



SYMPOSIUM

Heightened Condition-Dependent Growth of Sexually Selected Weapons in the Rhinoceros Beetle, *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae)

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Synopsis The exaggerated weapons and ornaments of sexual selection are condition-dependent traits that often grow to exaggerated proportions. The horns of male rhinoceros beetles are extremely sensitive to the larval nutritional environment and are used by rival males in combat over access to females. In contrast to horns, other parts of the body, such as wings, eyes, and legs, scale proportionally with body size, whereas others, such as males' external genitalia, are invariant with body size, regardless of nutrition. We document how body parts of the Asian rhinoceros beetle, *Trypoxylus dichotomus*, exhibit plasticity and constraint in response to nutritional condition. We discuss the implications of these results for the evolution of condition-dependent and condition-independent traits in animals.

Introduction

The exaggerated ornaments and weapons of sexual selection are predicted to exhibit heightened condition-dependent expression (David et al. 2000; Cotton et al. 2004a; Bonduriansky and Rowe 2005; Emlen et al. 2012; Warren et al. 2013). That is, their growth is expected to be more sensitive to an individual's nutritional and/or physiological state than is the growth of other, non-sexually selected, parts of the body. Heightened condition-dependent expression is predicted to be critical in the evolution and maintenance of signals used in females' mate choice and male–male competition for at least two reasons. First, heightened sensitivity to condition of the body results in the development of unusually variable traits. Males in the best condition produce disproportionately large ornaments or weapons, whereas males in poor condition produce disproportionately smaller ones. This increase in variability in a trait can amplify differences in body size from male to male, making these traits excellent signals for choosy females

or rival males to discern otherwise subtle differences in a male's condition (Cotton et al. 2004a; Bonduriansky 2007; Bradbury and Vehrencamp 2011).

Second, condition-dependent expression of a trait is a mechanism ensuring that sizes of ornaments and weapons honestly reflect the underlying condition of their bearer (Pomiankowski 1987; Grafen 1990; Iwasa et al. 1991). If ornaments and weapons do not honestly reflect a male's quality or fighting ability, choosy females and rival males would not benefit from attending to the signals, and the signaling system would be expected to break down (Maynard-Smith and Harper 2003; Searcy and Nowicki 2005). Thus, condition-dependent expression of traits offers an important mechanism for maintaining the reliability of ornaments and weapons as indicators of a male's quality.

Numerous studies have documented that exaggerated sexual traits are sensitive to variation in individual condition (Andersson 1994; Johnstone 1995). For example, when restricted nutrition is used to

induce variation in condition among individuals, the extent of expression of sexual traits is almost always strongly positively correlated with condition of the male (reviewed by Andersson 1994; Johnstone 1995; Cotton et al. 2004b). However, rigorous demonstration of “heightened” condition-sensitive expression requires comparing the sensitivity of growth of exaggerated sexual traits with that of other, non-sexually selected parts of the body. To date, only a few studies have directly compared condition-dependency between sexual and non-sexual traits (David et al. 2000; Cotton et al. 2004b; Bonduriansky and Rowe 2005), and all of these involve flies. As a result, it is still unclear how often sexual ornaments and weapons do, in fact, exhibit the “heightened” condition-dependent expression predicted by handicap models of sexual selection (Cotton et al. 2004a). In this study, we examine how the expression of a suite of sexual and non-sexual traits is affected by perturbations in the diet of the giant Asian rhinoceros beetle, *Trypoxylus dichotomus*.

Like other rhinoceros beetles (Coleoptera: Dynastinae), *T. dichotomus* produce elaborate cuticular outgrowths from the head and pronotum that are used in direct combat with conspecific males over reproductive access to females (Siva-Jothy 1987; Hongo 2003, 2007). Horns are therefore sexually selected weapons that are critical in determining a male’s reproductive success. Recently, we showed that the horns of male *T. dichotomus* are more sensitive to RNAi knockdown of the insulin receptor (*T.d. InR*) than at least two other body parts: wings and genitalia (Emlen et al. 2012). Here, we extend these results by conducting a large-scale rearing experiment to explore how sensitive these sexual traits are, relative to a suite of non-sexual traits in both male and female *T. dichotomus*. Additionally, we provide baseline information about the growth and development of this species. We find that, as expected, the size of sexually selected traits (horns) is highly variable among individuals and hyper-sensitive—more sensitive to dietary manipulations—than other non-sexual traits (e.g., wings, elytra, and legs). In addition, we find that genitalia are largely invariable among individuals and unusually insensitive to dietary manipulations. Thus, traits differ dramatically and predictably both in their variability and in sensitivity to nutrition.

Methods

Collection of eggs

All experiments were conducted in a laboratory at the Fort Missoula Research Station of the

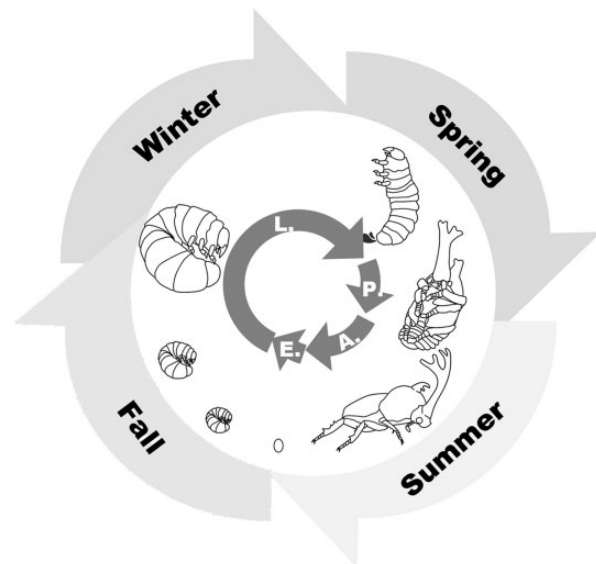


Fig. 1 Annual life cycle of the Asian rhinoceros beetle *Trypoxylus dichotomus*. The adults feed on sap flowing from oak, maple, and ash trees while larval nutrition is obtained from the consumption of decomposing detritus and hard wood on the forest floor. The larvae grow during three instars before molting into a pupa and then enclosing into a beetle. Just before the pupal molt, larvae create a pupal cell (a hard compact chamber in the soil), purge their gut and enter a 10-day prepupal phase. During this period, adult-type structures begin to grow under the larval cuticle as primordial discs. Cell proliferation of the discs occurs rapidly during the first several days of the prepupal period and then tapers off. *Trypoxylus dichotomus* is a non-model system for studying insect development with a very long (10–12 months egg to adult) generation time. However, *T. dichotomus* have an exaggerated weapon that plays an important role in sexual selection and evolution. (E., egg; L., larva; P., pupa; A., adult).

University of Montana. The life cycle of *T. dichotomus* is typical for holometabolous beetles with the egg–larval–pupa–adult cycle occurring over 10–12 months (Fig. 1). Adult *T. dichotomus* beetles were paired and mated using the methods described by Emlen et al. (2012). After mating, females were separated and placed individually in mulch-filled jars until they laid eggs. Eggs were collected 7 days after oviposition, weighed, and placed into individual plastic containers with 100% mulched maple leaves. Eggs were checked daily and dates of hatching were noted.

Rearing under high and low conditions of nutrition

Three days after molting into their second instar, larvae were randomly divided into two groups and kept in incubators set at 25°C on a 16:8 h light:dark cycle. In one group, larvae were provided with a high-nutrition diet, composed of a mixture of 25% leaf mulch and 75% quick-fermented hardwood

sawdust in 9-oz glass jars. This diet represents the maximum amount of fermenting and rotting wood to plant material that is found in the beetle pet food trade in Japan (H. Gotoh, personal communication) and from our previous experiments, we have consistently reared the largest males and females on this diet (stag beetles—Gotoh et al. 2011; rhinoceros beetles—Emlen et al. 2012). Larvae fed a low-nutrition diet were given 100% fast-fermented sawdust in 9-oz glass jars which contains less easily digestible plant material (no mulch); this food-limited diet consistently produces smaller *T. dichotomus* beetle larvae of both sexes (Emlen et al. 2012). “Low-nutrition” larvae received new food when their frass composed 90% of the jar. Food for “high-nutrition” individuals were changed as soon as frass content reached 40%. These differences in the amount of total food available also contributed to food limitation in the low-nutrition treatment. After molting into their third instar, high-nutrition larvae were moved into 1-gallon glass jars which maintained the food ad libitum treatment while low-nutrition animals remained in the 9-oz jars to maintain the food limited state of these individuals until pupation.

Measurements of larvae

To examine the effect of diet on growth trajectories, individuals were weighed repeatedly over the entire course of their development using an analytical balance. Eggs were weighed at day 0 and day 5, and then returned to their individual containers. Larvae were weighed 5 days after hatching and thereafter every 5 days during the first, second, and beginning of the third larval instars. Larvae grow very rapidly during this time, often gaining several grams of mass within a week. Approximately 40 days after the molt into the third instar, the larvae gain mass much more slowly, so then the larvae were weighed every 10 days until purging of the gut was complete.

When larvae showed signs of approaching gut-purge they were monitored daily for the presence of a pupal cell (a hard shell of compacted food that the larvae create around themselves before pupation). Once inside the pupal cell, they were removed, weighed, and placed into artificial cells carved out of floral foam. Once the pupae eclosed and the adults had fully sclerotized (10–14 days), they were sealed in airtight bags and euthanized by freezing.

Measurements of adults' traits

The head, elytra, wings, legs, and male genitalia were dissected from cold-euthanized adults and

photographed with a SPOT Insight Color camera mounted on a Leica M26 stereomicroscope. The photos were taken with SPOT Advanced software (Diagnostic Instruments, Detroit, MI, USA) and analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA). Width of the prothorax (a standard measure of body size), lengths of the elytra, wing, and hind femur, and diameter of the eye were measured both for males and females, and lengths of the cephalic horn, thoracic horn, and aedeagus (genitalia) were measured for males.

We assessed the magnitude of nutrition-dependent plasticity, our proxy for sensitivity to condition, by comparing the mean sizes of traits in individuals reared on high- and low-nutrition diets. Specifically, we calculated the percent change in response to nutrition as: $[(\text{mean trait size of high nutrition animals} - \text{the mean trait size of low nutrition animals}) / \text{overall mean trait size}] \times 100$. We measured the overall plasticity of body size as the percent change in width of the prothorax.

We also compared the allometric relationships between body size and the focal traits using the power equation $Y = \alpha X^b$, where Y is the log to base e of trait size, X is the log to base e of body size (prothorax width), α is the allometric coefficient, and b is the allometric slope. Values of $b < 1$ indicate negative allometry, $b > 1$ indicate positive allometry, and $b = 1$ indicate isometry. We estimated the allometric slopes using standardized major-axis regression with the SMATR package in R (Warton et al. 2006). Because our primary goal was to summarize the relationships between body and trait sizes (rather than to predict specific trait sizes), and because of measurement error in both measures of body (X) and trait (Y) sizes, we used standardized major axis instead of ordinary least squares regression (Warton et al. 2006).

Results

Nutritional effects on developmental trajectories

Males were significantly larger when raised on high-quality versus low-quality diets (Fig. 2; $T = 12.24$, $df = 28.53$, $P < 0.001$). Average mass was 10.89 ± 1.81 g (mean \pm SD) for males on a high-quality diet compared with 5.13 ± 1.02 g for those on a low-quality diet. Females were also significantly larger when raised at high versus low level of nutrition (Fig. 2; $T = 9.26$, $df = 20.09$, $P < 0.001$). Average mass was 9.33 ± 1.49 g for high-diet females on a high-quality diet compared with 4.97 ± 0.80 g for those on a low-quality diet.

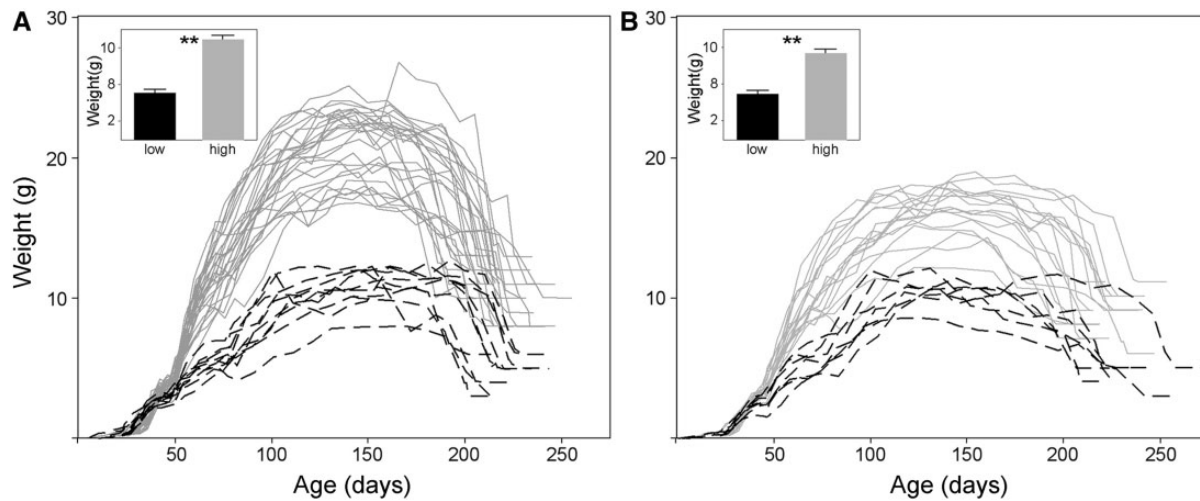


Fig. 2 Developmental trajectories for male (A) and female (B) *Trypoxylus dichotomus* over time under high and low conditions of nutrition. Males ($T = 12.24$, $df = 28.53$, $P < 0.001$) and females ($T = 9.26$, $df = 20.09$, $P < 0.001$) were significantly larger when raised on high-quality versus low-quality diets. High-nutrition individual trajectories are represented by gray lines and low-nutrition individual trajectories are represented by black dashed lines.

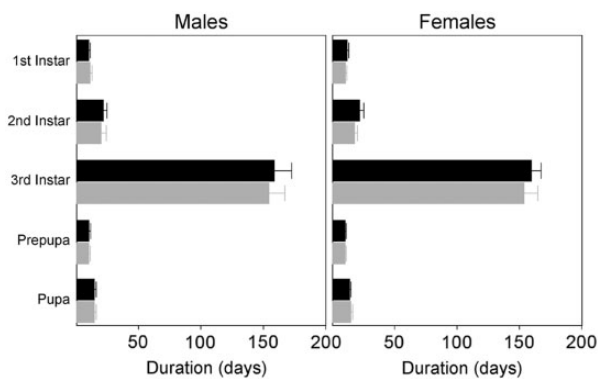


Fig. 3 The duration of the *Trypoxylus dichotomus* third instar is the longest developmental period in both sexes under high (gray bars) and low (black bars) conditions of nutrition. Individuals raised on high-nutrition diets had slightly shorter duration of development (208.7 ± 14.6 days) compared with individuals raised on low-nutrition diets (216.4 ± 11.9 days).

The length of each developmental stage did not differ significantly between males and females (Fig. 3; $T = 0.57$, $df = 52.98$, $P = 0.57$), but was significantly affected by diet (Fig. 3; $T = -2.08$, $df = 37.21$, $P = 0.04$). Individuals receiving high nutrition had slightly shorter duration of development (208.7 ± 14.6 days) compared with individuals receiving low nutrition (216.4 ± 11.9 days).

Traits respond differentially to variation in amount of food provided to larvae

The focal traits responded to the high-quality and low-quality diets differently, and varied from being

hyper-sensitive (head and thoracic horns), sensitive (elytra, wings, legs, and eyes), or insensitive (genitalia) to nutrition (Fig. 4). In males, length of the horns on the both head and thoracic were highly variable among individuals reared at high- versus low-nutrition states: there was a 61% change in length of the cephalic horn between individuals on high-quality diets and those on low-quality diets, and a 56% change in thoracic horn length (Fig. 4C). Additionally, head-horns and thoracic horns were positively allometric, as the allometric slopes were significantly >1 (head-horns: $r = 0.96$, $P < 0.001$; thoracic horns: $r = 0.94$, $P < 0.001$). In fact, the 95% confidence intervals around the allometric slopes were >2.5 for both head-horns ($b = 2.55$ [95% CI: 2.28, 2.85]) and thoracic horns ($b = 2.55$ [95% CI: 2.23, 2.93]), and the allometric slopes for head and thoracic horns were not significantly different from each other ($r < 0.001$; $P = 0.99$). These results indicate that large males had disproportionately long horns, whereas small males had disproportionately short horns.

Elytra, wings, and legs of males also were sensitive to dietary treatment, but there was less variation in the sizes of these traits between high and low levels of nutrition: there was a 21% change in elytral length, 22% change in wing length, and 25% change in femur length. These traits were also largely isometric. Specifically, the allometric slopes for elytra and legs were not significantly different from 1 (elytra: $r = -0.18$, $P = 0.35$; legs: $r = 0.09$, $P = 0.59$), and the 95% confidence intervals around the

allometric slopes for elytra ($b=0.95$ [95% CI: 0.84, 1.06]) and legs ($b=1.03$ [95% CI: 0.93, 1.13]) included 1. Although the allometric slope for wings was slightly <1 ($r=-0.45$, $P=0.015$), the 99% confidence interval around the slope for wings also included 1 ($b=0.82$ [99% CI: 0.66, 1.01]). These results indicate that elytra, wings, and legs scaled nearly proportionately with body size for all individuals. Contrary to our expectation, eyes were less sensitive to dietary treatment than were elytra, wings, and legs; there was only a 15% change in diameter of the eye between high- and low-dietary treatments for males. Additionally, eyes did not scale isometrically ($b=0.65$ [95% CI: 0.57, 0.74]). The allometric slope for eyes was significantly <1 ($r=-0.78$, $P<0.001$), indicating that eye size did not increase proportionately with body size.

Genitalia were much less responsive to nutrition than any other measured body part; there was only a 10% change in aedeagus length between high and low levels of nutrition. Genitalia were also negatively allometric ($b=0.53$ [95% CI: 0.41, 0.69]); the allometric slope was significantly <1 ($r=-0.67$, $P<0.001$), indicating that males had similarly sized genitalia regardless of their body size.

In females, elytra, wings, and legs were sensitive to nutrition and showed moderate variability between individuals reared at high- versus low-nutritional levels: there was a 21% change in elytral length, 19% change in wing length, and 18% change in femur length between females on high-quality and low-quality diets. These traits were also all isometric. Specifically, the 95% confidence intervals around the slope for all three traits included 1 (elytra: $b=1.00$ [95% CI: 0.91, 1.10]; wings: $b=0.95$ [95% CI: 0.85, 1.05]; legs: $b=1.07$ [95% CI: 0.93, 1.22]), indicating that elytra, wings, and legs scaled proportionately with body size. As in males, eyes were less sensitive to nutrition, and only exhibited a 14% change in size between individual on high-quality diets and those on low-quality diets. Eyes were also negatively allometric ($b=0.71$ [95% CI: 0.62, 0.81]), as the allometric slope was significantly <1 ($r=-0.72$, $P<0.001$).

Discussion

This study characterizes the extent and nature of nutrition-dependent plasticity on growth in the rhinoceros beetle *T. dichotomus*. Body size in insects is determined both by development time and growth rate (Stern 2001; Nijhout 2003; Davidowitz et al. 2005). In the enlarged mandibles of stag beetles, the nutritional state, or condition, of the developing

larvae affects both the larval period (Gotoh et al. 2011) and growth rate (H. Gotoh, unpublished data). In *T. dichotomus*, nutrition primarily affected growth rate, causing only minor shifts in the duration of the growth period (low nutrition extended the larval period by $\sim 3\%$; Figs 2 and 3). Karino et al. (2004) also found that low nutrition prolonged the larval periods of *T. dichotomus* (formerly *Allomyrina dichotoma*), especially in females, but these differences, too, were modest. It is likely that the growth period is constrained in this species by its life history; *T. dichotomus* is univoltine and emerges and reproduces only during the 3 months of summer (Fig. 1; Hongo 2007, 2012). Any change in duration of the growth period in larvae would result in adults' eclosing at an inappropriate time, thereby reducing overall fitness (Karino et al. 2004; Plaistow et al. 2005).

Variation in larval diet had profound effects on the variability and expression of adults' traits (Fig. 4). In both males and females, the final size of most body parts (eyes, wings, elytra, and legs) was sensitive to changes in larval nutrition, and these traits scaled isometrically, or proportionally, with among-individual variation in body size (Fig. 4). Thus, most body parts responded to nutrition to a comparable degree, both with each other, and with overall body size, such that the traits of large individuals were essentially scaled-up versions of the traits of smaller individuals.

A few traits, however, stood out. In particular, the cephalic and thoracic horns of males were exquisitely variable in size, and hyper-sensitive to dietary manipulations (Fig. 4). The allometric slopes for both cephalic and thoracic horns were large (>1), indicating that their expressions tend to be more variable from individual to individual than for other body parts. As a result, the range of horn sizes from male to male was more extreme for these traits than it was for other body parts. These results are entirely consistent with the predictions from sexual-selection theory for the heightened condition-dependent expression of sexual traits (Cotton et al. 2004a; Bonduriansky 2007; Bradbury and Vehrencamp 2011).

Trypoxylus dichotomus horns are used as weapons in direct fights with other males over reproductive access to females (Siva-Jothy 1987; Hongo 2003, 2007; Karino et al. 2005). Males with longer horns are more likely to win these fights and thus attain higher mating success (Karino et al. 2005; Hongo 2007), so horns are expected to experience strong directional selection for increased size. Females do not choose males based on the shape or size

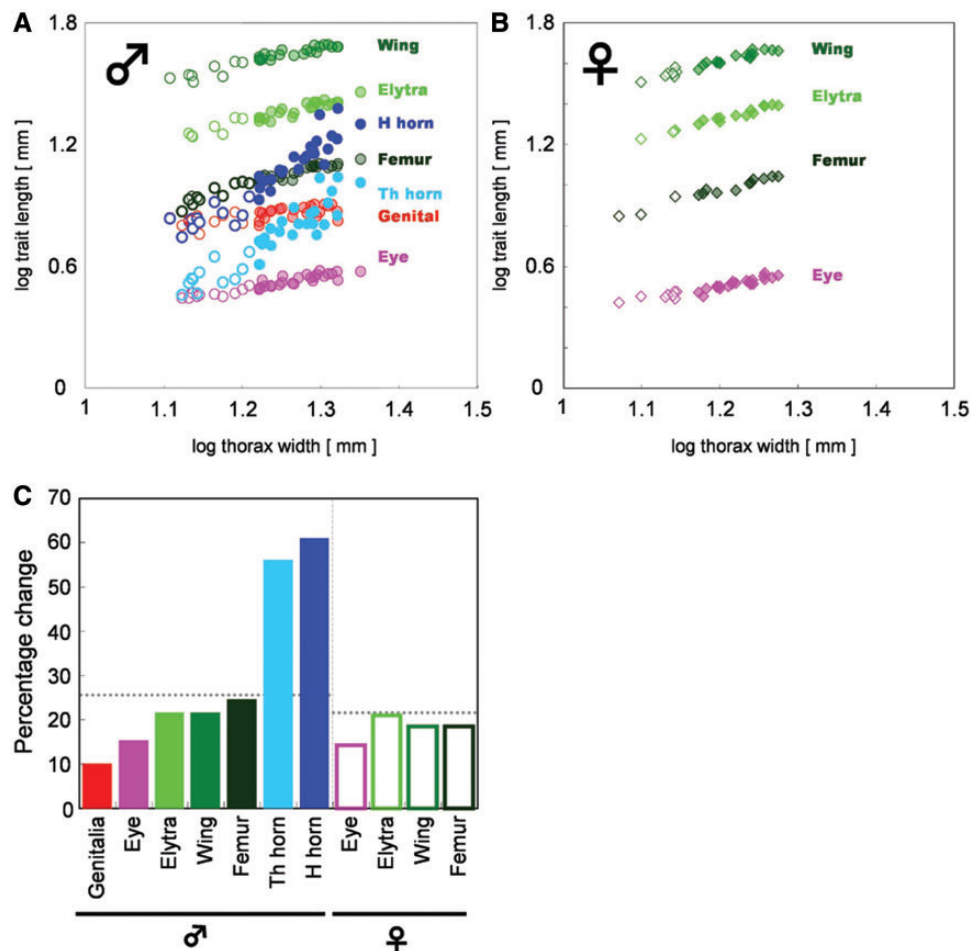


Fig. 4 Scaling relationships of body parts under high and low conditions of nutrition in male (A) and female (B) *Trypoxylus dichotomus*. Males' wings, elytra, femur, and eyes were measured and found to grow proportionally with body size whereas cephalic horns and thoracic horns have a much steeper allometric slope. (C) The genitalia, thorax, and cephalic horns of males show extreme changes in growth under high versus low conditions of nutrition in male *T. dichotomus* compared with changes in overall body size (dashed line). All other body parts both in males and in females show changes between high- and low-nutritional levels similar to overall changes in body size.

of their horns, and it is unknown whether horns function as signals prior to, or during, male–male combat. However, males only escalate to intense, fully-engaged fights with size-matched rivals (Hongo 2003; McCullough and Zinna 2013) and males often tap their opponents with their cephalic horn before and during fights, indicating that horns may indeed act as signals to potential rivals. Clearly, the function of horns in male–male combat in rhinoceros beetles is consistent with their role as conspicuous and reliable signals of a male's condition.

Genitalia were insensitive to the nutritional state of developing larvae, compared with other traits (Fig. 4). The allometric slopes for genitalia were half those for the wings and legs, two non-sexual traits, indicating that all males produced genitalia of similar size regardless of their nutritional state

or overall body size. Nothing is known about the effect of variation in the morphology of males' genitalia on mating or fertilization success in this species, but these results are consistent with the observation that male genitalia in many insects experience stabilizing selection for an intermediate, standard size that fits the average, and therefore most abundant, genitalia size of females (e.g., Eberhard 2009; Shingleton and Frankino 2013). Buffering growth of the genitalia from variations in the environment allows individuals across all body sizes to produce “one size fits all” genitalia that presumably maximizes a male's mating success across a broad range of body sizes of females (Eberhard et al. 1998; Eberhard 2009). Elegant studies by Tang et al. (2011) have found that genitalia in *Drosophila* are also insensitive to nutritional perturbations, and

they have linked this canalized pattern of growth to a trait-specific insensitivity to signaling through the insulin/IGF pathway.

This study reveals important differences in traits' sensitivity to nutrition, one widespread component of environmental variation that drives among-individual variation in physiological condition. In insects, individual nutritional state is tightly correlated with circulating levels of insulin-like peptides (ILPs) (for a recent review, see [Koyama et al. 2013](#)), and each organ uses ILP level as an indicator of overall body condition ([Shingleton and Frankino 2013](#); [Koyama et al. 2013](#)). In *Drosophila*, differential insulin-signaling activity among traits is responsible for plasticity of organ size ([Tang et al. 2011](#); [Shingleton and Frankino 2013](#); [Koyama et al. 2013](#)). Earlier we showed ([Emlen et al. 2012](#)) that horns, wings, and genitalia of male *T. dichotomus* responded differently to nutritional conditions, and that this appeared to be regulated in part by the insulin-signaling pathway; exaggerated, sexually selected traits (horns) exhibited greater sensitivity to this pathway than did wings or genitalia. These results suggest that the evolution of increased sensitivity to ILPs in specific developing parts of the body might be a general mechanism both for exaggerated growth of a trait and for enhanced, or heightened, condition-sensitive expression of ornaments and weapons of sexual selection ([Emlen et al. 2012](#); [Warren et al. 2013](#)).

Growth of most metazoan appendages is sensitive to nutrition, and the resulting plasticity of growth likely entails a dialog between whole-animal physiological signals of nutritional state, on the one hand, and appendage-patterning mechanisms on the other hand ([Koyama et al. 2013](#); [Shingleton and Frankino 2013](#)). Yet the details of these interactions remain poorly understood. We suggest that exaggerated sexually selected structures are well suited for teasing apart the nuances of the interactions of these pathways, due to their unusually high levels of sensitivity to nutrition. As [Bolker \(2012\)](#) succinctly points out, to study the impact of environment on genetics and development, it is critical to study species in which the selective context matters. We argue that animals with exaggerated sexually selected structures are a best fit for understanding condition-dependent growth because of the specific conditions under which they have evolved. How generalizable these mechanisms will be to other systems remains to be tested for most pathways but as long as the mechanisms remain unstudied, these data will never be available ([Bolker 2012](#)). However, in addition to the insulin-/IGF-signaling pathway, it is well known

that the JH/ecdyteroid pathways have vital roles in modulating nutrition-sensitive patterns of plastic growth in beetles' weapons ([Emlen and Nijhout 1999, 2001](#); [Gotoh et al. 2011, 2014](#); [Emlen et al. 2012](#)). Finally, exciting new results on the regulation of sexual differentiation ([Kijimoto et al. 2012](#); [Ito et al. 2013](#); [Gotoh et al. 2014](#)) and positional information ([Wasik et al. 2010](#)) on exaggerated growth of a trait strongly suggest that interactions between gene networks are ultimately responsible for condition-dependent phenotypic plasticity in these traits. Future studies will be needed to better understand the developmental mechanisms responsible for nutrition-dependent plasticity generally, and for the heightened (or attenuated) plasticity of specific body parts.

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References

- Andersson M. 1994. Sexual selection Princeton, NJ: Princeton University Press.
- Bradbury JW, Vehrencamp SL. 2011. Principles of animal communication. 2nd ed. Sunderland (MA): Sinauer Associates, Inc.
- Bolker J. 2012. Model organisms: there's more to life than rats and flies. *Nature* 491:31–3.
- Bonduriansky R, Rowe L. 2005. Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution* 59:138–51.
- Bonduriansky R. 2007. The evolution of condition-dependent sexual dimorphism. *Am Nat* 169:9–19.
- Cotton S, Fowler K, Pomiankowski A. 2004a. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond B Biol Sci* 271:771–83.
- Cotton S, Fowler K, Pomiankowski A. 2004b. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution* 58:1038–46.

- David P, Bjorksten T, Fowler K, Pomiankowski A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* 406:186–8.
- Davidowitz G, Roff DA, Nijhout HF. 2005. A physiological perspective on the response of body size and development time to simultaneous directional selection. *Integ Comp Biol* 45:525–31.
- Eberhard WG, Huber BA, Rodriguez RL, Briceno RD, Salas I, Rodriguez V. 1998. One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. *Evolution* 52:415–31.
- Eberhard WG. 2009. Static allometry and animal genitalia. *Evolution* 63:48–66.
- Emlen DJ, Nijhout HF. 1999. Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus Taurus* (Coleoptera: Scarabaeidae). *J Insect Physiol* 45:45–53.
- Emlen DJ, Nijhout HF. 2001. Hormonal control of male horn length dimorphism in *Onthophagus taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone. *J Insect Physiol* 47:1045–54.
- Emlen DJ, Warren IA, Johns A, Dworkin A, Lavine LC. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337:860–4.
- Gotoh H, Cornette R, Koshikawa S, Okada Y, Lavine LC, Emlen DJ, Miura T. 2011. Juvenile hormone regulates extreme mandible growth in male stag beetles. *PLoS One* 6:e21139.
- Gotoh H, Miyakawa H, Ishikawa A, Ishikawa Y, Sugime Y, Emlen DJ, Lavine LC, Miura T. 2014. Developmental link between sex and nutrition; *doublesex* regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles. *PLoS Genet* 10:e1004098.
- Grafen A. 1990. Sexual selection unhandicapped by the Fisher process. *J Theor Biol* 144:473–516.
- Hongo Y. 2003. Appraising behaviour during male–male interaction in the Japanese horned beetle *Trypoxylus dichotomus septentrionalis* (Kono). *Behaviour* 140:501–17.
- Hongo Y. 2007. Evolution of male dimorphic allometry in a population of the Japanese horned beetle *Trypoxylus dichotomus septentrionalis*. *Behav Ecol Sociobiol* 62:245–53.
- Hongo Y. 2012. Mating interaction of the Japanese horned beetle *Trypoxylus dichotomus septentrionalis*: does male excluding behavior induce female resistance? *Acta Etholog* 15:195–201.
- Ito Y, Harigai A, Nakata M, Hosoya T, Araya K, Oba Y, Ito A, Ohde T, Yaginuma T, Niimi T. 2013. The role of *doublesex* in the evolution of exaggerated horns in the Japanese rhinoceros beetle. *EMBO Rep* 14:561–7. (doi:10.1038/embor.2013.50).
- Iwasa Y, Pomiankowski A, Nee S. 1991. The evolution of costly mate preferences. II. The ‘handicap’ principle. *Evolution* 45:1431–42.
- Johnstone RA. 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biol Rev* 70:1–65.
- Karino K, Seki N, Chiba M. 2004. Larval nutritional environment determines adult size in Japanese horned beetles *Allomyrina dichotoma*. *Ecol Res* 19:663–8.
- Karino K, Niiyama H, Chiba M. 2005. Horn length is the determining factor in the outcomes of escalated fights among male Japanese horned beetles, *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae). *J Insect Behav* 18:805–15.
- Kijimoto T, Moczek AP, Andrews J. 2012. Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. *Proc Natl Acad Sci U S A* 109:20526–31.
- Koyama T, Mendes CC, Mirth CK. 2013. Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. *Front Physiol* 4:263.
- Maynard-Smith J, Harper DGC. 2003. *Animal signals*. Oxford: Oxford University Press.
- McCullough EL, Zinna RA. 2013. Sensilla density corresponds to the regions of the horn most frequently used during combat in the giant rhinoceros beetle *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae: Dynastinae). *Ann Entomol Soc Am* 106:518–23.
- Nijhout HF. 2003. The control of body size in insects. *Dev Biol* 261:1–9.
- Plaistow SJ, Tsuchida K, Tsubaki Y, Setsuda K. 2005. The effect of a seasonal time constraint on development time, body size, condition, and morph determination in the horned beetle *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae). *Ecol Entomol* 30:692–9.
- Pomiankowski A. 1987. Sexual selection: the handicap principle does work—sometimes. *Proc R Soc Lond B Biol Sci* 231:123–45.
- Searcy WA, Nowicki S. 2005. *The evolution of animal communication: reliability and deception in signaling systems*. Princeton (NJ): Princeton University Press.
- Shingleton AW, Frankino WA. 2013. New perspectives on the evolution of exaggerated traits. *Bioessays* 35:100–7.
- Siva-Jothy MT. 1987. Mate securing tactics and the cost of fighting in the Japanese horned beetle, *Allomyrina dichotoma* L. (Scarabaeidae). *J Ethol* 5:165–72.
- Stern DL. 2001. Body-size evolution: how to evolve a mammoth moth. *Curr Biol* 11:917–9.
- Tang HY, Smith-Caldas MSB, Driscoll MV, Salhadar S, Shingleton AW. 2011. FOXO regulates organ-specific phenotypic plasticity in *Drosophila*. *PLoS Genet* 7:e1002373 (doi:10.1371/journal.pgen.1002373).
- Warren I, Gotoh H, Dworkin IM, Emlen DJ, Lavine LC. 2013. A general mechanism for conditional expression of exaggerated sexually-selected traits. *Bioessays* 35:889–99.
- Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biol Rev* 81:259–91.
- Wasik BR, Rose DJ, Moczek AP. 2010. Beetle horns are regulated by the Hox gene, *Sex combs reduced*, in a species- and sex-specific manner. *Evol Dev* 12:353–62.