
Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach

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Alibert, P., Moureau, B., Dommergues, J.-L. & David, B. (2001). Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. — *Zoologica Scripta*, 30, 299–311. New morphometric methods, the geometrical morphometrics, offer promising perspectives to appraise morphological variation among organisms and open up, to a large extent, the field of morphometrics for the study of systematics and evolution. Until now, however, few studies have explored the potential of these methods at a microgeographical scale. In the present work, we applied them to quantify morphological (size and shape) differentiation among populations of two forest species of ground beetles: *Carabus auronitens* and *C. nemoralis*. We found a significant shape variation among sites, as well as among sexes, for both species. Additionally, for *C. auronitens*, we found significant positive correlations in both sexes between morphological (shape) and geographical distances between populations. In contrast, significant size differences were found between sexes, but not between sites. We conclude that geometrical morphometric methods provide valuable tools for the study of morphological variation among populations and therefore offer, on the whole, interesting perspectives for the study of biodiversity patterns.

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Introduction

Population differentiation is considered as an essential step in the process of speciation (Balon 1993; Margurran 1998), and its study is therefore crucial for the understanding of the processes underlying biodiversity. For several decades, geographical differentiation within species has essentially been viewed from a genetic viewpoint. This has been related to the concomitant development of efficient genetic markers as well as powerful statistical analyses of data obtained with these markers (Sunnucks 2000). All of these studies contributed to a significant increase in our knowledge of the definition of species and of their sensitivity to geographical dispersal and eventually to habitat clearance. In contrast, studies focusing on morphological differentiation within species are quite rare (but see Brown *et al.* 1992; Thomas *et al.* 1998). So far, morphological approaches have received only moderate attention, partly because of the lack of accuracy of traditional morphometric methods at the intra-specific level. However, the last few years have seen the development of new morphometric methods: the geometrical morphometrics (Bookstein 1991;

Rohlf 1993a; Rohlf & Marcus 1993). These methods, which allow the study of shape in addition to the study of size, offer powerful analytical and graphical tools for the quantification and visualization of morphological variation within and among samples of organisms. The principle of these methods can be briefly summarized as follows. Raw data corresponding to two- or three-dimensional Cartesian coordinates of landmarks describe the form of the morphological structure under study. Differences among individual configurations of landmarks are captured using mathematical functions which fit the differences in positions of the landmarks. Then, the variation in shape within and among samples can be appraised using the parameters of these functions as variables in classical uni- or multivariate statistical procedures (Rohlf & Marcus 1993; but see also Bookstein 1996 for some restrictions). In addition, as these methods preserve the geometrical relationships among landmarks, they provide the opportunity to represent the contribution of each landmark to the shape changes directly in the space of the original specimen, e.g. by the mean of displacement vectors or deformation grids.

In the last 10 years, the number of studies using geometrical morphometrics has increased considerably. These methods have now been used and proved to be relevant in a large spectrum of fields of morphometrics, including systematics, phylogeny, ontogeny or the study of developmental stability (Loy *et al.* 1993; Zelditch *et al.* 1993, 1995; Fink & Zelditch 1995; Auffray *et al.* 1996; David & Laurin 1996; Naylor 1996; Klingenberg & McIntyre 1998). Surprisingly, few studies have explored the potential of geometrical morphometric methods at a microevolutionary level (Loy 1996, but see, for example, Laurin *et al.* 1994; Auffray *et al.* 1996; Baylac & Daufresne 1996; Adams & Funk 1997).

In the present study, we apply geometrical morphometrics to assess morphological differentiation among populations of carabid beetles at the microgeographical scale. Ground beetles (Coleoptera, Carabidae) belong to one of the best studied invertebrate families (see Lövei & Sunderland 1996 for a review). Species richness (more than 40 000 described species), abundance and the large distribution of ground beetles makes this group particularly prone to investigation in numerous topics of ecology and evolution, including landscape ecology and conservation research programmes. Carabids have proved their usefulness as environmental indicators (Heijerman & Turin 1994; Maelfait *et al.* 1994), and studies at the community (Klein 1989; Blake *et al.* 1994; Eyre & Luff 1994; Davies & Margules 1998) or population (Liebherr 1986; Basedow 1994; Cardenas 1994; Baumgartner *et al.* 1997) levels are numerous. In order to explore morphological differentiation among populations at a regional scale of up to 50 km, we chose two forest species of ground beetles, *Carabus* (*Chrysocarabus*) *auronitens* Fabricius, 1772 and *Carabus* (*Archicarabus*) *nemoralis* Müller, 1764, that differ slightly in their habitat requirements, *C. auronitens* being more stenotopic and strictly bound to forest, and *C. nemoralis* more eurytopic and less strongly associated with forest (Assmann *et al.* 1994; Kennedy 1994; Niehues *et al.* 1996). This, and the fact that the two species are wingless, explain their small dispersal power (Wallin & Ekbohm 1988; Nève de Mévergnies & Baguette 1990). For instance, Mader (1984), after a 2-year mark–release–recapture experiment, reported that for several species of stenotopic forest carabids, but also for more eurytopic ones (including *C. nemoralis*), a 6-m-wide highway was almost never crossed during the whole period of the experiment and therefore constituted a very effective barrier to mobility. Similar results have been reported for *C. auronitens* (Nève de Mévergnies & Baguette 1990). At a microgeographical scale, connectivity, and its impact on population dynamics, is thus strongly related to the dispersal ability of individuals, i.e. their ability to cope with barriers as well as to move over long distances. This makes ground beetle populations particularly sensitive to geographical isolation. Therefore, our objectives were to use a geometrical morphometric

approach to estimate the morphological (size and shape) differentiation in *C. auronitens* and *C. nemoralis*: (i) among populations in relation to geographical distance and to the presence of barriers between sites; and (ii) within populations, between sexes.

Materials and methods

Study area and sampling

The study took place in the vicinity of Dijon (Burgundy area, eastern France) where six sampling sites were selected (Fig. 1). The sites CIT-A and CIT-B were both located in the large forest of Cîteaux, the two sites being spaced 10 km apart. Two sites, BRAZ and VERN, were located in two presumed fragments of the Cîteaux forest (Brazey forest and Vernot forest) and were 5 km and 10 km from CIT-A and CIT-B, respectively. According to the local archives housed at the Prefecture of Dijon, Brazey forest and Vernot forest were isolated from the Cîteaux forest before the 17th century. Both are separated today from the Cîteaux forest by various barriers, such as railroads, roads, creeks, canal or agricultural fields (Fig. 1). The two other sites, FERT and CHAUX, are located 45 km south and east, respectively, in large forests (Ferté forest and Chaux forest). The forest and study area size are summarized in Table 1. All the selected forests are copice dominated by common oak (*Quercus robur*), hornbeam (*Carpinus betulus*) and common beech (*Fagus sylvatica*). They are also similar in terms of soil and altitude. The surroundings are mainly agricultural land.

Animal capture took place from December 1997 to June 1998 using two different sampling methods according to the period. From December to February, as almost all beetles were sheltering for the winter (dormant period), they were collected directly after inspection of wood debris. From March to June, when animals became active, sampling was carried out with pitfall traps. Each trap was visited twice a week. Six hundred and sixty-two beetles were captured with, whenever possible, a minimum of 65 individuals for each site (Table 1). Both species, *C. auronitens* and *C. nemoralis*, were captured in all of the sampling sites, except for FERT, where only *C. nemoralis* was found, and CHAUX, where only *C. auronitens* was captured.

Data collection

Two-dimensional Cartesian coordinates of 50 landmarks were recorded on the ventral view of each specimen (Fig. 2) using an optic measuroscope (Nikon measuroscope 10×, Nippon KOGAKU K.K. model 0, 1/100 mm). All specimens were scored by one experimenter (BM) in a random order with regard to the site of origin. The location of the landmarks was chosen according to two criteria: reliability in terms of correspondence between specimens and the ability to best describe the geometry of the form under study. In

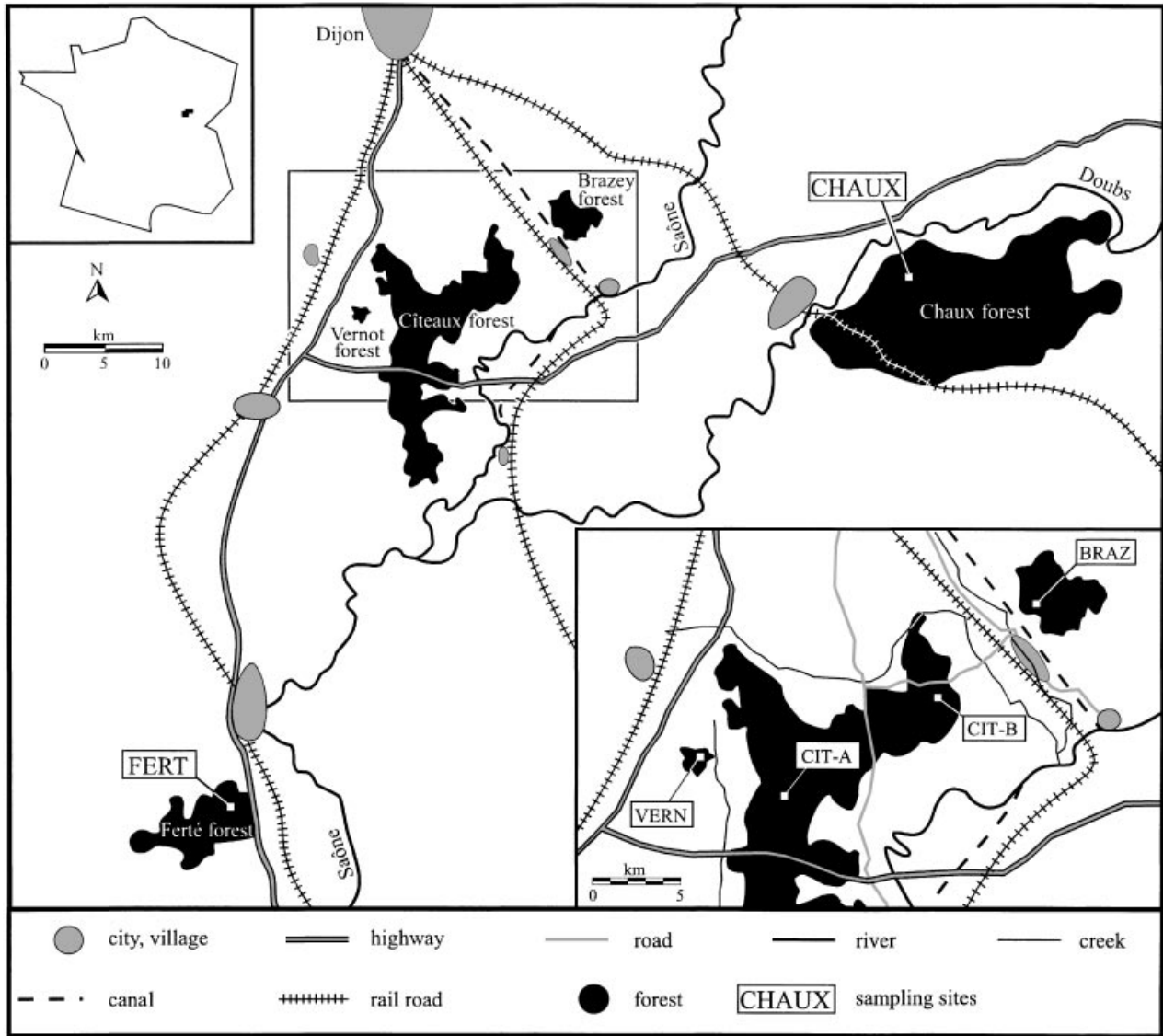


Fig. 1 Map of the study area and sampling sites.

Table 1 Location, area and size of samples.

Site	Forest	Locality	Total forest size (ha)	Study area size (ha)	Sample size			
					<i>C. auronitens</i>		<i>C. nemoralis</i>	
					Females	Males	Females	Males
CIT-A	Massif de Cîteaux	Argilly	10300	8	34	35	33	35
CIT-B	Massif de Cîteaux	Magny-lès-Aubigny	10300	9	36	34	31	36
BRAZ	Massif de Brazey	Brazey-en-plaine	1140	7	35	35	33	36
VERN	Bois de Vernot	Argilly	97	2.3	35	35	33	32
CHAUX	Forêt de Chaux	Bretenière	13000	5	33	10	—	—
FERT	Forêt de la Ferté	Saint-Ambreuil	7750	5	—	—	35	36
Totals					173	149	165	175
					322		340	

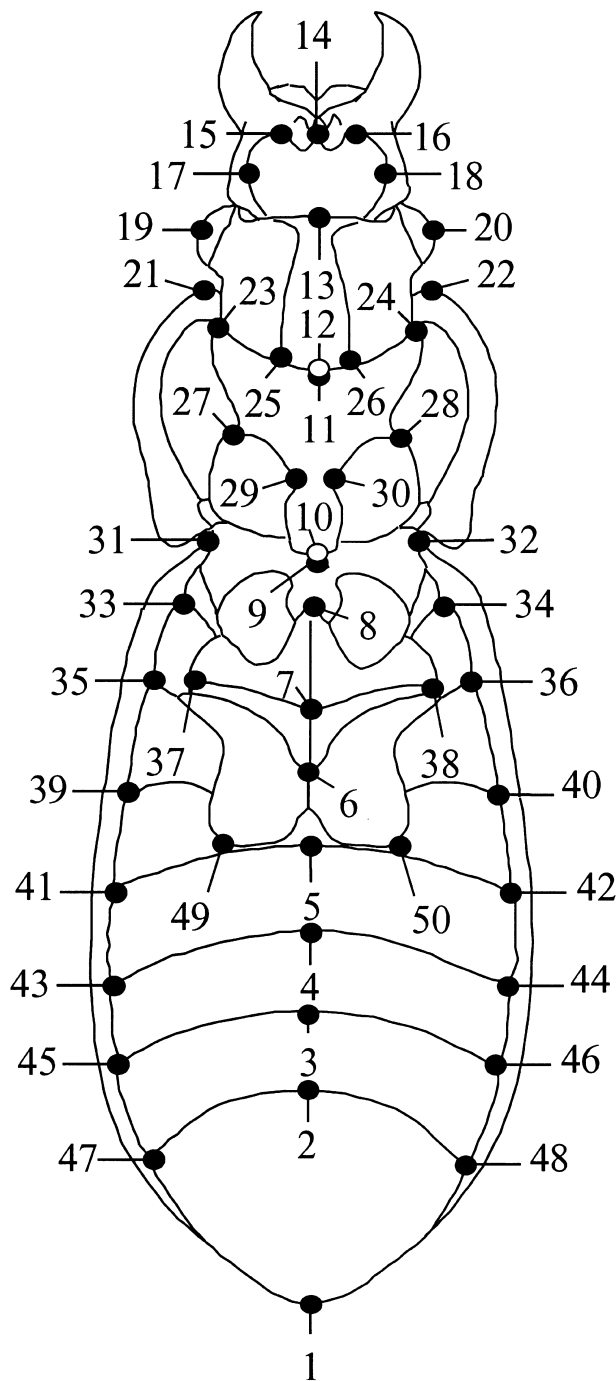


Fig. 2 Location of the 50 landmarks on the ventral view of the ground beetle. Legs, antennae and maxillae and labial palps were removed (modified from David *et al.* 1996).

order to make the location of landmarks easier, legs, antennae and maxillae and labial palps were removed. The raw data set therefore corresponded to 662 configurations of 50 (x,y) coordinates. There is no consensus among morphometricians

about the interest of considering landmarks on one or both sides in bilaterally symmetrical organisms (for a discussion, see morphmet forum at <http://life.bio.sunysb.edu/morph/>). It is argued that the study of symmetrical landmark configurations increases the degrees of freedom without adding much new information, and leads to high correlation values in the covariance matrices. However, because in our case the whole organism (and not only part of it) was studied, we considered that it was important to take into account the whole shape variation, including that related to differences between sides. In addition, from a biological point of view, the consideration of the full body allowed a more realistic visualization of the shape variations in the space of the original specimen when using the deformation grids.

Prior to morphometric analysis, a geometrical adjustment was performed on the configurations of all the specimens in order to correct for the possible imperfection in the alignment of the three articulated parts of the body, i.e. head, prothorax and abdomen, which could have occurred during the preservation of animals. This correction consisted in aligning the segments defined by the landmarks 12 and 14 (located on the head) and 10 and 11 (located on the prothorax) to the symmetry axis defined by the abdominal landmarks 6 and 8 (David *et al.* 1996).

Shape analysis

Shape differences among individuals and populations were investigated using generalized least-square (GLS) Procrustes superimposition methods (Rohlf & Slice 1990; Bookstein 1991; Rohlf & Marcus 1993). These methods allow the description and quantification of the differences between two or more specimens after their landmark configurations have been aligned according to a procedure which ensures the best overall fit. This is performed in several steps (Rohlf & Slice 1990). First, all the configurations of landmarks are scaled by standardizing the size to a unit centroid size, the centroid size corresponding to the square root of the sum of the squared distances between the centroid (i.e. centre of gravity of the landmarks) and each of the 50 landmarks of the configurations (Slice *et al.* 1996). Then, the centroids of all the landmark configurations are superimposed and translated to the origin. Finally, the landmark configurations are rotated against a reference configuration so that the sum of the squares of the residual distances between corresponding landmarks is at a minimum. The reference configuration corresponds to a computed configuration (called consensus configuration hereafter) expressing the shape that has the least summed squared Procrustes distances to all the configurations of the studied sample (Slice *et al.* 1996).

Within both species, shape differences between each of the aligned configurations (322 and 340 for *C. auronitens* and *C. nemoralis*, respectively) and the consensus configurations

were recorded in terms of residuals (so-called Procrustes residuals) at each of the 50 landmarks. To explore the shape variation among individuals and samples, it is possible to perform a principal component analysis (PCA) on the Procrustes residuals. If variations are considered in full shape space (i.e. both uniform and nonuniform transformations, *sensu* Slice *et al.* 1996), such an analysis corresponds to a version of a more complex geometrical morphometric method, the relative warps analysis (Bookstein 1991; Rohlf 1993a). More precisely, a PCA on Procrustes residuals is technically identical to version 1 of the relative warps analysis (Bookstein 1996). The relative warps correspond to the principal components, and define a shape space in which individuals are replaced. Here, we performed a relative warps analysis on both species using the software TpsRelw v. 1.18 (Rohlf 1993b). This approach allows the expression of shape variations along the relative warps in terms of the transformation of deformation grids.

For both species, shape differences between sexes and sampling sites were tested by a two-way multivariate analysis of variance (MANOVA) on the scores obtained for all of the individuals on the relative warps representing 95% of the total variance. We retained only 95% of the expressed variance in order to reduce the number of axes involved in the computation. To test whether morphological divergence among populations was related to geographical distances, matrices of pairwise shape differences (i.e. noneuclidian distances between populations in the shape space) were compared with the corresponding matrices of pairwise geographical distances between sampling sites using Mantel tests. These tests were conducted for both sexes and both species.

Size analysis

We investigated size differences among individuals and populations using two kinds of size estimators: the centroid size and the euclidian distance between the landmarks 1 and 14. Whereas the former constitutes the size parameter almost systematically used in geometrical morphometrics, the latter provides a widespread estimator of size in traditional morphometric studies on beetles. Both centroid size and distance between landmarks 1 and 14 were calculated and extracted prior to GLS superimposition. Sexual dimorphism and variation in size among sites were tested using ANOVA. When ANOVAs were significant, pairwise comparisons among means were performed using the T'-method of Spjøtvoll and Stoline (Sokal & Rohlf 1995) for nearly equal sample size. As such an unplanned multiple comparison test is conservative, the occurrence of type I error is limited.

Measurement error

Because of the large amount of work involved in taking duplicate measurements on the 662 individuals under study, errors

due to measurement were estimated from a subsample of 40 individuals of *C. auronitens* (20 males and 20 females) randomly chosen across all samples. Each of the 40 specimens was measured twice. As positioning has been shown to be an important source of error (Arnqvist & Mårtensson 1998), the second session of measurement was conducted after the specimens had been removed and replaced under the measuroscope in order to take the positioning error into account. The 80 landmark configurations obtained were then scaled, translated and rotated against the consensus configuration (computed as described above) by a GLS Procrustes superimposition method. Then, as for the study of the shape variation among individuals, a relative warps analysis was conducted. The variability in the position of the 80 configurations in the shape space was assessed using the scores obtained by each individual on the first three relative warps. For each of these axes, the variability in the scores was partitioned into 'within-individuals' (i.e. measurement error) and 'among-individuals' components using model II one-way ANOVAs, with individuals as the categorical factor (Bailey & Byrnes 1990; Arnqvist & Mårtensson 1998).

The percentage measurement error, estimated as the proportion of the total variance attributable to within-individuals variation, was found to be 0.4%, 36.5% and 4.2% for the first three relative warps, accounting for 26.3%, 12.1% and 10.7% of the total explained variation, respectively. If the proportions of measurement error for the first axis, and to a lesser extent for the third axis, are very low, the measurement error for the second axis remains fairly high, in comparison with other axes and also with other studies (Bailey & Byrnes 1990). However, it should be kept in mind that the imprecision of the measurements is expressed relative to the inter-individual variation, i.e. when variation among individuals is low, the within-individual variation (measurement error) becomes important. Thus, in studies conducted at the intraspecific level, as is the present study, higher values of measurement errors are expected. Overall, and owing to the low values of measurement error on the first and the third axes, we assumed that the variability due to the imprecision of measurements was not a source of bias in our study. Moreover, visual inspection of plots of the Procrustes residuals after GLS superimposition of the specimens measured twice revealed that the total variability was homogeneously distributed among the 50 landmarks (not shown here). Thus, none of the landmarks appeared to be associated with a greater measurement error.

Results

Figure 3 shows the result of the GLS superimpositions for the 322 specimens of *C. auronitens* (the result for *C. nemoralis*, which is quite similar, is not shown here). The relative

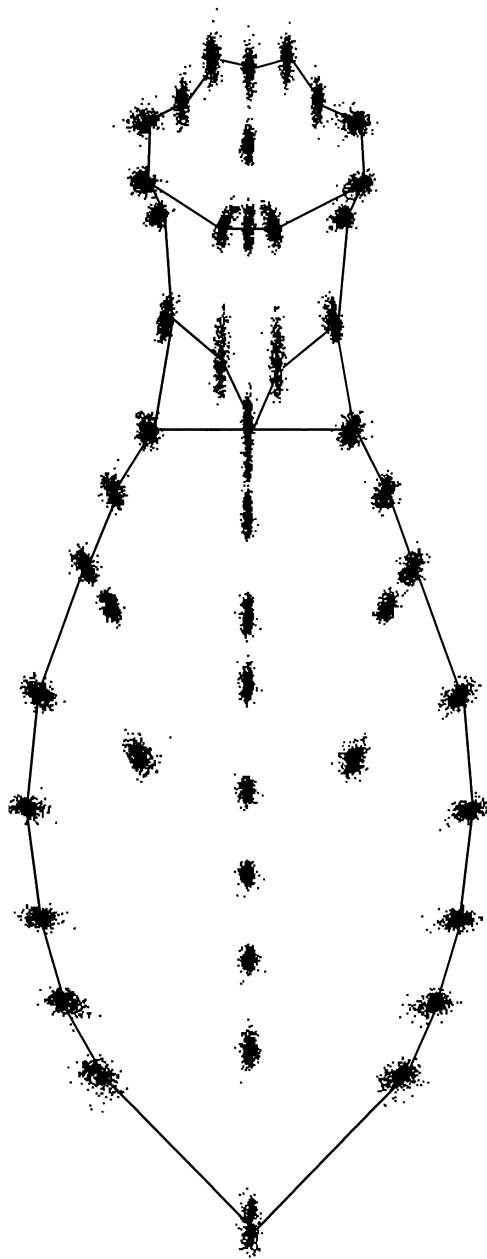


Fig. 3 Result of the generalized least-square superimposition for the 322 landmark configurations of *C. auronitens*. Points express the overall shape variation in landmark location around the consensus configuration.

warps analysis showed that 95% of the total variability was explained by the first 26 axes for *C. auronitens* and by the first 28 axes for *C. nemoralis*. For both species, much of the shape variation was captured by the first three relative warps, with 71.53% and 65.15% of the shape variation among specimens explained for *C. auronitens* and *C. nemoralis*, respectively.

Sexual dimorphism in shape

Two-way MANOVAS, performed on the scores obtained by the individuals in the relative warps analysis, revealed, for both species, a significant shape variation among sexes (Table 2). To better visualize the shape variation associated with sexual dimorphism, we only considered the consensus configuration for each sex in each sampling locality. Thus, the 10 consensus configurations per species were subjected to relative warps analysis. For both species, sexes are clearly separated in the shape space as shown by the MANOVA (Fig. 4). Interestingly, deformation grids expressing the range of shape variation along the axes indicate that sexual dimorphism concerns the same part of the body for both species. In particular, the heads of males are more elongated than those of females. In Fig. 4, grid a2 illustrates this elongation for males of *C. auronitens* and grid b2 shows the relative head contraction for the female of *C. nemoralis*. In addition, in both species, the posterior abdominal segments of females appear to be more enlarged than those of males. This trend is also visible for males in grid a2 and for females in grid b2 (Fig. 4).

Shape differentiation among populations

MANOVAS revealed, for both species, a significant shape variation among sites (Table 2). Figure 5 shows that, when the relative warps analysis is limited to the consensus configuration of each sampling locality, the individuals from the CHAUX site exhibit a clear shape differentiation. Deformation grids indicate a similar pattern of deformation for both sexes. In females, grid a1 displays strongly curved vertical lines at the level of the prothorax, which indicates a forward movement of the related landmarks and therefore the elongation of the prothorax for the specimens from the CHAUX site (Fig. 5). Correspondingly, a slight compression of the head occurred. The same morphological trends also pertained to males, as illustrated by grid b1, which expresses opposite deformations to those of specimens from the CHAUX site.

On the whole, Mantel tests revealed, for both sexes, a significant positive correlation between morphological and geographical distances between populations when considering the overall shape space (males: Mantel *t*-test: 1.798, $r = 0.84$, $P = 0.0361$; females: Mantel *t*-test: 2.091, $r = 0.95$, $P = 0.0183$). In other words, this means that geographically more distant specimens are more differentiated in terms of shape.

For *C. nemoralis*, the geographically most distant site (FERT) was not, morphologically, the most differentiated. Instead, the smaller site (VERN), and to a lesser extent the CIT-A site, appeared more distant in the shape space of the first three relative warps (Fig. 6). Deformation grids indicated a similar pattern of differentiation for both sexes, although weaker for males. As expected, Mantel tests did not reveal any significant correlation between morphological and geographical

Table 2 Results, for both species, of the two-way MANOVAs performed on the individual scores obtained on relative warps representing 95% of the total variation. Degrees of freedom of numerator and denominator are denoted 'Num. d.f.' and 'Den. d.f.', respectively.

Source of variation	<i>C. auronitens</i>					<i>C. nemoralis</i>				
	Wilks Λ	F	Num. d.f.	Den. d.f.	P*	Wilks Λ	F	Num. d.f.	Den. d.f.	P*
Sex	0.112	38.45	26	126	< 0.001	0.108	39.03	28	133	< 0.001
Site	0.004	15.0	104	502.41	< 0.001	0.008	11.10	112	530.81	< 0.001
Site \times sex	0.118	3.44	104	502.41	< 0.001	0.476	0.97	112	530.81	ns

*Significance levels: ns, not significant.

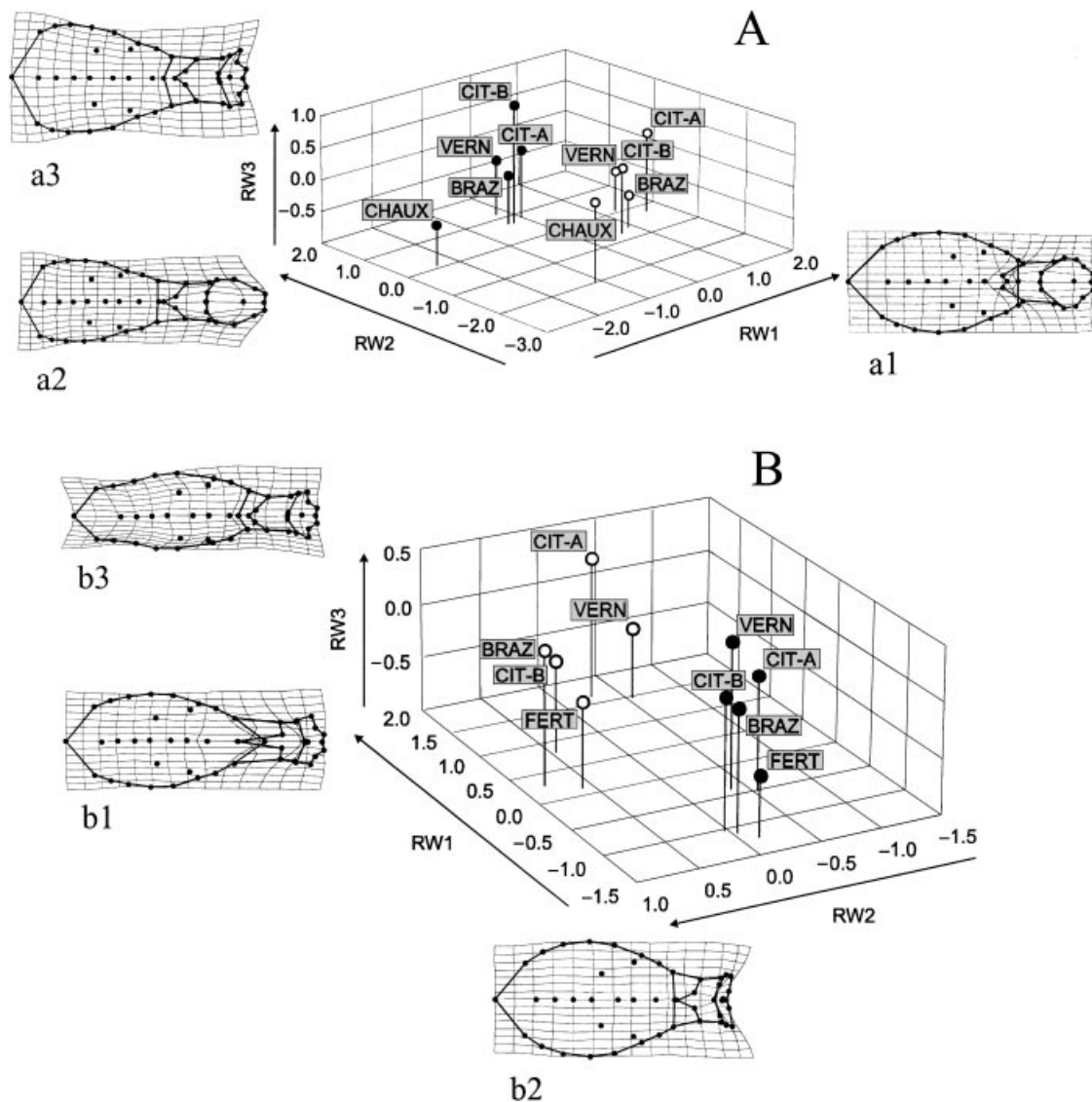


Fig. 4 Relative positions of the consensus configurations, for each site and sex, in the shape space defined by the first three relative warps. —A. *C. auronitens*. —B. *C. nemoralis*. Open circles correspond to the female samples and filled circles to the male samples. Deformation grids indicate which landmarks are implied for each axis definition and express the maximal shape variation along these axes in showing positive deformations. Negative deformations correspond to displacement of landmarks in the opposite direction on the grids. Scores on the relative warps are $\times 100$ and deformation grids are magnified three times.

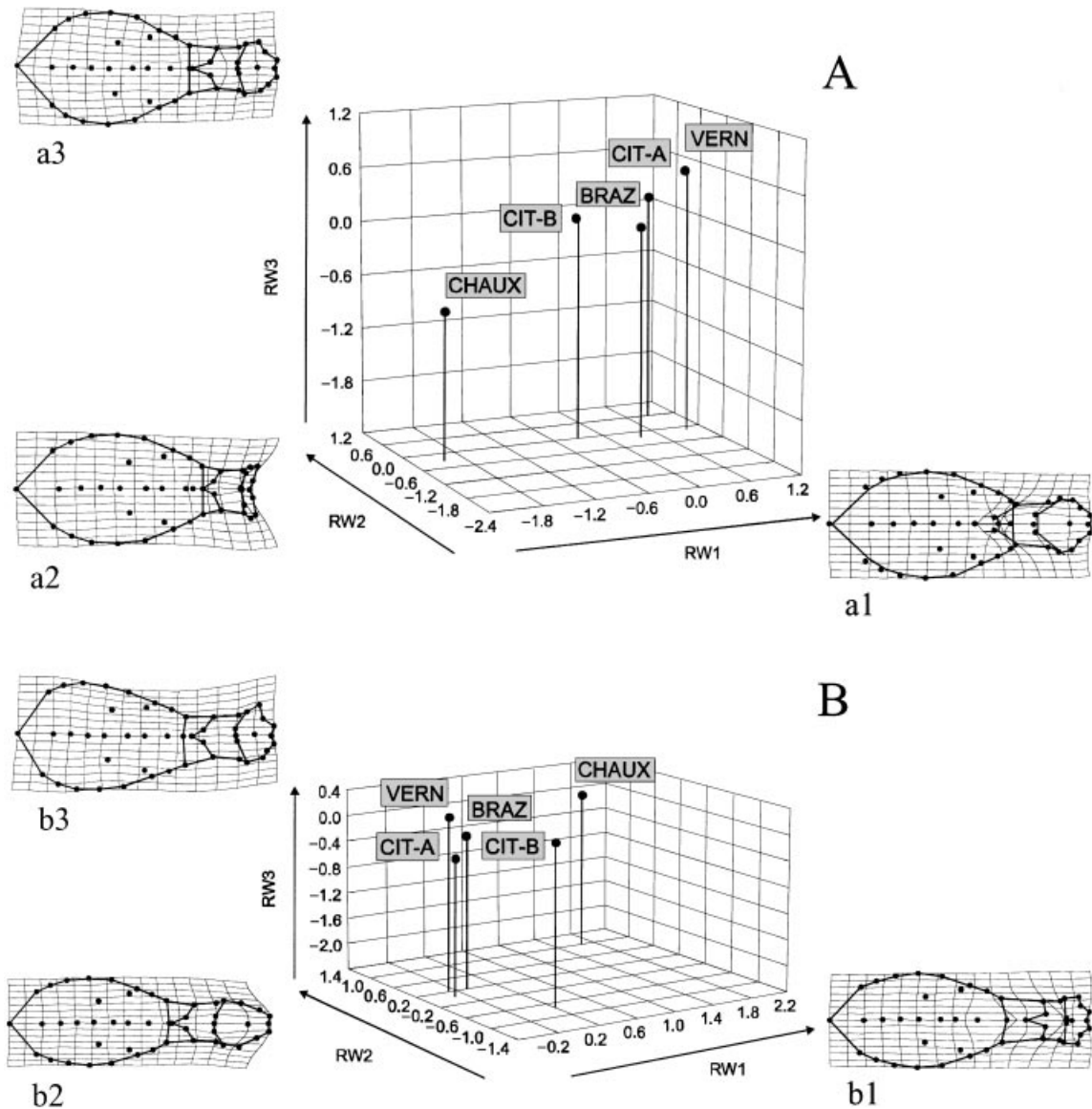


Fig. 5 Relative positions of the average configurations of the five sites for *C. auronitens* in the shape space defined by the first three relative warps. —A. Females. —B. Males. See legend of Fig. 4 for explanation of the deformation grids. Scores on the relative warps are $\times 100$ and deformation grids are magnified three times for females and twice for males.

distances between populations (males: Mantel *t*-test: 0.334, $r = 0.12$, not significant; females: Mantel *t*-test: -0.176 , $r = -0.043$, not significant).

Finally, as shown in Table 2, interaction between sex and site effects tested in the MANOVAS was not significant for *C. nemoralis*, whereas it appeared highly significant for *C. auronitens* (Wilks $\Lambda_{104,502.41} = 0.118$, $F = 3.44$, $P < 0.001$). This can be explained by a biased sex ratio for this species (see Table 1) in the most differentiated sample in terms of shape (CHAUX site).

Size variation

The two size estimators (centroid size and euclidian distance between the landmarks 1 and 14) produced rather similar results; therefore, only those concerning centroid size are presented here. Not surprisingly, a clear sexual dimorphism in size was detected. For both species, females were significantly larger than males when tested on the all-individual data set as well as within each studied site (Table 3). Figure 7 shows, for both species and both sexes, the variation in centroid size among sites. *C. auronitens* samples were homogeneous

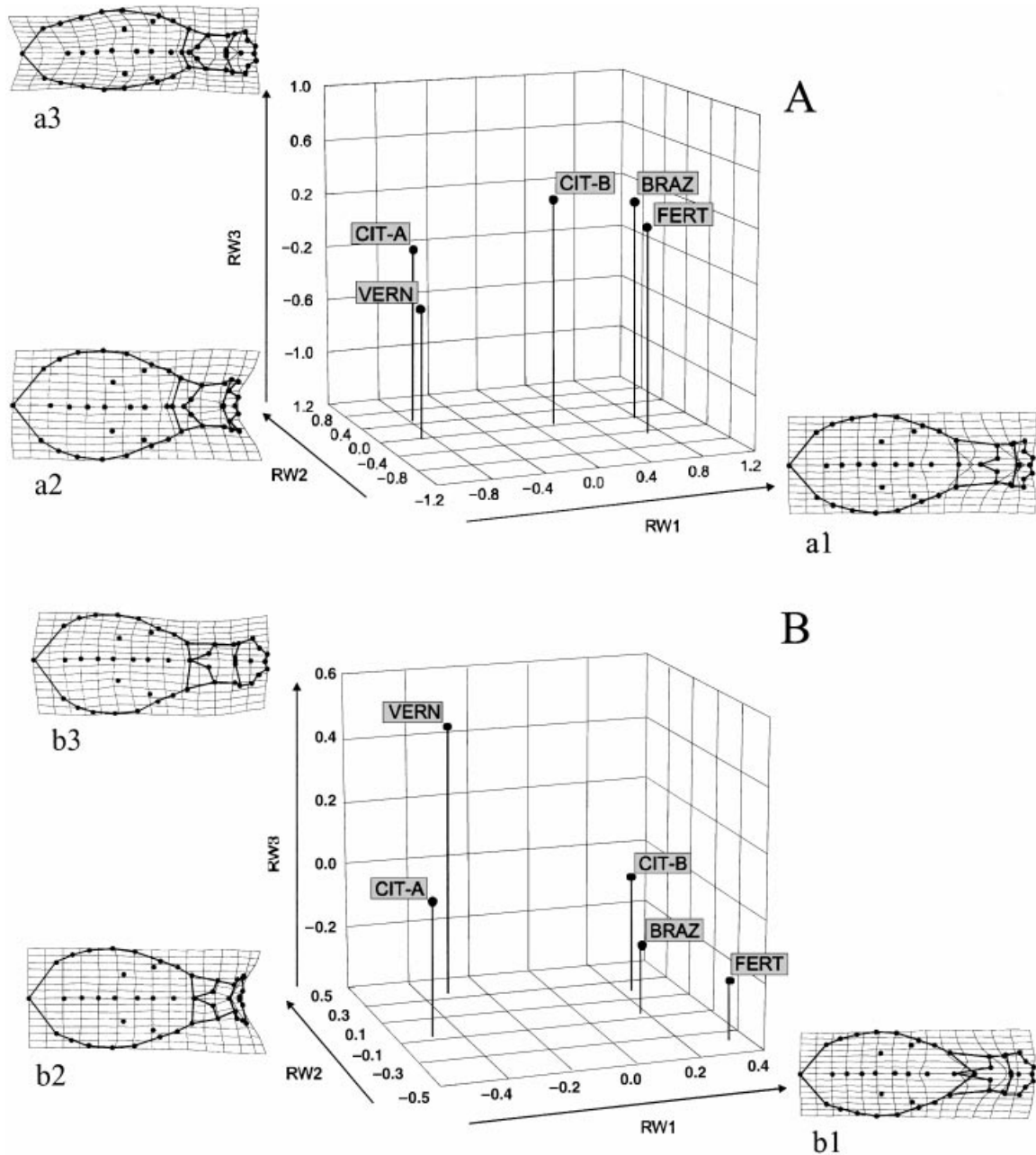


Fig. 6 Relative positions of the average configurations of the five sites for *C. nemoralis* in the shape space defined by the first three relative warps. —A. Females. —B. Males. See legend of Fig. 4 for explanation of the deformation grids. Scores on the relative warps are $\times 100$ and deformation grids are magnified 2.5 times for females and three times for males.

with regard to size (only one comparison among sites was significant), whereas a relatively high number of comparisons (seven out of 20) were significant for *C. nemoralis*. For this species, individuals from BRAZ, and to a lesser extent those from CIT-B, were larger than the others, but differences were more pronounced for females than for males (Fig. 7).

Discussion

Morphological differentiation and geographical distance

The main objective of this study was to use a geometrical morphometric approach to detect space-related morphological changes within two species of ground beetle. We found, at least for *C. auronitens*, measurable morphological changes between populations. The body shape of specimens sampled

Source of variation	<i>C. auronitens</i>				<i>C. nemoralis</i>			
	F	Num. d.f.	Den. d.f.	P*	F	Num. d.f.	Den. d.f.	P*
All specimens	260.77	1	312	< 0.001	237.56	1	330	< 0.001
CIT-A	54.77	1	67	< 0.001	21.90	1	66	< 0.001
CIT-B	64.18	1	68	< 0.001	70.15	1	65	< 0.001
BRAZ	65.37	1	68	< 0.001	58.76	1	67	< 0.001
VERN	118.51	1	68	< 0.001	51.39	1	63	< 0.001
CHAUX	16.26	1	41	< 0.001	—	—	—	—
FERT	—	—	—	—	50.43	1	69	< 0.001

*Significance levels.

Table 3 Results, for both species, of the ANOVAS testing differences between the centroid size of males and females. Degrees of freedom of numerator and denominator are denoted 'Num. d.f.' and 'Den. d.f.', respectively.

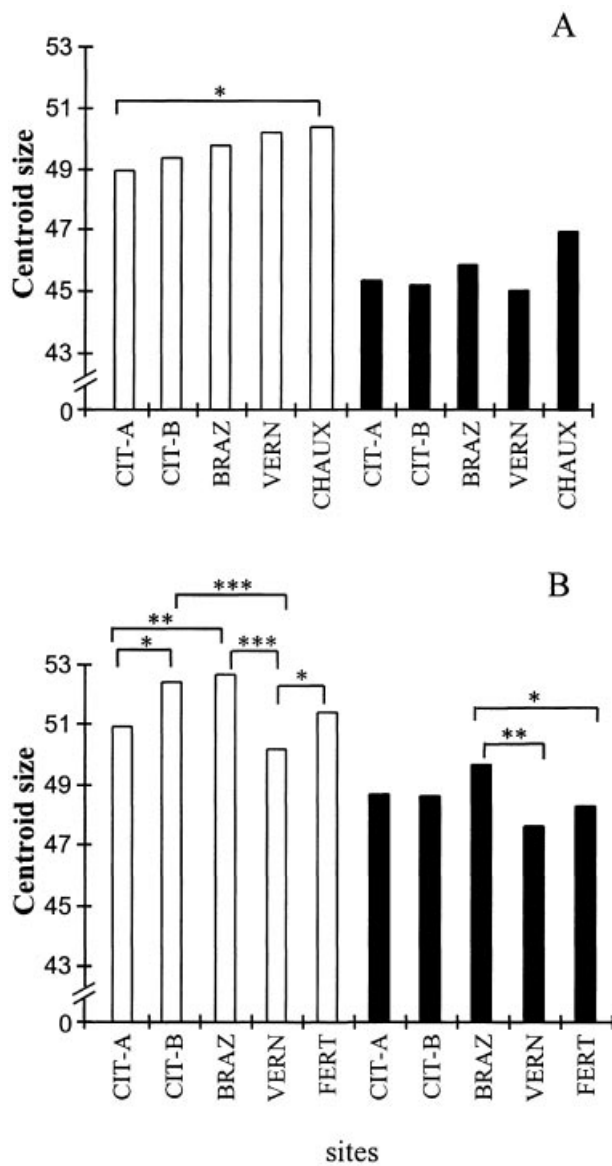


Fig. 7 Variation in centroid size among sites for both sexes. —A. *C. auronitens*. —B. *C. nemoralis*. Open bars, female samples; filled bars, male samples. Asterisks indicate when differences among sites are significant: * $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$.

in the geographically most distant site (CHAUX site) was clearly differentiated from that of specimens originating from sites located 45 km apart; on the whole, the amount of morphological divergence between populations was significantly correlated to their geographical distance. These results were found for both sexes. We believe that such shape differences are the consequence of isolation and fragmentation, rather than simply a response to local environmental differences between sampling sites. One argument in favour of our results is that morphological differences between significantly differentiated sites concern only shape, but not size. It is generally considered that a variation in size between populations largely depends on environmental conditions, whereas a variation in shape reflects variation in the genetic constitution (see, for example, Patton & Smith 1989; Adams & Funk 1997). It is thus reasonable to consider that the shape changes reported here reflect a genetic differentiation between the Chaux forest population and those from the Cîteaux forest and its surroundings. Moreover, our results are in agreement with the available data concerning the population genetics of *C. auronitens*. On the basis of a study of variability at four electrophoretic loci, Assman *et al.* (1994) have proposed a phylogeographical scenario for the colonization of Europe after the last glaciation period. These authors postulate several refuge areas in southern France from which populations expanded their area to northern France and middle Europe. During this postglacial recolonization, the populations of *C. auronitens* would have undergone several bottlenecks, which would have been responsible for the genetic differentiation and reduced genetic variability observed in populations in central and northern France (Assman *et al.* 1994). Nowadays, the use of hypervariable genetic markers offers promising perspectives for the investigation of the genetic structure and population dynamics of beetles. In a pioneering work focusing on the population genetics of the endangered species *Carabus solieri*, Rasplus *et al.* (2000) have found, using microsatellite markers, fairly high genetic differentiation between the populations of the southern French Alps, even for those not too far apart (around 11 km apart). Fragmentation of the habitat of *C. solieri* could explain these low levels of gene flow (Rasplus *et al.* 2000). The use of such molecular markers would

allow us to test the congruence between morphological and genetic differentiation for *C. auronitens*.

At a closer scale, although we found a significant correlation between geographical and morphological distances, it is noteworthy that the various barriers separating sites do not play a major role in the differentiation of populations. Indeed, sites which are separated from the Cîteaux forest by barriers (VERN and, more conspicuously, BRAZ; Fig. 1) are not morphologically more differentiated than those located inside this continuous forest (sites CIT-A and CIT-B). Such a result may have several nonexclusive interpretations. First, differentiation among populations exists but the morphometric approach used was not powerful enough to detect it. A study of genetic markers would be helpful to test such a hypothesis. Second, the time elapsed since the sites BRAZ and VERN were isolated from the main part of Cîteaux forest has not been long enough to allow a measurable divergence between populations. As mentioned above, these sites have been isolated for at least 300 years. Most of the barriers are, of course, much more recent as the highways, railways and canal shown in Fig. 1, and the intensive exploitation of agricultural fields, all occurred during the 20th century. Third, despite the brachyptery, both species studied have a non-negligible dispersal ability. The rare data concerning *C. nemoralis* indicate that this species does not present a strict association with forests and would be able to explore more open landscapes, such as set-aside or even arable habitats (Kennedy 1994). The fact that the geographically most distant site of our study (FERT) was not morphologically the most differentiated supports the idea that *C. nemoralis* would be able to move over relatively long distances in a heterogeneous environment. Concerning *C. auronitens*, although this species is more stenotopic and strictly bound to forest (Assman *et al.* 1994; Niehues *et al.* 1996), it could also be a better colonist than is generally believed (Niehues *et al.* 1996).

Sexual dimorphism

Another objective of the present study was to provide additional elements concerning morphological variation between sexes. As expected, we found an important female-biased sexual dimorphism in size for both *C. auronitens* and *C. nemoralis*. More interestingly, the geometrical morphometrics allowed us to show a sexual dimorphism of the shape, in particular for the posterior abdominal segments, which appeared to be more enlarged for the females of the two species. This sexual abdominal shape dimorphism, which has been reported for several groups of insects (Adams & Funk 1997), has been hypothesized to result from a positive correlation between fecundity and female abdomen size, and hence from a selection for the increase of fecundity (Wickman & Karlsson 1989; Adams & Funk 1997). This, as well as allometric trajectories, remains to be studied in the *Carabus* group.

Conclusions

Geometrical morphometrics, in identifying and quantifying biodiversity through the computation of morphospaces or disparity estimates, appears to be powerful enough to assess morphological variations at all taxonomic levels, including the intraspecific level. This latter characteristic offers a particularly interesting perspective, as intraspecific variation has until now mainly been assessed from a genetic viewpoint, and there is obviously a need to extend such an approach to phenotypic traits such as morphology. Our results demonstrate that geometrical morphometric methods provide valuable tools for the study of morphological variation among populations and thus offer promising perspectives for various problematics, such as the assessment of the impact of habitat fragmentation on species and, more generally, the study of biodiversity patterns. The present study provides additional support for the idea that geometrical morphometric methods have the potential to become one of the most powerful techniques for describing variation below the species level (Loy 1996). Moreover, in this work, we applied only one method of the several available in geometrical morphometrics. Other relative warps analyses, such as those offering the possibility to assign a given weight to large-scale or small-scale deformations on the specimen, may allow more accurate decomposition of the morphological variation in uniform and nonuniform shape transformations (Bookstein 1991; Rohlf 1993a). These techniques also allow a broader use of multivariate analyses (Bookstein 1996). Finally, it is worth reiterating that geometrical morphometrics also allows us to study conjointly numerous characters, as it proposes, in addition to the study of size, an overall assessment of the shape of organisms. The more traits taken into account, the better the estimation of morphological diversity.

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