Trade-off between horns and other functional traits in two Onthophagus species (Scarabaeidae, Coleoptera)

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Trade-off between horns and other functional traits in two horned beetle species (*Onthophagus*: Coleoptera, Scarabaeidae)

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Abstract

Beetle horns are extraordinarily diversified secondary sexual structures used for mate choice and male-male combat. Due to an interaction of nutritional, hormonal and genetic factors, their polyphenic development is metabolically expensive, and occurs in the virtually closed system of the pre-pupal stage, after the developing larva has stopped feeding. Previous studies showed the occurrence of resource competition resulting in a trade-offs between horns and other morphological structures. These studies also revealed functional associations between autoecology and horns, as a function of their physical location (i.e. head versus pronotum), and suggested that constraints imposed by trade-offs on adult morphology may have profound evolutionary consequences, such as ecological and reproductive isolation. In this study, we compared trade-off patterns between horns and other functional traits (eyes, antennae, legs, head, epipharynges and genitalia) in two congeneric species bearing horns located in the same anatomical area, but with different morphologies. Specifically, we considered *Onthophagus taurus* (Schreber, 1759)*, characterised by a pair of long, lunated cephalic horns, and *O. fracticornis* (Preyssler, 1790), expressing a single cephalic horn. We demonstrated that, even when horns are located in the same physical position on the insect's body, differences in horn morphology can bring about differences in how functional traits respond to horn investment. These differences are interpretable in light of the hierarchy of functions carried out by these structures and their component parts in each species.

Keywords: autoecology; genitalia; phenotypic plasticity; male polyphenism.

Introduction

The horns of scarab beetles (Coleoptera: Scarabaeidae) are secondary sexual traits used by males during mate choice and rival assessment. Within the *Onthophagus*, one of the most representative and studied group, the ~2000 species described so far exhibit hundreds of different types of horns, which have basically the same function despite their morphological diversity (Balthasar 1963; Matthews 1972; Howden and Young 1981; Emlen 2000).

Horn production primarily depends on the amount of larval food available (Emlen and Nijhout 1999, 2001), and is the result of irreducible interactions between genetic, developmental and environmental factors (Moczek 2010; 2011): only male exceeding a certain body size moult into major males bearing fully developed horns, while the others exhibit very reduced horns or remain hornless (minor males). Horn growth is metabolically expensive, and mainly occurs in the virtually closed system of the pre-pupal stage, after the developing larva has stopped feeding. The shared and limited pool of resources available at this stage can trigger competition between horns and other developing traits for nutrients, hormones, growth factors and morphogens (Nijhout and Wheeler 1996; Nijhout and Emlen 1998; Klingenberg and Nijhout 1998; Emlen 2001; Okada and Miyatake 2009). Nijhout and Emlen (1998) demonstrated that, in insects, some anatomical structures can grow to disproportionally lager sizes when the growth of other co-developing structures is experimentally inhibited. Studies on this subject have mainly reported trade-offs between horns and traits that grow in their physical proximity (Nijhout and Wheeler 1996; Nijhout and Emlen 1998; Klingenberg and Nijhout 1998; Emlen 2001). This fact might be explained considering that developing tissues primarily use local pools of resources to proliferate, and communicate through short range signals (e.g. paracrine morphogen) to regulated reciprocal growth (Gibson and Schubiger 2000). However, some chemical intermediaries that are known to regulate growth and size of insect appendages, such as hormones or insuline-like factors (Nijhout and Grunert 2002), can circulate freely in the emolymph (Nijhout 1994). Trade-offs can therefore be hypothesised to occur over longer distances, and more recent studies actually highlighted such an effect between horns and primary sexual traits (Moczek and Nijhout 2004; Simmons and Emlen 2006; Parzer and Moczek 2008). Furthermore, several studies (see Husak and Swallow 2011 for a review) showed that ornaments may negatively affect individual performance capacity, and there is some evidence from the dung beetles *Onthophagus taurus* that the increased moulting time required for the complete development of individuals with larger secondary sexual traits exposes them to a greater risk of predation (Hunt and Simmons 1997).

Developmental constraints that trade-offs impose on adult morphology are believed to have profound evolutionary consequences, i.e. the potential of promoting ecological and reproductive isolation, possibly leading to speciation as a by-product (Emlen 2001; Moczek and Nijhout 2004; Okada et al. 2007; Moczek 2009). Dung beetles dwell in different habitats and feed on a great variety of dung types, but all of them have to fulfil similar fundamental needs in every environment, such as finding food, detecting and flying to their feeding and nesting resources, locating potential partners. However, the relative effort that different species have to put to attain these goals may depend on their autoecology, and the variation of costs of horn development may interact with the ecology of beetles as a function of physical horn

location. For example, Emlen (2001) demonstrated that horn growth at the base of the head causes a reduction in eye size through a developmental trade-off mechanism. Such a horn type might therefore be more costly to produce in nocturnal species, that need to see in low-light conditions (McIntyre and Caveney 1998; Frederiksen and Warrant 2008), compared to diurnal ones. Nocturnal species were indeed significantly less likely to have horns at the base of the head (Emlen 2001), suggesting that the costs of horn production may actually be related to the species' ecology.

The study of Emlen (2001) focused on differences in physical horn location (head vs. pronotum), finding that horn growth reduces the size of the neighbouring morphological structures (animals with horns that extend from the base of the head have tradeoffs with eyes; animals with horns at the front of the head have tradeoffs with antennae, animals with horns on the pronotum have tradeoffs with wings) and that these trade-offs can therefore be linked to the species' ecology. In this paper, we used the same approach to investigate the consequences of different horn morphologies developing in the same anatomical area (the vertex carina of head) on other traits. We investigated trade-offs between horns and other functional traits (eyes, antennae, head, epipharynx, legs, and copulatory organ) and compared them between two congeneric species characterised by different types of horns extending from the basal part of the head (the vertex carina): *Onthophagus taurus,* bearing a pair of cephalic horns, and *O. fracticornis*, expressing a single cephalic horn – a horn morphology considered ancestral to the paired form (Emlen et al. 2005; Macagno et al. 2009). Aims of the study were 1) to determine whether small differences in horn morphology could still bring about detectable trade-offs, 2) to show a possible relationship between trade-offs and different aspects of species' autoecology, 3) to explore the consequences of investment in secondary sexual traits on primary sexual traits, and thus their possible effects on differentiation and reproductive isolation.

Materials and Methods

In this study, we analysed 104 male dung beetles: 18 major and 30 minor *O. taurus*, collected in N-W Italy (Pont Canavese, Piedmont), and 31 major and 25 minor *O. fracticornis*, sampled in N-W Italian Alps (Val Ferret, Aosta Valley). The two species share similar ecological features, both being diurnal (Psarev 2001; Emlen et al. 2005) and favouring open to closed habitats (Wassmer 1995; Macagno and Palestrini 2009). Both species are generalists with respect to feeding habits (Martín-Piera and Lopez-Colon 2000), although *O. fracticornis* has shown preference towards horse dung when allowed to choose (Dormont et. al. 2007). However, while *O. taurus* is generally widespread at middle and low altitudes, *O. fracticornis* is usually found in mountain habitats, and therefore has a more scattered distribution (Martin-Piera and Lopez-Colon 2000).

In the laboratory, individuals of both species were cleaned in 100°C distilled water for 10 min and then dissected. Pronota and heads were removed from the abdomen and separately fixed on horizontally-leveled plasticine supports by completely plunging their convex ventral sides. Epipharynges were extracted from the head and boiled for 5 min in 5% potassium hydroxide (KOH). After 5 min in distilled

water, they were dehydrated for 5 min in 70% and 5 min in 99% ethanol, and then cleared in BioClear (CIAB, Chemical Instruments AB, Lidingö, Sweden) for 3 min. Epipharynges were mounted in Canada Balsam on microscope slides and covered with coverslips. Balsam volume was standardized for all preparations. Antennae were removed from the heads and positioned horizontally on wet cotton wool. To extract the eyes, heads were placed in boiling 30% KOH for 10 min, until completely softened. Eyes were then collected, rinsed in distilled water and placed with a drop of water on microscope slides covered with coverslips. Copulatory organs (aedeagi) were extracted, cleared in boiling 5% KOH for 5 min and positioned on wet cotton wool, taking care to align their edges on the same horizontal plane. After being separated from the body, legs were likewise positioned on wet cotton wool.

Two-dimensional images of each structure were taken using a Leica Z16Apo stereoscopic dissecting scope (Leica Microsystems AG, Wetzlar, Germany). Pronota were photographed at 20x magnification, heads at 25x, horns at 31.4x, lower eyes at 62.5x, upper eyes, antennae, epipharynges and aedeagi at 57.5x, and legs at 39.4x. We measured the left side of symmetric structures (head, epipharynx and aedeagus phallobase) and the left part of paired structures (antenna, upper and lower eye and leg). Measurements were taken with the software LAS v 2.5.0 (Leica Application Suite), whereas landmark configurations were digitized with the software *TpsDig* 2.12 (Rohlf 2006). Fig. 1 and Fig. 2 show linear measurements, areas and landmark configurations chosen to estimate the size of each morphological structure. Centroid sizes (the squared root of the sum of squared distances of the set of landmarks from their centroid) were used as an estimate of the size of head, epipharynx, paramere (the distal portion of aedeagus) and leg (Alibert et al. 2001; Rosenberg 2001; Ubukata 2003). Centroid size values of each landmark configuration were calculated using the software *TpsRelw* 1.46 (Rohlf 2008).

To evaluate the effect of horn size on the other structures in analysis, we used two methods.
1) *Regression residuals approach* (Emlen 2001). In both species, pronotum width (Fig. 2) was taken as a proxy for body size to calculate the static allometries of horn, head, epipharynx, antenna, upper and lower eyes, aedeagus (separately for paramere and phallobase) and leg. After removing one conspicuous outlier (a male *O. taurus* with a large pronotum, but very small horns), the horn length/body size scaling relationships were determined by fitting to the log-transformed data the four-parameter sigmoidal regression

$$y = y_0 + \frac{ax^b}{c^b + x^b}$$

where x is pronotum width, y is horn length, y_0 specifies the minimum horn length, a defines the horn length range in the sample, b represents a slope coefficient, and c is body size at the inflection point of the sigmoidal curve (Eberhard and Gutierrez 1991; Emlen and Nijhout 2000; Moczek and Nijhout 2002; 2003). The scaling relationships between log-transformed pronotum width and log-transformed centroid size, length, width or area of the other traits were assessed with linear regression models ($y = y_0 + bx$) (Al-Wathiqui and Rodríguez 2011). When significant, negative angular coefficients of linear regressions between the residuals of static allometries of horn and other traits were regarded as evidence of a trade-off

between horns and each trait considered. Analyses were performed with SigmaPlot (Systat Software Inc., Richmond, CA).

2) *General Linear Model*. Considering some criticism towards methods based on regression residuals (Garcia-Berthou 2001; Freckleton 2002), using the same data we fitted a general linear model (GLM) for each trait with pronotum width, horn length and morph as explanatory variables, plus all second-order interactions (Knell and Simmons 2010). Given Knell's (2009) indications on the appropriateness of switchpoint models for the description of horn static allometries in *Onthophagus* beetles, both asymptotic majors (*sensu* Tomkins et al. 2005a) and individuals with intermediate-sized horns were classified as majors. Starting from the complete model, model simplification was done by gradually removing nonsignificant terms until a minimal adequate model was obtained (Zuur et al. 2007).

Results

Scaling relationships between horn length and pronotum width for *O. taurus* and *O. fracticornis* are shown in Fig. 2, and parameters of sigmoidal regressions for both species are reported in E.S.M.1. Parameters of static allometries of the head, epipharynx, antennal parts, upper and lower eye areas, paramere, phallobase and leg are shown in E.S.M. 2. In both species, genital parts showed negative allometries, angular coefficients (b) of their scaling relations being <0.50. In *O. taurus*, phallobase length was uncorrelated to body size. Most non-sexual traits showed either an isometric ($b = \sim 1.00$) or a slightly positive ($b = \sim 1.30$) or negative ($b = \sim 0.70$) allometric trend. An interesting exception to this rule was the upper eye area of *O. taurus*: a negative allometry with an angular coefficient (0.18) even lower than those shown by the analysis of genital parts.

Angular coefficients of regressions between residuals of static allometries of horns and the other traits considered are reported in Table 1. In Table 2 we report the general linear models that best represent the effect of explanatory variables on each trait considered. According to the regression residuals approach, the relative investment in head horn length was positively correlated with the relative investment in leg centroid size and length in both species, and with paramere centroid size in *O. fracticornis* (Table 1). The GLM approach also showed a positive effect of horn length on leg centroid size in *O. taurus*, and on leg length in both species (although in *O. taurus* the scaling relation between leg length and pronotum width became less steep the more horn length increased, and in *O. fracticornis* the relation between horn length and leg length was steeper in minor than in major males). In *O. fracticornis*, major males also showed larger paramere and leg centroid sizes compared to minor males (Table 2).

The regression residuals method detected patterns in keeping with a trade-off between horn length and antennomere 1 and horn length and phallobase length in *O. fracticornis*, and between horn length and lower eye and horn length and phallobase width in *O. taurus* (Table 1). Accordingly, general linear models confirmed a negative effect of horn length on the area of antennomere 1 in *O. fracticornis* and on phallobase width in *O. taurus*, and also showed that lower eye area of *O. taurus* and phallobase length of *O. fracticornis* increased with horn length in minor males, while they decreased in major males (Table 2). Further effects of horn length and/or morph on the traits considered were only detected by the GLM method, including: 1) Positive effects: major males showed larger structures than minor males (lower eye

area and epipharynx centroid size in *O. fracticornis*, head centroid size and phallobase length in *O. taurus*), and a positive effect of horn length on paramere centroid size in *O. taurus* (where, however, the scaling relation between paramere centroid size and pronotum width became less steep the more horn length increased); 2) Negative effects: in *O. fracticornis*, horn length had a negative effect on phallobase width and head centroid size (although here the effect was nearly not significant) and, furthermore, major males had a shorter scapus length than minor males; in *O. taurus*, major males had a smaller antennomere 3 compared to minor males, although here horn length had a positive effect on this trait (Table 2).

Discussion

Sensory organs

Our results showed a differential effect of horn investment on sensory organs in the two species. Both methods strongly supported a pattern in keeping with a trade-off between horn length and antennomere 1 in *O. fracticornis*, and GLM results hinted at the possibility that scapus length was also affected by a trade-off in this species. On the contrary, none of antennal parts showed strong evidence of trade-offs in *O. taurus,* where GLM suggested that horn length have a positive effect on this trait, although major males have smaller antennomere 3 compared to minor males. As in other insects, beetles' antennae are believed to be the main long-distance chemoreceptive organs. They are primarily used to perceive odours, in particular to locate feeding resources (Halffter & Matthews 1966), but they can also be used to detect a conspecific beyond the usual home range of the beetle (Halffter & Matthews 1966) and to stimulate females during courtship (Moczek 1999). Our results indicated that, in *O. fracticornis*, at least the larger antennomere undergoes a trade-off with horn growth. This result was somewhat unexpected, because a trade-off with antennal parts was expected only for those horns that extend from the front of the head (Emlen 2001) and not for horns located more distantly at the basis of the head. This observation supports the hypothesis that some chemical intermediary implicated in the regulation of growth and size of insect appendages can reach longer distances by circulating in the emolymph (Nijhout 1994; Nijhout and Grunert 2002; Moczek and Nijhout 2004), therefore producing a trade-off between traits that are not in close physical proximity. Furthermore, the negative effect of horn growth on the antennal parts used for long distance perception hints at the possibility that this function may be not so crucial and central for *O. fracticornis*.

Insects use eyes mainly to head toward food, suitable habitat and females, to regulate their flying speed or altitude, to avoid obstacles, and to control their landing on objects (Floreano and Zufferey 2010). In dung beetle species, compound eyes are divided into two parts by the canthus (Byrne and Dacke 2011). Scholtz et al (2009) suggested that the upper part of the eye deals with functions related to flying activity and long-distance perception, while the lower part of the eye is more related to short-range and more refined visual functions (food selection, relational activities). The patterns of trade-off between horn length and eye area were different in the two species: evidence of a negative effect between horn length

and lower eye area in *O. Taurus* was confirmed by both methods, while *O. fracticornis* did not show any evidence of trade-off (GLM even suggested a potential positive effect on lower eye in this species). This finding allowed us to extend what Emlen (2001) reported: our results indeed confirmed the existence of a trade-off between a horn localised at the basis of the head and visual organs, but also indicated that not all of the eye's parts are involved at the same level. Upper and lower eye, indicated as functionally different parts (Scholtz et al 2009), may therefore also behave as separated developmental modules. Furthermore, the differences in the functional costs of horn development may interact with the ecology of the species, as previously pointed out for antennae. The trade-off with lower eye, discovered for *O. taurus* only, hints at the possibility that this species can accommodate a lower investment in short distance vision as a consequence of a horn growing in close proximity; on the contrary, long distance visual perception may be a crucial, adaptive function. Taken together, the results on antennal parts in *O. fracticornis* and on lower eye in *O. taurus* concur in depicting two species with different ecological features, i.e. a lower level of long distance mobility in *O. fracticornis* with respect to *O. taurus*. This result matches with the habitat selection of *O. fracticornis*, which has been described as rather stenoecious, being restricted to pastures in mountain areas (Petrovitz 1956, Avila and Pascual 1988; Baum 1989; Gangloff 1991; Sowig 1995), and with recent genetic studies showing that different *O. fracticornis* populations share little genetic flow, which is indicative of a low dispersal capacity and of a certain degree of habitat specialisation (Pizzo et al 2011; Macagno et al 2011a). By contrast, genetic studies have detected consistent gene flow across populations of *O. taurus* (Pizzo et al. 2006a), as a result of a higher mobility and a better tolerance to environmental conditions.

Other external structures

Previous studies showed that head shape varies in relation to horn size (Pizzo et al. 2006b; Macagno et al. 2009), which was interpreted as a balancing static system compensating barycentre backing due to horn protrusion in major males. The observed shape modifications also confer a relatively large and solid appearance on major males, which is likely to be advantageous in aggressive encounters. Whereas the residuals method showed no significant effects of horn length on head size, GLM suggested a possible polarized situation characterised by a positive effect of horns length on head size in *O. taurus* and a negative effect on *O. fracticornis* (even if only just significant). These results seem to indicate that paired horns, which are also longer and more unbalancing, need more support and indeed a reinforcement of cephalic size.

Ornaments may negatively affect individual performance, and it is known that males with larger weapons also possess larger compensatory (supportive or functional) traits to mitigate this effect (Husak and Swallow 2011). Moczek and Emlen (2000) and Madwell and Moczek (2006) showed that *Onthophagus* males with long horns suffered a significant decrease in running speed and manoeuvrability within tunnels, likely due to the horns impeding the beetles' movement within the narrow space, rather than to any intrinsic physiological capabilities. The increased investment in leg size with horn investment we highlighted here is in agreement with previous results of Tomkins et al (2005b), and might therefore be

interpreted as a compensatory system to reduce this disadvantage. If the evolution of some kind of compensation has the ability to reduce the costs associated with ornament development, then sexual selection may allow further exaggeration of ornaments over evolutionary time (Husak and Swallow 2011). It is also know that males bearing longer horns undergo morphological changes that confer higher success in male-male competition (Otte and Stayman 1979; Okada and Miyatake 2004; Pizzo et al. 2006b; Okada et al. 2007; Okada e Miyatake 2009, Macagno et al. 2009). After initial contact, fighting *Onthophagus* males assume a typical position with the head and thorax held low, the abdomen held high, and the forelegs braced against the tunnel walls (Moczek and Emlen 2000). Lailvaoux et al. (2005) studied the relationship between male armaments and physical variables that are likely to influence directly the outcome of male contests. According to their results, in *Euoniticellus intermedius* (Reiche, 1849) horn length, a known predictor of victory in male fights (Pomfret and Knell 2006; Knell 2011), is strongly correlated with the force required to pull a beetle out of a hole, and with the distance that a beetle is able to walk before reaching exhaustion, which are both variables that can also be correlated with leg size. The positive correlation between leg size and horn length, found in both species, could therefore be attributed to males with longer horns investing more in legs because, contrary to minors, they fight by pushing other males, the strength coming from their legs. Forelegs are also used for courtship, as the male drums over the back and side of the female until she reaches the right position for mating (Moczek 1999). Forelegs, like horns, can therefore act as a secondary sexual trait, and consequently be sexually selected for an increased size in major males.

Primary sexual traits

Previous studies showed a trade-off between head horns and genitalia (Moczek and Nijhout 2004; Simmons et al. 2006; 2007; Parzer and Moczek 2008), and have been corroborated by a growing number of researches showing that genetic manipulations directed at horn development generally also affect genitalic growth (Moczek and Rose 2009; Snell-Rood and Moczek 2011; Wasik and Moczek 2011). Our data supported these results, but in this study we also found that the parts that make up primary sexual traits might not all be subject to trade-offs in the same manner. The relative investment into head horns was never negatively correlated with the relative investment into parameres; instead, it had a negative effect on the phallobase.

These differences were not detectable in previous studies based on morphology (e.g. Parzer and Moczek 2008), where the authors used the length of paramere and phallobase combined to estimate male copulatory organ size. Recent studies provided evidence for mosaic evolution of genitalia (Song and Wenzel 2004; House and Simmons 2005; Werner and Simmons 2008) and suggested that size and shape of different portions of the same copulatory structures respond to different selective pressures depending on their function during copulation. Size and shape of genital structures and their component parts may be sufficiently developmentally and genetically decoupled to evolve rather independently of each other. Therefore, it seems that local, segment-specific regulation of differential growth and differentiation must underlie genitalic development and evolution, rather that genitalia-specific developmental processes (Mc Peek et al, 2008, 2009; Macagno et al. 2011b). During copulation, male parameres interact closely with

specific regions of the inner side of female pygidia (pygidial flaps: Macagno et al. 2011b) in order to gain stability that subsequently allows sperm transfer (Werner and Simmons 2008). Although it is not clear whether the pygidial flap could act as a proper 'lock' able to exclude heterospecific matings, it appears likely that proper coupling of the two structures can make heterospecific matings less effective and therefore promote reproductive isolation. Such a mechanical constraint, along with sexual selection (House and Simmons 2003; Simmons et al 2009), could indeed limit the effect of trade-off on parameres; the phallobase, by contrast, might be freer to accommodate trade-offs due to horn growth because it is not constrained by the necessity of contacting the female during mating. Parzer and Moczek (2008) suggested that, when acting on the level of primary sexual traits, trade-offs might have the potential of promoting differentiation and reproductive isolation as a byproduct. To confirm this hypothesis, further studies should relate the effect of trade-offs on different parts of the genitalia with the functions that those parts hold during the copula.

Conclusions

On the basis of the results obtained here, we suggest that not only physical location, but also smaller differences in the morphology of horns located in the same anatomical areas can determine differences in functional traits affected by trade-offs with horn investment in different species. We have also demonstrated that traits that are not in strict physical proximity to the horn (e.g. the antenna) can be affected negatively by horn growth, and have shown that different parts of the same organs (antennae, eyes and genitalia) can be affected differently by a trade-off. We also suggest that differences in how horn investment affects functional traits may depend on the hierarchy of importance of the functions accomplished by these organs, or their component parts, in each species.

Acknowledgements

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Fig 1 Morphological traits analysed and measures taken. A: landmark configurations taken on the left side of the head and used to compute centroid sizes (CS). B: landmark configurations taken on the left side of the epipharynges and used to compute centroid sizes (CS). C: left side of aedeagus, showing landmark configurations used to compute the centroid size of left paramere (CS), length of left paramere (Lpar) and width of phallobases (Wpha). D: measures taken on the left antennae: scapus length (Ls), and area of the first (Aa1), second (Aa2) and third (Aa3) antennomere. E: area of lower (Ale) and upper (Aue) eye. F: left leg, showing linear lengths (Ll) and landmark configurations used to compute centroid sizes (CS).

Fig 2. Log- log horn length/pronotum width scaling relations of *O. taurus* and *O. fracticornis*. Pronotum width is used as a proxy for body size. The figure also shows linear measures taken on horns and pronota.
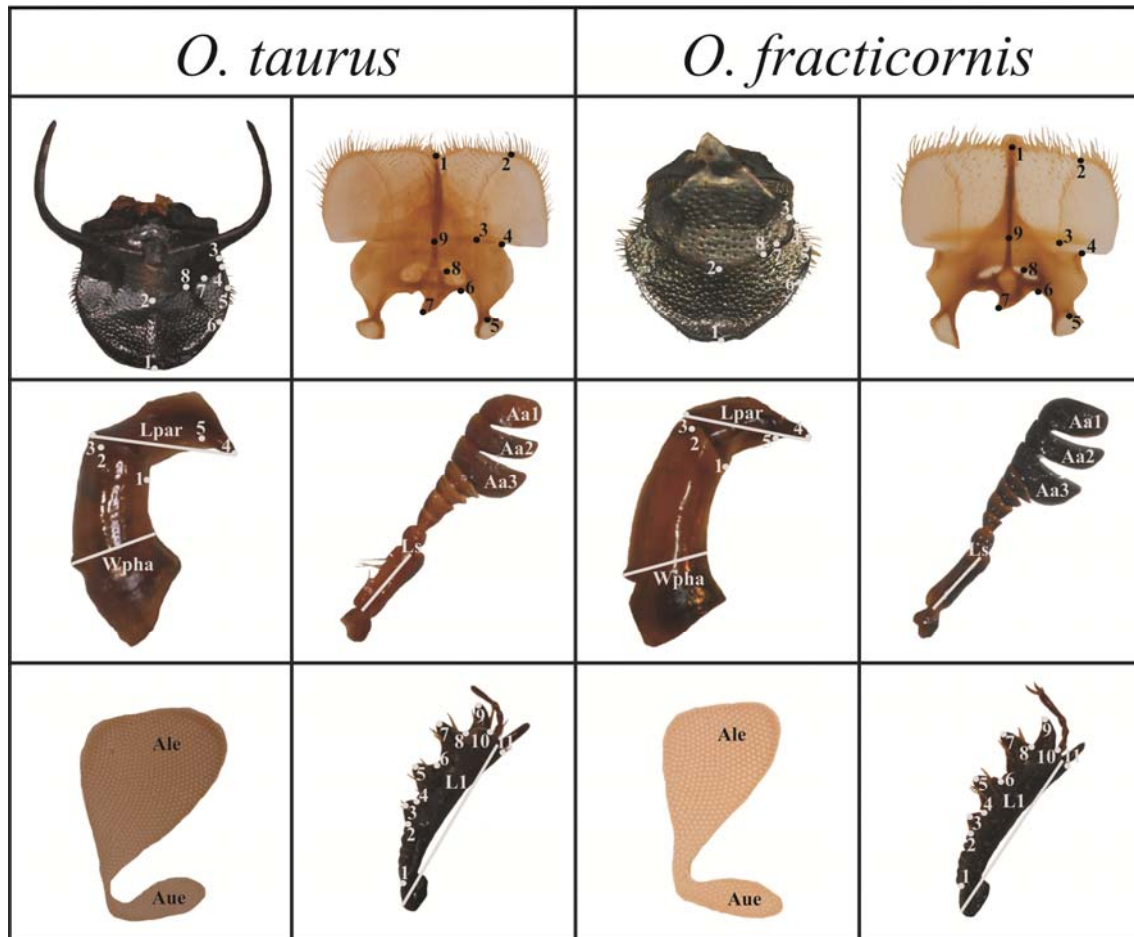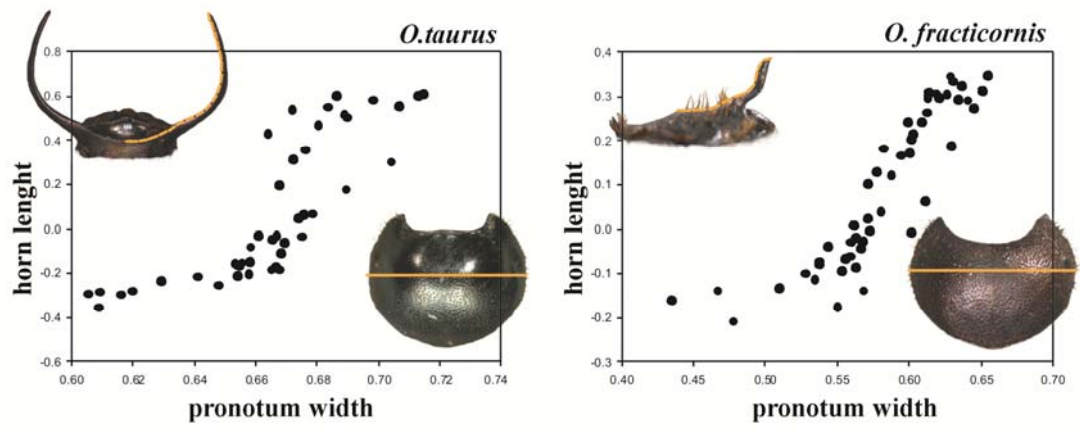

Figure 1

Figure 2

Tables

Table 1. Angular coefficients (b) of regressions between residuals of static allometries of horns and the other traits considered. Negative, significant terms were considered as evidence of trade-off between horns and traits affected. Ant=antennomere, Phall=phallobase, CS=centroid size, S.E.=standard error, R^2=coefficient of determination of regressions, n.a.=not applicable (because phallobase length in uncorrelated with body size – see E.S.M 2)

Trait	*Onthophagus taurus*				*Onthophagus fracticornis*			
	b ± S.E.	t	Sig.	R^2	b ± S.E.	t	Sig.	R^2
Lower eye area	-0.070 ±0.034	-2.031	0.049	0.092	0.106±0.081	1.304	0.198	0.032
Upper eye area	-0.042 ±0.049	-0.863	0.393	0.018	-0.259±0.249	-1.039	0.304	0.021
Ant 1 area	0.015 ±0.029	0.513	0.611	0.006	-0.220±0.100	-2.255	0.029	0.090
Ant 2 area	0.021±0.0309	0.6738	0.504	0.011	-0.070±0.128	-0,547	0.587	0.006
Ant 3 area	0.028±0.029	0.9746	0.336	0.023	-0.150±0.139	-1.108	0.273	0.024
Head CS	0.016±0.013	1.203	0.236	0.034	0.028±0.028	-0.459	0.648	0.004
Epipharynx CS	0.019±0.009	2.000	0.052	0.089	0.041±0.039	1.037	0.305	0.021
Paramere CS	0.012±0.013	0.866	0.391	0.018	0.069±0.033	2.081	0.042	0.078
Leg CS	0.024±0.011	2.336	0.025	0.117	0.088±0.030	2.972	0.005	0.148
Scapus length	0.001±0.019	0.0531	0.958	0.000	-0.006±0.060	-0.101	0.920	0.000
Leg length	0.036±0.011	3.275	0.002	0.207	0.071±0.032	2.255	0.029	0.091
Phall width	-0.063±0.023	-2,767	0.008	0.157	-0.062±0.040	-1.556	0.126	0.045
Paramere length	0.045±0.010	1.448	0.155	0.049	0.031±0.034	0.897	0.374	0.016
Phall length	n.a.	n.a.	n.a.	n.a.	-0.172±0.040	-4.281	0.000	0.260

Table 2. General linear models that best represent the effect of explanatory variables or their interactions on each trait considered. Morphs were coded as 1=major and 2=minor. Ant=antennomere, Phall=phallobase, CS=centroid size, Std. Error=standard error, pronotumW=pronotum width, hornL=horn length.

Trait	*Onthophagus taurus*					*Onthophag*			
	Parameter	B	Std. Error	Wald Chi-Square	Sig.	Parameter	B		
Lower eye area	(Intercept)	-1.071	0.288	13.798	0.000	(Intercept)	-1.417		
	[morph=1]	-0.023	0.023	0.989	0.320	[morph=1]	0.030		
	pronotumW	0.804	0.418	3.699	0.054	pronotumW	0.984		
	hornL	0.206	0.134	2.356	0.125				
	[morph=1] * hornL	-0.284	0.125	5.204	0.023				
Upper eye area	(Intercept)	-1.773	0.247	51.353	0.000	(Intercept)	-2.303		
	[morph=1]	-0.036	0.020	3.063	0.080	pronotumW	1.189		
	pronotumW	0.656	0.387	2.876	0.090				
Ant 1 area	(Intercept)	-2.048	0.111	342.793	0.000	(Intercept)	-2.159		
	pronotumW	1.118	0.167	44.628	0.000	pronotumW	1.499		
						hornL	-0.169		
Ant 2 area	(Intercept)	-1.895	0.118	258.689	0.000	(Intercept)	-2.129		
	pronotumW	0.967	0.178	29.404	0.000	pronotumW	1.377		
Ant 3 area	(Intercept)	-0.807	0.387	4.347	0.037	(Intercept)	-2.065		
	[morph=1]	-1.150	0.491	5.482	0.019	pronotumW	1.339		
	pronotumW	-0.495	0.562	0.776	0.378				
	hornL	0.432	0.156	7.713	0.005				
	[morph=1] * pronotumW	1.660	0.723	5.274	0.022				
	[morph=1] * hornL	-0.406	0.158	6.603	0.010				
Head CS	(Intercept)	2.399	0.064	1392.420	0.000	(Intercept)	2.273		
	[morph=1]	0.013	0.005	6.193	0.013	pronotumW	0.865		
	pronotumW	0.676	0.100	45.355	0.000	hornL	-0.308		
						pronotumW * hornL	0.555		
Epipharynx CS	(Intercept)	2.269	0.037	3669.793	0.000	(Intercept)	2.356		
	pronotumW	0.903	0.057	254.053	0.000	pronotumW	0.774		
						[morph=1]	0.015		
Paramere CS	(Intercept)	2.340	0.129	328.905	0.000	(Intercept)	2.529		
	[morph=1]	0.013	0.011	1.490	0.222	[morph=1]	0.015		
	pronotumW	0.584	0.185	9.963	0.002	pronotumW	0.129		
	hornL	0.586	0.228	6.624	0.010				
	[morph=1] * hornL	0.162	0.075	4.677	0.031				
	pronotumW * hornL	-1.087	0.405	7.211	0.007				
Leg CS	(Intercept)	2.595	0.064	1630.284	0.000	(Intercept)	2.495		
	pronotumW	0.777	0.098	62.819	0.000	[morph=1]	0.011		
	hornL	0.024	0.008	8.705	0.003	pronotumW	0.944		
Scapus length	(Intercept)	-0.870	0.073	143.101	0.000	(Intercept)	-0.879		
	pronotumW	0.733	0.110	44.406	0.000	[morph=1]	-0.059		
						pronotumW	0.764		
						hornL	0.022		

Trait	Parameter	Estimate	SE	Wald	p	Parameter	Value
						[morph=1] * hornL	0.201
Leg length	(Intercept)	-0.299	0.073	16.852	0.000	(Intercept)	-0.368
	pronotumW	0.861	0.110	60.840	0.000	[morph=1]	0.004
	hornL	0.385	0.153	6.307	0.012	pronotumW	0.970
	pronotumW * hornL	-0.528	0.225	5.533	0.019	hornL	-0.070
						[morph=1] * hornL	0.121
Phall width	(Intercept)	-0.860	0.137	39.247	0.000	(Intercept)	-0.541
	pronotumW	1.076	0.209	26.412	0.000	[morph=1]	0.025
	hornL	-0.066	0.017	14.463	0.000	pronotumW	0.531
						hornL	-0.130
Paramere length	(Intercept)	-0.238	0.040	35.234	0.000	(Intercept)	-0.258
	pronotumW	0.344	0.061	32.032	0.000	pronotumW	0.194
Phall length	(Intercept)	-0.131	0.009	208.004	0.000	(Intercept)	-0.761
	[morph=1]	0.029	0.012	5.546	0.019	[morph=1]	0.851
						pronotumW	1.147
						hornL	-1.934
						[morph=1] * pronotumW	-1.398
						pronotumW * hornL	2.971