do fight vigorously enough to break their horns (Siva-Jothy 1987; E. L. McCullough, unpublished data), which suggests that horns are sometimes pushed to their upper performance limits. We are currently measuring the safety factors and structural properties of *T. dichotomus* horns to explore whether mechanical constraints set an upper bound on maximum horn size. Although fitness costs may be important in limiting the size and form of ornaments and weapons in some systems, we expect that the evolution of sexually selected traits will often be limited by other factors (e.g. developmental, genetic and mechanical constraints) before reaching the theoretical cost–benefit equilibrium.

Rhinoceros beetles vary dramatically in the number, location, shape and size of their horns (Arrow 1951; Mizunuma 1999). Although these traits have captured the attention of biologists for more than a century (Darwin 1871; Arrow 1951; Eberhard 1977, 1979), the diversity of horn morphologies is still poorly understood (Emlen 2008). We suggest that the lack of costs offers a simple, yet unexpected, explanation for why these structures are both elaborate and diverse. That is, if our results from *T. dichotomus* are typical for rhinoceros beetles, and horns are indeed cheap to produce and carry, then horns may be free to diverge in size and form. Other researchers have similarly argued that the diversity of bird tails and fiddler crab claws may be attributed to the fact that these structures also incur low costs. Specifically, modifications in the shape and size of bird tails have only minor effects on flight performance because the tail 'hides' in the wake of the bird's body (Clark & Dudley 2009), and the morphology of dedicated weapons, such as the major claws of fiddler crabs, may be unconstrained given that these appendages are used exclusively for fighting (Bonduriansky 2007). Thus, structures that are not constrained by strong fitness costs may be particularly evolutionarily labile.

While the lack of important fitness costs may help explain why rhinoceros beetle horns are so diverse, it provides little insight into specific patterns of weapon diversity. To understand the differences in horn morphology among species, future studies will need to examine whether particular horn designs may perform better than others depending on where and how the horns are used. If true, selection to maximize the performance of horns during fights may have favoured the divergence in the shape and size of horns. Additional integrative studies are still needed to fully understand the selective forces driving (and limiting) the exaggeration and diversification of weapon size and form.

Acknowledgments

We thank Creagh Breuner for access to the spectrophotometer, and Megan Hamilton and Katherine Dorsett for their help analysing the immune data. We are grateful to Art Woods, Keaton Wilson and two anonymous referees for their comments on earlier versions of this manuscript. This project was supported by the Ford Foundation and the National Science Foundation (DGE-0809127 to E.L.M.).

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