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ELABORATION AND DIVERSIFICATION OF RHINOCEROS BEETLE HORNS

By

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Dissertation

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Elaboration and diversification of rhinoceros beetle horns

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Sexual selection is a potent driver in the evolution of male morphology and has given rise to bright, flashy ornaments that attract females, and big, bulky weapons that aid in male-male combat. Although previous studies have focused extensively on how female choice can drive the evolution and diversification of male ornaments, surprisingly little work has been done on the evolution of male weapons. Thus, we simply cannot convincingly explain why sexually-selected weapons are so diverse.

The few studies that have attempted to explain patterns of weapon diversity have focused on one of two driving forces: 1) selection to minimize physiological costs, or 2) selection to maximize fighting performance. While researchers have found evidence that both are important in understanding patterns of weapon diversity, each selective pressure alone only explains a small portion of the overall variation in weapon form. My dissertation evaluates the importance of these two selective forces on driving the evolution and diversification of a class of particularly extravagant male weapons: rhinoceros beetle horns.

In chapters one, two, and three, I examine the costs of producing and carrying horns. I find that beetle horns are surprisingly inexpensive to produce and carry, which suggests that differential costs cannot explain the variation in horn morphology among species. Instead, because low-cost structures are expected to be highly evolutionarily labile, the lack of fitness costs may have permitted both the elaboration and diversification of rhinoceros beetle horns. In chapters four and five, I examine the structural performance of horns to test whether horn morphology reflects a history of selection for improved fighting performance. I find that longer horns are more susceptible to breaking during fights, indicating that mechanical limits may place an upper bound on horn size. I also find that different horn types do in fact perform best under species-specific fighting loads, which suggests that structural adaptations that improve horn performance under different fighting styles have driven the diversification of weapon form. Collectively, my research expands our understanding of the selective pressures driving the diversity of rhinoceros beetle horns, and offers new insight into the interplay between organismal form and function.

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INTRODUCTION

One of the most exciting challenges facing evolutionary biologists is to understand the selective pressures that drive morphological diversity. Competition for mates, in particular, is a potent driver in the evolution of male morphology and has given rise to many of nature's most extreme body forms, including the striking ornaments of female choice, and the enlarged weapons of male-male combat. Although male weapons are equally as impressive and diverse as ornaments (Emlen 2008), sexual selection studies have focused almost exclusively on the process of female choice and the evolution and diversification of ornaments (Fisher 1930; O'Donald 1967; Lande 1981; Kirkpatrick 1982; Pomiankowski 1987; Iwasa, Pomiankowski, and Nee 1991; Pomiankowski, Iwasa, and Nee 1991). As a result, we still lack a theoretical understanding of the evolution of male weapons, and we simply cannot explain why sexually-selected weapons are so diverse (Emlen 2008). Understanding diversity in animal weapon morphology is the overarching aim of my dissertation.

The few studies that have attempted to explain patterns of weapon diversity have focused on one of two driving forces: 1) selection to minimize physiological costs, or 2) selection to maximize fighting performance. While researchers have found evidence that selection to minimize costs and maximize performance are both important in understanding patterns of weapon diversity, each selective pressure alone only explains a small portion of the overall variation in weapon form.

For example, selection to minimize the costs of producing horns has been successful in explaining some of the variation in the location of horns among dung beetles. Because horns stunt the growth of neighboring structures (e.g., eyes, antennae, wings) in many dung beetle species, and reduced eye, antennae or wing sizes are likely to have very different fitness consequences depending on the behavioral ecology of each species, selection has favored the evolutionary loss of horns that are the most costly (Emlen 2001; Emlen et al. 2005). In particular, horns on the back of the head that stunt the growth of eyes are expected to be especially costly to nocturnal species that depend on large eyes to see in low light conditions, and selection has indeed favored the evolutionary loss of head horns at the base of the head among nocturnal species. However, production costs only help to explain variation in the location of horns, and the vast diversity in horn shape remains a mystery (Emlen 2001; Emlen et al. 2005).

Similarly, in the case of ungulates, maximizing the fighting performance of weapons has been successful in explaining some of the variation in horn and antler morphology among species. In particular, species that stab rivals during male-male competitions have short, smooth horns; species that wrestle have long horns with well-developed catching arches; and species that ram together head-on have thick, coiled horns. However, correlations between weapon morphology and fighting behavior cannot explain weapon diversity among species that use nearly identical fighting tactics (Geist 1966; Lundrigan 1996; Caro et al. 2003).

Given that male weapons are typically large, bulky structures that are likely to incur substantial costs to produce and carry, and that these weapons are frequently tested in male-male combats, I suggest that both selection to minimize costs and selection to maximize fighting performance are important in driving weapon diversity. As a result, an integrative approach that evaluates the importance of both agents of selection promises a broader understanding of the variation in weapon form. My dissertation evaluates the importance of these two selective forces on the evolution and diversification of rhinoceros beetle horns.

Rhinoceros beetles (Coleoptera: Dynastinae) are an ideal system for studying weapon diversity because of the incredible variability in the architecture of their horns (Arrow 1951; Mizunuma 1999). Species not only vary in the number and size of their horns, but also in the shape of their horns – with species wielding long pitchforks (*Trypoxylus dichotomous*), robust pinchers (*Dynastes hercules*), or skinny spears (*Golofa porteri*) to name just a few of the morphologies found in this group. While some males develop only short, stubby horns, other males wield horns that are longer than the rest of their body. Darwin himself noted, "If we could imagine a male *Chalcosoma*... with its polished bronzed coat of mail, and vast complex horns, magnified to the size of a horse or even a dog, it would be one of the most imposing animals in the world" (Darwin 1871). Thus, the unparalleled variation and the sheer magnitude of their horn sizes makes rhinoceros beetles ideal for exploring the selective drivers of morphological divergence in weapon form.

In chapters one, two, and three, I examine whether beetle horn diversity may reflect selection to minimize the physiological costs of producing and carrying horns. Previous researchers have found clear associations among dung beetles (Scarabaeinae) between the costs of producing horns and the beetles' habitat and ecology (Emlen 2001; Emlen et al. 2005). I predicted that similar relationships between the physiological costs of horns and the beetles' ecology would be important in understanding the divergence of horn morphology among rhinoceros beetles (Dynastinae). To this end, I measured a number of potential costs expected to be important in the development and maintenance of beetle horns, and that are likely to differ depending on each species' habitat, behavior, and ecology. I assumed that large horns would be the most costly to produce and carry, so I chose to measure physiological costs in a species with particularly large horns, *Trypoxylus dichotomus*, in which horn length may reach two-thirds the length of the rest of the body in the largest males.

Surprisingly, I found no evidence that horns were costly to produce or carry. Specifically, horns do not significantly increase the overall force required to fly (chapter 2), and have no effect on biologically-relevant measures of flight performance in the field (chapter 1). Horns also do not stunt the growth of other body structures; horns do not weaken the males' immune function; and most importantly, horns do not reduce male survival (chapter 3). Because low-cost structures are expected to be highly evolutionarily labile, the fact that horns do not incur important fitness costs may offer a simple explanation for both the elaboration and diversification of rhinoceros beetle horns. Other researchers have similarly argued that the stunning diversity of bird tails and fiddler crab claws may be attributed to the fact that these also are relatively low cost structures. In particular, modifications in the shape and size of bird tails have fairly minor effects on flight performance because the tail "hides" in the wake of the bird's body (Clark and Dudley 2009), and the morphology of fiddler crab claws may be particularly labile given that these appendages are used exclusively for fighting (Bonduriansky 2007). Thus, structures that are inexpensive to produce and carry may be free to diverge dramatically in size and form.

The lack of important physiological costs may help explain why rhinoceros beetle horns are so diverse in general, but provide little to no insight into the specific patterns of weapon diversity, or why each species exhibits its own unique type of horn. In chapters four and five, I assess the structural performance of horns to test whether beetle horn diversity reflects a history of selection to maximize fighting performance. I find that longer horns are more susceptible to breaking during fights (chapter 4). These results suggest that mechanical limits are important in setting an upper bound on horn size, and may also help explain the variation in horn size among species depending on how intensely and predictably each species uses its horns during fights. I also find that different horn types perform best (i.e., are stronger and more efficient at transmitting forces) under species-specific fighting loads (chapter 5). These results provide some of the most rigorous evidence to date for any animal that weapon shape is indeed adapted to meet the functional demands of fighting, and that structural adaptations that improve horn performance under different fighting styles have driven the diversification of weapon form.

Collectively, my research expands our understanding of the selective pressures that have facilitated (and constrained) the elaboration and diversification of some of nature’s most impressive body forms. By using diverse methods and applying an integrative approach, this work offers exciting new tools for answering enduring questions of biological diversity, and fresh insights into the interplay between organismal form and function.

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CHAPTER 1:

Costs of elaborate weapons in a rhinoceros beetle: how difficult is it to fly with a big horn?

ABSTRACT:

The giant horns of rhinoceros beetles exemplify the extreme morphologies that can result from sexual selection. Ornaments and weapons help males obtain mates, but may also impose fitness costs. Intuitively, exaggerated sexually selected traits should impair locomotion, yet compensatory morphologies often make it difficult to detect locomotor costs. Here we tested whether horns of the rhinoceros beetle *Trypoxylus dichotomus* impair flight, and whether males compensate by developing larger wings or flight muscles. Contrary to our expectation that horns are costly for flight, males flew as fast as females, and among males, horn length was not correlated with flight speed or distance flown. We found some evidence for compensations in the male flight apparatus; males had relatively larger wings and flight muscles than females, and males with long horns for their body size had larger wings than males with relatively short horns. Flight muscle mass, however, was unaffected by horn length. We conclude that *T. dichotomus* horns may have been costly in the past and led to morphological compensations in wing and flight muscle size, but they do not currently impose significant flight costs. Fitness costs are a central tenet of sexual selection theory, and the large horns of rhinoceros beetles are expected to impose particularly strong costs on locomotion. Given our finding that *T. dichotomus* horns are surprisingly easy to carry, future work will be needed to identify the potential costs that have been important in shaping the evolution of elaborate horn morphologies.

Keywords: Rhinoceros beetles, horns, flight, costs, compensations, sexual selection

INTRODUCTION

Competition for mates is a potent driver in the evolution of animal morphology, and has led to many of Nature's most impressive and beautiful forms: the massive antlers of elk, the elongated tail feathers of birds, and the colorful dewlaps of lizards (Darwin 1871; Andersson 1994). Although sexually selected ornaments and weapons help males obtain mates, they may also come with fitness costs (Kirkpatrick 1982; Grafen 1990; Iwasa et al. 1991). Specifically, sexually selected traits may impair locomotion (Barbosa and Møller 1999; Moczek and Emlen 2000; Basolo and Alcaraz 2003; Madewell and Moczek 2006; Allen and Levinton 2007), increase risk of predation or parasitism (Møller and Nielsen 1997; Zuk and Kolluru 1998; Godin and McDonough 2003), weaken the immune system (Folstad and Karter 1992; Saino and Møller 1996; Verhulst et al. 1999; Rantala et al. 2003), or stunt the growth of other body structures (Kawano 1995, 1997, 2006; Emlen 2001). Importantly, the very reason why choosy females and rival males are expected to pay attention to ornaments and weapons is because they are costly, as traits that are difficult to produce and maintain are likely to be honest indicators of a male's quality (Zahavi 1975; Kodric-Brown and Brown 1984; Nur and Hasson 1984; Zeh and Zeh 1988; Grafen 1990; Searcy and Nowicki 2005).

Given the large size of many male ornaments and weapons, impaired locomotion may be a particularly important cost of bearing sexually selected traits (Oufiero and Garland 2007). Yet empirical evidence for locomotor costs is equivocal. For example, the long tail ornaments of certain hummingbird species increase flight metabolic costs and reduce maximum flight speed. However, these costs are most evident only at high flight speeds, which are rare for flying hummingbirds. As a result, long tails probably impose a relatively minor increase in daily energetic costs (Clark and Dudley 2009). The elongated eye-stalks of sexually dimorphic stalk-eyed flies also do not appear to impose substantial flight costs. Although long-eyed males ascended at shallower angles and slower velocities than short-eyed females, males and females did not differ in horizontal flight velocity, and males actually flew tighter turns and turned more frequently than females (Swallow et al. 2000; Ribak and Swallow 2007). Even the tails of barn swallows – a classic and well-studied example of an exaggerated sexually selected trait – may not be costly, as long tail feathers increase drag (Evans and Thomas 1992; Barbosa and Møller 1999), but also enhance maneuverability (Thomas 1993; Norberg 1994; Buchanan and Evans 2000). Thus, although the costs of male ornaments and weapons are central to sexual selection theory, strong empirical support for locomotor costs is lacking (Kotiaho 2001; Husak and Swallow 2011).

The costs of sexual traits may be difficult to detect because organisms evolve as integrated units. As a result, elaborated traits can become developmentally integrated with other traits that are not the primary targets of sexual selection, but that enable individuals to use, display, or bear their sexually selected traits more effectively (Tomkins et al. 2005). In some cases, these correlated traits may offset the negative performance consequences of bearing sexually selected traits, and thereby complicate the relationships between sexual trait size and locomotion (Møller 1996; Oufiero and Garland 2007; Husak and Swallow 2011). For example, in both long-tailed birds and stalk-eyed flies, males have proportionately longer wings than females, which may help them compensate for the aerodynamic costs of their sexual ornaments (Balmford et al. 1994; Ribak et al. 2009). These modifications presumably allow males to "have their cake and eat it too": to display sexually selected traits without sustaining undue costs on locomotion. However, compensatory morphologies also make performance costs difficult to detect, and may help explain why gender differences in performance, or functional tradeoffs between sexual traits and performance, oftentimes are not found (Ribak and Swallow 2007; Worthington and Swallow 2010). It is now clear that studies examining the potential costs of sexually selected traits must also consider the possibility for compensatory mechanisms, because ignoring the consequences of these correlated traits may lead to erroneous conclusions about the costs of sexually selected traits (Oufiero and Garland 2007).

Rhinoceros beetles are an ideal system for examining the locomotor costs of a sexually selected trait due to the large size and elaborate architectures of their horns. For example, in the Japanese horned beetle *Trypoxylus dichotomus* (formerly known as *Allomyrina dichotoma*), males have a pitchfork-like horn that extends forward from the head, and horn length can be nearly two-thirds the length of the rest of the body (fig. 1). The benefit of having an exaggerated horn is clear: males with longer horns are more likely to win fights against rival males and gain mating opportunities (Siva-Jothy 1987; Karino et al. 2005; Hongo 2007). However, little is known about the costs of bearing large horns in rhinoceros beetles (but see Hongo 2010), despite their important implications for potential evolutionary, developmental, and physiological constraints on weapon size and form.

Here, we examine the costs of beetle horns by comparing flight speeds and distances flown by free-flying male and female *T. dichotomus*. Intuitively, the large, branched head horn should impair a male's ability to fly. We therefore predict that horned males will fly slower and shorter distances than hornless females, and that among males, horn size will tradeoff with flight performance. However, even if horns impair locomotion, males and females may fly equally well if males have evolved compensatory adaptations in their flight apparatus. We therefore compared investment in wing size and flight muscle to examine if and to what degree males minimize the net cost of their giant horns.

METHODS

Trypoxylus dichotomus is a large rhinoceros beetle common throughout mountainous regions in East Asia. The species exhibits wide variation in both body size and horn size. Large males have a long, branched head horn and sharp thoracic horn; small males have a short head horn and tiny thoracic horn; and females have no horns at all.

Flight performance

Field experiments were conducted on the National Chi Nan University campus in central Taiwan. The campus grounds contain many (>120) *Fraxinus* trees, which are the exclusive host plant of *T. dichotomus* in Taiwan. Beetles chew into the bark and feed on the exuding sap. The study was conducted in June and July when adults are abundant and most active. Beetles were collected from their natural sap sites, and individually marked with quick drying paint markers. Each beetle was given a unique color combination on its elytra and number on its prothorax for identification. Horn length of all males, and body size (measured as prothorax width) of both males and females were measured to the nearest 0.01 mm with dial calipers (Mitutoya). [See Emlen (1997) for justification for using thoracic width as a measure of body size.] All beetles were released to their original feeding trees after being marked and measured.

We examined two measures of the beetles' flight performance: speed and distance. Flight speed was measured directly on free-flying beetles to the nearest 0.1 km/h using a high performance speed sensor (Stalker ATS Performance Radar Gun). Beetles were observed taking off from the ground on their own accord, and flight speed was recorded once the beetle reached a stable cruising speed flying directly away from the observer. To control for effects of ambient wind, flight speeds were only measured on clear, windless nights. We measured flight speeds of 137 males and 74 females. Flight speeds were averaged in cases where an individual was measured more than once (males *n* = 48; females *n* = 7).

To measure flight distances, we scanned all feeding trees within the study site every night, and recorded the locations of any marked beetles. Distances between trees were measured to the nearest 1 m with a global positioning system (Garmin GPSMAP 60CSx). To verify that our scan samples were an accurate measure of the beetles' flight distances, we equipped a subset of marked beetles (males $n = 15$; females $n = 6$) with radio transmitters to measure flight distances directly. The small 0.2 g radio transmitters (Advanced Telemetry Systems, Series A2405) did not appear to affect the beetles' flight performance or behavior. Flight speeds did not differ between beetles with radios (2.21 ± 0.33 m/s) to the same beetles without radios (2.16 ± 0.39 m/s; $T = -0.28$, $df = 14$, $P = 0.78$), and radio-tagged beetles appeared to feed and mate normally.

Radio telemetry revealed that beetles usually hide during the daytime in the canopy of their feeding tree or a neighboring non-host tree, or sometimes will bury themselves in the grass, leaf litter, or dirt near their feeding tree. A few radio-tagged males were occasionally found hiding in a non-host tree up to 400 m from their nighttime feeding site. Because these trips to non-host trees could not be detected by scanning the feeding trees, our scan samples may have underestimated some beetle movements. However, these hiding trees were all located within the study site, and the distances to and from these non-host trees were well within the range of flight distances observed among feeding trees. We are therefore confident that the nightly scan samples represent an accurate measure of the typical distances flown by the beetles in our study.

We recorded flight distances of 399 males and 28 females. Because the sample size for female distances was so small, and because we suspect that our scan samples may significantly underestimate female flight distances, we did not test for a sex difference in flight distances. Unfortunately, because we were only able to track a small number of females via scan sampling and radio-telemetry, we do not yet know how far females typically travel. The maximum flight path recorded for each individual was used in our statistical analyses.

Compensatory traits

We examined beetles for two potential compensatory traits: increased wing size and increased flight musculature. The beetles used in these morphological measurements were purchased as final instar larvae from a commercial insect distributor (Yasaka Kabuto Kuwagato World, Japan), and reared to adulthood in our laboratory at the University of Montana. Horn length of all males, and prothorax width of both males and females were measured to the nearest 0.01 mm with dial calipers (Mitutoya). Body mass of all beetles was measured to the nearest 0.001 g with an analytical balance (Mettler Toledo). After being measured and weighed, the beetles were placed in airtight containers and euthanized by freezing.

To measure wing size, we severed both left and right hind wings from the thorax at the base of the wing hinge, and pressed the wings between two glass plates. We took digital photographs of the wings, and imported the images into imaging software (ImageJ v1.41, NIH) for calibration and analysis. Wing length was measured as the distance between the base of the wing hinge to the most distal point on the curved wing tip. Wing area was measured as the area inside a manually-traced outline of each wing.

To measure flight musculature, we isolated the pterothorax (hereafter referred to simply as the thorax) from each frozen specimen, and cut it in half sagittally. Any visible esophageal contents and non-muscle tissues were removed, and both halves were weighed immediately. The procedure took less than two minutes, and there was no measurable water loss over this time period. The thorax was then soaked in 1M NaOH for 24 hours, rinsed with water to remove all dissolved muscle tissue, dried at room temperature for 24 hours, and reweighed. Flight muscle mass was found by subtracting the empty exoskeleton mass from the original thoracic mass (Marden 1987).

Analysis of male dimorphism

Although previous studies have described *T. dichotomus* as male dimorphic (Iguchi 1998; Hongo 2003, 2007; Karino et al. 2005; Plaistow et al. 2005), there are no obvious behavioral differences between the two morphs. Both major males and minor males use their horns to pry rival males away from sap sites that attract females, and both morphs exhibit sneaking behavior (Hongo 2003, 2007). Nevertheless, visual inspection of the relationship between male horn length and body size suggests that it is non-linear. We therefore analyzed horn versus body size measures for possible non-linear allometries (Knell 2009). To statistically test for the presence or absence of male dimorphism, we fitted the quadratic model: $y = \alpha_0 +$

$\alpha_1 x + \alpha_2 x^2 + \varepsilon$, where y is the natural log of horn length, x is the natural log of body size (measured as prothorax width), α_i is a regression coefficient, and ε is the random error. The regression coefficient α_2 was significantly different from zero ($\alpha_2 = -2.33$; $T = -8.64$; $df = 980$; $P < 0.001$), and the quadratic model described the data significantly better than the simple linear model: $y = \alpha_0 + \alpha_1 x + \varepsilon$ ($F_{981,980} = 74.61$, $P < 0.001$). Further analyses were therefore performed to characterize the non-linear relationship between horn length and body size.

Briefly, we compared three models on the basis of their goodness of fit (Akaike's information criterion, AIC) to determine which model best described the relationship between horn length and body size in our data: a switch-point model using the procedure outlined by Eberhard and Gutierrez (EG) (1991), a switch-point model using the Segmented package in R (Muggeo 2003), and a quadratic model. The EG switch-point model fit the data significantly better than the other two models (EG model AIC = 3631; Segmented model AIC = 3643; quadratic model AIC = 3712). According to this model, the switch-point in body size that delineated major males from minor males was 23.41 mm, and the relationship between horn length and body size differed in both slope ($T = -5.79$; $P < 0.001$) and intercept ($T = 6.59$; $P < 0.001$) among major and minor males. Males with a pronotum width ≥ 23.41 mm were classified as major males, and males with a pronotum width < 23.41 mm were classified as minor males.

Statistical analyses

We examined the effect of body size on the beetles' flight performance using ordinary least-squares linear regressions. To test for the effect of horn size on male flight performance, we then fitted general linear models (GLM) to the male dataset with horn length, body size (measured as pronotum width), and morph as explanatory variables. Models were initially fitted with all second-order interactions included, and simplified to the minimum adequate model by sequentially removing non-significant terms on the basis of deletion tests (Crawley 2007). We tested for a sex difference in flight speed using Student's t-test.

The allometric relationships between body size and the two potential compensatory traits (wing size and flight musculature) were examined using the power equation: $y = ax^b$, where x is body mass, and y is wing length, wing area, or flight muscle mass. We log-transformed all morphological variables before analyses. We assumed that wing size and flight musculature in females were near the naturally-selected optima (Lande 1980), and tested for compensations by comparing the scaling exponents (b) and scaling factors (a) of the allometric relationships between males and females. Because horn length exhibits positive allometry (i.e. large males have disproportionately long horns), we expected large males to incur relatively higher flight costs than small males. If males compensate for the cost of bearing horns, and wing size and flight muscle mass is commensurate with the magnitude of these costs, we expected the scaling exponents (i.e. allometric slopes) for wing size and flight muscle mass to be greater for males than females. We conducted our analyses using standardized major axis (model II) regression with the SMATR package in R (Warton et al. 2006).

To further explore the effect of horn length on wing and flight muscle size, we fitted a GLM to the male dataset with horn length, body mass, and morph as explanatory variables, and all second-order interactions. The minimal adequate model was selected by deletion tests (Crawley 2007).

RESULTS

Flight performance

The elaborate horns of male *T. dichotomus* do not inhibit the beetles' flight performance, at least in terms of how fast or how far beetles fly. There was no correlation between flight speed and body size in either males ($R^2 = 0.002$, $F_{1,135} = 0.22$ $P = 0.64$) or females ($R^2 = 0.008$, $F_{1,72} = 0.54$, $P = 0.46$). Among males, flight speed was unaffected by horn length, body size, morph, or any interactions among these variables. Model simplification indicated that the minimum adequate model predicting male flight speed was the null model (table 1). There was no difference in average flight speed between males and females ($T = 0.53$, $df = 127$, $P = 0.60$, fig. 2). All beetles flew between 1 and 4 m/s (mean = 2.27, SD = 0.44).

Males were typically seen on repeated nights, in some cases for up to almost two months after their initial capture, and several males flew close to 1 km between feeding trees. Females, on the other hand, were typically seen for only one or two days after their initial capture (if at all), and rarely moved far from the feeding tree on which they were first sighted. However, most of the radio-tagged females flew out of the detection range of our receiver (approximately 800 m, depending on terrain). These results suggest that females also fly long distances between feeding and oviposition sites, and may even fly farther distances than males.

There was no correlation between flight distance and body size in either males ($R^2 = 0.008$, $F_{1,397} = 3.37$, $P = 0.07$) or females ($R^2 = 0.002$, $F_{1,26} = 0.04$, $P = 0.85$). Among males, distance flown was unaffected by horn length, body size, morph, or any interactions among these variables (table 2).

Compensatory traits

Our results suggest that male *T. dichotomus* compensate for their horns by investing relatively more in their flight apparatus. The scaling exponents (i.e. allometric slopes) did not differ between males and females for wing length (Likelihood ratio test $lr = 2.10$, $P = 0.15$), wing area ($lr = 0.60$, $P = 0.44$), or flight muscle mass ($lr = 0.006$, $P = 0.94$; fig. 4). However, the scaling factors (i.e. allometric intercepts) did differ significantly between males and females for all three flight-related morphologies. In all cases, the scaling factor was significantly higher for males than females (wing length: Wald test $W = 42.7$, $P < 0.001$; wing area: $W = 39.9$, $P < 0.001$; flight muscle mass: $W = 34.8$, $P < 0.001$; fig. 4). Specifically, compared to females, males have a 4% mass-specific increase in wing length, a 10% increase in wing area, and a 7% increase in flight muscle mass.

When we examined patterns of morphological variation among males, body mass was the only significant predictor of flight muscle mass (table 3). In contrast, wing length (table 4) and wing area (table 5) were affected by body mass, horn length, and morph. Males with long horns for their body size had longer and larger wings than males with relatively short horns, and major males had slightly longer and larger wings than minor males, even after accounting for differences in horn and body size.

DISCUSSION

Sexually selected ornaments and weapons are assumed to be costly. *Trypoxylus dichotomus* offers an ideal system for measuring the locomotor costs of a male weapon. In this species, males must fly to and from sap sites every night to mate with females, yet males have a long, branched head horn that presumably makes flying difficult. Contrary to our predictions, males flew as well as hornless females, and among males there was no correlation between horn length and either flight speed or flight distance. We offer three possible explanations for these unexpected results. First, horns may be costly to beetles for other, unmeasured aspects of locomotion. Second, horns may impair flight, but beetles offset these costs by producing larger wings and flight muscles. Third, the exaggerated horns of rhinoceros beetles simply may not be as costly for flight as we imagined.

The speed at which a beetle flies and the distance over which it typically travels are just two of the many proxies of beetle flight performance. Although we found no sex difference in flight speed, and no tradeoffs between horn size and flight speed or distances flown, the horns of *T. dichotomus* may affect other aspects of flight performance (e.g. take-off ability, horizontal or vertical acceleration, maneuverability) that were not measured in this study. Alternatively, horns may affect the beetles' terrestrial locomotion. In dung beetles, for example, long horns reduce running speed and maneuverability inside underground tunnels (Moczek and Emlen 2000; Madewell and Moczek 2006), and the long horns of *T. dichotomus* may similarly impair the males' ability to maneuver around sap sites on the trunks and branches of trees. Nevertheless, flying (rather than walking) is the primary mode of locomotion for *T. dichotomus*, so flight speed and distances flown are likely to be the most ecologically relevant measures of performance for this species. Whether or not a male rhinoceros beetle mates depends on his ability to protect resource sites that attract females. In *T. dichotomus*, males guard wounds on the sides of trees where females come to feed (Hongo 2007). A male's reproductive success therefore depends on his ability to find and fly to sap sites, to return to a sap site if he is kicked off it by a rival male, and to travel to new sap sites and mate with additional females. Furthermore, flight speed is likely to directly affect a beetle's ability to evade aerial predators (Hongo and Kaneda 2009). As a result, flight speed and distances flown may represent the aspects of performance that have the most direct effects on a beetle's fitness. We were unable to measure male reproductive success in this study, however, so future work is warranted to assess the actual fitness consequences of variation in locomotor performance.

A second explanation is that males compensate for the cost of large horns by investing more in their flight apparatus. We tested this hypothesis in two ways: by comparing wing and flight muscle allometries between horned males and hornless females, and by examining among-male variation in wings and flight musculature after accounting for differences in body size. We found evidence for partial, but not perfect compensation from both of these analyses. Specifically, we found that the scaling factors for wing size and flight muscle mass were significantly higher for males than females. At any given body size, males had significantly larger wings and flight muscles than females. However, males did not have steeper allometries than females. Thus, large males did not have disproportionately large wings or flight muscles, as we would expect if they fully compensated for their disproportionately long horns. Additionally, among males, we found that horn length significantly affected wing length and wing area, even after controlling for variation in body size. In contrast, there was no effect of horns on flight muscle mass. As a result, males with long horns for their body size compensate by developing longer and larger wings, but do not adjust the size of their flight muscles. Future studies, however, should examine whether mitochondrial density, tracheal density, or other aspects of flight muscle physiology vary between males and females, or between major and minor morphs.

A third explanation for why males and females do not differ in their ability to fly is that horns simply are not that costly. That is, if horns do not make flying generally more difficult, then males and females would not be expected to differ in flight performance. We find this hypothesis counterintuitive, especially given the striking size of horns in *T. dichotomus* and other rhinoceros beetle species. Nevertheless, the fact that males and females fly equally well, and that males are preyed upon less frequently than females (Hongo and Kaneda 2009) suggest that horns are not especially costly to carry. In fact, in a similar study comparing the flight ability of *T. dichotomus* males, Hongo (2010) found that larger males flew slightly faster than smaller males, which is the opposite trend of what would be expected if horns impeded flight.

It is important to note, however, that even if horns are not costly to carry in present-day beetles, the fact that males invest more in their flight apparatus by producing relatively larger flight muscles and wings suggests that horns were costly to flight in the past. For example, in many long-tailed bird species, there is a positive relationship between male wing size and tail length, which probably reflects selective pressure to overcome the added drag of the male tail ornaments (Evans and Thomas 1992; Andersson and Andersson 1994; Balmford et al. 1994; Møller et al. 1995). Similarly in stalk-eyed flies, the positive correlation between sexual dimorphism in wing length and eye-span most likely reflects selection to offset the increased rotational inertia imposed by long eye-spans, as longer wings allow males to generate stronger torques per wing-beat (Swallow et al. 2000; Ribak et al. 2009). If long tails did not increase drag, or if long eye-spans did not reduce maneuverability, we would not expect birds and flies to evolve compensatory increases in male wing size. We intuitively expect compensatory changes to imply costs. Thus, the simple observation that *T. dichotomus* males have relatively larger flight muscles and wings than females suggests that the locomotor costs of horns were once strong enough to act as agents of selection on the beetles' flight apparatus. We suggest that horns historically imposed substantial flight costs and led to compensations in wing and flight muscle size, but that these costs have been mitigated in present-day beetles. Similar to the narrowed tips of ornamental tail feathers in male birds that have evolved to minimize aerodynamic drag (Møller and Hedenström 1999), we suggest that the structure and composition of the horn itself has become modified over time to significantly reduce horn mass and minimize flight costs (McCullough and Tobalske, in prep).

We recognize at least two potentially important limitations to this study. First, we compared wing size and flight muscle mass between males and females in order to test whether males compensate for the costs of bearing horns. Female *T. dichotomus* are hornless, so they are a convenient, natural control for examining the effects of horns on the beetles' flight apparatus. However, these comparisons may be misleading because females certainly are not males that simply lack horns. In particular, males and females may differ substantially in their reproductive investments (e.g. Stearns 1992), so females may deviate from the naturally-selected optima in wing size and flight musculature due to their own resource allocation constraints. As a result, any differences observed between males and females in their wing and flight muscle allometries cannot be attributed solely to the presence or absence of horns. Nevertheless, without *a priori* knowledge of the optimal scaling relationships for wing and flight muscle size, we argue that the naturally hornless females represent an appropriate (albeit not perfect) baseline for assessing whether males compensate for their handicap of bearing exaggerated horns.

A second limitation is that the beetles used in this study may have experienced relatively benign conditions: the beetles used in our flight measurements were collected from a university campus, and the beetles used in our morphometric analyses were purchased as final instar larvae from a commercial supplier and raised to adulthood in the lab. However, we doubt that the conditions experienced by our beetles were sufficient to mask the costs of carrying or producing horns. In particular, we found no differences in the relationships between horn length and body size among our lab-reared beetles, the beetles collected on the campus grounds, and beetles from a rural population that was monitored briefly as a side project. We therefore suspect that the wing and flight muscle allometries that we measured in the lab-reared beetles are representative of the allometries found in natural beetle populations. Additionally, we have no evidence that the beetles living on campus grounds fly differently than beetles that developed in more natural conditions. The flight speeds reported here on our urban population of *T. dichotomus* are similar to the flight speeds measured on a wild beetle population (Hongo 2010). Although the wild-caught beetles flew slightly slower than our urban beetles, the previous study measured flight speed on tethered males, while we measured flight speed on unencumbered, free-flying beetles. As a result, the differences in flight performance between the two studies are probably primarily due to different methodologies, rather than inherent differences in the beetles' condition. More importantly, the basic conclusion of both studies is the same: long horns do not make it more difficult to fly.

A central tenet of sexual selection theory is that ornaments and weapons are costly (reviewed in Andersson 1994). Rhinoceros beetle horns are among the largest sexually selected traits found in Nature (Darwin 1871; Eberhard 1980; Andersson 1994), and we expect these extreme morphologies to be associated with particularly high fitness costs. Contrary to our expectations that the long, pitchfork-like horn of male *T. dichotomus* makes flying more cumbersome, here we have shown that the horns do not impose significant flight costs. Given the importance of costs for both maintaining the reliability of ornaments and weapons as signals of male quality, and ultimately for limiting maximum trait size, future work will be needed to investigate other potential costs (e.g. weakened immunity, increased predation, resource allocation tradeoffs) that may have been important in shaping the evolution of these extreme horn morphologies.

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

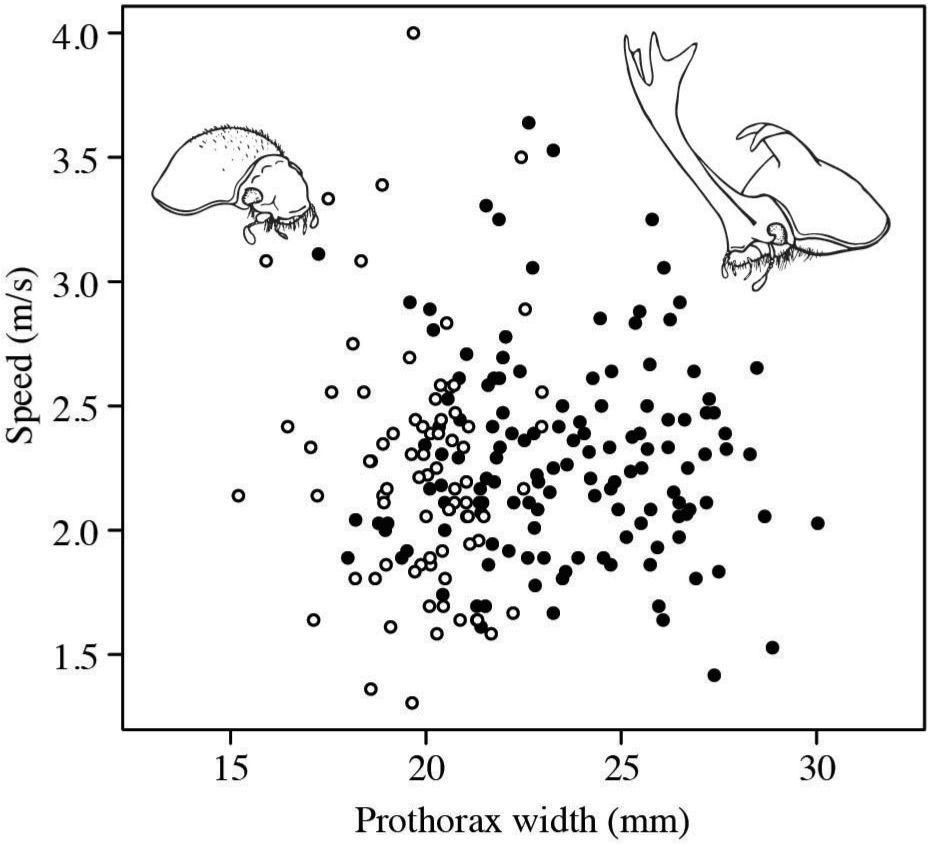

Figure 2. Relationships between prothorax width and flight speed for males (closed circles, $R^2 = 0.002$, $F_{1,135} = 0.22$ $P = 0.64$) and females (open circles, $R^2 = 0.008$, $F_{1,72} = 0.54$, $P = 0.46$)

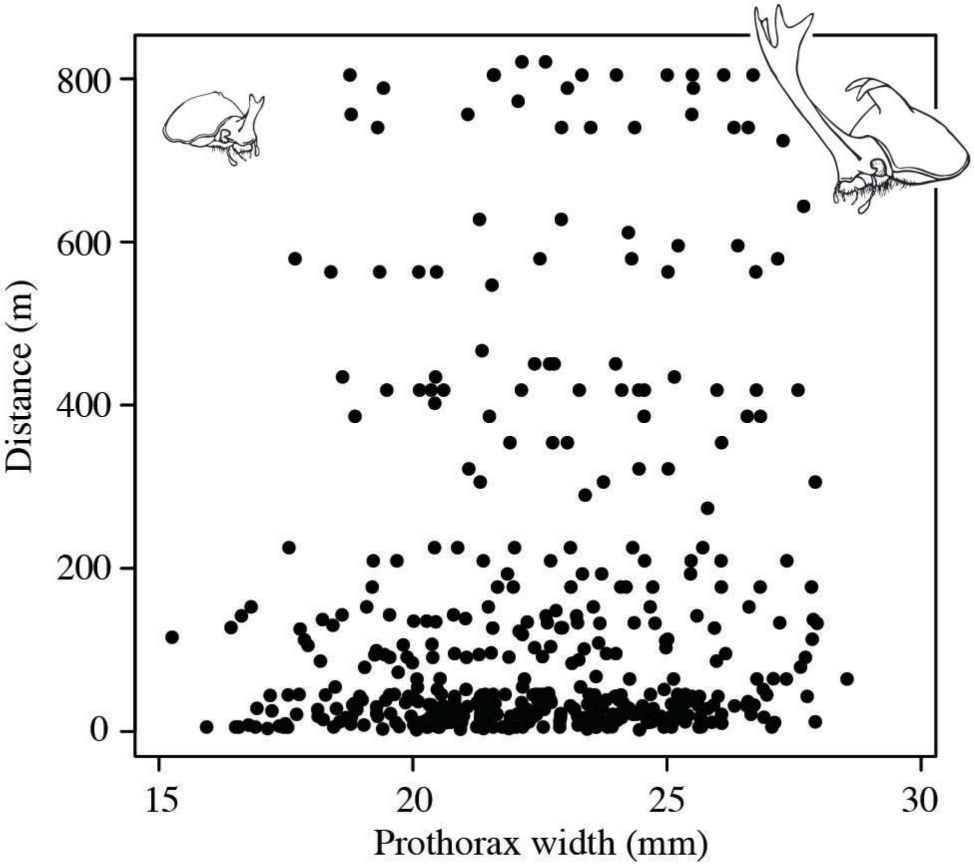

Figure 3. Relationship between prothorax width and distance flown for males (R^2 = 0.008, $F_{1,397}$ = 3.37, P = 0.07).

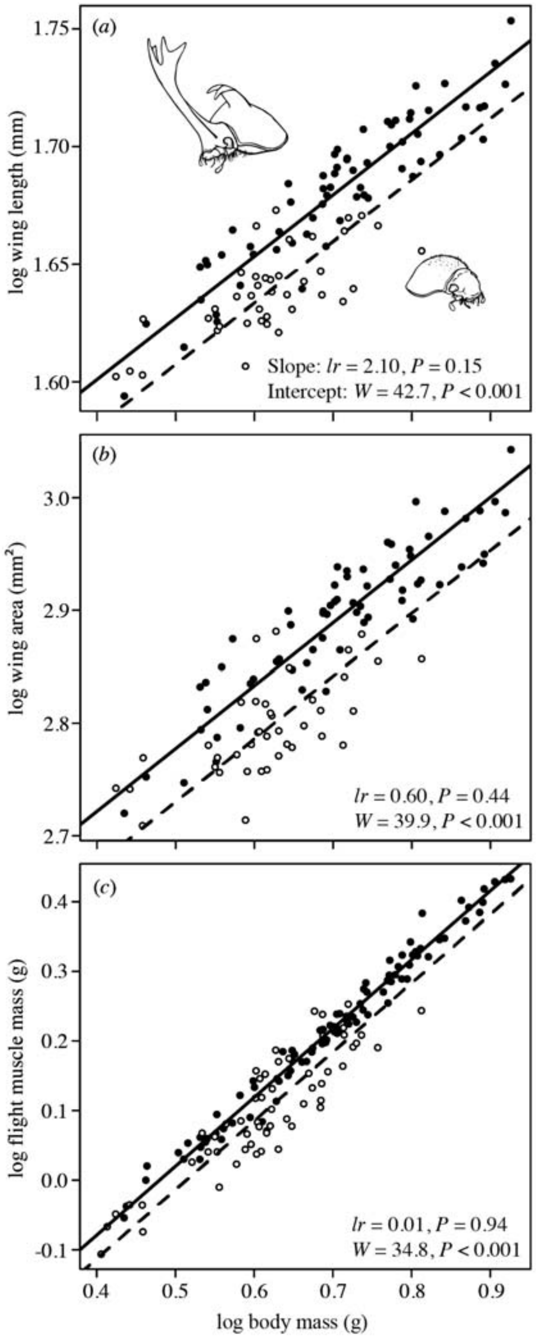

Figure 4. Allometric relationships between body mass and (*a*) wing length, (*b*) wing area, and (*c*) flight muscle mass. Males = closed circles, females = open circles. Lines represent the standardized major axis regression lines for males (solid lines) and females (dashed lines).

Table 1

Explanatory variables retained and removed (shown in italics) for the general linear model predicting male flight speed

Explanatory variable	Coefficient	Standard error	*F*	*P*
Intercept	2.28	0.035		
Horn length (HL)			*1.17*	*0.282*
Prothorax width (PW)			*2.66*	*0.106*
Morph			*0.305*	*0.582*
HL:PW			*2.86*	*0.093*
HL:Morph			*2.66*	*0.106*
PW:Morph			*0.279*	*0.598*

The 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.

Table 2

Explanatory variables retained and removed (shown in italics) for the general linear model predicting male flight distance

Explanatory variable	Coefficient	Standard error	*F*	*P*
Intercept	158.8	11.06		
Horn length (HL)			*2.26*	*0.133*
Prothorax width (PW)			*2.32*	*0.128*
Morph			*1.86*	*0.174*
HL:PW			*1.27*	*0.260*
HL:Morph			*2.24*	*0.135*
PW:Morph			*1.33*	*0.250*

The 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.

Table 3

Explanatory variables retained and removed (shown in italics) for the general linear model predicting male flight muscle mass

Explanatory variable	Coefficient	Standard error	*F*	*P*
Intercept	-0.481	0.010		
Log(Mass)	0.996	0.014	4751.1	< 0.001
Log(HL)			*1.34*	*0.251*
Morph			*2.31*	*0.108*
Log(Mass):Log(HL)			*0.349*	*0.557*
Log(Mass):Morph			*0.331*	*0.567*
Log(HL):Morph			*2.31*	*0.108*

The 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.

Table 4

Explanatory variables retained and removed (shown in italics) for the general linear model predicting male wing length

Explanatory variable	Coefficient	Standard error	*F*	*P*
Intercept	2.28	0.05		
Log(Mass)	0.218	0.048	302.33	< 0.001
Log(HL)	0.349	0.056	40.75	< 0.001
Morph	0.017	0.008	5.23	0.026
Log(Mass):Log(HL)			*1.79*	*0.186*
Log(Mass):Morph			*1.09*	*0.301*
Log(HL):Morph			*0.936*	*0.337*

The 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.

Table 5

Explanatory variables retained and removed (shown in italics) for the general linear model predicting male wing area

Explanatory variable	Coefficient	Standard error	*F*	*P*
Intercept	1.40	0.023		
Log(Mass)	0.115	0.022	350.1	< 0.001
Log(HL)	0.151	0.025	37.63	< 0.001
Morph	0.009	0.003	6.57	0.013
Log(Mass):Log(HL)			*0.658*	*0.421*
Log(Mass):Morph			*0.453*	*0.504*
Log(HL):Morph			*0.883*	*0.351*

The 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.

CHAPTER 2:

Elaborate horns in a giant rhinoceros beetle incur negligible aerodynamic costs

ABSTRACT

Sexually-selected ornaments and weapons are among nature's most extravagant morphologies. Both ornaments and weapons improve a male's reproductive success; yet unlike ornaments that need only attract females, weapons must be robust and functional structures because they are frequently tested during male-male combat. Consequently, weapons are expected to be particularly costly to bear. Here, we tested the aerodynamic costs of horns in the giant rhinoceros beetle, *Trypoxylus dichotomus*. We predicted that the long, forked head horn would have three main effects on flight performance: increased body mass, an anterior shift in the center of mass, and increased body drag. We found that the horns were surprisingly lightweight, and therefore had a trivial effect on the male beetles' total mass and mass distribution. Furthermore, because beetles typically fly at slow speeds and high body angles, horns had little effect on total body drag. Together, the weight and the drag of horns increased the overall force required to fly by less than 3%, even in the largest males. Because low-cost structures are expected to be highly evolutionarily labile, our finding that horns incur very minor flight costs may have permitted both the elaboration and diversification of rhinoceros beetle horns.

Keywords: rhinoceros beetles, horns, aerodynamic costs, sexual selection

1. INTRODUCTION

From foraging for food, to avoiding predators, to finding and securing mates, locomotion is critical to the survival and reproductive success of nearly all animals. Selection for efficient locomotion, particularly among animals that fly, favors streamlined body forms, yet competition for mates has driven the evolution of an array of flashy ornaments and exaggerated weapons that often make males anything but streamlined [1,2]. Thus, the elaborate morphologies that are favored by sexual selection typically oppose the simple, streamlined morphologies favored by natural selection, which has led to a number of compensatory changes to ameliorate the costs of bearing large, sexually-selected traits [3–5].

For example, the outermost feathers of long-tailed, sexually dimorphic male birds are often narrowed at the tips to help offset the aerodynamic drag that these tail ornaments accrue [6,7]. Similarly, in sexually dimorphic stalk-eyed flies, males have significantly longer eye-spans than females, yet overall head mass does not differ because males have smaller eye bulbs and thinner eye stalks [8–10]. In contrast to ornaments (e.g., eye-stalks and tail streamers), however, that merely need to be attractive to females, sexually-selected weapons must be robust and functional structures because they are often tested during male-male combat [11–15]. Consequently, structural modifications that reduce the costs of carrying large weapons may not be favored if these changes compromise the weapons' structural integrity and performance during fights. Sexually-selected weapons are therefore expected to be particularly costly to bear, yet we still know surprisingly little about the costs of carrying these exaggerated structures.

Here, we investigate the aerodynamic costs of elaborate horns in a large Asian rhinoceros beetle, *Trypoxylus dichotomus*. Rhinoceros beetles are an ideal system for investigating the costs of carrying sexually-selected weapons because of the impressive size of their horns: in some species the length of the horn is longer than the length of the rest of the body [16,17]. Moreover, flying is the primary mode of locomotion for rhinoceros beetles, with beetles flying to and from resource sites each night [18,19]. Recent studies have begun to examine the effects of horns on the flight performance of rhinoceros beetles in the field [20,21], yet no studies to date have examined the specific aerodynamic effects of beetle horns, or of any other sexually-selected weapon.

We predicted that the long, forked head horn of male *T. dichotomus* would have three primary effects on the beetles' flight performance. First, we expected that the large horns would significantly increase total body mass, thereby increasing the amount of lift required to support the beetle's weight to stay aloft [22–24]. Second, we expected that the long horns extending forward from the head would significantly shift the center of mass forward, which could alter stability and maneuverability [25,26]. And third, we expected that the pitchfork-like head horn would significantly increase total body drag, thereby increasing the amount of thrust required to drive the beetle forward [22–24]. We present a set of experiments that investigate the consequences of horns on the beetles' total body mass, mass distribution, and aerodynamic drag, and then discuss how these three factors contribute to the overall cost to fly with an elaborate sexually-selected horn.

2. METHODS

T. dichotomus is a large Asian rhinoceros beetle with a wide variation in body and horn size. Large males have a branched head horn that can reach nearly two-thirds the length of their body, and a smaller thoracic horn; small males have a short head horn and tiny thoracic horn; and females are hornless. Males use their horns to pry rival males away from wounds or sap sites on trees where females come to feed [27,28]. Males with the longest horns are more likely to win fights and gain access to these sap sites, and to achieve higher mating success [29,30].

Beetles were purchased as final instar larvae from a commercial insect distributor (Yasaka Dabuto Kuwagata World, Japan) and reared to adulthood at the University of Montana. Horn lengths of all males and prothorax widths (a standard measure of body size; [31]) of both males and females were measured to the nearest 0.01 mm with dial calipers.

The mass contribution of the horns was measured by placing males (n = 91) in airtight containers, euthanizing them by freezing, and weighing each beetle to the nearest 0.001 g with an analytical balance. The head and thoracic horns were removed using nail clippers, and then the horns and hornless body were re-weighed. Horns were hollow, air-filled, and surprisingly lightweight. We therefore measured the relative moisture content of the horns and three additional body parts – the legs, elytra, and head/pterothorax (hereafter referred to as the thorax) – in order to further investigate potential differences in the composition of the horns. Relative moisture content was measured by severing and weighing each body part, drying the samples to a constant mass for at least 72h, and then re-weighing them dry. Relative moisture content was calculated as the difference between wet and dry masses, divided by the original wet mass.

A plumb line method was used to measure the beetles' center of mass [24]. Freeze-euthanized beetles (males: *n* = 56; females: *n* = 40) were pinned dorso-ventrally through the anterior distal corner of their right elytron and balanced on two horizontal bars. A digital photograph of each beetle was taken after it had settled. The pin was then removed and reinserted through the outer corner of the left elytron. The beetle was balanced again on the bars, and a second photograph was taken. A line of gravity was drawn on each photograph through the suspension point, and the two photographs were superimposed. The center of mass was defined by the intersection of the two lines of gravity, and measured as a fractional position along the anteroposterior body axis between the clypeus (position = 0) and the tip of the abdomen (position = 1).

Total body drag was measured by mounting dried specimens onto a custom-made, calibrated force transducer (see [32] for details). Because we were interested in the aerodynamic effects of horns, we dried the beetles with their elytra closed and legs removed, which minimized the effects of other body parts on flow dynamics. Drag was measured on 10 large males, 10 small males, and 8 females. To determine the relative contribution of the horns to drag, half of the males (5 large and 5 small) were measured both before and after the head horn and thoracic horn were removed. Drag was measured for each beetle in a variable speed wind tunnel (see [33] for details) at body angles-of-attack relative to flight direction (hereafter referred to as body angle) ranging from 0 to 90°, and wind speeds ranging from 0.5

to 8 m/s. Throughout this paper, “wind speed” refers to *equivalent* wind speed, which is the true wind speed at sea level that would produce the same dynamic pressure given the observed air pressure. We preferred to adjust equivalent wind speed in our experiments in order to control for daily fluctuations in temperature and barometric pressure, and because it is equivalent wind speed, rather than true wind speed, that determines the magnitudes of forces acting on a flying beetle [34]. Typical flight speeds were determined previously [21] using a high performance speed sensor on free-flying beetles in the field. Typical body angles were measured by filming beetles flying down the laboratory hallway at 500 frames per second with a high-speed video camera (Photron SA3).

To control for the large variation in size among individuals, drag measurements were converted to drag coefficients (C_D):

$$C_D = \frac{2D}{\rho S u^2}$$

where D is the measured drag, ρ is air density, S is frontal surface area, and u is wind speed. Drag coefficients of manipulated males are based on frontal surface areas after the horns were removed. Frontal surface areas were measured using imaging software (ImageJ v1.41, National Institutes of Health) from digital photographs of the beetles at 0° body angle. Our results are qualitatively the same when the drag coefficients were calculated using the frontal surface area specific to each body angle. (See [35] for rationale for keeping frontal surface area constant.)

Finally, we used 2D particle image velocimetry (PIV) to visualize the effects of horns on the near-wake fluid dynamics of beetle specimens mounted in the wind tunnel with body angles of 50° and wind speeds of 3 m/s. Details on our PIV system are described elsewhere [32]. In brief, we seeded the air using sub-micron sized olive oil particles, positioned the laser to illuminate a parasagittal slice just off of the beetle's midline, and placed the camera perpendicular to the planar illumination field. We used cross-correlation of paired images (elapsed time between images = 210 μs) and adaptive multi-pass processing to calculate particle velocity. We computed average particle velocity and vorticity using 50 sequential image pairs. To reduce surface reflections, beetle specimens were coated with a thin layer of rhodamine B dissolved in acrylic laquer [36]. To quantify the effect of horns on the near-wake flow field, we measured average and minimum horizontal and vertical velocities 1.5 body lengths behind each beetle.

3. RESULTS

The horns of male *T. dichotomus* made a very minor contribution to the beetles' total body mass. Relative horn mass ranged between 0.5 to 2.5% (1.5 ± 0.6%, mean ± SD). The horns were significantly drier than other body parts (ANOVA: $F_{3,96} = 1156.7$, $P < 0.001$): relative moisture content was 63.8 ± 1.7% in thoraces, 54.2 ± 2.0% in legs, 39.8 ± 2.1% in elytra, but only 25.7 ± 3.6% in horns.

The center of mass was significantly closer to the head in males compared to females (Welch's t-test: $t = 10.35$, $df = 66.57$, $P < 0.001$); the fractional position was 0.48 ± 0.03 in males and 0.57 ± 0.05 in females. There was no relationship between center of mass and body size in females ($R^2 = 0.03$, $F_{1,38} = 1.18$, $P = 0.29$), but there was a significant correlation in males (Fig. 1, $R^2 = 0.34$, $F_{1,54} = 28.42$, $P < 0.001$); the center of mass was more anterior in large males than small males. However, the horns themselves had a trivial effect on the center of mass. The center of mass was significantly closer to the head in males with their horns intact compared to hornless males (Paired t-test: $t = -5.86$, $df = 55$, $P < 0.001$), but horn removal shifted the center of mass by only 1.7%. Furthermore, there was no relationship between body size and the change in center of mass between intact and hornless males ($R^2 = 0.001$, $F_{1,54} = 0.05$, $P = 0.82$), indicating that the long horns of large males did not shift the center of mass more than the short horns of small males.

Beetles flew at slow speeds and high body angles. Typical flight speeds were between 1 and 4 m/s (2.27 ± 0.44 m/s), and body angles ranged between 30 and 85° (54 ± 12°). As expected from aerodynamic theory, drag coefficients increased at higher body angles (Fig. 2). However, drag coefficients at body angles from 0 to 90° did not differ significantly between males with and without their horns (Repeated-measures ANOVA: $F_{1,96} = 2.29$, $P = 0.13$). Intact males had slightly higher drag coefficients compared to hornless males at shallow body angles (<30°), but they actually had slightly lower drag coefficients at high body angles (>30°); this is opposite what we would expect if horns significantly increased body drag. Moreover, at the beetles' typical flight speed (3 m/s) and body angle (50°), drag coefficients did not differ among large males with and without their horns, small males with and without their horns, and naturally hornless females (ANOVA: $F_{1,35} = 2.26$, $P = 0.14$).

Visualizations of the airflow around mounted beetles using PIV (Fig. 3) provided further evidence that the beetles generate substantial drag during flight, but that the horns have a relatively small effect. The bodies of horned males generated a wide, drag-based wake with strong vortex shedding dorsally and ventrally (Fig. 3a), as expected for a non-streamlined body at moderate Reynolds numbers ($Re \approx 10{,}500$). There was a large momentum deficit in the wake of the male's body, indicating nearly complete flow separation; average horizontal velocity in the near-field wake was only 70% of the free-stream velocity, and minimum horizontal velocity was only 27% of free-stream flow.

The male's horn also produced a drag-based wake, but the contribution of the horn was small compared to the rest of the body: the horn's wake width was only 20% of the width of the body wake. Moreover, the loss of horns did not substantially change the flow field. Males without their horns (Fig. 3b) and naturally hornless females (Fig. 3c) also produced a fully-separated, drag-based wake, and the momentum deficit in the wake of hornless males and females was similar to that of the intact males: minimum horizontal velocity was 31% of free-stream flow in the wake of hornless males, and 30% in females. Furthermore, even in the hornless males and females, the air stream detached from the surface of the beetles at the maximum width of the body, as expected for a non-streamlined object. As a result, the lack of horns did not delay flow separation or help streamline the beetle's body. There was, however, a slight net downward induced flow for all beetles. Average vertical velocity in the near-field wake was -0.62 m/s for horned males, -0.52 m/s for hornless males, and -0.48 m/s for females. These results indicate that, despite the non-streamlined bodies and high body angles, beetles produced a small amount of body lift [32].

4. DISCUSSION

The horns of rhinoceros beetles are among the most elaborate traits found in nature, and intuitively we expect these structures to impair locomotion. However, our data show that the aerodynamic costs of bearing large horns are exceptionally small, and probably biologically negligible. In particular, the drag coefficients and patterns of airflow around beetles were essentially the same for males flying with and without their horns. Drag increases exponentially with flight speed [35], so minimizing drag may be relatively unimportant for beetles that typically fly at slow speeds. Furthermore, because beetles fly at very high body angles, the large projected area and prominent flow separation in the wake of the beetles' body swamp the relatively small drag contribution from the horn.

Large horns also contributed surprisingly little to the beetles' total body mass. Even in the largest males, horns represented only 2.5% of the beetles' total mass. While the center of mass was closer to the head in large males compared to small males, this difference did not stem from the disproportionately long horns of large males. Rather, the center of mass appears to be shifted towards the head in large males because they have larger prothoracic muscles (McCullough, unpublished data), which are critical for generating sufficient torque to dislodge rivals off the trunks and branches of trees during male-male combats [27,30,37].

In order to fly at a constant velocity, an animal must generate enough lift to overcome its body weight, and enough thrust to overcome its body drag. Horns represent a very small increase in total body weight and body drag, and therefore result in a trivial increase in the overall force required to fly. Among the largest males, less than 3% more force is required to fly with a horn compared to without a horn; among the smallest males, the added force requirement is less than 2%.

Many insects are able to carry loads substantially heavier than their body weight [38]. For example, vespid wasps often carry animal prey that weigh 20-70% of their body mass [39,40], and foraging bumblebees may return to the nest with nectar and pollen loads that double their body mass [41]. As a result, it is unlikely that the beetles are significantly burdened by their lightweight horns. Moreover, because the horns of *T. dichotomus* are among the largest found in rhinoceros beetles, and the only horns with such a broad, forked tip [17], it is unlikely that aerodynamic costs will be significant in other species with smaller horns.

Rhinoceros beetles exhibit an impressive diversity in the shape, size, number, and location of their horns [16,17]. We suspect that this morphological variation largely reflects species differences in the tactics used during male-male fights. Males fight on various substrates (e.g., broad tree trunks, narrow bamboo shoots, and inside burrows), and wield their horns in different ways to pinch, push, or pry their opponents away from valuable resource sites [13,14,28]. As a result, sexual selection may have favored divergent horn designs, as different types of weapons are likely to perform best depending on where and how they are used.

Given that variations in the shape and size of horns appear to have very minor consequences on the beetles' flight performance, the exaggeration and diversification in horn morphology driven by male-male competition may have been largely unopposed by natural selection for efficient locomotion. Previous authors have similarly argued that the diversity of tail ornaments among birds may reflect the fact that tails are hidden by the wake of the body and thereby have minimal aerodynamic effects [42]. We suggest that, as in bird tail ornamentation, the large horns of rhinoceros beetles may effectively "hide" from selective pressures because of the enormous wake of the body due to the beetles' slow flight speeds and high body angles. However, whether the evolution of horns has been constrained by selection to minimize costs in other naturally-selected tasks remains to be tested. Future studies are also necessary to determine if horns approach their mechanical limits during intense fights, and whether functional limitations play a role in determining the shape and size of the beetles' elaborate horns.

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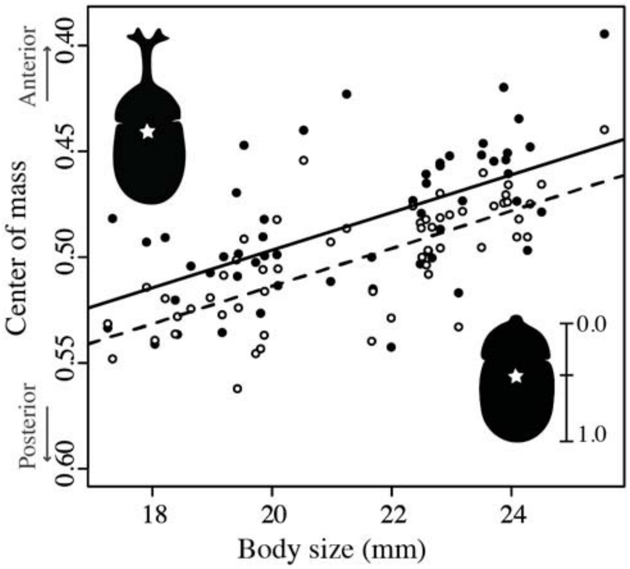

Figure 1. Relationships between body size and center of mass for males with horns (closed circles, solid line) and without their horns (open circles, dashed line). The center of mass was significantly closer to the head in large males than small males ($R^2 = 0.34$, $F_{1,54} = 28.42$, $P < 0.001$), and in horned males compared to hornless males ($T = -5.86$, $P < 0.001$). Horn removal, however, resulted in only a 1.7% shift in the center of mass.

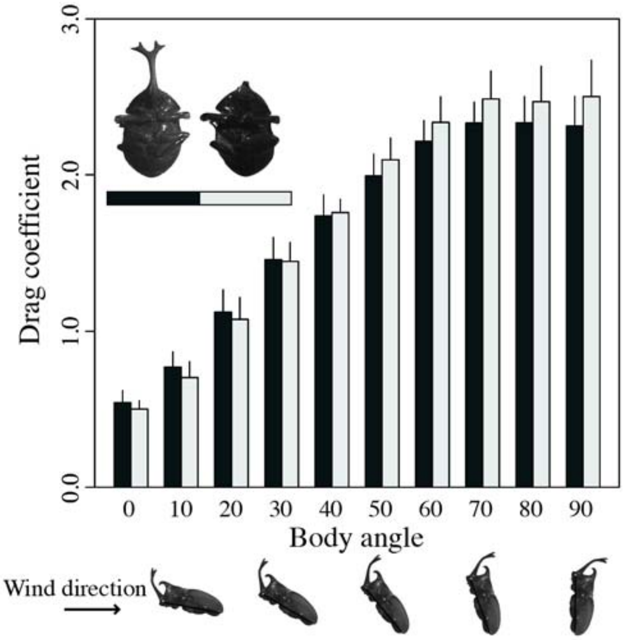

Figure 2. Drag coefficient as a function of body angle for males with horns (closed bars) and without their horns (open bars). Drag coefficients increased with increasing body angle, but did not differ between males with and without their horns (repeated-measures ANOVA: $F_{1,96} = 2.29$, $P = 0.13$). Inset: Representative frontal pictures of an intact and hornless male at 50° body angle.

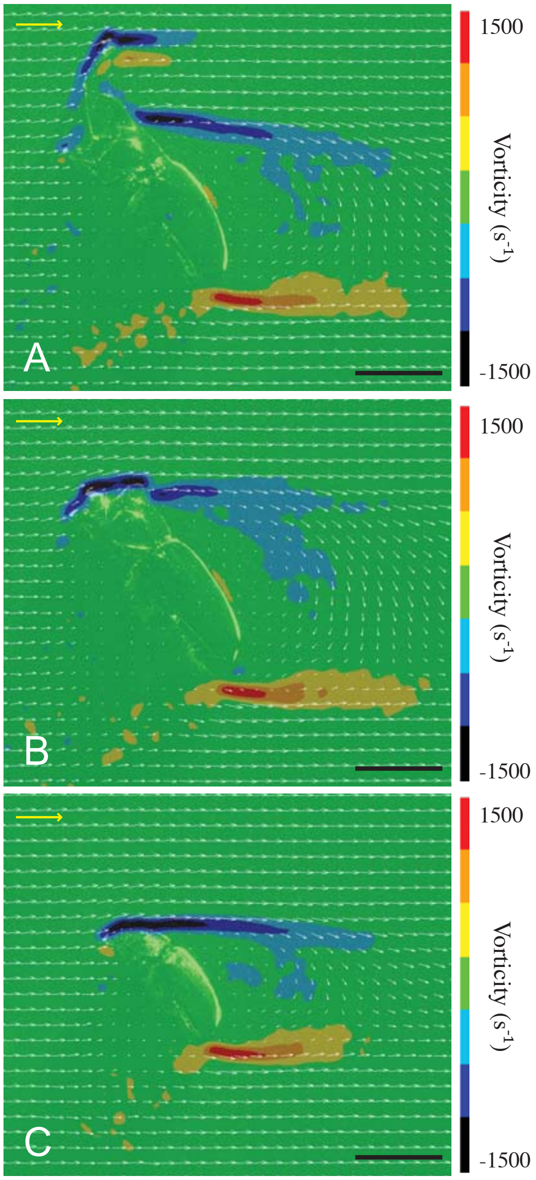

Figure 3. Time-averaged flow fields of a parasaggital PIV sample around mounted beetles in a wind tunnel. Wind speed = 3 m/s and body angle = 50°. White vectors in the foreground indicate average velocity; coloured background indicates average vorticity. Large males with their horns (A), without their horns (B), and naturally hornless females (C) all generated drag-based wakes with complete flow separation. Horns also produced a strong drag wake, but their contribution to overall drag was small compared to the rest of the body. All beetles generated a slight net downward induced flow, indicating a small amount of body lift production. Yellow scale vector = 10 m/s; Black scale bar = 20 mm.

CHAPTER 3:

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

ABSTRACT:

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 signaling 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 find no evidence for fitness costs. Previously we found that horns do not impair flight performance, and here we show that horns do not stunt the growth of other body structures or weaken the beetles' immune response. Finally, and most importantly, horns do 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.

Keywords: rhinoceros beetles, horns, sexual selection, male-male competition, costs, *Trypoxylus dichotomus*

INTRODUCTION

Sexual selection is a powerful evolutionary force that has given rise to many of nature's largest, most colorful, 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 signaling 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 signaling systems.

The prevailing explanation for honest signaling is that cheating is prohibitively costly (Zahavi 1975; Zahavi & Zahavi 1997). Specifically, if the marginal costs of signaling are greater for individuals of low quality than 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 see [Wilson & Nussey 2010; Lailvaux & Kasumovic 2011] for discussions on the difficulties in defining and measuring individual quality and condition). 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 signaling equilibrium (Hurd 1995; Lachmann et al. 2001; Számadó 2011). This situation may be uncommon for ornaments because choosy females cannot easily verify if 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 signaling may be favored 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 Japanese horned beetle. *Trypoxylus dichotomus* is a giant rhinoceros beetle common in mountainous regions in East Asia. 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 tradeoffs 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 tradeoffs in rhinoceros beetles (Kawano 1995), and tradeoffs 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).

Here, we tested for tradeoffs in resource allocation between horns and four morphological traits (wings, eyes, fore-legs, and genitalia) and three measures of immune response (encapsulation response, phenoloxidase activity, and hemocyte density). Additionally, we tested whether these potential tradeoffs 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, fore-tibia length, and aedeagus (genitalia) length were measured to the nearest 0.01 mm with dial calipers. Wing length was measured using imaging software (ImageJ v1.46) from digital photographs of flattened wings.

Horns are disproportionately long in large males and disproportionately short in small males. We therefore tested for morphological tradeoffs by comparing the relationships between horn length and the four morphological traits after controlling for the possible confounding variation in body size. Specifically, we fit 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 and et al. 1997). We investigated both responses by measuring the beetles' encapsulation response, hemocyte density, and phenoloxidase (PO) activity. Cellular encapsulation is the main line of defense against multicellular pathogens, such as nematodes, fungi, and parasitoids (Gillespie and et al. 1997). The process occurs when a pathogen is surrounded by layers of hemocytes (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 hemocoel 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 hemocyte 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 hemocytes circulating in the hemolymph (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 tradeoffs 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 (5.35 ± 1.55 g; mean ± sd; $N = 44$) compared to the unfed group (5.03 ± 1.39 g; $N = 45$), but body mass did not differ significantly between the two treatments ($T = 1.03$, $df = 85.56$, $P = 0.31$). Beetles were weighed to the nearest 0.001 g with an analytical balance, and horn length was measured to the nearest 0.01 mm with dial calipers. Both morphological measurements were log transformed before analyses.

To measure encapsulation response, beetles were anaesthetized by placing them in the freezer for 10 minutes, and two sterile 3 mm-long pieces of nylon monofilament were inserted into their body cavities through punctures into the left and right sides of the abdomen. The beetles' immune system was allowed to react to the implants for 24 hours, and then the implants were dissected from the body cavity. The implants were photographed under a stereomicroscope, and the gray value of reflected light was analyzed using imaging software (ImageJ v1.46). We compared the gray value of each implant to the gray value of a control, un-implanted piece of nylon monofilament, and used the difference between the two gray 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 hemolymph from the body cavity, so we used a “flush” method (Chino et al. 1987) to collect hemolymph for the PO activity assays and hemocyte counts. Before removing the implants, which is a destructive procedure, the beetles were slowly injected with phosphate-buffered saline (pH 7.4) into the 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 hemolymph from the wound. A 10 μL sample of buffered hemolymph was mixed with 20 μL of anticoagulant (Cotter et al. 2004) and reserved for the hemocyte counts. The remaining hemolymph was frozen immediately to disrupt the hemocyte membranes and release cellular PO for the PO assays.

PO activity was measured spectrophotometrically using dopamine as a substrate following the basic methods described in Cotter and Wilson (2002). We added 90 μL of 10 mM dopamine to 90 μL of the buffered hemolymph, 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 minutes. Preliminary results (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 minutes.

A sample of 12 μL of the hemolymph-anticoagulant mixture was pipetted onto each side of a Neubauer hemocytometer. Hemocytes were counted in four non-adjacent squares, and hemocyte density was expressed as the number of cells per mL of buffered hemolymph for each beetle.

We tested for immune tradeoffs 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 fit GLMs for encapsulation rate, PO activity, and hemocyte 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 non-significant 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 hemocyte 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 hemocyte density. Furthermore, if beetles invest additional resources towards their immune system by feeding as adults, we expected stronger immune responses for beetles in the fed group compared to the unfed group. We therefore expected a negative partial effect of treatment (unfed vs. 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 to 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 calipers, 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 four hours between 20:00h and 04:00h 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 tradeoffs 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 fore-tibiae, but was not correlated with aedegaus 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, fore-tibia, and aedeagus size (Table 1). These results indicate that males with relatively long horns for their body size also had relatively long wings, fore-tibiae, and aedeagi.

Immune responses

We found no evidence for resource allocation tradeoffs for encapsulation rate, PO activity, or hemocyte density (Tables 2 and 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 hemocyte density ($R^2 = 0.03$, $F_{1,76} = 2.12$, $P = 0.15$). Body mass and food treatment also were not significant predictors of either PO activity or hemocyte density; model simplification indicated that the minimum adequate models predicting both PO activity and hemocyte 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.

Survival analyses

The total period analyzed 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 ($\chi^2 = 0.03$, $df = 1$, $P = 0.87$) or horn length ($\chi^2 = 0.29$, $df = 1$, $P = 0.59$) as covariates 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 have measured four of the most relevant costs associated with carrying and producing an elaborate weapon. Remarkably, we find 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 horns 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 were significant predictors 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 tradeoffs

Contrary to our expectation for morphological tradeoffs, 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; Balmford et al. 1994; Andersson & Andersson 1994; Møller et al. 1995), and male stalk-eyed flies with exaggerated eye-spans (Swallow et al. 2000; Husak et al. 2011) have relatively larger wings than females, 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 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 horns as pitchforks to pry their opponents off the trunks and branches of trees (Siva-Jothy 1987; Hongo 2003). Long fore-legs 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 fore-tibiae 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 data 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 size 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. dichtomus* (Siva-Jothy 1987; 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 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 tradeoffs in beetles and other insects (Kawano 1995, 1997; Nijhout & Emlen 1998; Stevens et al. 1999; Emlen 2001; Moczek & Nijhout 2004; Parzer & Moczek 2008), these tradeoffs are not universal (Simmons & Emlen 2006). In some cases, the evidence for tradeoffs is weak, and in others, tradeoffs are nonexistent (CE Allen and DJ 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 tradeoffs 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 tradeoffs depends on individual variation in the ability to acquire resources (van Noordwijk & de Jong 1986). Indeed, tradeoffs 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 tradeoffs. We found no differences in the relationships between horn length and body size among our lab-reared beetles, beetles collected on the campus grounds for the survival analyses, and 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 tradeoffs.

No survival costs

We cannot rule out the possibility that horns incur significant, but as-yet-unidentified costs. However, in order 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). It is important to note, however, that both our study and Hongo and Kaneda's study 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 signaling, 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 ones, 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 one 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). *T. dichotomus* males do fight vigorously enough to break their horns (Siva-Jothy 1987; McCullough, in prep), 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 in order 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. In order 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 favored 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.

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Table 1. Results from GLMs examining the effects of body size and horn size on male morphological characters

Explanatory variable	Coefficient	SE	*t*	*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
Fore-tibiae:				
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.

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

Explanatory variable	Coefficient	SE	*F*	*P*
Encapsulation rate:				
Intercept	57.17	16.82		
Treatment	11.60	3.02	11.44	<0.01
Log(HL)	-46.75	13.30	12.36	<0.001
Log(mass)			*0.47*	*0.50*
Log(mass):Log(HL)			*1.99*	*0.16*
Log(HL):Treatment			*0.05*	*0.82*
Log(mass):Treatment			*0.34*	*0.56*
PO activity:				
Intercept	-1.85	0.03		
Treatment			*3.66*	*0.06*
Log(HL)			*0.31*	*0.58*
Log(mass)			*2.13*	*0.15*
Log(mass):Log(HL)			*3.91*	*0.05*
Log(HL):Treatment			*2.10*	*0.15*
Log(mass):Treatment			*0.61*	*0.44*
Hemocyte density:				
Intercept	6.35	0.03		
Treatment			*2.07*	*0.15*
Log(HL)			*1.87*	*0.18*
Log(mass)			*3.09*	*0.08*
Log(mass):Log(HL)			*0.09*	*0.77*
Log(HL):Treatment			*0.55*	*0.46*
Log(mass):Treatment			*0.07*	*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.

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

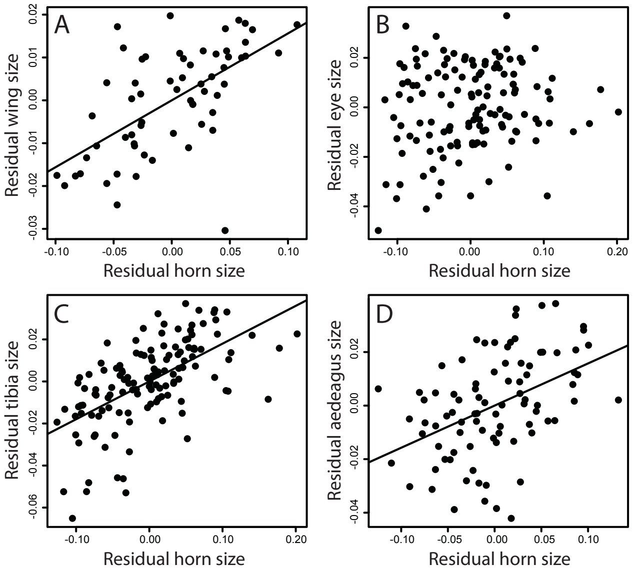

Figure 2. Partial regression plots showing the relationship between horn length and (a) wing length, (b) eye diameter, (c) tibia length, and (d) aedeagus length after controlling for variation in body mass. Lines represent significant partial correlations.

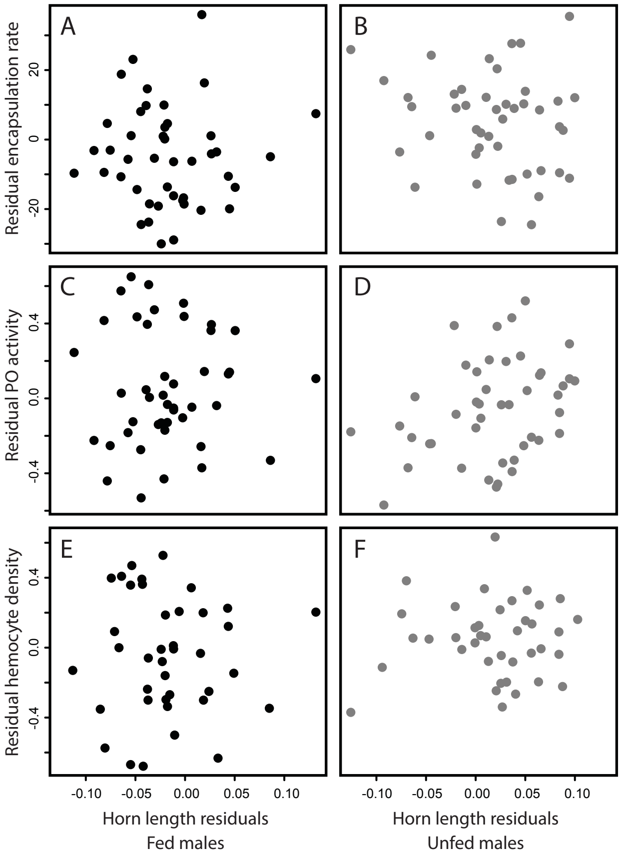

Figure 3. Partial regression plots showing the relationship between horn length and (a,b) encapsulation rate, (c,d) PO activity, and (e,f) hemocyte density after controlling for variation in body mass. Left panels (black points) show partial correlations for fed males; right panels (gray points) show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

CHAPTER 4:

Mechanical limits to maximum weapon size in a giant rhinoceros beetle

ABSTRACT

The horns of giant rhinoceros beetles are a classic example of the elaborate morphologies that can result from sexual selection. Theory predicts that sexual traits will evolve to be increasingly exaggerated until survival costs balance the reproductive benefits of further trait elaboration. In *Trypoxylus dichotomus*, long horns confer a competitive advantage to males, yet previous studies have found that they do not incur survival costs. It is therefore unlikely that horn size is limited by the theoretical cost-benefit equilibrium. However, males sometimes fight vigorously enough to break their horns, so mechanical limits may set an upper bound on horn size. Here, I tested this mechanical limit hypothesis by measuring safety factors across the full range of horn sizes. Safety factors were calculated as the ratio between the force required to break a horn and the maximum force exerted on a horn during a typical fight. I found that safety factors decrease with increasing horn length, indicating that the risk of breakage is indeed highest for the longest horns. Structural failure of oversized horns may therefore oppose the continued exaggeration of horn length driven by male-male competition and set a mechanical limit on the maximum size of rhinoceros beetle horns.

KEYWORDS

rhinoceros beetles, horns, safety factors, mechanical limit, sexual selection

INTRODUCTION

Sexual selection drives the evolution of many of Nature's most conspicuous morphologies and displays [1,2]. A central tenet of sexual selection theory is that sexual traits are costly, and that these traits will evolve to be increasingly exaggerated until naturally-selected survival costs balance the reproductive benefits of further trait elaboration [2–4].

In many systems, the size or intensity of sexual traits and displays does indeed reflect an equilibrium between mating benefits and survival costs [5]. For example, male field crickets with longer calling bouts and higher call rates are favoured by choosy females, but are also more likely to be attacked by parasitoid flies that use these calls to locate their hosts [6]. Similarly, male guppies with brighter colour patterns are more attractive to females, but are also more conspicuous to predators [7]. As a result, these sexual traits represent a selective balance between mating benefits and survival costs.

In other systems, however, sexual traits do not appear to reflect a balance between benefits and costs. In fact, surprisingly few studies have found evidence that sexual traits incur evolutionarily significant costs [8–10], and there are now several examples of exaggerated ornaments and weapons that are not nearly as costly as we might expect [11–13]. These observations suggest that the evolution of sexual traits is constrained by other factors before reaching the theoretical cost-benefit equilibrium.

The horns of the Asian rhinoceros beetle, *Trypoxylus dichotomus*, are one such example of an elaborate, but inexpensive sexually-selected trait. Males have a long, forked head horn that they use to pry rival males away from wounds or sap sites on trees where females come to feed [figure 1; 14,15]. As in other rhinoceros beetles [16,17], *T. dichotomus* horns are positively allometric, such that large males have disproportionately long horns and small males have disproportionately short horns [14,15]. Males with longer horns are more likely to win fights and gain ownership of these sap sites, and therefore achieve higher mating success [18,19]. Surprisingly, although horns can reach nearly two-thirds the length of the body in the largest males, they appear to incur no costs. Specifically, horns do not impair the beetles' ability to fly [20–23]; horns do not stunt the growth of other body structures [24]; horns do not weaken the beetles' immune function [24]; and, most importantly, horns do not reduce male survival [24,25]. Thus, there appears to be directional sexual selection for increasing horn size, yet little or no counterbalancing selection due to low survival. What prevents *T. dichotomus* males from evolving even longer horns?

I hypothesized that mechanical limits set an upper bound on horn size. That is, the continued exaggeration of horn size may be constrained by the risk of structural failure of the horn itself. *T. dichotomus* males fight vigorously enough to break their horns [14; personal observation], which suggests that horns are sometimes pushed to their structural limits. Furthermore, because males cannot repair or replace a broken horn, injured males are effectively removed from the mating pool. Yet whether unusually large weapons are more susceptible to failure is currently unknown.

To test this mechanical limit hypothesis, I estimated the safety factors of horns across the full range of horn sizes, using both lab and field measurements. Safety factors quantify how "safe" a structure is relative to the loads it is likely to experience, and are defined in this study as the ratio between the load that causes a horn to fail and the maximum expected load [26].

During fights, a *T. dichotomus* male inserts his head horn underneath an opponent, and uses it like a pitchfork to pry the rival up and off the substrate. Thus, to win a fight, a male must overcome his rival's ability to hold on to the tree, or his grasping force. Game theory predicts that males will only escalate to intense, sustained fights when the contestants are of equal size [27–29], and field observations indicate that this is true for *T. dichotomus* [15,30]. Because the maximum loads a horn must withstand occur during fights with a size-matched rival, a male's own grasping force can be used as a proxy for the maximum fighting forces likely to be experienced by his horn. If mechanical limits are important in determining maximum horn size, safety factors should decrease with increasing horn length.

METHODS

Field observations were conducted at 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. The study was conducted in June and July when adults are most abundant. All males found in the study area were collected from their natural sap sites and individually marked with quick-drying paint pens. Head horn length (hereafter simply referred to as horn length) and body size were measured to the nearest 0.01 mm using dial calipers. Horn length was measured from the base of the head horn to the tip of the lateral-most tine, and body size was measured as prothorax width. Males were scored as having no visible injuries, moderate injuries (i.e., scrapes or small wounds on their horns, pronota, or elytra), or severe injuries (i.e., several broken tines or entirely broken horns).

I estimated how much force a male would exert and transmit through his horn to dislodge a typical size-matched rival (hereafter referred to as "fighting force") by measuring that male's own grasping force. I measured the fighting forces of field-collected males (n = 278) by clamping a spring scale onto their short thoracic horn (which is just above the beetle's centre of mass [23]), and slowly pulling on the scale until the beetle was dislodged from the tree. Fighting force was measured five times for each individual, with a one-minute rest between trials. Beetles were observed closely to ensure that no trials resulted in obviously submaximal performance, and there was no evidence that they fatigued over time (repeated-measures ANOVA: $F_{4,1230} = 0.62$, $p = 0.65$). The maximum force from these trials was used in the statistical analyses (see [31] for justification for using maximal performance values). All trials were conducted at natural sap sites between 20:00h and 04:00h when the beetles are most active.

To estimate the loads that cause horn failure, I purchased beetles as final instar larvae from a commercial insect vendor and raised them to adulthood in the lab. Lab-reared beetles were used in the breaking tests to control for differences in age and thus variation in the wear and fatigue of horns. Adult males were housed in individual glass containers, and experiments were conducted when beetles were approximately 10 days post-eclosion. Horn length and body size were measured to the nearest 0.01 mm using dial calipers. The heads of cold-euthanized males (n = 76) were dissected from the body and fixed at the base to a support block using fast-drying epoxy. The distal tip of the horn was also embedded in a thin strip of epoxy to minimize stress concentrations. To prevent desiccation, the horns were wrapped with wet paper towels while the epoxy hardened.

The maximum force supported by a horn before failure (hereafter referred to as “breaking force”) was measured using an Instron In-Spec 2200 mechanical tester. Horns were loaded in cantilever bending to mimic the loading regime experienced during a typical fight. The safety factor for each horn was then calculated as the measured value of breaking force divided by the estimated value of fighting force. Fighting force estimates for beetles used in the breaking force tests were derived from the best-fit line from ordinary least squares regression: *fighting force* = 0.12 × *horn length* + 0.94 (see also Results). The results are qualitatively the same when fighting forces were estimated as a function of body size instead of horn length, because body size and horn length are strongly correlated ($R^2 = 0.91$).

After horn failure, the maximum stress experienced within the horn cuticle before fracture was estimated using classic beam theory [32]. The distance along the antero-posterior axis of the horn between the tip (i.e., point of force application) and the fracture margin was measured, and then the free end of the horn was embedded in clear acrylic resin and sanded until smooth to measure the cross-sectional geometry of the horn (e.g., height and second moment of area) as close as possible to the site of failure. The outer and inner margins of the cuticle at this location were traced manually from digital photographs using ImageJ (National Institutes of Health), and the second moment of area (a shape factor that describes the distribution of mass in the cross-section about the neutral bending axis) was measured with the BoneJ plugin [33]. The ultimate bending stress (σ) of the horn cuticle at the site of failure was then calculated as:

$$\sigma = \frac{Frc}{I}$$

where *F* is the breaking force, *r* is the distance between the fracture margin and the point of force application, *c* is half the height of the horn cross-section, and *I* is the second moment of area.

RESULTS

Of the 1012 males collected and measured in the field, 174 (17%) showed some sign of injury, and 44 (4%) had severely damaged horns (figure 2). Injured males were significantly larger than uninjured males (ANOVA: $F_{1,1010} = 19.14$, $p < 0.001$). The average body size was 24.66 ± 2.27 mm (mean ± SD) for severely injured males, 23.89 ± 2.77 mm for injured males, and 23.04 ± 2.70 mm for uninjured males. These observations are consistent with those of Siva-Jothy [14], who also found that severe horn damage was most common among the largest males.

Maximum fighting forces increased with horn length (figure 3; $R^2 = 0.48$, $F_{1,275} = 252.2$, $p < 0.001$), indicating that longer horns experience higher loads. There was also a weak, but statistically significant relationship between breaking force and horn length (figure 3; $R^2 = 0.08$, $F_{1,74} = 6.05$, $p = 0.02$), indicating that longer horns can withstand higher loads. Horns broke at various locations between the middle and base of the horn shaft, but there was no relationship between horn length and the location of failure along the antero-posterior axis of the horn ($R^2 = 0.001$, $F_{1,74} = 0.04$, $p = 0.85$). The ultimate bending stress of the horn cuticle at the point of fracture ranged from 92.5 to 394.6 MPa (201.4 ± 75.2 MPa). There was no relationship between bending stress and horn length ($R^2 = 0.02$, $F_{1,70} = 1.07$, $p = 0.30$), but there was a significant relationship between bending stress and the location of failure along the length of the horn ($R^2 = 0.54$, $F_{1,70} = 83.28$, $p < 0.001$). Ultimate bending stress decreased from the proximal base to the middle of the horn shaft, which may be largely explained by the corresponding decrease in second moment of area (unpublished data).

Safety factors ranged from 3.5 to 10.3 (6.5 ± 1.6). There was a significant negative relationship between safety factor and horn length (figure 4; $R^2 = 0.13$, $F_{1,74} = 10.9$, $p < 0.01$), indicating that longer horns are more susceptible to failure.

DISCUSSION

The results presented here support the hypothesis that mechanical limits set an upper bound on horn size in the rhinoceros beetle *T. dichotomus.* The safety factors of horns, calculated as the ratio of absolute breaking force to maximum expected load, decreased with increasing horn length, indicating that the risk of breakage is highest for the longest horns. Furthermore, field observations indicate that males do indeed push horns to their structural limits, and that large males with long horns are the most susceptible to horn damage [figure 2; 14]. Mechanical failure of large horns may therefore “put the brakes on” the continued exaggeration of horns driven by male-male competition.

Although it is possible that horns also experience high loads when a male falls to the ground after an unsuccessful fight, these forces are likely to be much lower than the forces exerted on horns during fights. The estimated impact force of a “worst case scenario” fall, based on high-speed videos of beetles falling to the floor in the laboratory, is approximately 2.5 N, which is no greater than the measured fighting forces. In the field, beetles falling into leaf litter will experience significantly lower impact forces. I therefore expect that the fighting forces estimated here are the maximal loads exerted on horns, and thus the most biologically relevant forces for determining the safety factors of beetle horns.

Biological safety factors should vary depending on the structure’s contribution to fitness, the predictability of the loads it experiences, and how costly the structure is to produce and maintain [26]. Based on this theory, beetle horns are expected to have very high safety factors. Horns are critically important to a male’s reproductive success; males cannot repair or replace a broken horn; and the loads exerted on horns during fights are likely to be very unpredictable. Additionally, males should be able to afford a robust horn because they are inexpensive to produce and maintain. In agreement with these predictions, I found that *T. dichotomus* horns have high safety factors, ranging from 3.5 to 10.3. Nevertheless, these estimates suggest that even the horns with the lowest safety factors are at least three times stronger than needed to withstand the maximum forces incurred during fights, so the probability of fracture should be very low. The observation that horns do fail in the field at fairly high frequencies indicates that they must be subjected to loads or conditions that differ from those measured in this study.

It is important to note, however, that the safety factors of beetle horns are comparable to safety factors measured for other biological structures: 2.6 to 7.4 for crab claws [34,35], 4.8 for the leg bones of a galloping horse [26], 6 for the wing bones of a flying goose [26], and 9 to 17 for the flight feathers of a flying pigeon [36]. And, like beetle horns, these structures can and do fail. Approximately 6% of wild crab populations have broken claws [34], and the incidence of fracture for leg and wing bones of mammals and birds ranges from 0.2 to 3% [37]. Thus, even structures with high measured safety factors are susceptible to mechanical failure under natural conditions.

There are at least three aspects of loading in biological structures that often are not accounted for in safety factor estimates, and which may contribute to the seemingly high rates of failure under natural conditions: complex loading regimes, material fatigue, and viscoelasticity of biological materials. These factors may also explain the apparent mismatch between the measured safety factors of beetle horns and the relatively high incidence of breakage in natural populations.

First, in this study, breaking forces were measured by loading horns in pure cantilever bending, yet horns are likely to experience more complex loading regimes in typical fights. In particular, *T. dichotomus* horns – with their broad, forked tips – are expected to be both bent and twisted when a male tries to pry his opponent up and off of a tree. One of the broken horns collected in the field had a spiral-shaped fracture, indicating that horns can be subjected to significant torsional loads during fights.

Second, the horns used in the breaking tests were from males that were kept in individual containers, and that were therefore in nearly pristine condition. In natural populations, however, horns accrue scratches, abrasions, and other types of wear (figure 5) that can act as local stress concentrators and significantly lower the theoretical strength of a structure [38–40]. Furthermore, horns are probably very susceptible to material fatigue due to repetitive loading and the accumulation of micro-cracks. Fatigue failure has been reported for a number of biological structures, including mammalian bones [32] and mollusk shells [40], and recent work on the fatigue properties of locust wing cuticle indicates that cyclic loading can cause failure at loads that are only a third of the cuticle's ultimate breaking strength [41]. These observations indicate that older horns may fail at loads significantly lower than those that would cause failure the first time the horns are loaded [32,40,41]. This fatigue hypothesis is supported by seasonal patterns of horn damage in the field: broken horns were more common at the end of the breeding season, presumably after males had engaged in many nights of intense fighting, subjecting their horns to high, repeated loads.

Third, like other biomaterials, horn cuticle is expected to be at least somewhat viscoelastic, so a horn's ability to dissipate energy and withstand crack propagation during impacts may be highly sensitive to the rate at which it is loaded [32,38]. High loading rates are known to make viscoelastic materials more rigid and brittle [32], so a very rapid flick during a fight may put horns at a higher risk of failure.

Water content can also significantly affect the material properties of insect cuticle [42–44]. Specifically, desiccation increases the strength and stiffness of cuticle, but decreases fracture toughness, or the ability to withstand defects and crack propagation [42]. The horns of *T. dichotomus* males are significantly drier than other body parts; their relative moisture content is only 26%, compared to 40% in elytra, and 54% in legs [23]. Intriguingly, because horns are so dry, the material properties of "fresh" samples of horn cuticle are remarkably similar to those of "dry" samples of cuticle from other insects: ultimate bending stress is 201.4 ± 75.2 MPa for fresh horn cuticle, compared to 217.4 ± 48.2 MPa for dry locust leg cuticle [42]. Previous authors have suggested that insects could increase the stiffness and strength of their exoskeletons by simply reducing the cuticle's moisture content [42]. The low moisture content of *T. dichotomus* horns may be an example of such an adaptation. However, while the increase in strength and stiffness make horns more resistant to bending and thus more effective at transmitting fighting forces [45], these changes also make the horns more brittle, and therefore more likely to fail, particularly in the presence of surface defects and other types of wear [42]. Whether the horns of other rhinoceros beetle species also have low moisture contents, or whether other species trade off the strength, stiffness, and toughness of horns in different ways remains to be tested.

I propose that mechanical limits are important in setting the maximum size of horns in rhinoceros beetles in general. In all species studied to date, males use their horns as weapons in male-male competitions [17,46,47], and males with broken horns are not uncommon in museum collections (Figure 6). Thus, it appears that many rhinoceros beetle species push horns to their mechanical limits, so the competitive advantage of increased horn size may be opposed by the increased vulnerability of horn failure. Quantifying the structural properties of different horns, and identifying how intensely and predictably each species uses its horns during fights may be critical in understanding variation in horn size among species. Future studies should also consider other factors (e.g., genetic and developmental constraints) that may contribute to the limits on horn size. Furthermore, understanding how variation in horn shape, in addition to horn size, affects performance during combat is likely to provide important insights into patterns of diversity in rhinoceros beetle horns, and potentially other sexually-selected animal weapons [45].

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Figure 1. *Trypoxylus dichotomus* males use their long forked head horn to fight over a hornless female feeding at a natural sap site.

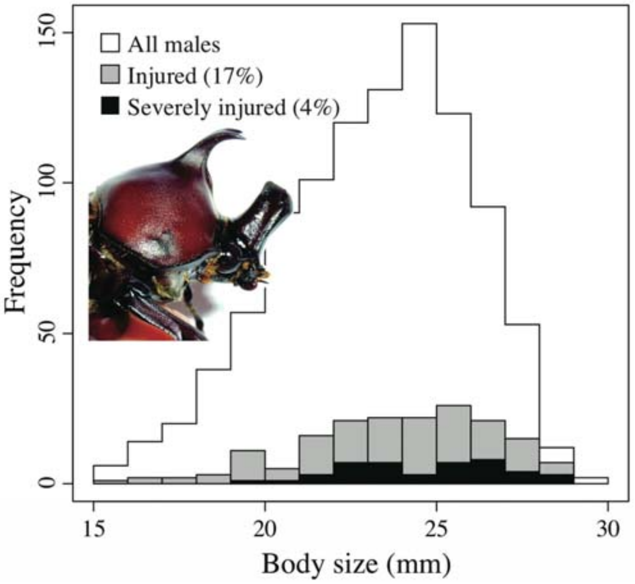

Figure 2. Body size histograms of field-collected males with varying degrees of injury; 17% of the population showed some sign of injury, and 4% had severely damaged horns. Inset: example of a male with a broken head horn.

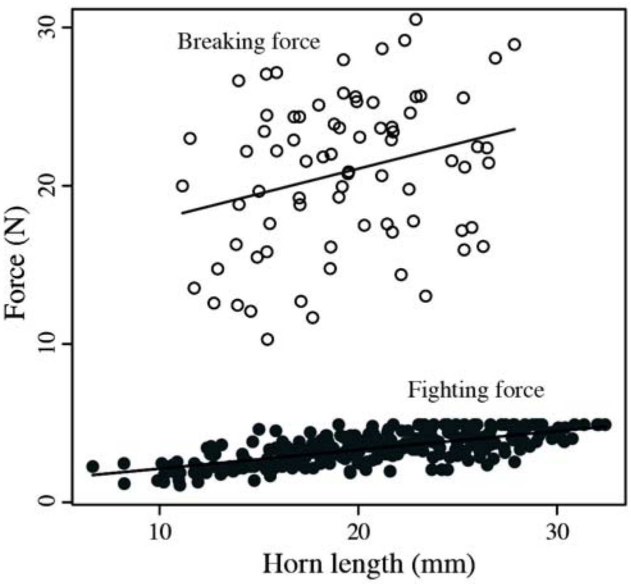

Figure 3. Relationships between horn length and fighting force (dark grey points; $R^2 = 0.48$, $F_{1,275} = 252.2$, $p < 0.001$; *fighting force* = 0.12 × *horn length* + 0.94) and breaking force (open circles; $R^2 = 0.08$, $F_{1,74} = 6.05$, $p = 0.02$; *breaking force* = 0.32 × *horn length* + 14.74).

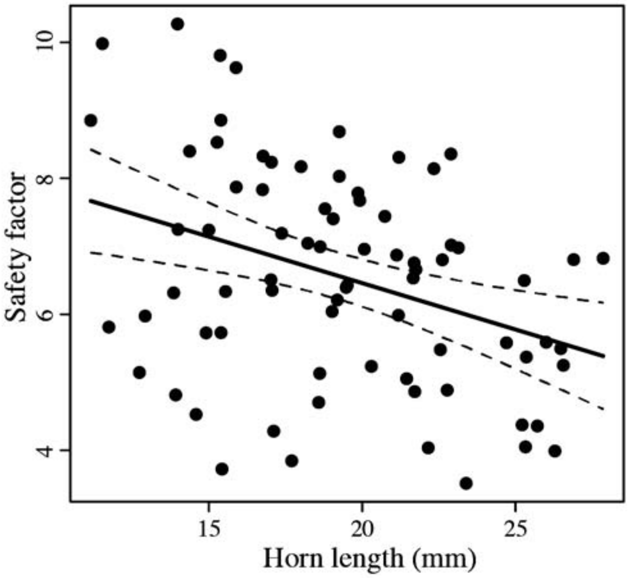

Figure 4. Relationship between horn length and safety factor ($R^2 = 0.13$, $F_{1, 74} = 10.9$, $p < 0.01$; *safety factor* = -0.14 × *horn length* + 9.19). The dashed lines indicate the 95% confidence intervals.

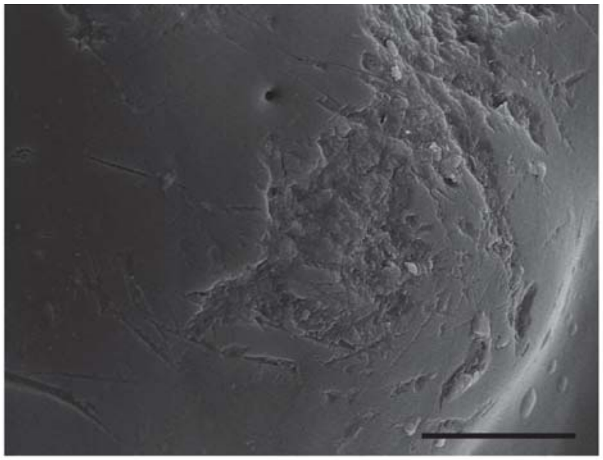

Figure 5. Scanning electron micrograph of the outer surface of a horn from a field-collected beetle, showing substantial abrasion and numerous scratches and micro-cracks. Scale bar, 10 μm.

Figure 6. Museum specimens of different rhinoceros beetle species with broken horns. Clockwise from top left: *Augosoma centaurus*, *Dynastes granti*, *Oryctes boas*, and *Dynastes tityus*.

CHAPTER 5:

Structural adaptations to diverse fighting styles in sexually selected weapons

Abstract:

The shapes of sexually selected weapons differ widely among species, but the drivers of this diversity remain poorly understood. Existing explanations suggest that weapon shapes reflect structural adaptations to different fighting styles, yet explicit tests of this hypothesis are lacking. Using finite element analysis, a powerful and standard engineering technique, we test whether functional specializations for increased performance under species-specific fighting styles have driven diversification of weapon form. We find that the horns of different rhinoceros beetle species are both stronger and stiffer in response to species-typical fighting loads, providing direct evidence that weapons are adapted to meet the functional demands of fighting. Our research establishes a critical link between weapon form and function, revealing one way male-male competition can drive the diversification of animal weapons.

Main Text:

Sexually selected traits are renowned for their extreme size and diversity (*1*, *2*). Some sexual traits, such as elaborate feathers in birds-of-paradise and widowbirds, are used as ornaments to attract choosy females, while others, such as giant elk antlers and stag beetle mandibles, are used as weapons in male-male battles over access to females. Numerous empirical (*3*–*8*) and theoretical (*9*–*14*) studies have shown how female choice can drive the diversification of male ornaments. Surprisingly few studies, however, have examined whether male-male competition drives the diversification of weapons, and the mechanisms responsible for weapon divergence remain largely unexplored (*15*). Consequently, although sexually selected weapons are just as diverse as ornaments, it is not clear why this should be so.

The most intuitive explanation for weapon diversity is that weapons are adapted to species-specific fighting styles. Specifically, differences in the way males fight, or where they fight, may favor corresponding changes in weapon shape (*15*). This hypothesis has been explored most thoroughly for the horns and antlers of ungulates (*16*–*19*). For example, males in species with short, smooth horns tend to be stabbers; males with robust, curved horns typically ram opponents; and males with long, reaching horns wrestle or fence (*17*, *18*). Although these broad comparative patterns provide evidence that different fighting styles have contributed to the divergence of weapon forms, all of the studies are correlative. Explicit tests of the functional performance of weapons in response to forces incurred during fights are still lacking, and no studies have tested whether animal weapons perform better at their own style of fighting than they do at others. Thus, although functional specialization of weapons for diverse styles of fighting remains the most intuitive and widely cited driver of weapon diversity, it has yet to be directly tested for any type of animal weapon.

Here, we perform a functional analysis of rhinoceros beetle horns to test whether structural adaptations for fighting have driven the diversification of weapon form. Specifically, we compare the structural performance of various beetle horn morphologies using finite element analysis – a standard and powerful engineering analysis technique used to predict how complex structures deform, and ultimately fail, in response to applied loads.

Rhinoceros beetles (Coleoptera: Dynastinae) are ideal for studying weapon diversity for three reasons. First, their horns are diverse (*15*, *20*); species vary in the number, size, and shape of their horns – with species wielding long pitchforks, robust pincers, or thin spears, to name just a few (Figs. 1, 2). Second, horns are used as weapons during combat with rival males over access to females; there is no evidence that females choose males based on the shape or size of their horns (*21*–*24*). Horn morphology is therefore expected to reflect differences in how horns are used during fights, without conflicting selective pressures from female choice. Third, species fight on a variety of substrates (e.g., on broad tree trunks or narrow shoots, inside tunnels) and use their horns in different ways, which may select for qualitatively different fighting structures (*15*, *22*, *23*). We test whether beetle horns are adapted to meet the functional demands of fighting by constructing finite element models of the head horns of three rhinoceros beetle species, and loading the model horns in ways that mimic the forces incurred during both species-typical, and atypical, fights (*25*).

The three species investigated in this study – *Trypoxylus dichotomus*, *Golofa porteri,* and *Dynastes hercules* – have very different horn morphologies, and distinct fighting styles. In all three species, males insert their head horn underneath an opponent to pry him from the substrate, but the specific maneuvers used – and therefore the forces that horns experience – are different (Fig. 1). The head horns of *Trypoxylus* males are long and forked, and function like a pitchfork, prying and twisting opponents off the trunks and branches of trees (*23*, *26*). The head horns of *Dynastes* males are long and work together with a long thoracic horn like the pincer arms of pliers to lift and squeeze opponents, tossing them off of trees to the ground (*21*). The head horns of *Golofa* males are long and slender, and are used like a fencing sword to both lift opponents, and push them sideways off balance, in chaotic scrambles for control of narrow shoots (*27*). Because of their distinctive fighting styles, head horns in these species are either: a) bent vertically and twisted (*Trypoxylus*), b) bent vertically only (*Dynastes*), or c) bent vertically and laterally (*Golofa*) during fights, which makes it possible to analyze the structural performance of horns in response to fighting loads that are both typical, and atypical, for each species.

Horns performed best when loaded under conditions that mimic species-typical fights, and more poorly when loaded under atypical conditions. We assessed two measures of horn performance: strength and stiffness (*25*). Horn strength was evaluated by comparing model stress. In all three species, horns had significantly lower stresses, and thus were less likely to break, under typical fighting loads (Fig. 2). Stiffness was evaluated by comparing total strain energies. In all three species, horns had significantly lower strain energies while transmitting forces under typical fighting loads (Fig. 3), which indicates that horns deformed less, and thus were more effective at transmitting forces to dislodge an opponent, when deployed in a fashion typical of fights for each species. These findings provide direct evidence that horn shape is adapted to meet the functional demands of fighting.

One aspect of horn morphology that likely explains variation in performance is cross-sectional shape. Given that a male's goal during fights is to dislodge opponents (*22*, *23*, *26*), an optimal horn should resist deflection in response to typical loads. According to standard beam theory, a structure's resistance to bending is directly related to its second moment of area, or how its mass is distributed about the neutral bending axis (*28*). Structures that are bent predictably in a single direction (e.g., pliers, *Dynastes* horns) are the most resistant to bending when the mass is distributed far from the bending axis, such that elliptical cross-sections are ideal (*19*, *28*). By contrast, structures that are bent unpredictably from many different directions (e.g., fencing swords, *Golofa* horns) perform best with circular cross-sections, because circular cross-sections distribute mass equally around all possible bending axes (*19*, *28*).

Biological structures that are both bent and twisted should benefit from non-circular cross-sections (such as the triangular cross-sections of sedge petioles or the u-shaped cross-sections of feather rachises) as these shapes allow the structures to twist before being bent out of shape (*29*). Because triangular cross-sections have both a high second moment of area (which confers high flexural stiffness) and high polar moment of area (which confers high torsional stiffness) (*28*), triangular cross-sections may be ideal for structures that must resist bending and twisting (e.g., *Trypoxylus* horns). Thus, if variation in performance among the three species reflects differences in their resistance to bending and twisting, then we predict *Trypoxylus* horns will have triangular-shaped cross-sections; *Dynastes* horns, elliptical cross-sections; and *Golofa* horns, circular cross-sections. Transverse sections from our micro-computed tomography (micro-CT) scans of the three beetle horns confirm these predictions (Fig. 4), suggesting that differences in cross-sectional morphologies alone may explain much of the variation we observed among species in horn performance. If and how additional aspects of horn shape (e.g., tubercles in the middle of the shaft, ridges along the edges, or bifuractions at the tip) contribute to structural performance during combat remains to be explored, and finite element analysis promises to be an important tool in these endeavors.

Recent improvements in three-dimensional imaging have now made it possible for evolutionary biologists to use mature engineering technology to rigorously test the form and function of complex biological structures. We capitalized on these new applications of standard engineering tools to answer old questions about biological diversity. Using finite element analysis, we were able to directly test the functional performance of weapons in response to both species-typical and species-atypical fighting loads for the first time in any taxa. This study illustrates the critical link between the form and function in rhinoceros beetle horns, and indicates that variation in weapon shape reflects a history of selection for improved performance during species-typical fights. More broadly, the findings highlight the potential for male-male competition to drive the divergence of animal weapons, some of nature's most spectacular body forms.

References and Notes

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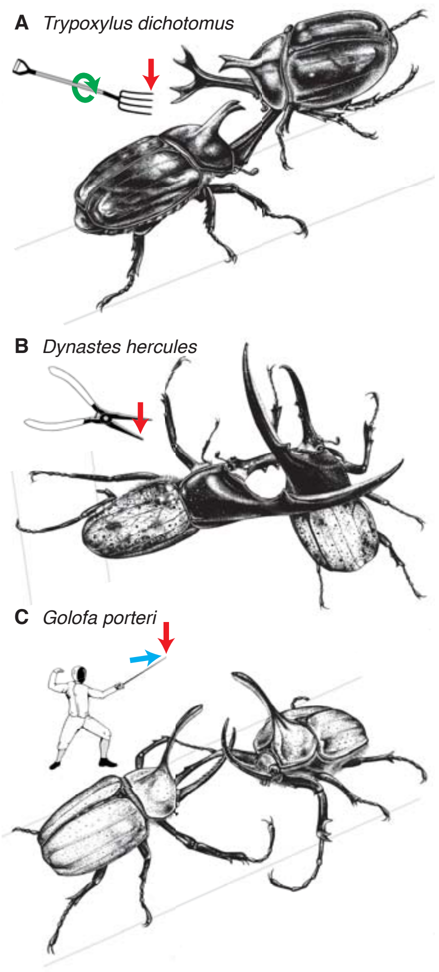


Fig. 1. Variation in horn morphology and fighting styles in rhinoceros beetles. (**A**) *Trypoxylus dichotomus* males have a long, forked head horn that is used like a pitchfork to lift and twist opponents off tree trunks during fights. (**B**) *Dynastes hercules* males have a long head horn and long thoracic horn that are used together like pliers to lift, squeeze, and then toss opponents to the ground. (**C**) *Golofa porteri* males have a long, slender head horn that is used like a fencing sword to both lift opponents off narrow shoots and push them sideways off balance. Vectors represent the typical forces experienced by horns during fights: vertical bending (red), lateral bending (blue), twisting (green). Illustrations by David J. Tuss.

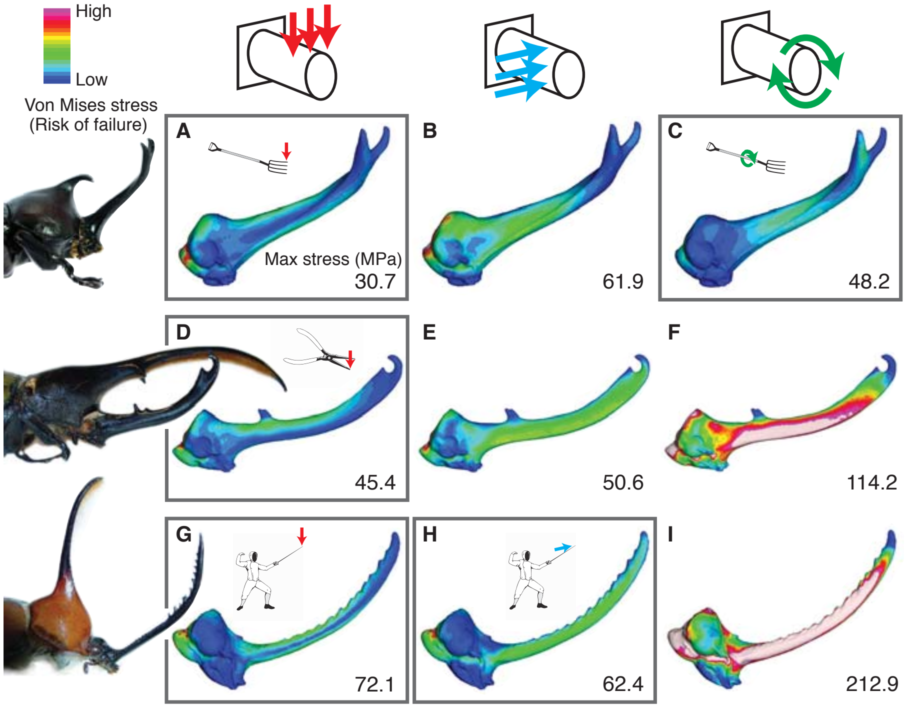
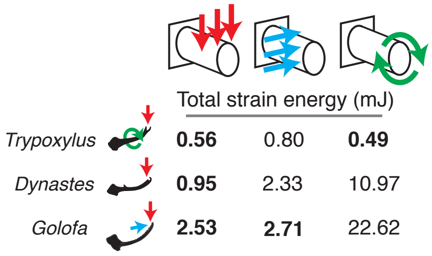


Fig. 2. Horns are stronger under species-specific fighting loads. von Mises stress distributions and maximum stress values from finite element models of *Trypoxylus* (**A-C**), *Dynastes* (**D-F**), and *Golofa* (**G-I**) horns under vertical bending (**A, D, G**), lateral bending (**B, E, H**), and twisting (**C, F, I**) loads. Typical fighting loads for each species are outlined in grey; atypical fighting loads are not outlined. In all three species, maximum von Mises stresses in the horn are higher (warmer colors) under atypical loading conditions, indicating a higher likelihood of breaking. Contour plots are scaled to 80 MPa peak von Mises stress. The high stresses at the base of the head horn are artifacts from constraining the models and are not included in calculating the maximum stress values in the horn.



	Total strain energy (mJ)		
Trypoxylus	**0.56**	0.80	**0.49**
Dynastes	**0.95**	2.33	10.97
Golofa	**2.53**	**2.71**	22.62

Fig. 3. Horns are stiffer under species-specific fighting loads. In all three species, total strain energies from finite element models were lower under typical loading conditions (bold) compared to atypical loading conditions (not bold), indicating that horns are stiffer and thus more effective at transmitting forces to dislodge opponents during species-specific fights.

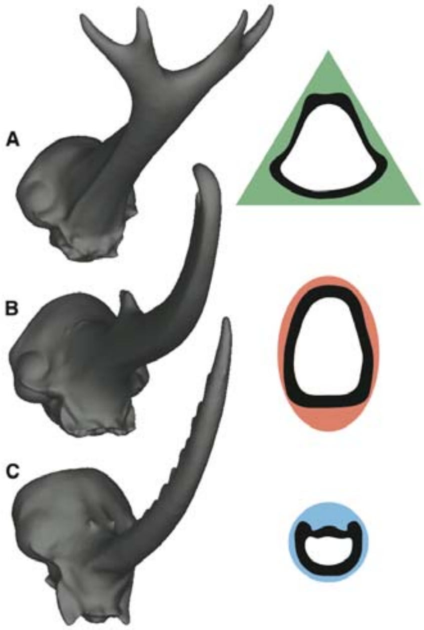


Fig. 4. Variation in horn performance is attributed to different cross-sectional morphologies. Three-dimensional finite element models and outlines from transverse micro-CT slices from the middle of each horn. The cross-sectional morphology of horns is triangular in *Trypoxylus* (**A**), elliptical in *Dynastes* (**B**), and circular in *Golofa* (**C**), as predicted if horns are adapted to resist deflection under their typical fighting loads.

Supplementary Materials:

Materials and Methods

Model construction

Finite element models were based on high-resolution three-dimensional reconstructions of the head horns of three rhinoceros beetle species (*Trypoxylus dichotomus*, *Golofa porteri,* and *Dynastes hercules*) created from micro-CT scans. The specimens were scanned with a SkyScan 1173 micro-CT scanner at 70 kV and 110 µA. The spatial resolution of the files was 20.0 µm for *Trypoxylus* and 26.6 µm for *Golofa* and *Dynastes*.

We simplified the initial reconstructions using Geomagic (Geomagic, Inc.) to remove extraneous morphological details. Specifically, we corrected artificial holes and surface irregularities, and deleted the antennae, maxillary and labial palps, and the apodemes and tentoria inside the head capsule that significantly increased the geometric complexity (and thus the computational demands) of the models, but were unlikely to affect the results of the loading experiments on the horn. The simplified models were then imported into Mimics (Materialise) to be cleaned and edited further (e.g., by improving the aspect ratios and reducing the number of the triangular surface elements) to generate the final solid element models. There were 44,459 nodes and 178,755 elements in the final *Trypoxylus* model, 44,032 nodes and 192,133 elements in the *Golofa* model, and 63,832 nodes and 259,918 elements in the *Dynastes* model*.* The final models were then imported into Strand7 (Strand7 Pty Ltd) for linear static finite element analysis.

Finite element analysis

We compared the results of finite element models consisting of 4 versus 10-noded tetrahedral elements. The difference in mean stress values was less than 10%, which indicates that our models are robust (*30*). Due to the substantially longer computing time required for the 10-noded tetrahedral models, we chose to conduct our analyses based on models composed of 4-noded tetrahedral elements.

Because the goal of the study was to compare horn performance based on differences in shape, we scaled all three models to the same size and assigned identical material properties. For modeling simplicity and in the absence of more precise reference data, the models were assigned homogeneous and isotropic material properties based on Young's modulus values for the clypeus cuticle of horned dung beetles ($E = 15.98$ GPa) (*31*) and an average Poisson's ratio ($\nu = 0.30$). The models were constrained from rigid body motion by fixing three nodes around the occiput, where the head attaches to the pronotum.

We simulated the three most important loading conditions that beetle horns experience during fights: vertical bending, lateral bending, and twisting. For the vertical and lateral bending models, we maintained the same force to surface area ratios in order to control for differences in model size and thereby compare the strength of the models based solely on differences in shape (*32*). The three models were therefore scaled to the same surface area (723 mm^2, or the surface area of the *Trypoxylus* model), and the same total force (4N) was applied to the horn tip, based on field estimates of the forces exerted by *Trypoxylus* males during fights (McCullough, in press). For the twisting models, we removed the confounding effect of model size by maintaining the same torque to volume ratio. The models were therefore scaled to the same volume (116 mm^3, or the volume of the *Trypoxylus* model), and the same total torque (32.8 Nmm) was applied to the widest points of the horn tip, based on the torque resulting from a 4N force applied at the lateral-most tine in our *Trypoxylus* model (moment arm = 8.2 mm).

Results from the finite element models indicate that the highest stresses generated in a horn during fights occur in the middle of the shaft, which matches the actual location of horn fractures for beetles in the field (McCullough, in press). Our finite element models therefore accurately predict the responses of horns to the forces incurred during fights.

Comparing model performance

The structural performance of horns in response to the various loading conditions was evaluated based on two criteria: strength and stiffness. First, we evaluated the strength of the horns by comparing model stress. Von Mises stress is a good predictor of failure due to ductile fracture (*32*), so for a given load, structures with lower Von Mises stress are less likely to fail and therefore are interpreted as being a stronger structure. We evaluated model strength qualitatively by visually comparing the Von Mises stress contour plots, and quantitatively by comparing the maximum Von Mises stress values in the shaft of the horn.

Second, we evaluated the performance of horns in terms of stiffness by comparing total strain energy. Assuming the function of a horn is to transmit forces to dislodge an opponent (*22*), horns that are stiffer (less compliant) for a given volume of material are more optimal than those that are less stiff (more compliant) (*32*). The work expended by an applied load in elastically deforming a structure is stored as elastic strain energy, so for a given load, the smaller the model's strain energy, the less work that is lost to deformation, and thus the more effective the model is at transmitting fighting forces (*32*). Strain energy in bending is proportional to force squared and inversely proportional to the cube root of volume (*33*). We therefore controlled for the effect of horn size in our vertical and lateral bending models by scaling the measured strain energies to conserve the same force squared to cube root of volume ratio. Similarly, because strain energy in twisting is proportional to torque squared and inversely proportional to volume (*33*), we removed the confounding effect of horn size in our twisting models by scaling the measured strain energies to conserve the same torque squared to volume ratio. (See ref. (*32*) for details on scaling finite element models.)

The actual values for stresses and strains should be interpreted with caution because of our simplifying assumptions of homogeneous and isotropic material properties. However, by applying identical material properties and scaling the models appropriately, we can interpret the relative performance of different horns with confidence (*32*).