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Research Article

Ecological character displacement in mandibular morphology of three sympatric horseshoe bats

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Abstract

To understand how interactions among three medium-sized rhinolophid species, *Rhinolophus blasii*, *R. euryale* and *R. mehelyi* affect the evolution of their phenotype we studied the variation of morphological patterns in allopatric and sympatric populations. We used 2D landmark-based geometric morphometrics to test whether shape and size of the skull and the mandible change when in sympatry. To disentangle interspecific interactions from sexual dimorphism and effects of environmental gradients the dataset was controlled for sex and geographical variables (e.g. longitude). Our study revealed two main morphological change patterns: (i) ecological character displacement in mandible shape and size and (ii) ecological sexual dimorphism in mandible shape. No patterns of morphological change in size or shape of the lateral or ventral skull views were detected either in sympatry or allopatry or along the geographical gradient. Our results suggested the coexistence of *R. blasii*, *R. euryale* and *R. mehelyi* is likely due to dietary separation but we cannot rule out that it might be facilitated by a combination of factors including different habitat use, commuting distances, behavioural strategies and prey-capture methods.

Introduction

Morphological characters in relation to species ecology have been frequently used to examine interspecific interactions, such as resource partitioning that could be an evolutionary response to competition between species. According to the competitive exclusion principle (Grinnell, 1917; Gause, 1934), the same ecological niche cannot be occupied at the same time by two or more species. Often the niche concept is defined as the multivariate set of environmental features used by one species within a certain biotic setting (Crow et al., 2009). Thus, species occurring in sympatry are expected to display resource partitioning either by different habitat use or by food partitioning. The phenotypic outcomes of this differentiation may be reflected by divergent echolocation call frequencies in insular rhinolophids (Russo et al., 2007) or subtle differences in the external morphology e.g. shape of the beaks in Darwin's finches from the Galapagos, (Abbott et al., 1977), or the mandibles of tiger beetles (Hori, 1982).

The evolution of traits under selective pressure towards reduced competition for resources is called character displacement (Pfennig and Pfennig, 2009) and it explains why species with the same ecological needs have different morphologies in the contact zone (Brown and Wilson, 1956). The study of character displacement among closely related species can provide a better understanding upon (i) the evolutionary mechanism of species coexistence, (ii) the changes that occur during or shortly after the speciation processes, and (iii) the way diversity is maintained and the manner in which the interspecific interactions are influencing geographic distribution of species and assembly of communities (Grant, 1994; Pfennig and Pfennig, 2009).

Differences in skull size and/or morphology can be explained by adaptation to ecological factors (Aldridge and Rautenbach, 1987; Evans and Sanson, 2005; Freeman, 1981) or by competition (Dayan and Simberloff, 1998; Weinbeer and Kalko, 2004). Mammalian skull performs multiple functions, e.g., feeding and brain and sensory organs protection (Cheverud, 1981; Hallgrimsson et al., 2007); bats are ideal group to investigate the drivers of mammal skull diversity since bats represent 20% of the mammal species and encompass the full spectrum of sensory ecologies and diets of mammals. Studies have shown that at least among some *Myotis* species there is strong competition for food resources (Arlettaz, 1995; Gannon and Rácz, 2006; Husar, 1976) which may result in character displacement. Thus, one would expect that traits experiencing strong selection to reduce the competition between sympatric populations of different species are trophic traits (Gannon and Rácz, 2006). The cranial size of *Plecotus auritus* was found to vary in the presence of a congeneric competitor, *P. austriacus*, supposedly as a result of a change in the hardness of the diet (Postawa et al., 2012).

In the case of the European horseshoe bat species, studies have shown that differences in wing morphology (Dietz et al., 2006; Salsamendi et al., 2005), echolocation calls (Heller and von Helversen, 1989; Russo et al., 2001; Salsamendi et al., 2005; Siemers et al., 2005), habitat use (Aihartza et al., 2003; Bontadina et al., 2002; Goiti et al., 2003, 2008; Jones and Rayner, 1989; Russo et al., 2002, 2005; Salsamendi et al., 2005) and body-size parameters such as mandible length (Popov and Ivanova, 2002) evolved to allow coexistence. However,

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the mechanism by which niche differentiation takes place in sympatric horseshoe bats remains elusive.

In Europe three medium-sized rhinolophid species (*Rhinolophus blasii*, *R. euryale* and *R. mehelyi*) occur either in sympatry or in allopatry. The sibling species *R. mehelyi* and *R. euryale* have diverged from the same ancestor 3 My ago (Guillén et al., 2003). However, molecular studies (Zhou et al., 2009) have shown that *R. blasii* falls within a distinct clade despite morphological similarities with the former species. *R. euryale* is known from the Early Pliocene (Salari, 2011) while *R. blasii* and *R. mehelyi* appear in the fossil records in the Early and Middle Pleistocene, respectively (Tata and Kotsakis, 2005). Likewise, a paleontological record shows that the three species have co-existed in Turkey beginning with the Middle Pleistocene (Lindenau, 2005). All three species are mainly cave-dwellers (Dietz et al., 2007) and prey predominately on moths (Goiti et al., 2004; Whitaker and Black, 1976).

In ecological and behavioural studies *R. euryale* and *R. mehelyi* have received more attention than *R. blasii*. Russo et al. (2005) and Salsamendi et al. (2012b) found support for interspecific divergence of foraging habitats of *R. euryale* and *R. mehelyi* caused by differences in wing morphology. Radio-telemetry studies revealed that these sibling species are both foraging in and along forest edges in allopatric conditions (Goiti et al., 2008; Salsamendi et al., 2012b), while in sympatry they tend to segregate with *R. mehelyi* foraging in less complex habitats of savannah-type (Russo et al., 2005; Salsamendi et al., 2012b). It was also shown that differences in echolocation call characteristics of *R. euryale* and *R. mehelyi* are presumably too small to allow any dietary partitioning (Salsamendi et al., 2012b).

R. euryale and *R. mehelyi* are well documented in terms of both diet (Sharifi and Hemmati, 2001; Goiti et al., 2004; Sharifi and Hemmati, 2004; Salsamendi et al., 2008, 2012a) and habitat use (Russo et al., 2002; Aihartza et al., 2003; Russo et al., 2005; Goiti et al., 2008; Salsamendi et al., 2012b). The diet of *R. euryale* and *R. mehelyi* was also studied in sympatry vs allopatry. Salsamendi et al. (2008, 2012b) and Goiti et al. (2008) found a low divergence in the diet and variation in prey volume between allopatric and sympatric populations of *R. euryale* and *R. mehelyi*. *R. mehelyi* consumes more moths than *R. euryale* (95% and 98% vs. 85% and 93%), whereas the latter shows a more diversified diet in sympatry. However, *R. mehelyi* may select larger prey or hard-bodied insects like Coleoptera (Sharifi and Hemmati, 2004) facilitated by differences in skull and jaw morphology.

The third morphologically similar species, *R. blasii*, is seldom included in ecomorphological studies (see Dietz et al., 2006; Popov and Ivanova, 2002) and its habitat preference remains unknown (Dietz et al., 2007; Siemers and Ivanova, 2004). *R. blasii*, probably because is the rarest horseshoe bat species in Europe (Krystufek and Dulic, 2001) remained over the years an enigmatic species. No new data on its dietary preferences have been reported since the first investigation of Whitaker and Black (1976) who established that it preys almost exclusively on moths.

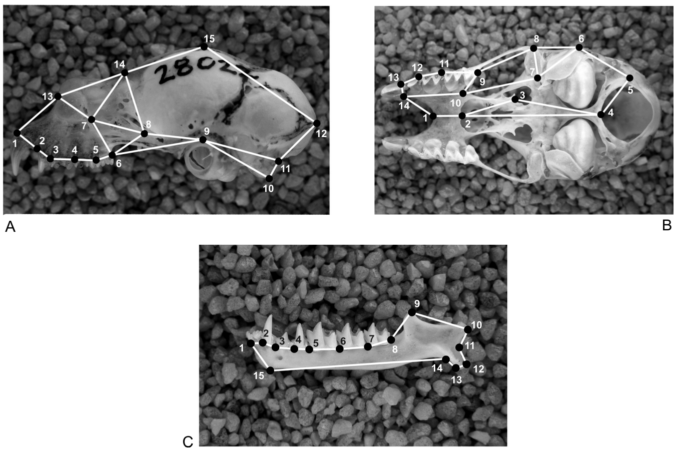

Figure 1 – Landmarks of the lateral (A) and the ventral (B) skull, and of the mandible (C) in *Rhinolophus*.

However, these three medium-sized horseshoe bats — having very similar external and craniodental characters, foraging strategy and extensively overlapping distribution in the Mediterranean Basin — are good model organisms to be studied both in sympatry and allopatry. To understand how interactions among the three medium-sized rhinolophid species affect the evolution of their phenotype we studied the variation of morphological patterns in allopatric and sympatric populations of *R. euryale* and *R. mehelyi* and sympatric population of *R. blasii*. Using 2D landmark-based geometric morphometrics we tested whether the shape and the size of the skull (in lateral and ventral views) and the mandible change when in sympatry. Change in cranial morphology, i.e. character displacement among these species would suggest trophic separation, whilst the lack of character displacement would suggest that the species' coexistence is rather possible due to behavioural differences.

Material and methods

Specimens and geometric morphometrics

We used 181 adult specimens of the three medium-sized horseshoe bat species *R. euryale* (n=83), *R. mehelyi* (n=58) and *R. blasii* (n=40) from 53 localities throughout their geographic range. Excepting a few specimens kept in private collections all specimens are stored in the collections of the Naturhistorisches Museum Wien (NHMW) and in the Hungarian Natural History Museum (HNHM) (see Supplemental Material S1 for details). For each bat species we attributed the specimens from areas where one species is present and the other two species are absent to allopatric populations and specimens from areas where one species co-occur with the other two species to sympatric populations. This resulted in one allopatric (Allo) and one sympatric (Symp) population for *R. euryale* (Allo: n=46; Symp: n=37) and *R. mehelyi* (Allo: n=21; Symp: n=37) and one sympatric population for *R. blasii* (Symp: n=40). Since the distribution range of the three medium-sized horseshoe bats is shrinking to establish areas of allopatry and sympatry we used the distribution map generated based on both the sampled localities and data from literature (see for example Benda et al., 2003, 2006; Dobson, 1876, 1878; Gaisler, 2001a,b,c; Hanak et al., 2001; Harrison and Bates, 1991; Niazi, 1976).

To test for morphological change among the three bat species we used landmark-based 2D geometric morphometrics of the cranium. For each specimen we took digital photographs of the buccal view of the left mandible and of the left lateral and the ventral side of the skull. All photographs were taken using the same settings and we applied the same scale factor (i.e., equal 1) to all of them. Using thin-plate digitising analysis software (`tpsDig2` – Rohlf, 2004) we digitised fifteen landmarks on the mandible and the lateral side of the skull and fourteen landmarks on the ventral side of the skull (Fig. 1 and Tab. 1). However, the results for the lateral skull views should be considered with caution given that the cranium is a highly 3D structure and the lack of coplanarity may affect in particular landmarks 5–9–14 (Cardini, 2014). For each specimen the landmarks were digitised three times on the same set of photographs. For the analysis we used the average of the three replicates.

We conducted a Generalized Procrustes Analysis (GPA) to remove translational differences, minimize the rotation and standardize the size using the package "`geomorph`" (Adams et al., 2018) for R (R Core Team, 2017). The GPA involves an optimal superimposition of landmark configurations based on a least-squares algorithm whereby differences in landmark coordinates due to the position of specimens during the digitisation process were minimised and the size was standardised. The GPA was done separately for the mandible, the lateral and ventral skull data set. The new Cartesian coordinates obtained after the superimposition are the shape variables. To test for the presence of allometry the resulting shape variables were used in a multivariate regression against the centroid size (Drake and Klingenberg, 2008). Centroid size (i.e. the square root of the sum of squared distances of each landmark from the centroid of the configuration) and the Procrustes distance

Table 1 – Description of landmarks used to evaluate cranium and mandible size and shape variation.

Landmark	Description
Cranium, lateral skull	
1	Most anterior point on the upper canine alveolus
2	Most anterior point on the first upper premolar alveolus
3	Point on the lateral margin of the maxilla at the anterior end of the second premolar
4	Point on the lateral margin of the maxilla at the anterior end of the first molar
5	Point on the lateral margin of the maxilla at the anterior end of the second molar
6	Most postero-lateral point on alveolus of the third upper molar
7	Lacrimal foramen
8	Optic foramen
9	Widest point of the cranium where zygomatic arch originates from the squamosal bone
10	Most ventral point of occipital process
11	Most ventral point of occipital bone, posterior to the paroccipital process
12	Intersection of the sagittal and lamboid crests
13	Anterior most point of nasal bones along dorsal midline
14	Anterior most point of sagittal crest on the midline of the skull
15	Most dorsal point of the sagittal crest
Cranium, ventral skull	
1	Anterior border of the hard palatine excluding the premaxilla along the midline
2	Suture between the palatines at the midline
3	Most posterior point of the foramen ovale
4	Most anterior point on the foramen magnum at the midline
5	Most lateral point of the occipital condyle
6	Posterior edge of external auditory meatus
7	Posterolateral border of the vomer
8	Widest point of the cranium at the zygomatic arch
9	Most postero-lateral point on alveolus of the third upper molar
10	Anterolateral border of the vomer
11	Most posterior and lateral point of the first molar
12	Most anterior point on the second premolar
13	Most anterior point on the first premolar
14	Most posterior point on the first premolar
Mandible	
1	Anterior border of canine alveolus
2	Posterior border of canine alveolus
3	Anterior border of the first molar alveolus
4	Anterior border of the second molar alveolus
5	Anterior border of the third molar alveolus
6	Anterior border of the fifth molar alveolus
7	Anterior border of the seventh molar alveolus
8	Posterior border of the last molar alveolus
9	Lateral most extension of the coronoid process
10	Anterolateral tip of condylar process
11	Point of the extreme curvature at the sigmoid notch
12	Anterolateral tip of angular process
13	Posterolateral border of angular process
14	Posterior-most point on the baseline
15	Anterior lower border of the mandible

(i.e. the square root of the sum of the squared distances between the corresponding pairs of landmarks) of each specimen were calculated using "`shapes`" R package (Dryden, 2017). The size and shape measurement error for each dataset separately on bat species was estimated using one-way analysis of variance (ANOVA) of centroid size of all replicates and Procrustes ANOVA of the shape coordinates of all replicates, respectively, considering individuals as the source of variation (Klingenberg and McIntyre, 1998). Both ANOVAs were performed in Morpho J 1.05d software.

Shape analyses

We ran a principal components analysis (PCA) to evaluate general trends in shape space of the lateral and ventral skull views and the mandible across species; the within-species covariances were generated and PCA was performed on the latter. Only the first two PCs were considered since the other PCs included less than 8% of total variance. We performed a Canonical Variates Analysis (CVA) to test for significant interaction between pre-defined groups: (i) species (*R. euryale*, *R. mehelyi* and *R. blasii*) and population (Allo vs Symp), which implies ecological interactions among bat species and reflects morphological change, i.e. character displacement; and (ii) species, sex (female vs male) and population, which indicates whether character displacement differs between sexes. To visualise the magnitudes and the overall shape change we plotted the wireframe graphs in Morpho J 1.05d software. We performed linear models (LMs) on the Procrustes distance (PD) to investigate the role of the geographical gradient. In the models PD was introduced as a response variable and longitude (range: from -5.99 to 45.05) as a covariate.

Size analyses

We ran LMs on the centroid size (response variable) of lateral and ventral skull and mandible where species, sex and population were treated as predictor variables and the longitude was included as a covariate. When significant differences were found the least-significant difference (LSD) analysis was used for post-hoc multiple comparisons. The LMs were fitted using the "`stats`" package (R Core Team, 2017) and LSD were carried out using the "`lsmeans`" package (Lenth, 2016).

Results

Shape and size measurement error (among replicates variation) was lower than intraspecific (among individuals) variation both for the lateral and ventral skull and mandible (Tab. 2). The shape variables were not significantly correlated with centroid size ($p>0.05$) either for lateral and ventral skull or mandible and consequently, the size-non corrected shape variables were used for further analyses (Klingenberg and McIntyre, 1998).

PC1 and PC2 of the analysis of the lateral skull explained 35.66 and 12.30% of the total variance, respectively. The PCA scatterplot showed a broad superposition of species. However, it is important to note the placement of *R. euryale* on the upper left quadrant, *R. blasii* on the lower right quadrant, and *R. mehelyi* in intermediate positions of the graph, in general terms tended to be separated by *R. euryale*. PC1 and PC2 from the analysis of the ventral skull explained 36.00 and 8.62% of the total variance, respectively (Fig. 2). Compared to the lateral skull views, *R. blasii* was placed in the negative end of the PC1, *R. mehelyi* in the positive end of the PC1 and *R. euryale* was placed in intermediate position, broadly overlapping with the other two species (Fig. 2). In the analysis of the mandible, PC1 and PC2 explained 22.92 and 14.83% of the total variance, respectively. All the three species broadly overlapped (Fig. 2). Wireframe graphs (Fig. 3) showed a significant displacements of landmarks from the mean shape in both lateral and ventral skull and mandible.

The CVA to test for significant differences between population within species showed significantly differences in mandible shape but not in the lateral or ventral skull shape (Tab. 3). This pattern was also observed between populations within species and sexes (Tab. 4). The wireframe graphs showed also little overall shape change of the lateral and ventral skull between allopatric and sympatric populations both within species and sexes (Fig. 4, 5 and 6). For mandible in *R. euryale*, in both sexes the average mandible shape of allopatric populations compared to that of sympatric populations displayed differences in the relative position of: (i) the canine, i.e. in allopatric populations this particular tooth was positioned more anteriorly than in sympatric population; (ii) coronoid process, i.e. in allopatric populations the coronoid process was lower than in sympatric populations and (iii) articular process, i.e. in allopatric populations the articular process was positioned lower than in sympatric populations (Fig. 4 and 5). For mandible in *R. mehelyi*, in both sexes the average mandible shape of allopatric populations compared to that of sympatric populations displayed differences in the relative position of: (i) the canine, i.e. in allopatric populations the tooth was positioned more posteriorly than in sympatric population; (ii) coronoid process, i.e. in allopatric populations the coronoid

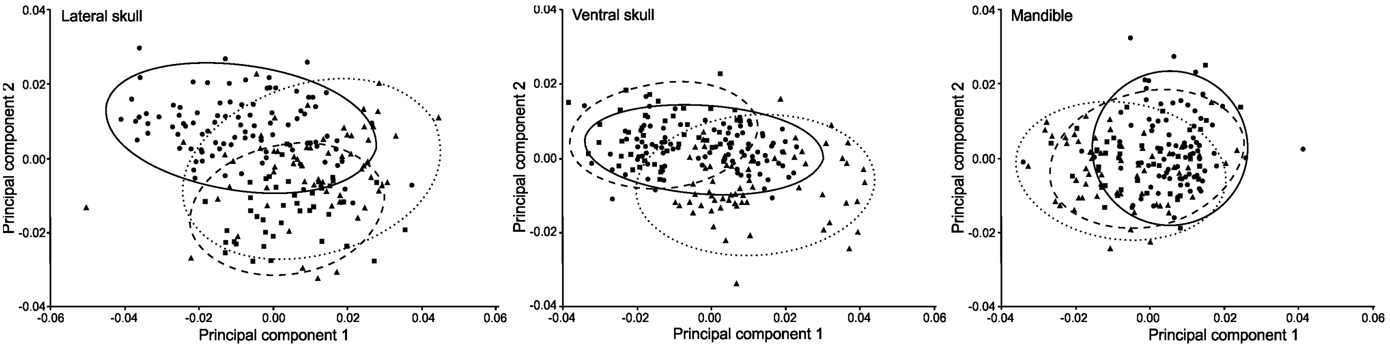

Figure 2 – Principal components analysis of the lateral and ventral skull views and the mandible for all specimens included in our analysis showing shape separation between *Rhinolophus euryale* (closed circle), *R. mehelyi* (triangle) and *R. blasii* (square). Ellipses indicate 95% confidence morphospace boundaries for each species, *R. euryale* (solid line), *R. mehelyi* (dashed line) and *R. blasii* (dotted line).

Table 2 – Procrustes ANOVA for testing the significance of intraspecific (among individuals) variation and measurements error (among replicates variation) for the lateral and ventral skull and mandible of *Rhinolophus*.

		Procustes distance				**Centroid size**			
		MS	**df**	**F**	*p*	**MS**	**df**	**F**	*p*
Mandibule									
R. euryale	Individual	0.000 064 779 6	2470	35.22	<0.0001	<0.000 000 000 1	95	0.67	0.984
	Error	0.000 001 839 3	4992			<0.000 000 000 01	192		
R. mehelyi	Individual	0.000 080 955 0	1638	14.41	<0.000 17	<0.000 000 000 1	63	1.18	0.230
	Error	0.000 005 617 0	3328			<0.000 000 000 01	128		
R. blasii	Individual	0.000 063 454 0	1092	31.76	<0.0001	<0.000 000 000 1	42	0.91	0.619
	Error	0.000 001 997 9	2236			<0.000 000 000 01	86		
Ventral skull									
R. euryale	Individual	0.000 083 396 0	2470	14.98	<0.0001	<0.000 000 000 1	95	1.14	0.222
	Error	0.000 005 566 0	4992			<0.000 000 000 01	192		
R. mehelyi	Individual	0.000 115 099 1	1638	4.98	<0.0001	<0.000 000 000 1	63	1.23	0.160
	Error	0.000 023 110 5	3328			<0.000 000 000 01	128		
R. blasii	Individual	0.000 061 563 8	1092	29.86	<0.0001	<0.000 000 000 1	42	0.78	0.811
	Error	0.000 002 061 6	2236			<0.000 000 000 01	86		
Lateral skull									
R. euryale	Individual	0.000 068 615 2	2280	26.74	<0.0001	<0.000 000 000 1	95	1.13	0.234
	Error	0.000 002 566 4	4608			<0.000 000 000 01	192		
R. mehelyi	Individual	0.000 080 553 1	1512	8.73	<0.0001	<0.000 000 000 1	63	0.71	0.938
	Error	0.000 009 229 3	3072			<0.000 000 000 01	128		
R. blasii	Individual	0.000 062 312 5	1008	14.18	<0.0001	<0.000 000 000 1	42	1.74	0.016
	Error	0.000 004 395 9	2064			<0.000 000 000 01	86		

process was higher than in sympatric populations and (iii) articular process, i.e., in allopatric populations the articular process was positioned higher than in sympatric populations (Fig. 4 and 6).

Longitude had no significant effect on PD (lateral skull: $F_{(1,170)}=0.470$, $p=0.494$; ventral skull: $F_{(1,170)}=1.526$, $p=0.230$; mandible: $F_{(1,170)}=0.327$, $p=0.568$) or CS (lateral skull: $F_{(1,170)}=0.276$, $p=0.6$; ventral skull: $F_{(1,170)}=0.771$, $p=0.38$; mandible: $F_{(1,170)}=0.380$, $p=0.539$) suggesting no changes in shape or size along a geographical gradient.

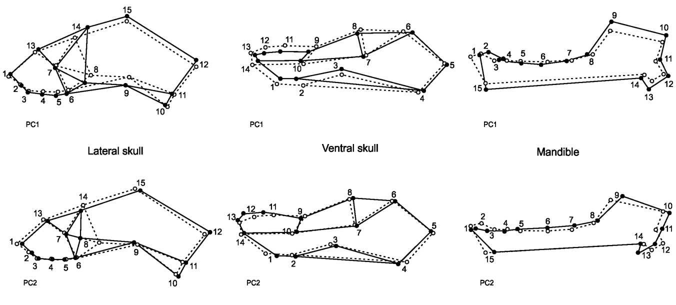

Figure 3 – Wireframe representations of lateral and ventral skull views and the mandible showing shape changes from the average (solid line) to the target shape (dotted line) for the first two Principal Component axes.

LMM showed that for the lateral and ventral skull none of the main or interaction effects on CS were significant (Supplementary Table S2). For mandible the analyses revealed an almost significant interaction effect between species and population on centroid size ($F_{(1,170)}=37.264$, $p=0.055$).The LDS analysis on centroid size showed that the mandible was significantly larger in allopatric populations than in sympatric populations for *R. euryale* (0.0000000365±0.0000000231, df=177, t=1.581, $p=0.05$) and not significantly smaller for *R. mehelyi* (-0.0000000291±0.0000000286, df=177, t=-1.018, $p=0.310$) (Fig. 7).

Discussion

Our study revealed two main morphological change patterns: (i) ecological character displacement in mandible shape and size and (ii) ecological sexual dimorphism in mandible shape. No patterns of morphological change in size or shape were supported for the lateral or ventral skull views in either sympatry or allopatry or along geographical gradients.

Character displacement is typically demonstrated as greater morphological differences among species in sympatry than in allopatry. We found that interspecific interactions among the three sympatric species of medium-sized horseshoe bats contributed significantly to differences in mandible morphology, i.e. shape and size for *R. euryale* and shape for *R. mehelyi*. The most likely hypothesis is that competitive interactions for food cause interspecific differences in mandibular morphology

Table 3 – Pairwise Procrustes distances (below the diagonal) and *p*-values for the null hypothesis of equal means (above the diagonal) for combination of species *Rhinolophus euryale, R. mehelyi* and *R. blasii* and population: allopatric (Allo) and sympatric (Symp) for the lateral and ventral skull and mandible.

Lateral skull	*R. euryale* Allo	*R. euryale* Symp	*R. mehelyi* Allo	*R. mehelyi* Symp	*R. blasii* Symp
R. euryale Allo		0.4739	<0.0001	<0.0001	<0.0001
R. euryale Symp	0.0056		<0.0001	<0.0001	<0.0001
R. mehelyi Allo	0.0293	0.0302		0.8209	<0.0001
R. mehelyi Symp	0.0326	0.0333	0.0066		<0.0001
R. blasii Symp	0.0322	0.0344	0.0374	0.039	

Ventral skull	*R. euryale* Allo	*R. euryale* Symp	*R. mehelyi* Allo	*R. mehelyi* Symp	*R. blasii* Symp
R. euryale Allo		0.7947	<0.0001	<0.0001	<0.0001
R. euryale Symp	0.0041		<0.0001	<0.0001	<0.0001
R. mehelyi Allo	0.0242	0.0225		0.1631	<0.0001
R. mehelyi Symp	0.0232	0.0214	0.0078		<0.0001
R. blasii Symp	0.0389	0.0383	0.0429	0.041	

Mandible	*R. euryale* Allo	*R. euryale* Symp	*R. mehelyi* Allo	*R. mehelyi* Symp	*R. blasii* Symp
R. euryale Allo		<0.0001	<0.0001	<0.0001	<0.0001
R. euryale Symp	0.0124		<0.0001	<0.0001	<0.0001
R. mehelyi Allo	0.0219	0.019		<0.0001	<0.0001
R. mehelyi Symp	0.0279	0.0243	0.0154		<0.0001
R. blasii Symp	0.0269	0.024	0.0303	0.0301	

of *R. euryale* and *R. mehelyi*. This hypothesis is supported also by the lack of change in the morphology of the lateral or ventral skull views in either sympatric or allopatric areas. The mammalian skull performs multiple functions, e.g., feeding and protection of brain and sensory organs. Thus, the differential variation of the cranium if compared to the mandible, is likely a consequence of a different role of the cranium.

Evidence of character displacement in our study is in line with the results of similar studies on *Myotis evotis* and *M. auriculus* in North America (Aldridge and Rautenbach, 1987) and *Plecotus auritus* and *P. austriacus* (Postawa et al., 2012) in Europe. These studies have reported morphological character displacement in sympatric populations of these insectivorous bats due to a possible change in their diet. Studies examining the diet of the target bat species have shown a general specialization towards a diet composed of moths. Moreover, since the interspecific interactions were present when the effect of environmental gradients was controlled, the observed changes in mandible morphology are unlikely caused by different environmental features or by different food resources in sympatric than in allopatric areas. Thus our results challenges the findings of previous studies which suggested that the coexistence of these sympatric medium-sized horseshoe bats is determined by different local conditions derived from different habitat types available for foraging. Studies conducted in the Iberian Peninsula concluded that the two sympatric sibling species (*R. euryale* and *R. mehelyi*) coexist rather due to displacement in the spatial niche dimension than food niche differentiation (Salsamendi et al., 2012b). Additionally, in an experimental study Voigt et al (2010) advanced the idea that energy cost during flight is an important determinant of a bat's feeding niche. Certainly, the higher wing loading of *R. mehelyi* not only reduces its manoeuvrability but it also influences the species' feeding behaviour. Compared to *R. euryale*, *R. mehelyi* hunts predominantly by perch-hunting, an energy-conserving manner (Dietz et al., 2009). Regarding wing morphology, *R. blasii* and *R. euryale* are very similar suggesting comparable flight capabilities (Dietz, 2007). Yet, *R. blasii* shows a higher behavioural flexibility in prey-capture than *R. euryale* and *R. mehelyi* (Siemers and Ivanova, 2004), and consequently has the highest flexibility in the habitat use among the three studied horseshoe bat species. Thus we cannot rule out that the coexistence these sympatric medium-sized horseshoe bats might be facilitated by a combination of other factors such as habitat use, commuting distances, behavioural strategies and prey-capture methods.

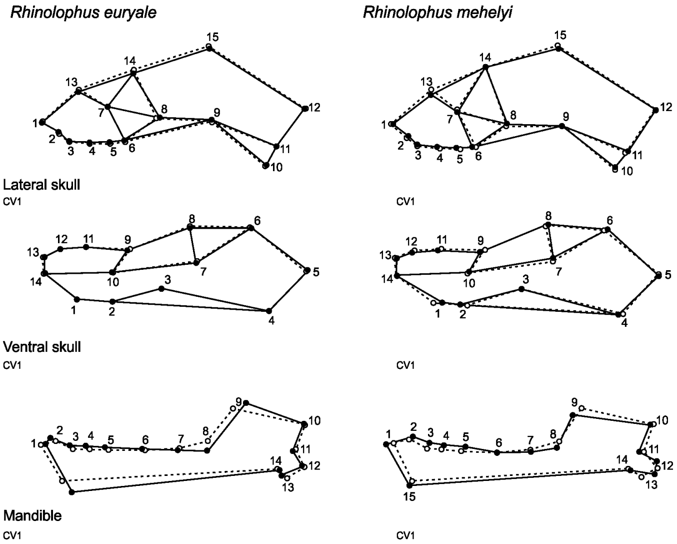

Figure 4 – Wireframe representations of lateral and ventral skull views and the mandible shape differences between allopatric (solid line) and sympatric (dotted line) populations of *Rhinolophus euryale* and *R. mehelyi.* Shape variations are magnified ca. 7 times.

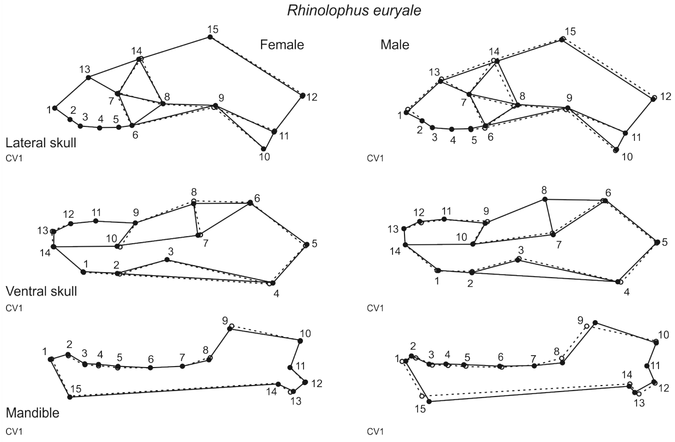

Figure 5 – Wireframe representations of lateral and ventral skull views and the mandible shape differences between allopatric (solid line) and sympatric (dotted line) populations of *Rhinolophus euryale,* with sexes separated. Shape variations are magnified ca. 7 times.

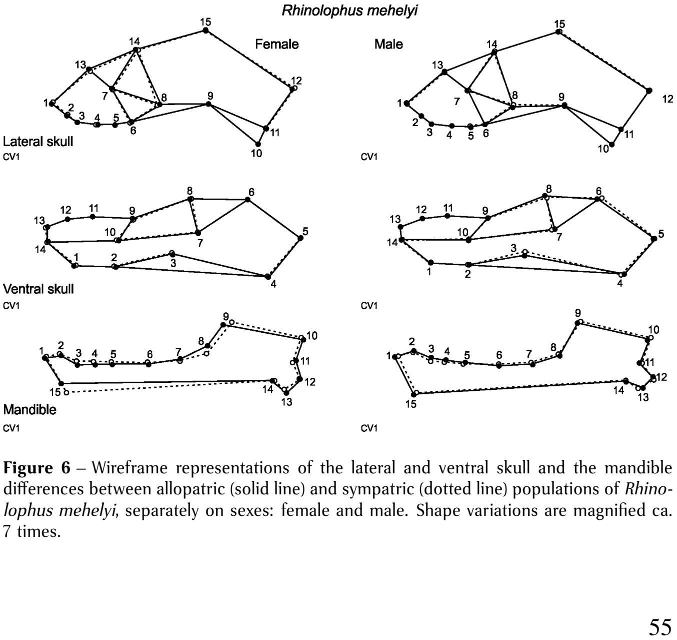

Figure 6 – Wireframe representations of the lateral and ventral skull and the mandible differences between allopatric (solid line) and sympatric (dotted line) populations of *Rhinolophus mehelyi,* separately on sexes: female and male. Shape variations are magnified ca. 7 times.

Table 4 – Pairwise Procrustes distances (below the diagonal) and P - values for the null hypothesis of equal means (above the diagonal) for combination of species: Rhinolophus euryale, R. mehelyi and R. blasii, sexes: female (F) and male (M) and population: allopatric (Allo) and sympatric (Symp) for the lateral and ventral skull and mandible.

Lateral skull	*R. euryale* F Allo	*R. euryale* F Symp	*R. euryale* M Allo	*R. euryale* M Symp	*R. mehelyi* F Allo	*R. mehelyi* F Symp	*R. mehelyi* M Allo	*R. mehelyi* M Symp	*R. blasii* F Symp	*R. blasii* M Symp
R. euryale F Allo		0.2639	0.1908	0.2143	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale F Symp	0.0093		0.5711	0.2372	0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale M Allo	0.0098	0.0073		0.3015	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale M Symp	0.0097	0.0096	0.0089		0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. mehelyi F Allo	0.0243	0.0262	0.0252	0.025 47		0.3043	0.0285	0.0775	<0.0001	<0.0001
R. mehelyi F Symp	0.0315	0.0345	0.034	0.0322	0.0157		0.9412	0.6449	<0.0001	<0.0001
R. mehelyi M Allo	0.0328	0.0362	0.0359	0.0333	0.0182	0.008		0.5376	<0.0001	<0.0001
R. mehelyi M Symp	0.0329	0.0352	0.035	0.0329	0.016	0.0093	0.0094		<0.0001	<0.0001
R. blasii F Symp	0.0333	0.036	0.0319	0.0336	0.0352	0.0402	0.041	0.0398		0.9068
R. blasii M Symp	0.0336	0.0358	0.0317	0.0332	0.035	0.0389	0.04	0.0386	0.0058	

Ventral skull	*R. euryale* F Allo	*R. euryale* F Symp	*R. euryale* M Allo	*R. euryale* M Symp	*R. mehelyi* F Allo	*R. mehelyi* F Symp	*R. mehelyi* M Allo	*R. mehelyi* M Symp	*R. blasii* F Symp	*R. blasii* M Symp
R. euryale F Allo		0.3092	0.4872	0.8199	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale F Symp	0.0079		0.7589	0.0506	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale M Allo	0.0076	0.0057		0.1813	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
R. euryale M Symp	0.0058	0.0097	0.0096		0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. mehelyi F Allo	0.0245	0.0204	0.0237	0.0251		0.2963	0.7533	0.7948	<0.0001	<0.0001
R. mehelyi F Symp	0.0253	0.022	0.0253	0.0258	0.0106		0.1736	0.4354	<0.0001	<0.0001
R. mehelyi M Allo	0.0266	0.0222	0.0251	0.0275	0.0084	0.0114		0.259	<0.0001	<0.0001
R. mehelyi M Symp	0.0227	0.0193	0.0224	0.0236	0.0071	0.008	0.0098		<0.0001	<0.0001
R. blasii F Symp	0.0395	0.0412	0.041	0.0376	0.0437	0.0406	0.0433	0.0425		0.8646
R. blasii M Symp	0.0371	0.0392	0.0388	0.0348	0.0429	0.0401	0.0429	0.0416	0.006	

Mandible	*R. euryale* F Allo	*R. euryale* F Symp	*R. euryale* M Allo	*R. euryale* M Symp	*R. mehelyi* F Allo	*R. mehelyi* F Symp	*R. mehelyi* M Allo	*R. mehelyi* M Symp	*R. blasii* F Symp	*R. blasii* M Symp
R. euryale F Allo		0.0003	0.3858	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale F Symp	0.0079		0.0011	0.31	0.0003	<0.0001	0.0073	<0.0001	<0.0001	<0.0001
R. euryale M Allo	0.0076	0.0057		0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. euryale M Symp	0.0058	0.0097	0.0096		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R. mehelyi F Allo	0.0245	0.0204	0.0237	0.0251		0.0054	0.3486	0.0048	<0.0001	<0.0001
R. mehelyi F Symp	0.0253	0.022	0.0253	0.0258	0.0106		0.0007	0.0596	<0.0001	<0.0001
R. mehelyi M Allo	0.0266	0.0222	0.0251	0.0275	0.0084	0.0114		0.0096	<0.0001	<0.0001
R. mehelyi M Symp	0.0227	0.0193	0.0224	0.0236	0.0071	0.008	0.0098		<0.0001	<0.0001
R. blasii F Symp	0.0284	0.0248	0.0259	0.0229	0.03	0.0304	0.0295	0.0275		0.3612
R. blasii M Symp	0.0283	0.0267	0.0254	0.024	0.0332	0.0345	0.0317	0.0304	0.0076	

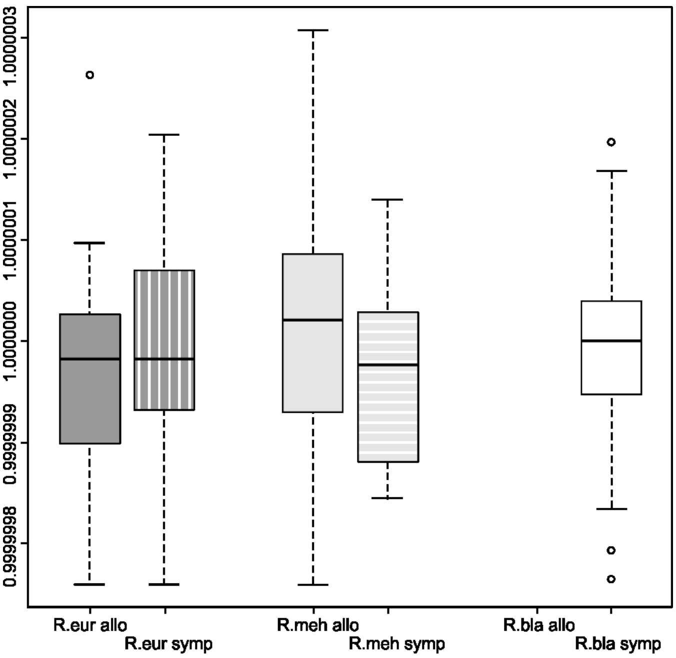

Figure 7 – Pairwise centroid sizes of the mandible for combination of allopatric and sympatric populations for *Rhinolophus euryale* (R.eur allo (dark grey); R.eur symp (dark grey with white vertical stripes)) and *R. mehelyi* (R.meh allo (light grey); R.meh symp (light grey and white horizontal stripes)) and sympatric population for *R. blasii* (R.bla symp (white)).

We found evidence for sex-specific divergence between allopatry and sympatry. Resource completion acts as a driver of sexually antagonist selection. Thus the presence of ecological sexual dimorphism implies that competition-driven character displacement had occurred between sexes (De Lisle and Rowe, 2015). Several studies focusing on insectivorous mammals indicated an association of the prey size (Aldridge and Rautenbach, 1987), food consistency (Evans and Sanson, 2005), or both factors (Freeman, 1981; R Core Team, 2017) with differences in the morphological features. These structures may evolve differently in similar species when distribution of the species overlaps and the species compete for the same resources. Jaws are the primary trophic structures on which natural selection acts. A study examining the morphological differences between *M. evotis* and *M. auriculus* detected character displacement both in size and shape of the jaws (Gannon and Rácz, 2006). The jaw morphology differences were emphasized in sympatry, *M. evotis* shifting to trophic architecture that allow better capturing hard-bodied prey whilst *M. auriculus*, on the contrary, soft-bodied prey. In two extremely similar insectivorous bats, *M. evotis* and *M. auriculus*, competition for food in sympatry was found to be avoided by change in food preferences of *M. evotis*. Furthermore, both species exhibited differences in food preferences between sexes in allopatry, suggesting that in both species niche subdivision occurs within species only when the potential competitor is absent. On the contrary, in our study the differences in mandible shape within sexes between allopatric and sympatric populations of both *R. euryale* and *R. mehelyi* indicate that the intraspecific niche subdivision occurs in these species only in the presence of the potential competitor. However, our study fails to take into account the more parsimonious hypothesis (Shine, 1989) for the evolution of sexual dimorphism, i.e., sexes have diverged as the result sex-biased selection with no direct outcome from resource competition.

We conclude that ecological character displacement and ecological sexual dimorphism due to competition for food are likely the causal mechanisms underlying the morphological differences between sympatric and allopatric populations of these three horseshoe bat species. Nevertheless, to provide empirical evidence the direct link between food preferences and mandibular morphology should be tested. Thus we suggest that the coexistence of *R. blasii*, *R. euryale* and *R. mehelyi* is due to dietary separation but we cannot rule out that it might be facilitated by a combination of factors including different habitat use, commuting distances, behavioural strategies and prey-capture methods.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Supplemental Material S1 List of specimens investigated.

Supplementary Table S2 Influence of species, sex, population and two and three-way interactions on centroid sizes.