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# Mating tactics determine patterns of condition dependence in a dimorphic horned beetle

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The persistence of genetic variability in performance traits such as strength is surprising given the directional selection that such traits experience, which should cause the fixation of the best genetic variants. One possible explanation is ‘genetic capture’ which is usually considered as a candidate mechanism for the maintenance of high genetic variability in sexual signalling traits. This states that if a trait is ‘condition dependent’, with expression being strongly influenced by the bearer’s overall viability, then genetic variability can be maintained via mutation-selection balance. Using a species of dimorphic beetle with males that gain matings either by fighting or by ‘sneaking’, we tested the prediction of strong condition dependence for strength, walking speed and testes mass. Strength was strongly condition dependent only in those beetles that fight for access to females. Walking speed, with less of an obvious selective advantage, showed no condition dependence, and testes mass was more condition dependent in sneaks, which engage in higher levels of sperm competition. Within a species, therefore, condition dependent expression varies between morphs, and corresponds to the specific selection pressures experienced by that morph. These results support genetic capture as a general explanation for the maintenance of genetic variability in traits under directional selection.

**Keywords:** performance; condition dependence; strength; sperm competition; mating tactics; horned beetle

## 1. INTRODUCTION

Performance traits such as strength, endurance and speed (Lailvaux & Irschick 2006) are astonishingly variable: we are all familiar with this variability in human athletic performances, but fewer people are aware that the same pattern is found across the animal kingdom. A study of 447 common lizards (*Lacerta vivipara*), for example, found that time to exhaustion for animals on a running track ranged from 36 to 1677 s: a 45-fold range (Le Galliard *et al.* 2004). Some of this variability arises from environmental factors, but there is also a substantial contribution from underlying genetic variance (Bennett & Huey 1990; Garland *et al.* 1990; Dingle 1991; Gu & Barker 1995; Schumacher *et al.* 1997; Rupert 2003; Le Galliard *et al.* 2004; Ketola & Kotiaho 2009). In many cases, this genetic variability persists despite directional selection: Irschick *et al.* (2008) reviewed 23 studies that have measured selection on performance traits and found that 12 of them reported significant directional selection. Directional selection on performance is likely to be especially important in those species where males engage in contests for access to mates. In taxa as diverse as lizards and shore crabs, high values of performance traits are either directly associated with contest victory

or correlated with high levels of expression of signals that are used during contest resolution (Lailvaux & Irschick 2006): being directly associated with mating success, these performance traits must be under strong directional selection, yet they remain highly variable.

A possible explanation for the persistence of genetic variation in performance traits is the ‘genetic capture’ hypothesis (Rowe & Houle 1996; Tomkins *et al.* 2004). Under this model, trait expression is determined by the overall viability and health of an animal, which is itself determined not only by the environment experienced by an organism but also, and crucially, by the effects of a large number of different genes. Genetic variation can therefore be maintained by mutation-selection balance because the large number of genes that influence condition present a large mutational target (Rowe & Houle 1996; David *et al.* 2000; Cotton *et al.* 2004a; Tomkins *et al.* 2004; Birkhead *et al.* 2006). This model can apply to the maintenance of genetic variability in all traits under directional selection (Rowe & Houle 1996; Houle 1998), but until now its use has been mostly restricted to explaining the maintenance of genetic variation in sexual signalling traits (Rowe & Houle 1996; Cotton *et al.* 2004a; Tomkins *et al.* 2004; Birkhead *et al.* 2006; see also Van Homrigh *et al.* (2007) for a study challenging this model). Performance traits are thought to be highly polygenic (Williams & Folland 2008) and so are good candidates for genetic capture effects.

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An important prediction of genic capture is that performance traits should show strong ‘condition dependence’, where ‘condition’ refers generally to an animal’s viability, or more narrowly to ‘the amount of resources available for allocation to fitness-enhancing traits’ (Rowe & Houle 1996). Strong condition dependence is most easily tested for by experimentally reducing resource availability, and comparing the response of the trait in question with one or more other traits that are not believed to be under directional selection (Cotton *et al.* 2004a). Here, we examine patterns of condition dependence in two performance traits; strength and walking speed, and also in testes mass using a model species where the males are dimorphic and pursue different mating tactics. These different mating tactics lead to different forms of selection on two of our three traits in the different morphs, allowing us to make comparisons of trait expression within the one sex.

Our model species is a horned dung beetle, *Onthophagus taurus*. Long-horned ‘major’ males of this species guard females within tunnels beneath dung pats, fighting with intruding rivals and mating repeatedly with the females they guard. Short-horned ‘minor’ males gain matings by adopting ‘sneak’ tactics such as entering tunnels when the major male is accompanying the female to collect dung, and then mating with the female on her return, or entering a tunnel via a side tunnel and bypassing a guarding male, thereby gaining access to the female (Moczek & Emlen 2000). These different behaviours generate different selective pressures for the two morphs. Major males are likely to experience selection for strength because they fight by physically pushing rivals (Moczek & Emlen 2000; see Lailvaux *et al.* (2005) for a study of performance and horn length in a different species of horned beetle). In minor males, on the other hand, strength is unlikely to be under directional selection, and these animals are expected to gain fitness from investing in traits such as testes mass instead: each minor male is likely only to mate once with a given female, who will also be repeatedly mated by a guarding male or males, so minor males are likely to experience stronger sperm competition (Simmons *et al.* 1999, 2007; Simmons & García-González 2008). Two of the three traits we studied, strength and testes mass, are therefore believed to be under strong morph-dependent directional selection in males of this species. Walking speed has less of an obvious selective advantage, although manoeuvrability in tunnels could be associated with mating success in both morphs, and minor morphs have been reported to have higher manoeuvrability in both *O. taurus* (Moczek & Emlen 2000) and *Onthophagus nigriventris* (Madewell & Moczek 2006).

## 2. MATERIAL AND METHODS

Beetles were collected from a dairy farm adjacent to the Mundijong Road approximately 25 km southwest of Perth, Western Australia, and kept in a controlled temperature room at 28°C with excess food for between 10 and 25 days. Individual male beetles were then allocated at random to one of three food treatments: no food, poor food or good food. Beetles were kept individually in 8 × 15 cm plastic containers with approximately 1 cm of damp sand. Those allocated to the ‘good food’ treatment were provided with a

generous teaspoonful of cow dung (approx. 10 ml). Beetles allocated to the ‘poor food’ treatment were given a similar quantity of dung that had been processed by rinsing it under a tap to remove most of the liquid component of the dung: this is the component that adult dung beetles feed on (Miller 1961), so the procedure should reduce the quality of the food. Beetles allocated to the ‘no food’ treatment were given a ball of wet cotton wool of roughly the same size as the amount of dung given to the other beetles. After 5 days, the beetles were weighed and their pronotum width (a standard measure of body size for these animals) measured with digital callipers. The experiment was carried out in five temporal blocks with either six or eight beetles per treatment per block, with the exception of the walking speed measurements that, for logistical reasons, were only performed with beetles from the first four blocks. Both walking speed and strength were measured in a second controlled temperature room, also at 28°C.

### (a) Walking speed

Each beetle was placed under an inverted 7 cm diameter Petri dish lid, on top of a sheet of clean white paper, and after a settling-down period of 1 min the animal’s movements were recorded at a rate of 12 frames per second for 10 min using a Logitech Quickcam S5500 webcam attached to an Apple Macbook Pro. All recordings were made between 10.00 and 12.00 to minimize any effects from diel behaviour. After recording, each movie was imported into the image analysis package IMAGEJ (Abramoff *et al.* 2004) using the Quicktime Movie Player plugin (<http://rsb.info.nih.gov/ij/plugins/qt-capture.html>), converted to greyscale and then a threshold transformation applied using parameter values that left the beetle as black while the background was completely converted to white. This allowed the use of the Multitracker plugin (<http://rsbweb.nih.gov/ij/plugins/multitracker.html>) to calculate the centroid position of the beetle for each frame of the movie. Distance moved per frame was then calculated as the distance between the animal’s position in two adjacent frames. Because preliminary studies found that even with constant lighting the calculated position for a stationary beetle could vary by up to 0.2 mm, any frame with movement of less than 0.25 mm was classed as stationary and discarded from the analysis, and median walking speed was calculated from the remaining data. Median speed was used in the analysis to minimize any influence from the rare occasions when the beetles attempted to fly.

### (b) Strength and testes weight

Following the video recording, each beetle had a length of cotton thread attached to the rear of its elytra using cyanoacrylate adhesive (‘Tarzan’s Grip’ brand) and maximum strength was measured using the method described in Lailvaux *et al.* (2005) as follows. The beetle was allowed to enter an artificial tunnel formed of two glass plates clamped together around two glass spacers, giving a tunnel 4 mm high and 7 mm wide. Two sheets of fine sandpaper (300 grade wet-and-dry paper) were placed between the sheets of glass and the spacers to give a rough surface for the beetles to grip. The glass sheets were supported such that the tunnel was inclined at an angle of approximately 60° from the horizontal.

Once the beetle had fully entered the tunnel, the cotton thread was pulled gently. This stimulates the beetle to brace its legs against the tunnel in the manner described for fighting beetles (Moczek & Emlen 2000). The thread

was then passed over a pulley and a plastic pot hung from the end with a hook. Water was dripped into the pot at a roughly constant rate until the beetle was unable to support its weight and was pulled out of the tunnel, and the pot plus water and hook was then weighed. Weights were converted into Newtons to give a measure of the force required to pull the beetle from the tunnel. Each beetle underwent three trials separated by at least 10 min, during which time the animal was kept in an  $8 \times 8 \times 8$  cm plastic box containing a piece of damp cotton wool. The highest measurement of pulling force was used as a measure of maximum strength (Losos *et al.* 2002; Lailvaux *et al.* 2005; although the results are qualitatively the same if the median measurement is used instead).

Following these procedures, the beetles were killed by freezing. Their heads were removed and photographed with a digital SLR camera fitted with a macro lens and the images were imported into IMAGEJ. Measure 4 from Tomkins *et al.* (2006) was used to give an estimate of horn length. The testes were then dissected out and weighed on a Mettler AG245 balance.

### (c) Data analysis

Allocation to morph was done by fitting a switchpoint regression to the log of horn length plus one, and log pronotum width (Knell 2009) using the 'segmented' library for R (Muggeo 2003). This gave a switchpoint at a log pronotum width of 1.57 (s.e. 0.007). All beetles with a pronotum width less than or equal to this value were classified as minors, the remainder being classed as majors.

Weight, walking speed, maximum strength and testes mass data were analysed by fitting general linear models with the measurement in question as the response variable and block, pronotum width, horn length, morph and treatment as explanatory variables, plus all second-order interactions. Model simplification was done by the removal of non-significant terms identified by deletion tests (Crawley 2002; Zuur *et al.* 2009) to give a final minimal adequate model. Model goodness of fit was assessed using the usual diagnostics, and in two cases (walking speed and testes mass), this led to the model being refitted using a log-transformed response variable in order to control for heteroscedasticity.

## 3. RESULTS

### (a) Beetle weight

Pronotum width (a measure of body size) was an important predictor of weight ( $F_{1,85} = 384$ ,  $p < 0.0001$ ) as was block ( $F_{4,85} = 4.48$ ,  $p = 0.002$ ) and horn length, with longer horned beetles weighing more ( $F_{1,85} = 29.68$ ,  $p < 0.0001$ ). Morph did not remain in the model as a significant predictor of weight, nor was it retained in the models fitted to walking speed, strength and testes mass. There was a significant effect of treatment ( $F_{2,85} = 18.59$ ,  $p < 0.0001$ ), with animals fed the good diet being the heaviest, those given the poor food having intermediate weights and those given no food weighing the least. The effects of the good food and the poor food are significantly different: a model with both 'food' treatments combined into one gives a significantly worse fit than the original model ( $F_{85,86} = 7.91$ ,  $p = 0.006$ ). The final minimal model with coefficients is given in the electronic supplementary material, table S1.

### (b) Walking speed

Model simplification left a minimal adequate model (electronic supplementary material, table S2) with a near-significant negative effect of pronotum width ( $F_{1,64} = 3.55$ ,  $p = 0.0641$ ), a significant positive effect of horn length ( $F_{1,64} = 6.52$ ,  $p = 0.0131$ ) and a significant block by treatment interaction ( $F_{6,64} = 6.03$ ,  $p = 0.0005$ ). The treatment by block interaction arose because in blocks 1 and 2 the beetles given no food had the highest median walking speeds, in block 3 the beetles given good food were the fastest and in block 4 the beetles given poor food were the fastest; thus walking speed showed no consistent condition dependence: a qualitatively similar result was found if the mean walking speed, rather than the median, was used as a response variable.

### (c) Maximum strength

Removal of non-significant terms resulted in a model with significant main effects of block and pronotum width, plus a horn length by treatment interaction. Further model simplification was achieved by replacing the treatment levels for good food and poor food with a single combined factor (Crawley 2002; Zuur *et al.* 2009): this did not result in a significant decrease in explanatory power ( $F_{83,85} = 0.19$ ,  $p = 0.828$ ), indicating that there is no difference between the effects of the two food treatments. Finally, examination of the relationship between horn length and maximum strength led to a model being fitted with horn length squared rather than horn length as an explanatory variable, which gave a better fit than the previous one: because both models have the same number of explanatory variables, they cannot be compared with an  $F$ -test, but the Akaike Information Criteria values differ by more than 2 ( $-166$  versus  $-161$  for the model using horn length squared and the model without, respectively). The final minimal model therefore has a significant main effect of block ( $F_{4,85} = 4.34$ ,  $p = 0.003$ ) and pronotum width ( $F_{1,85} = 57.34$ ,  $p < 0.0001$ ), and a significant interaction between horn length squared and treatment ( $F_{1,85} = 32.18$ ,  $p < 0.0001$ ). Full details are in the electronic supplementary material, table S3. Strength, therefore, was strongly influenced by food availability, but only in those males that fight for access to females (figure 1): the males with the longest horns showed a substantial increase in strength when given either class of food, but the males with the shortest horns showed no increase.

### (d) Testes mass

Model simplification led to a model with a significant main effect of pronotum width ( $F_{1,90} = 4.60$ ,  $p = 0.035$ ), plus a significant treatment by horn length interaction. As was the case with the maximum strength data, replacing the factor levels for good food and poor food with a combined food level did not result in a significant reduction in explanatory power ( $F_{90,88} = 1.50$ ,  $p = 0.229$ ), giving the final minimal adequate model summarized in the electronic supplementary material, table S4. Testes mass, therefore, followed the opposite pattern to maximum strength: in animals given no food, testes mass was not related to horn length, but in both fed treatments, testes mass declined in a similar way with horn length (figure 2); after controlling for body size, the heaviest testes were found in the beetles with the shortest

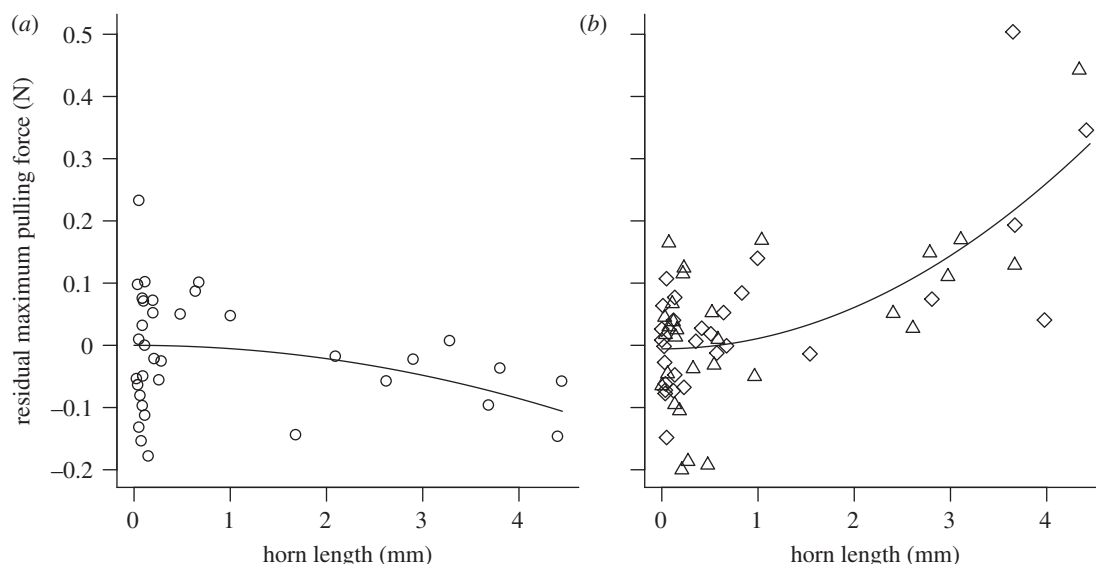


Figure 1. Strength is strongly condition dependent, but only in beetles with large horns that use a mate-guarding tactic. (a) Residual maximum pulling force (strength) following removal of the effects of body size (pronotum width) and block plotted against horn length for male beetles given no food for 5 days. The line shows the relationship between horn length and strength arising from the significant interaction term from the fitted model described in the electronic supplementary material. (b) Residual strength plotted against horn length for beetles given food for the 5 day period. Diamonds represent animals given the poor food treatment, triangles those given the good food treatment. The fitted line shows the relationship between horn length and strength from a model with both treatments pooled because there was no difference between the effects of the two food treatments on strength (see the electronic supplementary material). While the relationship between horn length and strength shows a slight decline (not statistically distinguishable from a slope of zero) for the unfed beetles, beetles that had access to food show a strong positive relationship between horn length and strength: sneak strategists with small horns do not increase their strength when fed, but there is a dramatic increase in strength when the beetles that fight for access to females, the ‘guard’ strategists with long horns, are fed.

horns, while those with the longest horns did not seem to have testes any larger than beetles that were kept without food.

#### 4. DISCUSSION

In total, we have estimates of condition dependence for three performance traits in this species. Walking speed is unlikely to be under strong directional selection and shows no condition dependence. Strength, a trait that major males use in contests over females but which is unlikely to confer a mating advantage on minor males, is strongly condition dependent in major males, but not in minors, with the degree of condition dependence itself depending on horn length. Condition dependence in courtship rate was measured in a previous study (Kotiaho *et al.* 2001). Males of this species court females by drumming on their elytra and abdomen with their head and forelegs (Kotiaho *et al.* 2001), so as a trait that involves dynamic muscular movements (Lailvaux & Irschick 2006), it can also be regarded as a performance trait. Kotiaho *et al.* found courtship rate to be correlated with mating success in both male morphs in this species (and therefore under directional selection in all males) and to show condition dependence independent of morph. These patterns correspond to what would be predicted from the simple model outlined in Rowe & Houle (1996), where traits under stabilizing selection are affected little by condition, but strong directional selection causing costly expression of an extreme value of a trait leads to condition dependence. This supports a genic capture model for the maintenance of genetic

variation in performance traits under persistent directional selection.

Like maximum strength, testes mass also shows morph-dependent condition dependence. In the case of strength, the nonlinear change in condition dependence with horn length could well reflect a substantial selection differential between the morphs, given that the sneak mating tactics employed by the minor beetles are unlikely to require strength for mating success, whereas the outcome of contests between major males is probably strongly influenced by this trait. In the case of testes mass, while selection will be stronger in minor beetles, there will still be some selection arising from sperm competition even in the largest majors, and in this case condition dependence changes linearly with horn length.

The relationship between the different tactics used by the two morphs and the degree of condition dependence, suggests that in addition to behavioural differences, there must be other differences between the morphs that are closely tied to their different approaches to mating. An obvious avenue for future research is a comparison of the anatomy, physiology and gene expression patterns for the testes and musculature of these two morphs: such work might well lead to important improvements in our understanding of the biological mechanisms by which condition dependence arises. While it has been suggested that the difference between morphs of these beetles is a consequence of a ‘developmental threshold’ occurring at a certain body size (Emlen & Nijhout 1999), some recent publications have claimed that the observed pattern arises from rapidly changing but continuous variation (Tomkins *et al.* 2005, 2006).

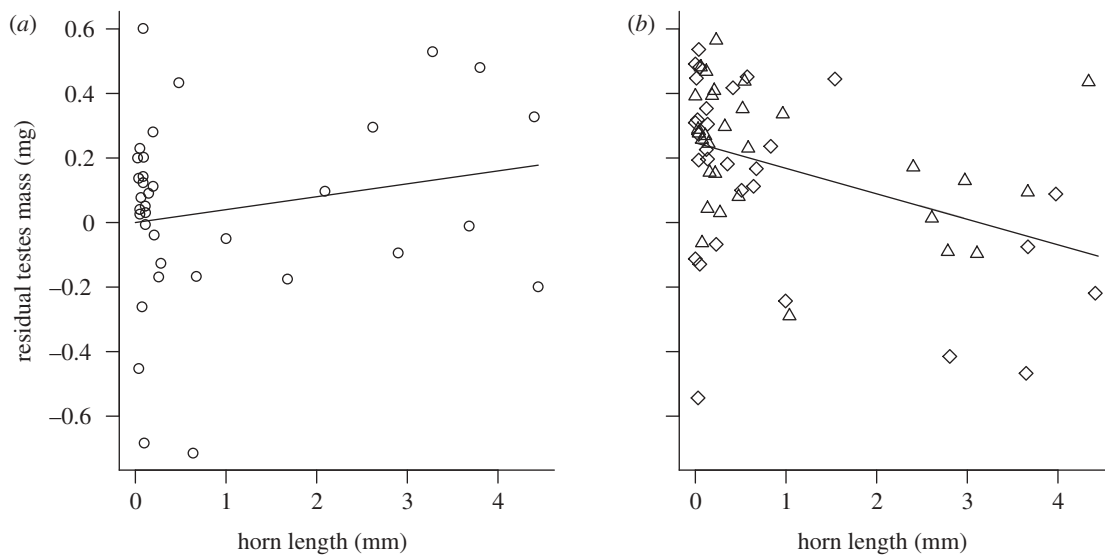


Figure 2. Testes mass is condition dependent, but more so in beetles with small horns that use sneak tactics. (a) Residual log testes mass following removal of the effects of body size (pronotum width) plotted against horn length for male beetles given no food for 5 days. The line shows the relationship between horn length and log testes mass arising from the significant interaction term from the fitted model described in the electronic supplementary material. (b) Residual log testes mass plotted against horn length for beetles given food for the 5 day period. Diamonds represent animals given the poor food treatment, triangles those given the good food treatment. The fitted line shows the relationship between horn length and log testes mass as for (a): the model was fitted with a pooled term for both food treatments because there was no statistically discernible difference between the two for this dataset. While the relationship between horn length and log testes mass shows a slight increase (not statistically distinguishable from a slope of zero) for the unfed beetles, beetles that had access to food show a strong negative relationship between horn length and testes mass. This is largely accounted for by an increase in testes mass in those beetles with the smallest horns: these are the minor morphs that follow a sneak strategy to acquire matings and do not enter into combat, and which are expected to be exposed to the highest levels of sperm competition.

A common theme to the results of the analyses carried out here is the lack of any significant predictive power from the 'morph' factor: in all of the models, fitted horn length, rather than morph, remains in the final minimal model. Horn length is a continuous variable with minor males at one end of the continuum, whereas morph is a dichotomous variable that does not distinguish between the longest horned (and presumably highest quality) majors and the other major males. The results presented here therefore all follow a pattern of continuous variation rather than sudden thresholds, implying that the differences between the morphs become evident gradually over a range of body sizes rather than suddenly at a set threshold. Further evidence of changes associated with longer horns comes from the analysis of weight, where it was found that horn length was a significant predictor of weight when body size was controlled for, with an increase in horn length of 1 mm leading, on average, to an increase in weight of 2.7 mg. This is unlikely to be simply a result of the added weight of the horns, because even a long set of horns only weighs around 0.5 mg (J. Tomkins 2010, personal communication).

Beetles that were given the poor food treatment were lighter than those given good food, suggesting that they had a poorer condition, yet the effect of this treatment on strength and testes mass was identical to the effect of the good food treatment, and condition dependence was only detectable in those animals that had been given no food at all. This implies that these traits are to some extent 'buffered' against nutritional stress, with beetles that have lost some weight because of a poor diet still being able to maintain high expression of strength or testes mass. A similar finding was reported from a study

of condition dependence of eyespan in Diopsid flies (Cotton *et al.* 2004b). It may be that those animals given the poor food are able to use stored resources such as their fat body to maintain their high expression of these traits, and that the beetles given no food were unable to do this.

Walking speed was the only response variable to show a significant block by treatment interaction effect. This is hard to explain with current data, but given that the animals in the later blocks were older than those in the earlier blocks, it is possible that this is an effect caused by younger animals responding differently to older animals. With regards to the other predictors of walking speed, it is noteworthy that horn length is more important than body size or any other variable tested here. Previous studies have found that longer horned male beetles 'run' more slowly in artificial tunnels (Moczek & Emlen 2000; Madewell & Moczek 2006), although not in *Euoniticellus intermedius* (Pomfret & Knell 2006). Our study was specifically designed to allow males to walk freely without any impediment from being in a confined tunnel, and it is interesting that under these conditions horn length predicts walking speed regardless of condition. It is possible that longer horned males make a greater effort when walking to partially counter the impediment they experience in tunnels: alternatively, they might walk faster because of physiological, anatomical or motivational differences arising for other reasons. To avoid confusion, we note that these animals do not seem to have an ability to run in a manner that is distinct from their ability to walk (R. Knell 2009, personal observations), and previous studies that have reported 'running speed' do not describe any attempt to motivate these

animals to move quickly (Moczek & Emlen 2000; Madewell & Moczek 2006), suggesting that our measures are comparable with those previously reported.

The result for maximum strength contrasts with that for walking speed: this trait is not believed to be under strong selection, and we find little evidence to suggest that condition dependence is operating here. This raises the possibility that different mechanisms are maintaining genetic variance in different performance traits in the same animals, with weak or fluctuating selection acting on walking speed in the same way as has been suggested for endurance in lizards (Le Galliard *et al.* 2004). Alternatively, it should be noted here that while walking speed is certainly a performance trait in that it involves dynamic movements, the extent to which animals are maximizing their effort (Irschick *et al.* 2008) is unclear for this trait. Walking speed is therefore possibly less likely than some other measures to expose links between performance and physiology or morphology (Irschick *et al.* 2008) and may be less sensitive to condition for this reason.

These patterns of condition dependence and selection also shed some light on how condition dependence should change as a trait becomes more exaggerated or as investment in it increases. A recent study (Johnstone *et al.* 2009) has questioned the assumption that increasing trait exaggeration should inevitably lead to increased condition dependence, and the authors suggest that in fact, enhanced expression of a trait could lead to no increase, or even a reduction in condition dependence. As Johnstone *et al.* discuss in their paper, it is hard to assess how likely this is because of a lack of empirical evidence regarding condition dependence in the same trait at different levels of exaggeration. The present examples provide two clear cases where increased expression of a trait is clearly associated with an increase in condition dependence.

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