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Male differentiation patterns in two polyphenic sister species of the genus *Onthophagus* Latreille, 1802 (Coleoptera: Scarabaeidae): a geometric morphometric approach

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Abstract

This paper focuses on morphological (both *shape* and *size*) differences that quite similar polyphenic sister species evolve during divergence processes. Traits were analysed using a geometrical morphometric approach, which has the ability to evidence also very subtle differences in shape. As a case study, we considered males of the dung beetle sister species pair *Onthophagus taurus* and *Onthophagus illyricus* (Coleoptera, Scarabaeidae); these species represent a typical example of polyphenic trait expression concerning the facultative development of horns and considerable body size differences. External shape morphology failed to discriminate *O. taurus* from *O. illyricus*, whereas the reproductive system shape showed significant interspecific discrimination power. However, the head of *O. taurus* was significantly larger than that of *O. illyricus* and the reverse was true for the elytra. The two species also showed different allometric values of the head with respect to body size. This complex pattern of interspecific morphological divergence is discussed in the light of the differential trait divergence rate hypothesis. In both species, differences between *major* and *minor* forms concern the overall shape of head and pronotum: we suggest that such different forms, which likely reflect morphological readjustment to accommodate horns of considerable bulk and disproportionate length, may be nevertheless advantageously used by the two male morphs in their alternative reproductive tactics. Male genitalia sizes were virtually constant with respect to body size; however, the ratio between phallotheca and body size was significantly higher in *minor* males, in keeping with the hypothesis of a higher investment in genitalia borne by this morph.

Key words: male genitalia – external morphology – allometry – differentiation between species and morphs – sperm competition – developmental investment

Introduction

Sister species are important models to study microevolutionary dynamics promoting speciation and an excellent material for qualitative and quantitative analyses of the divergence processes. Differentiation patterns can be pointed out by studying genetic, ecological and behavioural trait variation, but the morphological approach still remains prominent.

Onthophagus taurus Schereber, 1759 and *Onthophagus illyricus* Scopoli, 1763 (Balthasar 1963; Baraud 1992; Lohse and Lucht 1992; Moczek and Emlen 1999; Martín-Piera and Lopez-Colón 2000) are two closely related dung beetle species (Coleoptera, Scarabaeidae). Despite their external morphological similarity and the existence of individuals with apparent intermediate phenotypes, a species-level phylogeny, based on COI mitochondrial sequences from 18 *Onthophagus* species, have suggested that they are reproductively isolated sister species (A. Pizzo et al., unpublished data).

Onthophagus taurus showed, originally, a typical Turanic–European–Mediterranean distribution (Balthasar 1963); it was introduced to several Australian states and, in the late 1960s, also became introduced to Eastern USA. The chorology of *O. illyricus* is Turanic–European, and its distribution greatly overlaps that of *O. taurus*; due to the unreliability of several records and to the difficulty in distinguishing the species, its actual distribution is still uncertain (Martín-Piera and Lopez-Colón 2000). However, there is an extensive overlap zone where the two species often occur in syntopy. In these species, males exhibit discrete morphs. The existence of discrete morphological variants within populations, expressed facultatively in response to the internal or external environment experienced by the individual, is known as polyphenism, an extreme but common case of adaptive phenotypic plasticity

(Nijhout 1999; Moczek et al. 2002; Moczek and Nijhout 2003). The proximate factors determining which phenotype is produced are known for many polyphenisms. In some cases, the developmental and endocrine mechanisms adjusting developmental pathways to environmental conditions have been understood, in part at least. *Onthophagus taurus* and *O. illyricus* represent an interesting example of polyphenic trait expression concerning the development of horns, also known for other beetle species. Large *major* males produce horns, whereas smaller *minor* males are hornless or exhibit very reduced horns (Paulian 1935; Eberhard 1982; Paulian and Baraud 1982; Cook 1987; Eberhard and Gutierrez 1991; Rasmussen 1994; Kawano 1995). Male adult body size is primarily determined by larval feeding conditions, and only males with body size exceeding a critical threshold value develop horns (Emlen 1994; Hunt and Simmons 1997, 1998; Moczek 1998; Emlen and Nijhout 1999, 2001; Moczek and Emlen 1999; Moczek et al. 2002).

Eberhard (1982) first demonstrated that horned beetles use their horns as weapons in intrasexual competitions. The two different phenotypes use strikingly different reproductive tactics to acquire mating opportunities. The *major* phenotype is favoured in direct male–male competition. Hornless males have been found to generally avoid physical contact with other males, preferring a non-aggressive sneaking behaviour (Moczek 1999; Simmons et al. 1999; Moczek and Emlen 2000). Differences between *major* and *minor* also involve alternative mate-securing tactics (Siva-Jothy 1987; Cook 1990; Emlen 1997).

Polyphenic development is thought to play an important role in speciation and in the evolution of morphological and behavioural novelties (West-Eberhard 1989, 1992). Polyphenism give us another important tool to study differentiation

between sister species and to reveal differences in the response to environmental stimuli promoting phenotypic plasticity.

In this study, we used a geometric morphometric approach to analyse both the *shape* and the *size* components of morphological variations in males of *O. taurus* and *O. illyricus*. In recent years, geometric morphometrics (Bookstein 1991; Rohlf and Marcus 1993; Marcus et al. 1996) has proved to be a useful technique for solving a variety of biological problems; it is more powerful than traditional morphometrics and has the ability to visualize very subtle differences in shape.

We focused on three external morphological traits (head, pronotum and elytra) and one genital trait. The morphology and importance for species discrimination of genitalia in the genus *Onthophagus* are relatively well known (Zunino 1979; Palestrini 1992). The phallotheca is the only fully sclerotized male genital structure and thus it is the only part that can be reliably measured. We analysed the geometry of the left paramere.

By considering both shape and size, we first compared traits between species to evaluate the degree of interspecific differentiation, then we analysed differences of these same traits between the *major* and the *minor* morph in each species. External body characters that are not under sexual selection usually show slopes of the regression lines equal to or close to 1.0 (Harvey and Pagel 1991); on the contrary, the genital size of arthropods is not a good predictor of overall body size (Eberhard 1985; Wheeler et al. 1993; Eberhard et al. 1998; Palestrini et al. 2000). The slopes (allometric values) of log–log regressions of male genital size on indicators of body size are consistently less steep than those involving other body parts. Coefficients of variation for genital size also tend to be lower than those for the size of other body structures.

We studied allometric relationships among male body traits and compared allometric values between the two species.

General aim of this paper was to study morphological differentiation patterns in polyphenic sister species by means of a morphometric geometric approach. More in detail, the scope was twofold: first, to evidence and describe divergence patterns accompanying speciation and secondly, to point out, describe and compare between the species the divergence patterns between the two male morphs.

Materials and Methods

Geometric morphometric analysis was based on 62 males of *O. taurus* and 62 males *O. illyricus* (31 *minor* and 31 *major* in each species) collected at La Mandria Natural Park (Venaria Reale, Turin, Northwestern Italy, Italy; 45°8′N, 7°38′E) in 2003 and preserved in the collections of the Animal and Human Biology Department of the Turin University.

Images of the head, pronotum, right elytron, and phallotheca were captured using a digital camera Olympus DP11 (Olympus America Inc., Melville, NY, USA) connected to a stereoscopic microscope Leica MZ8 (Leica Microsystems AG, Wetzler, Germany). We made care to align the edges of each anatomical structure on the same horizontal plane. For the genitalia, we photographed the left paramere positioning the phallotheca on a thin film of glycerol to avoid deformation due to compression under cover slide.

In landmark-based morphometric analyses, the morphology of an object is represented by coordinates of sets of landmarks points (Bookstein 1991). In this study, landmarks were digitized using the software TpsDig 1.37 (Rohlf 2003a). Their position is shown in Figs 1 and 2. They were chosen for their quite easy identification, their homology in the two species and in the two morphs, and for their ability to capture the general shape of each morphological structure.
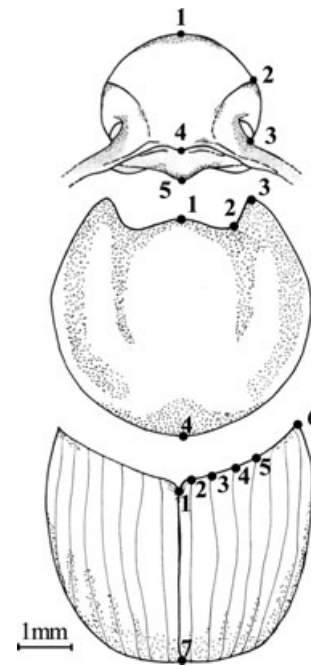

Fig. 1. Location of the landmarks on head ($n = 5$), pronotum ($n = 4$) and elytra ($n = 7$) of *Onthophagus taurus* and *Onthophagus illyricus* males. Photographs of pronota and elytra were taken at 12.5× magnification, while heads at 32× magnification. Landmarks were digitized on half of each structure to remove the variability introduced by an eventual asymmetry
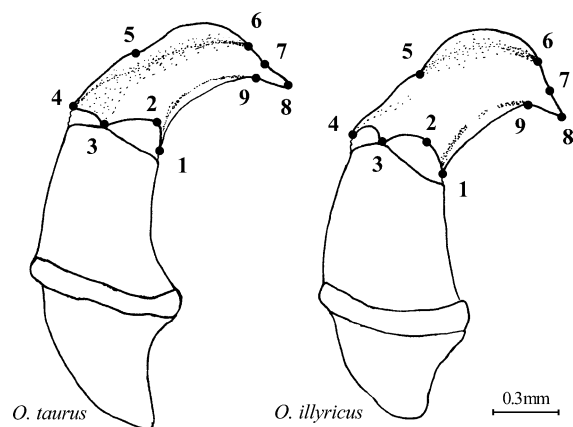

Fig. 2. Location of the landmarks ($n = 9$) on the side of the left paramere of the phallotheca. Most of *Onthophagus taurus* and *Onthophagus illyricus* individuals possessed genitalia which were simply distinguishable at a microscopic visual inspection, but some exhibited genital morphology in which intraspecific differences were not very marked. The two most extremely different morphologies are shown in the figure. Genitalia were held with a pair of tweezers and positioned on a thin film of glycerol. Photographs were taken at 50× magnification

To evaluate the confidence of the landmark configuration, a repeatability test was conducted by repeating 10 times the digitization of the landmarks on the same specimen. Then, we computed the ratio between the variance on the same specimen and the variance of the total sample [variance $= \sum$ (procrustes distances)$^2/N-1$, where N is the number of objects considered in each set of measures]; we accepted the landmark configuration only if the ratio was minor or equal to 0.05.

The landmarks of each specimen were optimally aligned using a Generalized Procrustes Analysis (GPA) to remove the non-shape effects of translation, rotation, and scale (Rohlf 1990; Rohlf and Slice 1990; Rohlf 1999). After superimposition, each landmark configuration corresponds to a single point in a non-Euclidean multidimensional space known as Kendall's shape space (Kendall 1981, 1984). Because of the difficulty in performing standard multivariate statistics in non-Euclidean space, each data point is then projected into a Euclidean space tangential to a reference point (the mean form) in the shape space (Kent 1994; Rohlf 1999). As long as variation in shape space is small, the data in tangent space are an almost perfect approximation of the data in shape space; we tested this approximation with the program TPsSmall 1.20 (Rohlf 2003b). Multivariate descriptions of the data in tangent space (the shape variables) can be generated through a variety of methods (Rohlf 1999); in the present study, we used the thin-plate spline (TPS) approach (Bookstein 1989, 1991), that allows to map the deformation in shape of an object into another. This approach translates in a mathematically rigorous way Thompson's (1917) idea of transformation grids, where one object is 'warped' into another. This method decomposes the data in uniform and non-uniform shape change components (called partial warps) (Bookstein 1991). GPA, multivariate descriptions of the shape variables, relative warp analysis (the principal component analysis of the partial warp scores) and visualization of transformation grids, that allowed us to describe shape variations, were performed using TPsRelw 1.33 (Rohlf 2003c). We plotted on an axis system the two first relative warp scores calculated for each specimen and assigned different symbols to each group of specimens using NTSYSpc 2.11 software (Rohlf 1998–2002). Parallel survey of relative warp plots and transformation grids allowed us to give some indication about the trend of interspecific and intraspecific shape variation in these structures.

Discriminant analysis was carried out on the relative warp scores of each structure to obtain a classification matrix based on shape variation; we used the percentages of correct classification to evaluate the interspecific discriminatoring power of each anatomical structure. To find out whether a certain amount of intraspecific shape variation was related to differences in size of each structure (Rosenberg 2001), we correlated the values of the centroid size and the value of the first relative warp (Dobigny et al. 2002; Cardini 2003) of head, pronotum, elytra and phallotheca in each species. Discriminant analysis and correlation were computed using the package Systat 8.0 (Wilkinson 1998).

In geometric morphometrics, values of the centroid size (the squared root of the sum of squared distances of the set of landmarks from their centroid) are used as estimation of the size of a structure (Alibert et al. 2001; Rosenberg 2001; Ubukata 2003). Log-transformed values of the centroid size were used to investigate differences in size of each morphological structure between species for each morph and between morphs within each species. When our centroid size data were found to be heteroscedastic between groups, we used an approximate t-test to compare differences in mean between groups as described in Sokal and Rohlf (1995).

Linear regression has been used to quantify allometric relationships in previous works (Gould 1966; Eberhard et al. 1998; Palestrini et al. 2000). To estimate functional relationships between variables (the sizes of elytra, pronotum, head and phallotheca), regressions of log–log transformed data were computed. We used both elytra and pronotum centroid size as indicator of overall body size. The slopes of these regressions on elytra or pronotum centroid size are referred to as allometric values and the regression lines themselves as allometric lines. Similar analyses were previously performed on species of the genus *Uca* (Crostacea, Ocypodidae) (Rosenberg 2001).

Results

Differentiation between species

When the shape of external morphological traits was considered, males of *O. taurus* and *O. illyricus* were not distinguishable. In the relative warp plots, individuals of the two species were mixed and did not form any distinct cluster (see Fig. 3a as
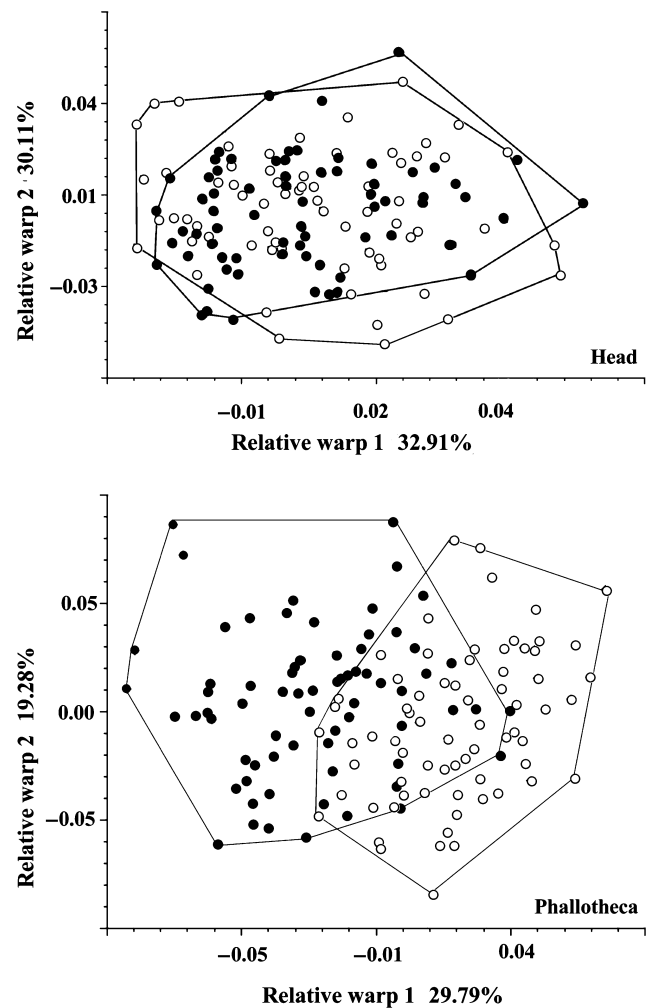
Fig. 3. Shape differentiation between species. Scatterplots of the two first relative warps scores obtained from the relative warp analysis of head and phallotheca are shown. Samples include both *major* and *minor* males, but similar results were also obtained when *major* and *minor* were separately considered. The head plot is presented as an example of the relative warp plots resulting from the analysis of the shape of external morphological structures, which do not permit any interspecific discrimination. The amount of variation explained by each relative warp (axis) is expressed as percentage. Black symbols (•) represent *Onthophagus taurus* and white symbols (○) *Onthophagus illyricus* males

an example). To the contrary, male genitalia showed a certain interspecific discrimination power (Fig. 3b). Discriminant analysis conducted on the relative warp scores of genitalia evidenced that 91% of *O. taurus* and 93% of *O. illyricus* were correctly classified, whereas percentages of correct classification were much lower for external morphological traits (ranging from 54% to 70%). Similar results were also obtained when the two male morphs were considered separately (results not shown).

The t-test conducted on centroid size showed that head was significant larger in *O. taurus* than in *O. illyricus* (*O. taurus* = 3.003 ± 0.039, *O. illyricus* 2.867 ± 0.024, variance $t = -23.056$, df = 102.1, p < 0.001), whereas elytra were larger in *O. illyricus* (*O. taurus* = 2.599 ± 0.040, *O. illyricus* = 2.617 ± 0.036, variance $t = -2.547$, df = 121.1, p = 0.012) both in *major* and in *minor* males. Pronotum size

Table 1. Differentiation between species

	Major			Minor		
	O. taurus	*O. illyricus*	t_s	*O. taurus*	*O. illyricus*	t_s
Head	**3.016**	2.898	26.45	**2.989**	2.837	22.75
Pronotum	2.685	2.694	1.94	2.609	2.631	2.40
Elytra	2.629	**2.644**	4.18	2.569	**2.589**	2.46

Values given are centroid size means. Results of the approximate *t*-test conducted on the centroid sizes of external traits between *O. taurus* and *O. illyricus*, keeping separate *major* and *minor* morphs.
Only when $| t_s| > t_\alpha$ the two means are significantly different. When the test was significant the highest value of the centroid size mean was in bold type. Tests were significant at a probability p = 0.02 when $t_s > t_\alpha = 2.457$ and at p = 0.001 when $t_s > t_\alpha = 3.646$.

did not evidence any significant differences between species (Table 1). Notably, the head of *O. taurus* minor males was significantly larger, on average, than that of *O. illyricus* major males ($t_s = 18.67$, $t_\alpha = 3.664$ for p = 0.001). Because the shape of the phallotheca was significantly differentiated between the species, we did not proceed to the interspecific size comparison.

Allometry

Linear regressions of the log–log transformed data of the elytra centroid size and the values of centroid size of head, pronotum and phallotheca are shown in Fig. 4. In both species, pronotum and elytra centroid sizes are in isometric relationship, with allometric values slightly higher than 1.0
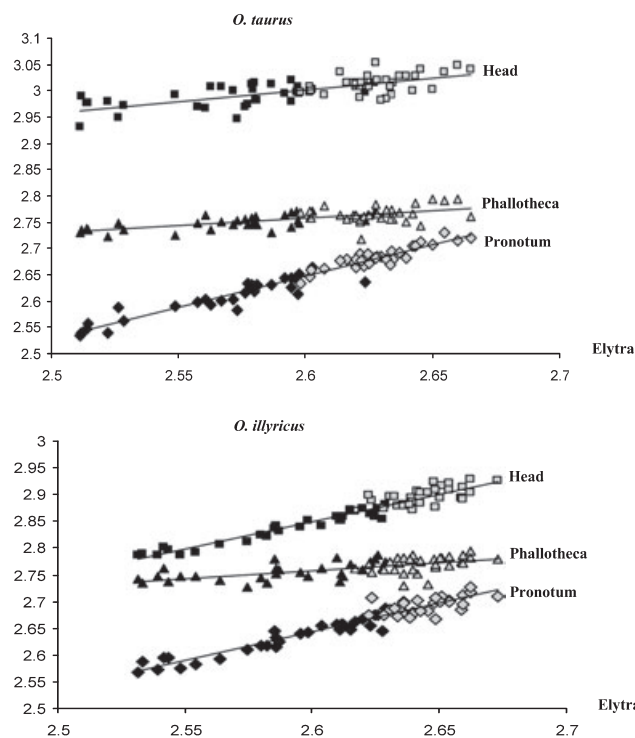

O. taurus

O. illyricus

Fig. 4. Regression lines obtained by regressing the two external body traits (head and pronotum) and the genital trait (phallotheca) on elytra centroid size. Black symbols correspond to *minor* males, grey ones to *majors*. Note that the slopes (i.e. allometric values) for the head differ significantly between *Onthophagus taurus* and *Onthophagus illyricus*. Regressions on pronotum centroid size (not shown) show similar trends, but lower allometric values

(1.18 in *O. taurus* and 1.07 in *O. illyricus*). The isometric relationship keeps valid for head and elytra centroid sizes in *O. illyricus* (allometric value is 1.03), but not for the head of *O. taurus* (whose allometric value is 0.44). Allometric values for the genital trait were low (0.28 for *O. taurus* and 0.33 for *O. illyricus*), and suggest that primary sexual traits and body size are uncorrelated and, in particular, that genital sizes do not change substantially with body size. We also used pronotum size as indicator of overall body size, because it has been shown to be particularly good for this purpose (Emlen 1994; Moczek 1998; Moczek and Emlen 1999; Palestrini et al. 2000): results of these regressions were similar to those obtained using elytra as regressor, but allometric slopes were lower (*O. illyricus*: 0.93 for the head, 0.87 for the elytra and 0.27 for the phallotheca; *O. taurus*: 0.35 for the head, 0.79 for the elytra and 0.23 for the phallotheca).

Differentiation between morphs

Relative warp analysis evidenced that head and pronotum shapes showed a good discriminatory power between *major* and *minor* males. We examined transformation grids corresponding to shape changes along the first relative warp axis. Apparently, *minor* males have longer, narrower and trapezoidal head, whereas *major* males tendentially show a head which is more rounded, larger and longitudinally compressed. This differentiation pattern keeps true in both species, but in *O. taurus* the amount of shape variation explained by the first relative warp (72.08%) is higher than in *O. illyricus* (39.12%) (Fig. 5). The pronotum shape variation allows morph discrimination as well; *minor* males seem to have a more tapered pronotum, with sharper anterior angles, whereas *major* males exhibit a larger and more solid pronotum. Moreover, variation of the reciprocal position of landmarks 1, 2 and 3 on the transformation grids seems to suggest that in *major* males the anterior edge of the pronotum is more compressed, curved or undulating, with a convexity at landmark 1 (Fig. 6). Relative warp plots of elytra and phallotheca, instead, failed to discriminate between the two morphs (Fig. 7).

A significant correlation between the value of the centroid size and the first relative warp scores was found for the head ($r = -0.551$, p < 0.0001 for *O. taurus* and $r = -0.742$, p < 0.0001 for *O. illyricus*).

As for the size, in both species all traits were significantly larger in *major* than in *minor* morphs (centroid size *t*-test); however, the ratio between phallotheca and body size (represented indifferently by pronotum or elytra centroid size), was higher in *minor* than in *major* males in each species (Table 2).
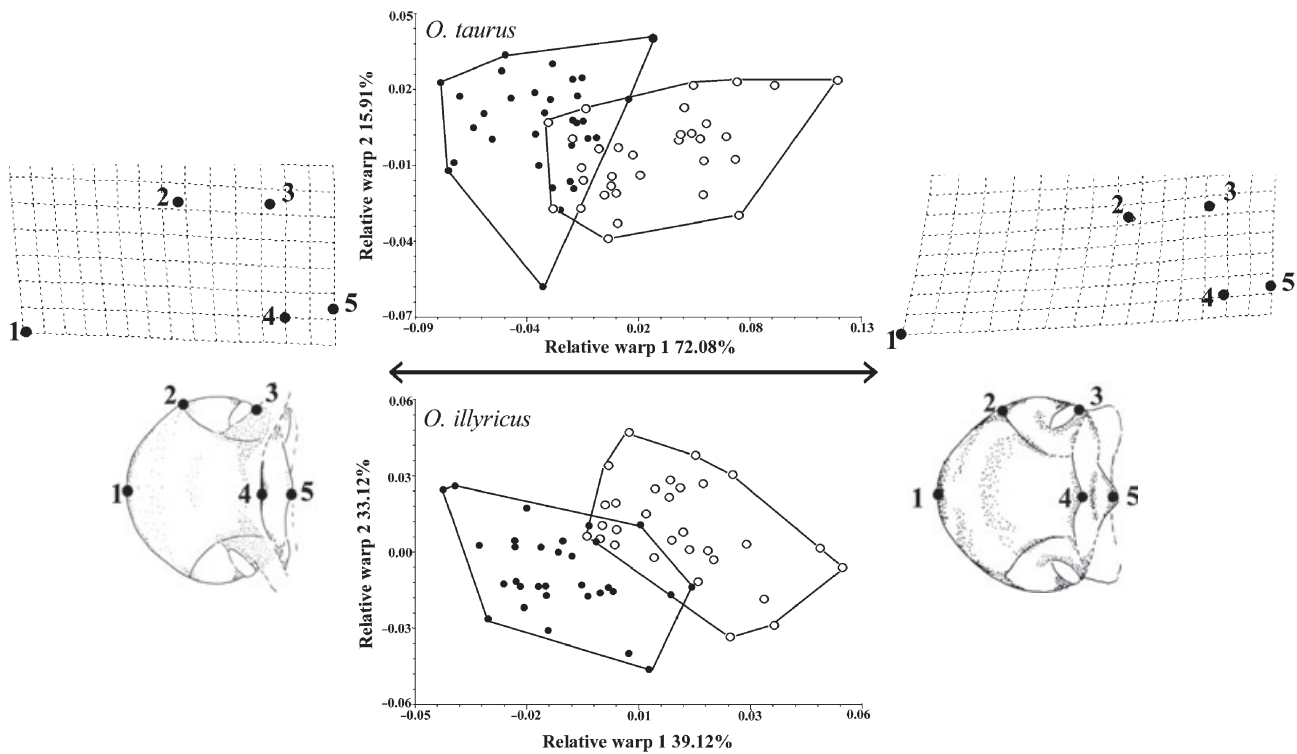
Fig. 5. Shape differentiation between morphs. Scatterplots of the two first relative warp scores obtained from the relative warp analyses of the head are shown. The amount of variation explained by each relative warp (axis) is expressed as percentage. Black symbols (•) represent *major* males and white symbols (▫) *minors*. Thin-plate spline (TPS) transformation grids on each side of the scatterplot refer to specimens plotted on the left (mostly *majors*) and right (mostly *minors*) extremity of the first relative warp axis; drawings below these 'warp' grids, illustrate the hypothetical overall head shape as suggested by landmark configurations on each grid
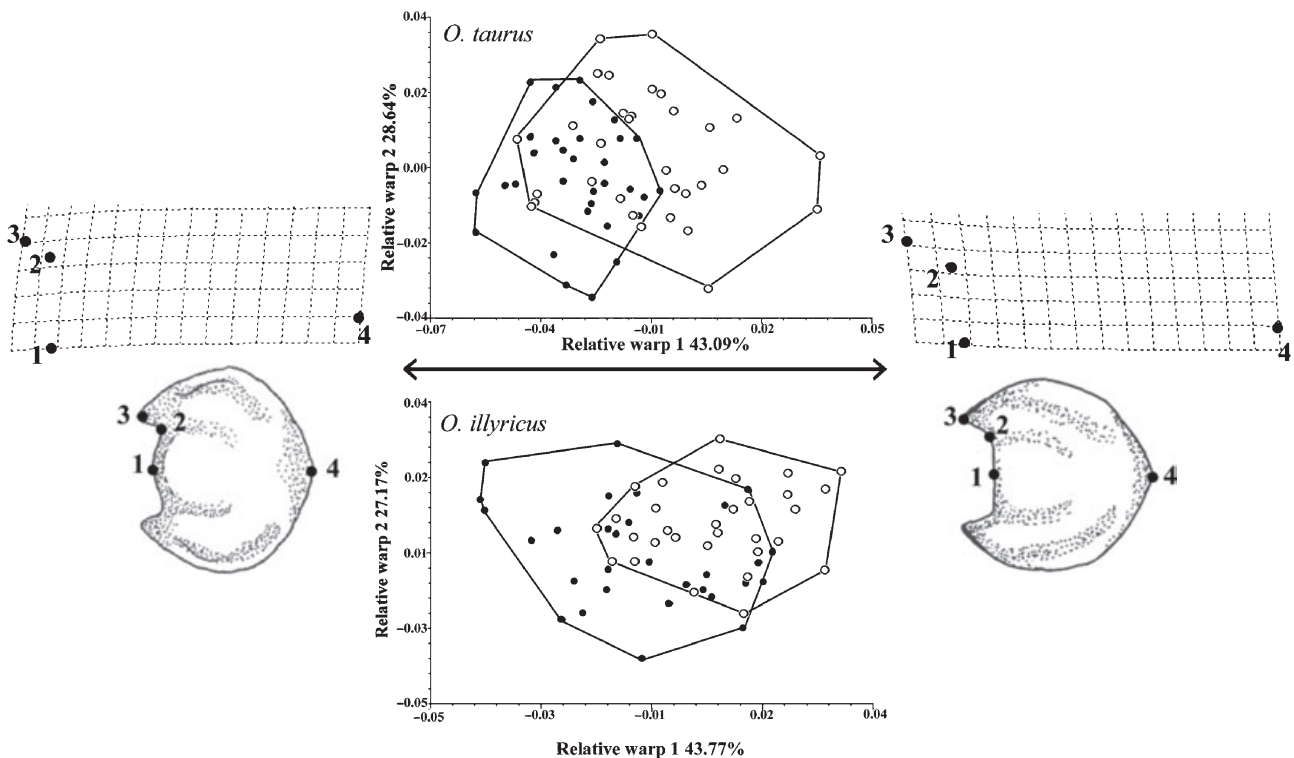
Fig. 6. Shape differentiation between morphs. Scatterplots of the two first relative warp scores obtained from the relative warp analyses of the pronotum are shown. The amount of variation explained by each relative warp (axis) is expressed as percentage. Black symbols (•) represent *major* males and white symbols (▫) *minors*. Thin-plate spline (TPS) transformation grids on each side of the scatterplot refer to specimens plotted on the left (mostly *majors*) and right (mostly *minors*) extremity of the first relative warp axis; drawings below these 'warp' grids, illustrate the hypothetical overall pronotum shape as suggested by landmark configurations on each grid
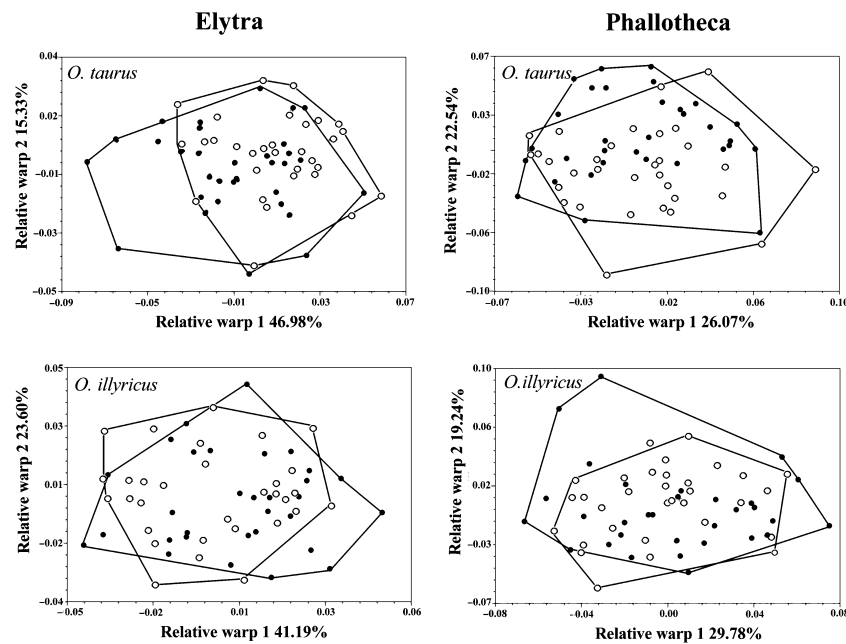
Elytra **Phallotheca**

Fig. 7. Lack of shape differentiation between morphs for male elytra and phallotheca. The figure shows the scatterplots of the two first relative warp scores obtained from the relative warp analysis. Black symbols (●) represent *major* males, white symbols (○) indicate *minors*. The amount of variation explained by each relative warp (axis) is expressed as a percentage

Table 2. Differentiation between morphs

	O. taurus			*O. illyricus*		
	Major	Minor	t_s	Major	Minor	t_s
Head	**3.016**	2.989	5.17	**2.898**	2.837	9.99
Pronotum	**2.685**	2.609	9.74	**2.694**	2.631	9.73
Elytra	**2.629**	2.569	9.10	**2.664**	2.589	9.21
Phallotheca	**2.766**	2.749	4.59	**2.773**	2.754	3.85
Phallotheca/pronotum	1.209	**1.380**	8.11	1.201	**1.332**	7.32
Phallotheca/elytra	1.052	**1.070**	7.37	1.049	**1.064**	11.37

Values given are centroid size means. Results of the approximate *t*-test conducted on the centroid sizes of head, pronotum, elytra and phallotheca between *major* and *minor* males in *O. taurus* and in *O. illyricus*. The last two rows show the *t*-test results conducted on the ratio between phallotheca centroid size and body size (represented, alternatively, by pronotum or elytra centroid size); higher value of the centroid size mean are given in bold type.
In all analysis differences were highly significant (p = 0.001) t_s values being higher than $t_\alpha = 3.646$.

Discussion

Geometric morphometrics failed to discriminate *O. taurus* from *O. illyricus* males when the shape of external morphological traits was taken into account. Morphological interspecific similarity was also observed in other animal (and plant) sister species (Yamazaki et al. 1997; Arlettaz 1999; Hardig et al. 2000; Dobigny et al. 2002; Mathews et al. 2002). It may be assumed that after speciation, there were not significantly different selective pressures on outer phenotype to promote differentiation of these morphological traits, which appear to be very homogeneous and conservative in the two species. However, both the relative warp and the subsequent discriminant analyses of the genital shape suggest that reproductive system morphology of the two species was differentiated. All the above is in keeping with the hypothesis that genitalia diverge at a higher rate than external morphology (Eberhard 1985). Despite the fact that external traits of the two species share the same shape, information provided by the analysis of

the centroid size suggests a divergent size differentiation pattern: head was significantly larger in *O. taurus*, while elytra was significantly larger in *O. illyricus*.

Many external body characters are known to show allometric values equal or similar to 1.0 (Harvey and Pagel 1991). In *O. taurus* and *O. illyricus* pronotum and elytra sizes showed isometric relationships, with allometric values slightly different than 1.0. In *O. illyricus* the allometric value for the head was also quite close to 1.0, whereas in *O. taurus* it was very low. This means that *O. taurus* head size is more constant among individuals and size differences between *minor* and *major* males are therefore less marked. This pattern suggests that in *O. taurus* this trait undergoes developmental process and is subject to selective pressures that are quite different from those affecting *O. illyricus*.

Moczek and Nijhout (2003) showed that males of *O. taurus* and *O. illyricus* differed highly significantly in the average horn length-body size allometry. Differences between the two

species were largely attributable to highly significant differences in the body size threshold between alternate morphs. This means that there are *O. taurus* males that, with respect to *O. illyricus*, exhibit horns and a *major* phenotype at smaller body size; Palestrini et al. (2000) demonstrated that also the allometric values for internal epipharynx traits in *O. taurus* were significantly lower than those for external body traits and only slightly higher than those for the genital traits.

All considered, these two species present a very complex pattern of morphological divergence. They can be distinguished for body size threshold between morphs (Moczek and Nijhout 2003), for the shape of male genitalia, the male size of elytra and head and the allometry of the head with body size (this paper). Differences in the shape of genitalia were expected and confirm that genitalia quickly diverge during the speciation process (Eberhard 1985). Interspecific differences in head and elytra size and in the allometric relationship of head with respect to body size indicate that also external body traits, despite the fact that they have the same shape in the two species, diverged at some level during the speciation process.

In each species head and pronotum shape are clearly differentiated between the two male morphs. As for the head, such a difference may be partly due to size variation between horned and hornless males, as suggested by the significant correlation between centroid size and the first relative warp. Concurrently, both the head and the pronotum must undergo morphological readjustment and constraints to accommodate horns of considerable bulk and disproportionate length. Whatever the cause of this differentiation pattern, in both species *minor* males have longer, narrower head, and a more tapered pronotum, with sharper anterior angles. We suggest that *minor* males may take advantage of this morphology when they run through lateral tunnels to reach a female. It has been demonstrated that smaller horned males, adopting the alternative tactic of sneaking copulation, are able to travel, on average, 1.1 s faster within a tunnel of standardized length (Moczek and Emlen 2000). In *major* males, instead, head and pronotum are more rounded, larger, compressed in the fore-hind direction and with the anterior edge of the pronotum presenting a convexity. These characteristics, conferring on animals a more massive and solid appearance, could be advantageous in a fighting tactic. Elytra shape does not vary between morphs, their morphology being in fact more related to the flying activity or, at least, more liable to aerodynamic constraints and not directly involved in different reproductive tactics or in the growth of the horns.

As suggested by the relative warp analyses of external body traits (Figs 5–7), the two species maintained very similar shape differentiation patterns between the two kind of polyphenic males. Phenotypic plasticity provides the opportunity for the independent evolution and adaptation of different phenotypes to different sets of environmental circumstances (West-Eberhard 1989, 2003). Moreover, a given phenotype may disappear and be replaced by a different alternate phenotype when the inducing environment changes (Moczek 2005). We could suggest that after speciation *O. taurus* and *O. illyricus* have probably maintained the same ecological niche and the same reaction norm to environmental stimuli.

Relative warp analysis of the phallotheca showed that there are no substantial differences in the shape of genitalia between *major* and *minor* forms. Independent of external phenotype, all males of a species must have a reproductive structure warranting the same mechanical opportunity of mating. This is stressed by the likeness of genital shapes revealed in this study. Our results showed a very weak linear increase in the size of genital traits with body size; this means that parameres are virtually invariant with respect to body size. The very shallow slope for the male genitalia is in accordance with the results obtained by Eberhard et al. (1998), who showed that male genitalia of 20 species of insects and spiders had shallower slopes than other body parts. Unless there is size-assortative mating, selection acts on males to adjust their stimulation so that it is appropriate for the most typical female size, favouring intermediate, standard size of the male genitalia (and thus low allometric values) (Eberhard et al. 1998; Palestrini et al. 2000). These results seem to suggest that males of *O. taurus* and *O. illyricus* have evolved developmental machinery that ensures constant genital size and shape independently of adult body size and phenotype.

The dynamics of developmental processes restrict the range of morphological variation that can be produced; when a limiting resource is shared among several body parts, the degree to which it is used by one part diminishes its availability to another (Reznick 1985). Beetle horns are exaggerated extensions of exoskeleton, constituting a substantial investment for a developing animal. Horn development is associated with a (negative) compensatory response in other neighbouring parts (Nijhout and Emlen 1998) and distant ones, like genitalia (Moczek and Nijhout 2004), and with an increased larval mortality (Hunt and Simmons 1997). Remaining small and hornless, *minor* males save resources to be invested in genitalia, sperms and ejaculate. Parker's game theoretical models of sperm competition predict that hornless males adopting a sneak tactic should invest more heavily in sperm production and in genital size because they will always be subject to sperm competition (Parker 1990). Small, hornless males develop, in fact, significant greater amounts of testicular tissue and produce larger ejaculate volume than *major* males (Cook 1990; Simmons et al. 1999; Tomkins and Simmons 2002). Simmons et al. (1999) found that the ratio of testis mass to body mass was higher in *minor* males. In our study, we also found that the ratio between the phallotheca size and the body size was higher in *minor* males, in keeping with the hypothesis of a greater expenditure in sperm competition and a higher investment in genitalia of *minor* males.

Résumé

Différenciation morphologique dans deux espèces voisines polyphéniques du genre ONTHOPHAGUS (Coleoptera, Scarabaeidae): apport des méthodes de morphométrie géométrique.

Cet article porte sur les différences morphologiques (au niveau de la forme et de la taille) que deux espèces voisines polyphéniques ont évolué au cours des processus de divergence. L'étude utilise les méthodes de la morphométrie géométrique, ayant la capacité de mettre en évidence des différences subtiles entre les formes. Comme modèle d'étude nous avons considèré les males de deux espèces voisines de coléoptères, *Onthophagus taurus* et *Onthophagus illyricus* (Coleoptera, Scarabaeidae), qui représentent un exemple typique d'expression polyphénique des traits morphologiques concernant des différences importantes de la taille du corps et le développement facultatif des cornes. L'analyse a mis en évidence que la forme des traits de la morphologie externe ne permette pas de distinguer *O. taurus* par rapport à *O. illyricus*, alors que la forme des structures génitales masculines montre un signifiant pouvoir de discrimination interspécifique. Cependant, la tête de *O. taurus* a une taille plus grande par rapport à *O. illyricus*, et l'inverse est vrai pour la taille des élytres. Les

deux espèces montrent aussi une différence au niveau des valeurs allometriques de la taille de la tête respect à la taille du corps. Ce complexe pattern de différences morphologiques interspécifiques est discuté sous l'hypothèse d'un taux de divergence et d'évolution différentiele entre traits morphologiques différentes. Dans chaque espèce, les différences entre les males *minor* et *major* concernent la forme générale de la tête et du pronotum: on suggère que ces différences de forme, qui reflétent probablement des changements morphologiques nécessaires à accommoder des cornes d'un poids considérable et d'une longueur disproportionnée, sont, peut-être, cependant avantageuses dans les différentes stratégies reproductives utilisées par le deux morphes. La taille de la phallotheca est résultée virtuellement constant respect à la taille du corps; cependant, les résultats de la standardisation entre la taille de la phallotheca et la taille du corps sont significativement plus hauts dans les mâles *minor*, en accord avec l'hypothèse d'un plus important investissement dans le système reproductif soutenu par cette morphe.

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