Artificial Selection on Horn Length-Body Size Allometry in the Horned Beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae)

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ARTIFICIAL SELECTION ON HORN LENGTH-BODY SIZE ALLOMETRY IN THE HORNED BEETLE *ONTHOPHAGUS ACUMINATUS* (COLEOPTERA: SCARABAEIDAE)

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Abstract.—Males of the horned beetle *Onthophagus acuminatus* Har. (Coleoptera: Scarabaeidae) exhibit horn length dimorphism due to a sigmoidal allometric relationship between horn length and body size: the steep slope of the allometry around the inflection of the sigmoid curve separates males into two groups; those larger than this inflection possess long horns, and those smaller than this inflection have short horns or lack horns. I examined the genetic basis of the allometric relationship between horn length and body size by selecting males that produced unusually long horns, and males that produced unusually short horns, for their respective body sizes. After seven generations of selection, lines selected for relatively long horns had significantly longer horn lengths for a given body size than lines selected for relatively short horns, indicating a heritable component to variation in the allometry. The sigmoidal shape of the allometry was not affected by this selection regime. Rather, selected lines differed in the position of the allometry along the body size axis. One consequence of lateral shifts in this allometric relationship was that the body size separating horned from hornless males (the point of inflection of the sigmoid curve) differed between selection lines: lines in which males were selected for relatively long horns began horn production at smaller body sizes than lines selected for relatively short horns. These results suggest that populations can evolve in response to selection on male horn length through modification of the growth relationship between horn length and body size.

Key words.—Allometry, artificial selection, dimorphism, horned beetle, *Onthophagus acuminatus,* threshold.

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Males of many animal species possess elaborate structural ornaments or weapons not present in females (e.g., tails: Andersson 1986; Møller 1991; spurs: Johnson et al. 1993; antlers: Clutton-Brock et al. 1989; Balmford et al. 1992; uropods: Shuster 1989). Variation in the expression of male ornaments can lead to variation in reproductive success, and thus in fitness (Andersson 1986; Conner 1988; Nicoletto 1993), and understanding the evolution of these traits requires some knowledge of their genetic determination.

Beetle "horns" are a common and dramatic class of male secondary sexual traits. Several thousand beetle species have horns (Arrow 1951; Enrodi 1985), and these structures have enthralled many prominent naturalists including Bates (1863), Darwin (1871), Wallace (1878), Fabre (1899) and Beebe (1944). Recent studies of horned beetle species have begun to reveal both the functions of horns and the natural circumstances important to selection on horns (Eberhard 1977, 1979, 1981, 1982; Palmer 1978; Goldsmith 1987; Siva-Jothy 1987; Conner 1988, 1989; Cook 1990; Rasmussen 1994). However, the genetic bases of beetle horns remain largely unexplored (but see Emlen 1994a).

For many horned beetle species, male horn length covaries linearly with body size. Because the slopes of these relationships often differ from unity (allometry; Huxley 1932), variation in horn length also constitutes variation in male shape (i.e., horn length expressed as a proportion of total body size; Arrow 1951). Consequently, populations of horned beetle species generally contain a continuous range of male morphologies.

In some horned beetle species variation in male morphology is not continuous, and males occur in two relatively discrete shapes (Eberhard 1982; Cook 1987; Rasmussen 1994). Male dimorphism in horned beetles generally results from nonlinearities in the allometric relationship between horn length and body size (Eberhard 1982; Eberhard and Gutierrez 1991). In particular, horn length in most dimorphic species increases sigmoidally with increases in body size. As with head size in polymorphic species of Hymenoptera (e.g., Wilson 1971; Wheeler 1991), steep slopes near the middle of the allometry (around the point of inflection of the sigmoid) cause male horn length to increase rapidly over a very narrow range of body sizes. This causes natural frequencies of horn lengths to be bimodally distributed, and separates males into two, approximately discrete shapes: males larger than the inflection of the allometry have very long horns, and males smaller than this size have short horns, or no horns.

Adult males of *Onthophagus acuminatus* Har. (Coleoptera: Scarabaeidae), a dung beetle commonly found in tropical lowland forests of Panama, Central America, possess two frontal horns that extend vertically from the base of the head. In static samples of natural populations of *O. acuminatus,* male horn length varies sigmoidally with body size, and males can be separated into "horned" and "hornless" morphologies on the basis of horn length (Fig. 1). Earlier, I conducted genetic studies on horns of *O. acuminatus,* but I did not detect heritable genetic variation for horn length. That is, the horn lengths expressed by individual males did not resemble the horn lengths of their fathers (father-son regression for horn length: $b \pm SE = 0.003 \pm 0.10$, $r^2 = 0.000$, $P = 0.9773$, $n = 39$; power ($\propto = 0.05$) > 99 if actual $r \geq 0.6$; power = 95 if $r = 0.5$; power = 15 if $r = 0.10$; Emlen 1994a). Rather, male horn lengths reflected nutritional conditions experienced during larval development (Emlen 1994a). Because these nutritional conditions also determine adult body size in *Onthophagus* (Lee and Peng 1981; Risdill-

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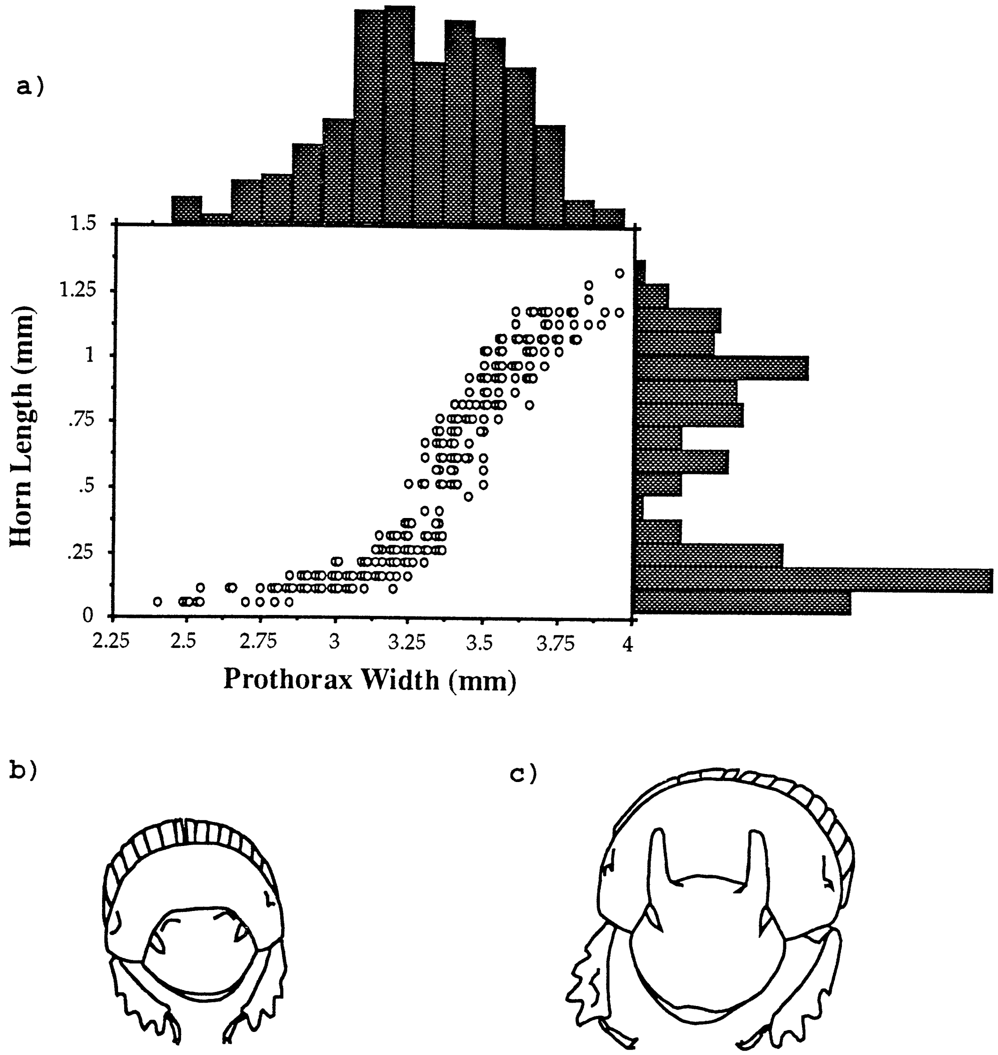

FIG. 1. (a) Allometric relationship between horn length and body size (Prothorax Width) for 561 male *Onthophagus acuminatus* (Coleoptera: Scarabaeidae) from Barro Colorado Island, Panama. The sigmoidal shape of the allometry produces a bimodal male horn length frequency distribution (right margin), and results in the separation of males into "hornless" (b) and "horned" (c) male morphologies.

Smith et al. 1982; Emlen 1994a), male horn lengths scale with body size. This results in natural populations having very precise relationships between horn length and body size despite large environmental components to body size variation.

The condition-sensitive mechanism of horn production in *O. acuminatus* suggests that evolutionary changes in horn morphology might result from genetic changes in the developmental relationship between horn length and body size (i.e., the allometry), rather than from changes in genes specifying horn length per se (Fig. 2).

Here I test the hypothesis that heritable variation exists for the horn length-body size allometry in *O. acuminatus* by artificially selecting on male horn length in relation to body size. I did this because several recent studies show that it is possible to select on allometric relationships between metric traits (e.g., selection on wing dimensions in *Drosophila* [Weber 1990], and selection on eye-stalk allometry in stalk-eyed flies, [Wilkinson 1993]). However, this study is the first of which I am aware to select on a nonlinear allometry. Because the sigmoidal shape of the horn length-body size allometry produces dimorphism among males, evolutionary changes in the allometry could alter the structure of *O. acuminatus* populations. In particular, changes in the allometric relationship between horn length and body size could alter the proportions of horned and hornless males by changing the body size associated with the onset of horn growth. I discuss results from this experiment in the context of horn evolution and male horn length dimorphism in *O. acuminatus,* and suggest a role for non-linear allometries in the evolution of costly male secondary sexual ornaments.

MATERIALS AND METHODS

Biology of O. acuminatus

Onthophagus acuminatus are common, diurnal inhabitants of the forest understory of Barro Colorado Island, Panama, where they feed primarily on dung from howler monkeys

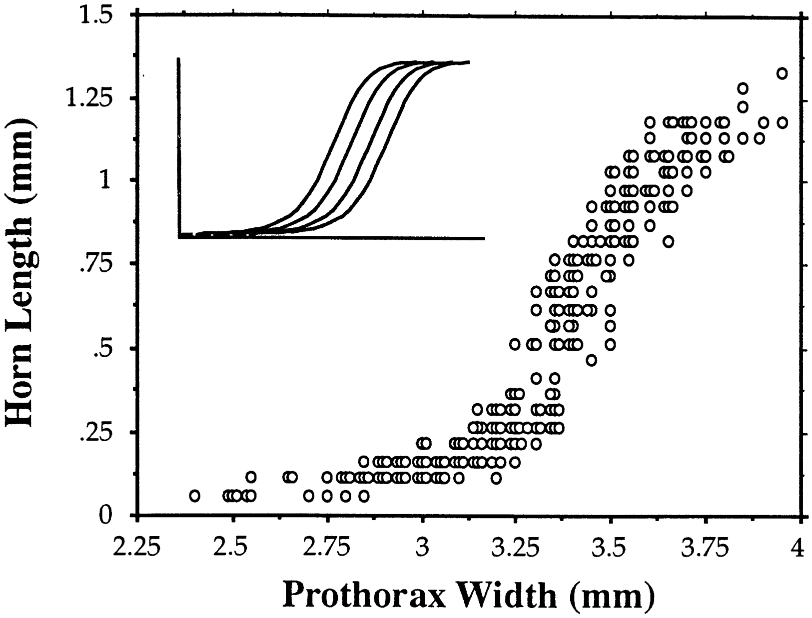

FIG. 2. Sigmoidal allometric relationship between male horn length and body size (as in Fig. 1), showing hypothetical genetic variation in the underlying allometry (insert). Each curve represents the allometry specified by a single genotype. While individuals only ever express a single horn length and body size, they may contain within them relationships specifying horn length across a range of possible body sizes. In this case, genetic variation in horn morphology would reflect genetic differences in the shape or position of the horn length-body size allometry.

(*Alouatta palliata*; Howden and Young 1981; Gill 1991). Beetles fly to dung seconds after it falls to the forest floor, and females dig vertical tunnels in which they sequester dung for larval provisioning (Emlen 1994a,b). Dung is pulled to the ends of these tunnels and packed to form dense "brood balls" ("brood masses" of Halffter and Edmonds 1982), and a single egg is laid in each brood ball (Emlen 1994a). Individual larvae develop and metamorphose in isolation within these brood balls, and dig to the soil surface as adults.

In the wild, horned and hornless males utilize different behaviors to encounter and mate with females, and these differences in behavior result in disruptive selection for horns (Emlen 1994b, unpubl. manuscript). Large males guard entrances to tunnels beneath dung, and males successful at tunnel defense mate repeatedly with resident females. Smaller males are unable to effectively defend tunnels, and instead sneak into tunnels either by sliding past the guarding males, or by digging side tunnels that intercept primary tunnels beneath the soil surface (Emlen 1994b, unpubl. manuscript). Horns significantly improve male ability to guard tunnels, but appear to hinder sneaking performance both by impeding movement in tunnels, and by making it more difficult to slide past guarding males (Emlen 1994b, unpubl. manuscript).

Beetles can be reared in the laboratory (Emlen 1994a,b) by placing adults in deep containers filled with potting soil and supplied with howler monkey dung. After adults have dug tunnels in the containers and pulled dung below ground, the soil can be sifted and all brood balls extracted. These are buried individually in soil-filled cups for the duration of development.

Quantifying Differences in the Horn Length-Body Size Allometry

To select on the allometric relationship between horn length and body size, I needed to incorporate measures of both horn length and body size into a single value effectively describing male shape. In particular, I needed to compare the relative horn lengths of individual males after accounting for their respective body sizes. To do this, I used data from wild-caught beetles to describe an average relationship between horn length and body size. The best-fit curve was obtained by fitting the modified logistic equation:

$$\text{horn length} = \text{P5} + \frac{\text{P1}}{1 + \left(\frac{\text{P1}}{\text{P3}} - 1\right)\exp(-\text{P2}[\text{body size} - 2.35]^{\text{P4}})} \tag{1}$$

(where Pi = the i^{th} parameter of the model) to the data using a simulated annealing algorithm (Metropolis et al. 1953; Szymura and Barton 1986). This equation was chosen because it accurately described horn length across the natural range of body sizes. The equation giving the best fit for the horn length-body size relationship:

$$\text{horn length} = 0.743 + \frac{2.24}{1 + \left(\frac{2.24}{0.04} - 1\right)\exp(-0.892[\text{body size} - 2.35]^{2.02})} \tag{2}$$

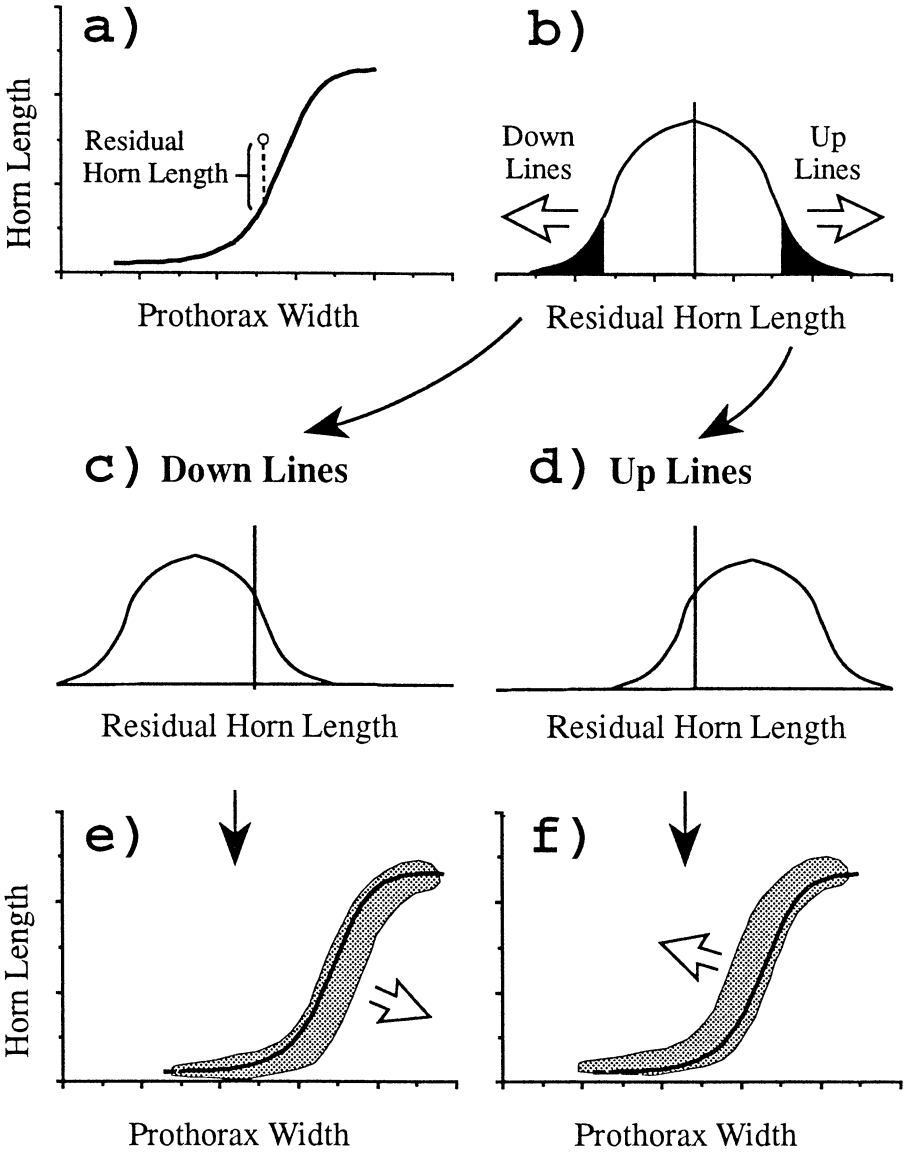

FIG. 3. Schematic of the method used to artificially select on horn length-body size allometry in *Onthophagus acuminatus*. Residual horn lengths were calculated for all males as the difference between actual horn length and the horn length expected for an average male of the same body size (a; see text). The curve is from the nonlinear regression model with estimators (equation 2), and the open circle is a hypothetical individual from one of the selected populations. Bidirectional selection was performed by selecting males with the largest (Up lines) and smallest (Down lines) residual horn lengths (b). Changes in the residual horn length distribution brought about by selection (c, d) would shift the horn length-body-size allometry (e, f).

was used to calculate average horn lengths for males of all possible body sizes. Individual males were then characterized as having unusually long or short horns for their respective body sizes by comparing their actual horn length to the horn length expected for an average male of the same body size (i.e., by calculating residual horn lengths from equation (2); Fig. 3a).

Selection Regime

Selection for the allometric relationship between horn length and body size was performed by selecting individuals with the largest and smallest residual horn lengths (Fig. 3b; see Conner 1988, for a natural analog). Selection was applied to six genetically separate lines. In two lines (Up 1 and Up 2), I selected for relatively long horns by breeding only males with the largest positive residual horn lengths. In two other lines (Down 1 and Down 2), I selected for relatively short horns by breeding only males with the largest negative residual horn lengths. The final two lines were used as controls, with breeding males chosen at random. Because I was able to characterize the horn length-body size relationship for individual males (by calculating residual horn lengths), I used an "individual" selection design (Falconer 1989). In each genetic line, five males were selected every generation as sires. Females do not have horns, so all females used in the crosses were chosen randomly from within each line. To increase the number of progeny sired by each male, and hence both the population size and the strength of selection at each generation, each male was paired with two females.

Rearing Procedure

Beetles used to initiate the selected and control lines were wild-caught from Barro Colorado Island, Panama. Five males were selected (see above) from samples of 50 males to start each selection and control line. Breeding trios (1 male + 2 females) were placed in 25 cm × 10 cm cylindrical buckets three-quarters filled with moist potting soil and supplied with fresh dung from howler monkeys (*A. palliata*). All dung was collected from the forest floor and stored for no more than five days in a refrigerator. In case dung from different days and/or individual monkeys varied in nutrient constitution, all dung was mixed before being administered to beetles. This did not control for any seasonal fluctuations in dung nutrient quality, but seasonal variation will have affected treatments equally since all beetles from all lines were fed from homogeneous dung mixtures at each feeding.

Every three days the soil in each of the cups was sifted, all brood balls were removed, and beetles were supplied with new howler monkey dung (details: Emlen 1994a). All brood balls from each male were buried in separate, soil-filled plastic cups and stored in darkness for the duration of larval development ($\bar{x} \pm \mathrm{SD} = 29.6 \pm 2.37$ days, $N = 191$; Emlen 1994b). Cups were labeled for sire, replicate and treatment, and cups from all treatments were arranged randomly in the laboratory.

Progeny were collected on emergence and separated by sex. All males were measured for horn length and body size (prothorax width) to the nearest 0.05 mm with a stereo-microscope ocular micrometer. Beetles from each sex and each line were kept in flat plastic containers with potting soil and howler monkey dung, until sexually mature (approximately seven days; Emlen 1994b).

Treatment males were selected on the basis of their residual horn lengths, paired randomly with two females each from the same generation of the same line, and allowed to breed as above. Control males were chosen randomly and reared similarly. This process was repeated for seven generations of selection between August 1992 and August 1993. After five generations, survivorship within lines was so poor (< 16%, down from 75%) that all sample sizes were small, and two lines (Up 1 and Control 1) were in danger of extinction. To restore these lines and reduce possible detrimental effects from inbreeding, I combined the two replicated lines from each treatment to form single lines for the final two generations. This improved offspring survivorship (30% and 49% for generations 6 and 7, respectively), but removed the replication of treatments. Consequently, generations 6 and 7 included only a single population each for Up, Down and Control lines.

Analyses

Horn length-body size allometries of treatment and control lines were compared using Analyses of Variance (ANOVAs). Because all beetles were reared in separate cups, they represent independent estimates of the selection treatment (i.e. individuals within each line were not interacting in any way that would cause the growth of one individual to be influenced by the presence of other individuals). Residual horn lengths of all male progeny from each line were pooled, and ANOVAs were performed at each generation to describe differences between treatment (Up and Down lines) and control residual horn length distributions (all residual horn length distributions were tested for normality using Kolmogorov-Smirnov and Chi-square tests, and none were significantly different from normal). Nested ANOVAs were used for generations 1 through 5, with replicates nested within treatment. Sample variance ratios (*F*-values) presented are for the effect of treatment on residual horn length. After generation 5 (when the replicate lines were combined), one-factor ANOVAs were used to compare the three remaining distributions (Up, Down and Control), and *F*-values for generations 6 and 7 are for the ANOVA model.

Consecutive generations of this selection experiment were not statistically independent because the individuals forming each generation were sampled non-randomly from the previous generation. Therefore, it was inappropriate to test for differences among lines at every generation, and *P*-values are presented only for generations 5 (the last generation with line replication) and 7 (the final generation).

Realized heritabilities were estimated as twice the slope of the regression of weighted cumulative selection differential on generation mean response (Falconer 1989). Selection differentials were calculated for each line as the mean residual horn length of selected parents minus the mean residual horn length of the population. To correct for unequal contributions to subsequent generations, parent means were weighted by the numbers of offspring each parent produced (Falconer 1989). Selection intensities were estimated by dividing selection differentials for each generation of each line by the phenotypic standard deviation of residual horn length (Falconer 1989). To remove between-generation environmental effects from estimates of response to selection, all responses were measured as the difference between treatment and control means. Weighted averages of replicate lines were used for both treatment and controls when calculating cumulative selection differential and response values.

Average selection intensities for the Up and Down treatments were calculated as the total selection intensity for each direction of selection, averaged across all seven generations. For generations 1 through 5 (when each treatment consisted of two replicate lines), this value was calculated as if both replicates were pooled (i.e. the mean of all parents from both replicates minus the mean of the combined populations, divided by the standard deviation of the combined populations). For generations 6 and 7 (when each treatment consisted of only a single population), selection intensities were calculated as above.

To identify how evolutionary changes in residual horn length were brought about, horn length-body size allometries were compared for Up, Down and Control lines. Nonlinear regressions were fitted to the final (generation 7) distributions of each line using the equation:

$$\text{horn length} = \text{P2} - \frac{\text{P4}}{\text{P4} + 1} + \frac{\text{P4}\left(\frac{\text{body size}}{\text{P1}}\right)^{\text{P3}}}{\text{P4} + \left(\frac{\text{body size}}{\text{P1}}\right)^{\text{P3}}} \quad (3)$$

and the same algorithm as above. This equation (a sigmoidal modification of the Michaelis-Menten equation) was chosen instead of equation (2) because parameters were biologically interpretable as components of allometry position and shape. Parameter 1 specifies the lateral position of the allometry (body size at the point of inflection), P2 the vertical position (horn length at the point of inflection), P3 the steepness and P4 the asymptote. Parameter values were estimated for the Up, Down and Control line populations, and compared using likelihood ratio tests (Zar 1984; Szymura and Barton 1986). Significant differences in any of the parameters would be measured as a reduction in log-likelihood of greater than one-half chi-square (1.92 for df = 1, at $P = 0.05$) when the value of the parameter from one of the populations was substituted into the best-fit equation describing the other population. Estimating this "effect of substitution" for each of the parameters provided a quantitative measure of how the horn length-body size allometry responded to artificial selection on residual horn length.

RESULTS

The allometric relationship between horn length and body size responded significantly to bidirectional selection on residual horn length in seven generations (Table 1). The average selection intensities for the experiment were: Up lines, $\bar{x} \pm$ SD = 0.853 ± 0.575; Down lines, $\bar{x} \pm$ SD = −1.237 ± 0.268. Both lines which were selected for relatively long horns (Up 1 and Up 2) had larger residual horn lengths than lines selected for relatively short horns (Down 1 and Down 2) from generation 3 onward (Fig. 4). Differences between Up and Down lines were visible but not significant at generation 5 (Nested ANOVA: $F = 6.180$, df = 2, 3, 78, $P = 0.0863$), and significant at generation 7 (one-factor ANOVA: $F = 45.572$, df = 2, 188, $P = 0.0001$). By generation 7, both treatment populations were significantly different from the control population (Scheffe tests: Up versus Control, $P = 0.0001$; Down versus Control, $P = 0.006$). All lines showed large, synchronous fluctuations in residual horn length across generations (Fig. 4; pairwise correlation coefficients (r) were all ≥ 0.53), indicating that variation in the horn length-body size allometry had a large environmental component of expression as well.

Realized heritabilities for residual horn length were 0.178 (Up lines) and 0.162 (Down lines) as estimated by twice the slope of the regression line of response on weighted cumulative selection differential (Up lines: b ± SE = 0.089 ± 0.02; Down lines: b ± SE = −0.081 ± 0.02; Fig. 5). However, because subsequent selection differentials were added to form the cumulative selection differentials, they were not statistically independent. Therefore, I also estimated realized heritabilities using only the total selection differential and total response at generation 7, giving estimates of 0.280 and 0.132 for Up and Down lines, respectively.

Selection for residual horn length inadvertently produced

TABLE 1. Results from seven generations of selection on residual horn length. Values are population means ± SE for each generation of the selection and control lines. Nested ANOVAs (replicates nested within treatments) were used to describe population differences for generations 1 through 5. F-values are for the effect of selection treatment on residual horn length. After generation 5, treatment replicates were combined, leaving one population each for Up, Down and Control lines. One-factor ANOVAs were used to describe population differences for generations 6 and 7, and F-values are for the ANOVA model. Because of the nonindependence of consecutive generations, P-values are presented only for generations 5 (the last generation with replication) and 7 (the final generation). In generation 7, different small case letters $^{(a,b,c)}$ represent significant differences between lines using Scheffe's test for multiple contrasts.

Line	1	2	3	4	5	6	7
Up 1	−0.005 ± 0.022	−0.007 ± 0.050	−0.009 ± 0.080	0.055 ± 0.053	0.073 ± 0.041	0.151 ± 0.025	0.264 ± 0.015^a
Up 2	−0.003 ± 0.032	0.209 ± 0.042	0.070 ± 0.051	0.238 ± 0.072	0.056 ± 0.026		
Control 1	−0.027 ± 0.043	0.071 ± 0.052	−0.112 ± 0.059	0.194 ± 0.069	0.013 ± 0.049	0.063 ± 0.025	0.105 ± 0.105^b
Control 2	0.033 ± 0.043	0.085 ± 0.042	−0.032 ± 0.035	0.169 ± 0.053	−0.016 ± 0.032		
Down 1	−0.035 ± 0.032	0.006 ± 0.032	−0.214 ± 0.050	−0.040 ± 0.068	−0.053 ± 0.032	0.030 ± 0.023	0.010 ± 0.027^c
Down 2	0.016 ± 0.029	−0.027 ± 0.029	−0.093 ± 0.031	0.015 ± 0.046	−0.157 ± 0.046		
$N =$	133	171	101	101	84	90	191
$F =$	0.075	0.917	0.917	2.706	6.180	6.786	45.572
$P =$					0.0863		0.0001

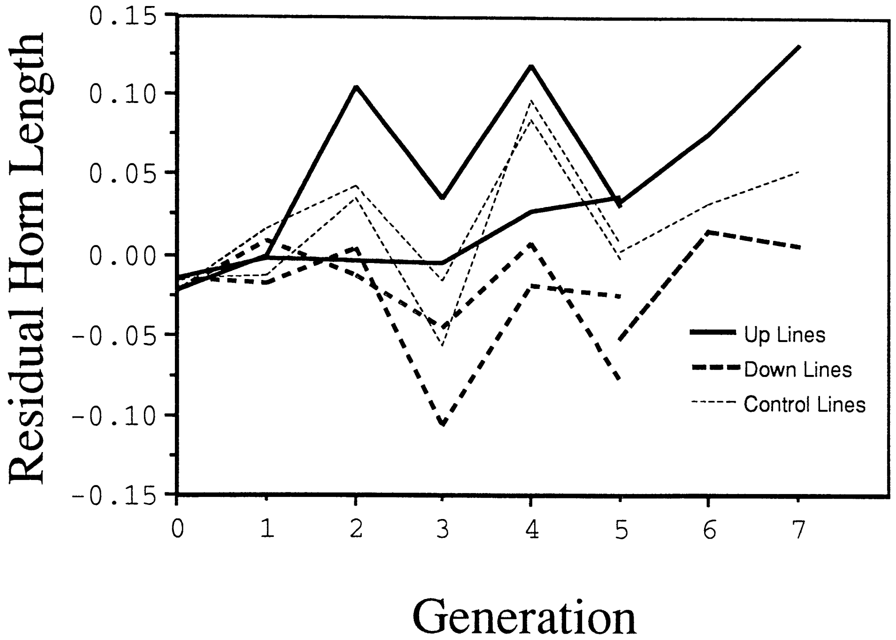

FIG. 4. Responses to selection on horn length-body size allometry in *Onthophagus acuminatus*. Residual horn length responded consistently and significantly to direct selection. From generation 3 onward, lines selected for increased residual horn length (solid lines) had larger residual horn lengths than lines selected for decreased residual horn length (wide, dashed lines). Significant differences between selection treatments were present for both generation 6 and 7, and by generation 7 both treatment lines were significantly different from the control line (see text, and Table 1). Residual horn length showed significant across-generation covariation, indicating a large environmental (seasonal) component to allometry variation.

some directional selection for both horn length and body size, when each trait was considered independently of the other (average selection intensities for horn length: Up lines, $\bar{x} \pm$ SD = 0.636 ± 0.509; Down lines, $\bar{x} \pm$ SD = −0.021 ± 0.631; average selection intensities for body size: Up lines, $\bar{x} \pm$ SD = 0.399 ± 0.460; Down lines, $\bar{x} \pm$ SD = 0.525 ± 0.395). Because prior genetic studies did not detect heritable variation for either horn length or body size (Emlen 1994a), I did not expect these traits to respond to selection. Results for horn length were consistent with this expectation: horn length showed no significant response to either direction of selection (regression of response on weighted cumulative selection differentials: Up lines, $F = 1.286$, $P = 0.2942$; Down lines, $F = 0.408$, $P = 0.5467$). Body size, on the other hand, responded to selection in the Down lines, but not the Up lines (regression of response on weighted cumulative selection differential: Down lines, $F = 13.103$, $P = 0.0085$; Up lines, $F = 0.005$, $P = 0.9465$), indicating some heritable variation for adult body size.

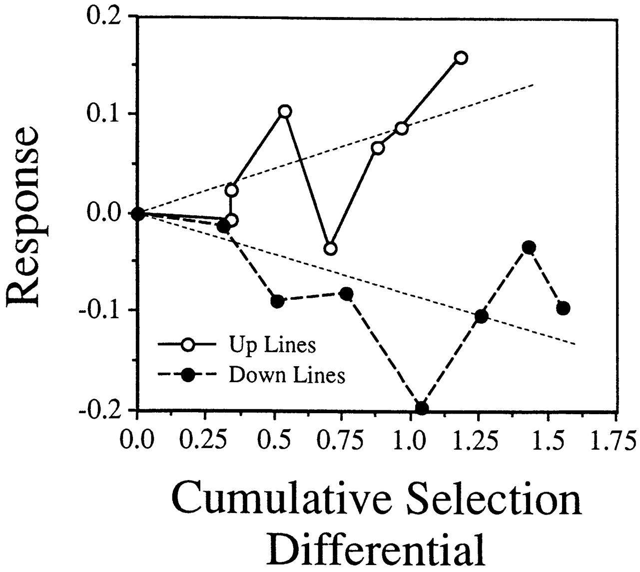

FIG. 5. Linear regressions of mean response to selection on weighted cumulative selection differential, for residual horn length (Up lines: b ± SE = 0.089 ± 0.02; Down lines: b ± SE = −0.081 ± 0.02). Realized heritabilities estimated by twice the slope of the above regressions were 0.178 and 0.162 for the Up (open circles) and Down (closed circles) lines, respectively.

Differences in the shape and position of the horn length-body size allometry resulted primarily from changes in the location of the point of inflection (i.e., the body size associated with the steepest part of the curve), and only minimally from changes in the vertical position of the curve and asymptote (Fig. 6; Table 2). Because the control line produced no males larger than the size of the inflection of the allometry, parameter estimates for this line were not robust. Consequently, I tested for parameter differences between the Up and Down lines, but not between these lines and the Control. Substitution of inflection point values from the Up line into the equation for the Down line, and vice versa, dramatically reduced curve fit in both directions (Table 2 presents the effect of substitution averaged over both directions). Curve height and asymptote were also affected, but to a lesser degree (log-likelihood effects of curve height and asymptote substitutions were more than 14-fold lower than for substitution

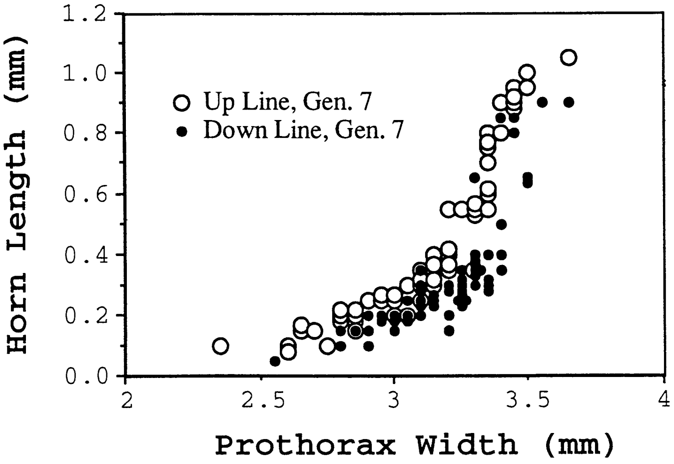

FIG. 6. Final horn length-body size distributions of lines selected for increased (open circles) and decreased (closed circles) residual horn length for seven generations. Changes in relative horn length resulted primarily from lateral shifts of the sigmoidal allometry along the body size axis.

TABLE 2. Effect of selection for residual horn length on allometry position and shape. Nonlinear regressions were fit to the horn length-body size distributions of the final (seventh generation) populations of lines selected for increased (Up) and decreased (Down) residual horn length, as well as the Control population. A simulated annealing algorithm provided parameter estimates for the curve best describing each of the distributions using equation (3) (see text). To test for significant differences in each of the curve parameters, values from one of the distributions were substituted into the equation for another, and vice versa. Differences in fit between the original models (using all parameter values for that population) and modified models (one parameter substituted) were compared using log-likelihood ratio tests. However, control males did not span the full range of body sizes (all were smaller than the inflection of the sigmoid), and parameter estimates for this line were not robust. Consequently, parameter values were compared between the Up and Down lines, but not between these lines and the Control.

				Up vs. Down	
Parameter	Up	Down	Control	Δ in log-likelihood	*P*-value
Inflection point	3.35	3.47	3.42	−103.91	< 0.0000
Curve height*	0.688	0.662	0.604	−6.72	< 0.001
Steepness	26.87	28.05	26.87	−0.67	ns
Asymptote	1.049	0.965	0.90	−3.48	< 0.01

* Horn length at the inflection point.

of the inflection point; Table 2). This implies that most of the evolutionary change brought about by the present selection regime can be attributed to lateral shifts in the position of the allometry along the body size axis (i.e., changes in the location of the point of inflection), and not to major changes in allometry shape.

DISCUSSION

Genetic Basis of Horn Allometry in O. acuminatus

Male horn length-body size allometry responded significantly to bidirectional selection in seven generations. By selecting on residual horn length, a size-independent measure of male shape, I was able to alter the allometric relationship between male horn length and body size. Males from lines selected for increased residual horn length had longer horns for a given body size than males from lines selected for decreased residual horn length, and these differences were significant for both generations 6 and 7 (Fig. 4). The response of horn length-body size allometry to artificial selection demonstrates a heritable component to variation in the allometry, with realized heritabilities of 0.178 and 0.162 for increased and decreased residual horn length, respectively.

Earlier, I found no detectable heritable variation for horn length or body size, when each was considered in isolation from the other (Emlen 1994a). This suggested that male horns had little or no evolutionary potential in current populations. Here I show that beetle horns can evolve, but that changes in horn length are brought about through genetic changes in the underlying allometric relationship between horn length and body size, rather than through changes in genes directly affecting horn length. This suggests that the genetic "trait" of relevance to studies of beetle horns—and perhaps to studies of other conditionally expressed male ornaments—is not the length or magnitude of the ornament per se but is instead the shape of the allometry relating ornament size to a range of possible body sizes. This study demonstrates that one such allometry has a heritable basis and can evolve in response to short term changes in the selection regime.

Evolutionary lability of the horn length-body size allometry of *O. acuminatus* has several important ramifications. First, it constitutes an example of a "threshold" trait with a demonstrated genetic basis to variation in the threshold. Second, it suggests how *O. acuminatus* populations will respond to short term changes in natural selective conditions. Third, it suggests how some of the morphological differences between related species might have been brought about. Finally, it suggests a role for nonlinear allometries in facilitating the evolution of costly male secondary sexual traits. These points are further discussed below.

Beetle Horns as a "Threshold" Trait

Because the lengths of *O. acuminatus* horns are strongly influenced by nutritional conditions experienced during larval development (Emlen 1994a), male horns are a "plastic" phenotypic trait (Bradshaw 1965). Furthermore, because male horn lengths are bimodally distributed, *O. acuminatus* horns fall into the subset of plastic traits that are discontinuously expressed. Discrete plasticity, or polyphenism, has been modeled as a threshold response to continuous variation in the environment (Lees 1966; Vepsäläinen 1974; Harrison 1979; Roff 1986; West-Eberhard 1992), and several recent models predict that threshold traits evolve through genetic changes in the sensitivity of the threshold to the environment (Hazel et al. 1990; Roff 1994). In this study I changed the critical size separating horned from hornless males by selecting on horn allometry. This indicates an evolutionary lability to the "threshold" of this dimorphism, and suggests that male horn length dimorphism in *O. acuminatus* is similar in this respect to genetically-determined thresholds for amphibian paedomorphosis (Harris 1987; Semlitsch and Wilbur 1989; Semlitsch et al. 1990), Lepidopteran pupal color dimorphisms (Clarke and Sheppard 1972; Hazel and West 1982; Sims 1983; Gruner and Sauer 1988) and *Daphnia* spine production (Parejko and Dodson 1991).

Selection on Horn Morphology in Natural O. acuminatus Populations

Under natural conditions, populations of *O. acuminatus* probably experience disruptive selection on male horns (Emlen 1994b; Emlen, unpubl. manuscript). The fitness consequences of horns depend primarily on male reproductive behavior, and males employ two distinct sets of behaviors to encounter and mate with females. Larger males guard entrances to tunnels containing females. Males smaller than most competitors are unable to effectively defend tunnels, and instead sneak into tunnels either by sliding past guarding males, or by digging side-tunnels that intercept guarded tunnels below ground (Emlen 1994b; Emlen, unpubl. manuscript). Guarding and sneaking behaviors impose different selection on horns: horns improve male ability to guard tunnels, but hinder sneaking (Emlen 1994b; Emlen, unpubl. manuscript). This appears to produce disruptive selection on male morphology, with long horns selectively advantageous in guarding males, and complete hornlessness advantageous in sneaking males.

Disruptive selection in this species should favor a correlation between male morphology and male behavior, so that all guarding males have long horns, and all sneaking males are hornless. In *O. acuminatus,* this would occur whenever the horn length-body size allometry was positioned along the body size axis such that the body size separating horned from hornless male morphologies (i.e., the inflection point of the sigmoid curve) coincided with the body size where males changed from guarding to sneaking behaviors. At this optimum, all males larger than the inflection of the allometry would both guard tunnels and have fully developed horns, and all males smaller than this inflection would sneak and be hornless.

However, this optimum position for the horn length-body size allometry should change whenever the body size distribution of the population changes. Because male reproductive behavior depends on the size of an individual male relative to the sizes of other males in the population, males in populations comprised of large individuals should switch from guarding to sneaking behaviors at larger body sizes than males in populations comprised of smaller individuals. Any change in the critical body size where males switch behaviors would select for horn length-body size allometries that inflected at a correspondingly altered body size. In this fashion, environmental factors that change the composition of the population (i.e., the average body sizes of individuals in the population) are likely to select for shifts in the precise body size associated with the onset of horn growth.

Do these changes in horn selection actually occur? Preliminary evidence from field studies of *O. acuminatus* indicates that the body size distributions of natural populations change dramatically (Emlen, unpubl. data). Seasonal changes in the diet of the howler monkeys appear to produce large scale fluctuations in the body sizes of newly emerging adults in *O. acuminatus* populations. These change the distribution of body sizes in populations over time, and probably alter the optimal body size for switching between guarding and sneaking behaviors.

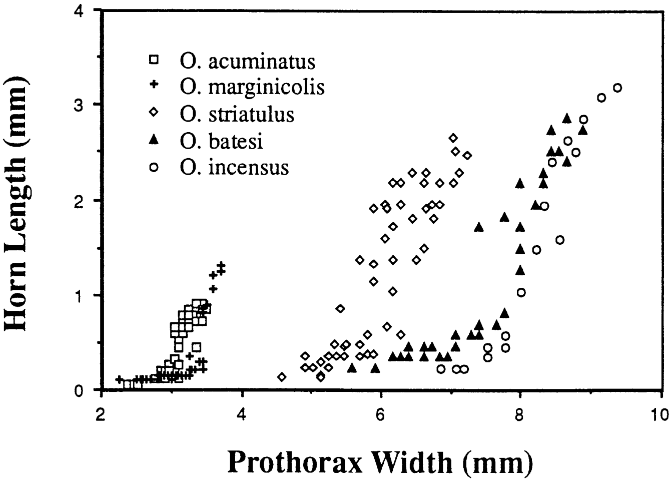

FIG. 7. Static allometric relationships between horn length and body size for five species of *Onthophagus,* indicating that evolutionary changes in body size appear to have been accompanied by lateral shifts in the position of the allometry. Measurements were taken from the Cornell University and Texas A&M University collections, as well as from the private collection of Dr. Henry Howden.

Here I have demonstrated that horn morphology in *O. acuminatus* populations can respond to these changes in selection. Although my artificial selection on residual horn length differed from the disruptive selection posited above, the responses to selection were identical: artificial selection changed the critical body size separating horned from hornless adult morphologies by shifting the position of the allometry along the body size axis. In lines where males were selected for relatively long horns, males began horn production at a smaller body size (3.35 mm prothorax width) than similar males from lines selected for relatively short horns (3.47 mm prothorax width; Fig. 6; Table 2). Consequently, I suggest that natural populations will experience short term changes in selection on horn morphology due to changes in the body size distribution, and that evolutionary responses to this selection will be brought about through shifts in the critical body size associated with the onset of horn growth. Furthermore, I suggest that for Panamanian populations of *O. acuminatus,* seasonal fluctuations in the body size distribution, and hence in the optimal relationship between horn length and body size, may contribute to the maintenance of selectable quantities of heritable variation for the horn length-body size allometry.

Horn Evolution in Onthophagus

Short-term changes in allometry brought about by artificial selection in *O. acuminatus* resemble macroevolutionary changes that occurred during onthophagine speciation. Several related species of *Onthophagus* differ primarily in body size, yet male horn lengths in these species do not fall along extensions of the same allometric relationship as *O. acuminatus.* Rather, each species exhibits its own sigmoidal allometry, but shifted with respect to body size (Fig. 7). In fact, the differences in allometry existing between species appear exactly the same as those generated between Up and Down lines in this study: horn allometries retained their sigmoidal shape, but shifted laterally along the body size axis, with some changes to both the height of the curve and the asymptote as well. This suggests that macroevolution of horns and horn allometry may have occurred through processes similar to those observed for microevolution in natural populations of *O. acuminatus.*

If we assume that ancestral species were behaviorally similar to *O. acuminatus,* we can speculate why these shifts in allometry might have occurred. Sexual selection on horn allometry results from size-dependent variation in male behavior (see above). Males employ two discrete behaviors to encounter females, and these select for different male shapes. Because one of these behaviors, sneaking, is parasitic on the other, the fitness payoffs for sneaking should depend on the frequency of sneaking males. In particular, sneaking should be more profitable when sneaking males are rare. Such negative frequency-dependence will help to maintain both behaviors within *Onthophagus* populations, and as long as both behaviors persist, selection will favor dimorphism in male horn morphology.

Horn length dimorphism occurs whenever the sigmoidal allometry relating horn length to body size inflects (i.e., begins horn production) at a body size within the range of body sizes present in the population, because some males fall on either side of the inflection separating horned from hornless adult shapes. Historical changes in the body size distribution of ancestral *Onthophagus* populations could have selected for shifts in the precise relationship between horn length and body size in the same way that seasonal fluctuations appear to select for shifts in the allometry of *O. acuminatus.* Heritable variation for the allometry, as demonstrated in this species, would have allowed the sigmoidal horn length-body size relationship to track macroevolutionary changes in body size, and could have led to the patterns of morphological variation visible today.

Nonlinear Allometries and the Evolution of Costly Secondary Sexual Traits

The results of this study indicate that some allometric relationships are metric, genetically based traits capable of quantitative evolutionary responses to selection (see also Weber 1990; Wilkinson 1993). However, the horn length-body size allometry examined here did not evolve equally in all directions: while allometry position changed rapidly in response to selection, allometry shape did not. It is possible that this reflects the nature of my selection regime. But it may also reflect underlying patterns of genetic variation. Examination of five related species of *Onthophagus* again suggests that allometry position is much more evolutionarily labile than aspects of allometry shape (see above; Fig. 7). If this proves to be a general feature of allometric relationships—that allometry shape is largely invariant—then this may afford fresh insight into questions concerning how and when costly secondary sexual ornaments evolve.

Linear and sigmoidal allometry shapes differ substantially in their patterns of allocation to ornaments. Genotypes specifying linear allometries express ornaments in males of all sizes, producing large numbers of males with intermediate shapes. In contrast, genotypes with sigmoidal allometries go from minimal to complete ornament expression over a narrow range of body sizes. These genotypes express ornaments only in large males, and produce fewer intermediate shapes.

Why should this difference matter, if both linear and sigmoidal allometries can generate exaggerated ornaments in large males? If an allometry represents the pattern of ornament expression for a single genotype across a range of body sizes (i.e., the horn morphologies produced by a genotype expressed in individuals with different body sizes), then it follows that the fitness of that genotype will be the average of the fitnesses of all individuals bearing the genotype. When genotypes differ in their patterns of ornament expression, selection should favor those genotypes with the greatest average fitnesses. Consequently, even if a genotype produces spectacularly ornamented (and therefore successful) males at large body sizes, the ornament will not spread in a population unless the average fitness of all males with that genotype exceeds the average fitnesses of other genotypes that do not produce the ornament. Because only a fraction of the males of any given genotype are likely to grow large enough to show full ornament expression, it is important to consider the morphologies and fitnesses of males of other size classes to fully appreciate the selective conditions favoring the evolution of an exaggerated male ornament.

Sexually selected populations are often characterized by high variance in male reproductive success: the few males with the largest ornaments or weapons generally acquire most of the available matings. In this situation, males with intermediate morphologies bear the burden of producing and wielding ornaments, but receive little, if any, of the reproductive payoffs. Because sigmoidal allometries produce almost no intermediate morphologies, the cost of expressing secondary sexual ornaments may be dramatically reduced. This would extend the range of selective circumstances favoring ornament evolution, and may increase the likelihood of their occurrence.

If the shapes of allometric relationships are relatively difficult to change under selection, as suggested by the results of this study (where the sigmoidal shape was retained despite changes in allometry position), then rare events altering allometry shape may have profound influences on the directions of subsequent morphological radiation. Specifically, lineages capable of generating sigmoidal growth patterns may be more likely to evolve costly male secondary sexual ornaments than similar lineages lacking this developmental potential. It is tempting to speculate that one or a few such historical events led to the evolution of the developmental capacity for sigmoidal allometric growth in beetles. This capacity, once established, may have facilitated the evolution of male horns, and could help to explain why beetles show such an extraordinary abundance and diversity of horn types, while similar appendages in other arthropods are generally rare or lacking.

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