

# Can functional traits help explain the coexistence of two species of *Apodemus*?

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Sustainable coexistence of similar, related species is generally expected to be achieved through character displacement, resulting in niche partitioning. The species *Apodemus sylvaticus* and *Apodemus flavicollis* are very similar both morphologically and ecologically, and have a large geographical overlap. Whether functional or biomechanical differences between these two species contribute to their coexistence remains unknown. A biomechanical model was created based on muscle data derived from dissections to estimate the maximum bite force. In addition, the dental microwear was analysed to test for evidence of a divergence in diet. Finally, geometric morphometric approaches were used to compare mandibular shapes. The results indicate that *A. flavicollis*, the slightly larger species, is optimized for biting at a larger gape angle. *Apodemus sylvaticus* appears to be slightly more specialized for grinding and biting at a narrower gape angle. However, the majority of shape variation in the mandible across both species follows the same pattern. No significant differences in microwear were observed between species, and thus, they appear to consume similar food types. These results suggest that character divergence resulting in niche partitioning has not occurred, possibly due to low resource competition. Alternatively, resource partitioning may occur through behavioural differences or differences in activity patterns.

ADDITIONAL KEYWORDS: biomechanics – ecology – mandible – morphology – rodent – sympatry.

## INTRODUCTION

The coexistence of similar, related species is expected to drive character displacement, and niche partitioning (Brown & Wilson, 1956; Schoener, 1989; Dayan & Simberloff, 1998). Some criteria have been suggested as necessary to allow sustainable sympatry of similar, related species such as the Hutchinson ratio of a linear size difference of at least 1.3 for mammals and birds

(Hutchinson, 1959). Related species may additionally require character divergence to sustainably coexist (Brown & Wilson, 1956; Okuzaki, Takami & Sota, 2010). Species often diverge along character axes that are directly related to the resources that are being partitioned (Adams & Rohlf, 2000; Grant & Grant, 2006). An example is the middle ground finch, *Geospiza fortis*, which diverged in beak size after the arrival of a competitor species (*Geospiza magnirostris*) on the same island (Grant & Grant, 2006). Competition for dietary resources is therefore an important selective pressure that may drive adaptive differences in feeding

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structures, resulting in differences in function that allow the exploitation of different resources (Adams & Rohlf, 2000; Lalis, Evin & Denys, 2009) unless resources are very abundant (Bauduin *et al.*, 2013).

The two species of wood mice considered in the present study, *Apodemus sylvaticus* (Linnaeus, 1758) and *Apodemus flavicollis* (Melchior, 1834), have overlapping ranges and are commonly found in sympatry, including syntopy, across Western Europe (Michaux *et al.*, 2001; Bugarski-Stanojević *et al.*, 2008). These species are morphologically very similar, such that molecular tests are required to confirm species identity (Michaux *et al.*, 2001). Although *A. sylvaticus* and *A. flavicollis* are closely related (Bugarski-Stanojević *et al.*, 2008), they do not appear to be sister species (Makova, Nekrutenko & Baker, 2000; Michaux *et al.*, 2002; Janzekovic & Krystufek, 2004). In addition, there is no evidence from either morphometric, molecular or experimental data that these species hybridize (Engländer & Amtmann, 1963; Jewell & Fullagar, 1965; Niethammer, 1969; Serizawa, Suzuki & Tsuchiya, 2000; Michaux *et al.*, 2001; Filippucci, Macholán & Michaux, 2002). In temperate Europe, the genus *Apodemus* is the dominant rodent present in most woodland and shrub habitats, even near their distribution limits (Kryštufek & Vohralík, 2007; Loman, 2008). These species preferentially feed on a variety of nuts and seeds, but will also eat fruit, insects and plant matter (Smal & Fairley, 1980; Rogers & Gorman, 1995; Loman, 2008). In Europe, *A. flavicollis* is a woodland species, inhabiting mature forests (Marsh & Harris, 2000), with some studies showing higher abundance in ancient woodland (Marsh, Poulton & Harris, 2001). This species is almost always found in sympatry with at least one other *Apodemus* species, the sympatric specie(s) varying throughout its range (Filippucci *et al.*, 2002; Kryštufek & Vohralík, 2007). *Apodemus sylvaticus* inhabits a wider range of habitats ranging from open grasslands and arable fields to early growth forests, anthropogenic forests and mature woodland (Amori & Contoli, 1994; Filippucci *et al.*, 2002; Barčiová & Macholán, 2006). It is more abundant in smaller forest islands as it prefers woodland edges and arable matrix close to woodland (García *et al.*, 1998). In woodlands, the height and cover of herbaceous plants affect its abundance (Montgomery, 1980; Marsh & Harris, 2000) and it is generally thought to prefer seeds from deciduous trees (Jensen, 1985).

A previous study on *A. flavicollis* and *A. sylvaticus* in Prague did not reveal character displacement for populations in sympatry. There were, however, differences between central urban and peripheral populations of *A. sylvaticus* (Mikulová & Frynta, 2001), probably due to the isolation and extreme conditions the central

populations experience due to urbanism. A study on the broad latitudinal differences in *A. sylvaticus* moreover suggested trends in mandible shape and size, with a reduced coronoid and angular processes in more northern populations (Renaud & Michaux, 2003). As the coronoid and the angular processes are important insertion sites for the jaw adductors, this suggests that these species may vary in the utilization of the available resources across their range based on differences in jaw function. Adaptive mandible shape change induced by diet as a selection pressure is also known to occur in other rodent species (Renaud, Chevret & Michaux, 2007).

Here, we investigate whether *A. flavicollis* and *A. sylvaticus* show differences in masticatory biomechanics that might facilitate their coexistence by allowing the use of different resources. We compared the mandible shape, the masticatory muscle morphology and dental microwear and used a biomechanical model to calculate functional traits that may help explain how these two species are able to coexist.

## MATERIAL AND METHODS

### SPECIMENS

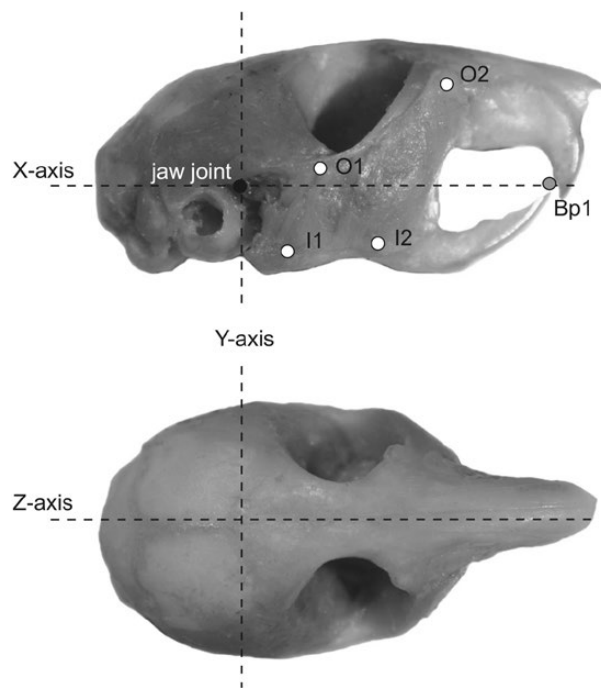
Three data sets are used in the present study: a larger sample to analyse mandible morphology, a subset of these to analyse dental microwear and an even smaller subset for which dissections were performed. All of the specimens in this study were adults and were genetically identified to species level. In total, 119 specimens were used for the morphometric analysis, 54 specimens were used for microwear analysis and 15 specimens were used for dissection and bite force modelling (Table 1).

Specimens that were used for dissection were trapped in the wild at two sites in France. The individuals from a site close to Orléans were genetically identified as *A. flavicollis* and were trapped over a period of 3 days in June 2008. The site is within a forest of tall, broad-leaved trees, mostly oak and beech. The specimens genetically identified as *A. sylvaticus* were trapped at the Parc de la Faisanderie within the forest of Sénart (3000 ha), about 26 km south-east of central Paris, over a 3-day period in May 2008. The park is a lawn area of about 1.5 acres with a few bushes and surrounded on all sides by forest. There are recreational fields about 0.3 km away through the forest from the park, with suburban area surrounding all sides of the forest. The wider sample of genotyped individuals used for morphometrics and microwear analyses included specimens from four sites across Belgium, France and Spain. The sites were Dalhem in Belgium, Montpellier in the south of France, and

**Table 1.** Three data sets of genotyped specimens were used in this study

Site	Species	Microwear	Coronoid	Non-coronoid
Belgium	<i>Apodemus sylvaticus</i>	12	14	na
Montpellier	<i>Apodemus sylvaticus</i>	10	19	na
Montseny	<i>Apodemus flavicollis</i>	7	22	na
	<i>Apodemus sylvaticus</i>	8	38	na
Murcia	<i>Apodemus sylvaticus</i>	10	14	na
Orleans	<i>Apodemus flavicollis</i>	3	4	5
Senart	<i>Apodemus sylvaticus</i>	4	8	10
Total		54	119	15

Specimens with undamaged mandibles were chosen to compare morphology ( $N = 119$ ) in a data set, which included a landmark on the tip of the coronoid. A subset of these ( $N = 54$ ) was used for a microwear analysis. A smaller set of specimens ( $N = 15$ ) from two sites from Northern France (Senart and Orleans) was used to obtain the muscle data needed for the biomechanical model. An additional morphological analysis was performed for these two sites, including some specimens with the coronoid tip missing ( $N = 15$ ). Microwear = number of specimens used for microwear analysis, coronoid = number of specimens included in the morphological analysis including landmark 29; non-coronoid = number of specimens included in the morphological analysis excluding landmark 29. na, not applicable.



**Figure 1.** Lateral view (top) and dorsal view (bottom) photograph of the skull and jaw muscles of *Apodemus sylvaticus*. Indicated are the X-, Y- and Z-axes, the jaw joint and a bite point on the incisor (Bp1). Also indicated are the origin and insertion of the anterior (O2, I2) and posterior (O1, I1) parts of the superficial masseter. The coordinates determined on dorsal and lateral view images were then used as input for a biomechanical model to estimate bite force.

Murcia and Montseny in Spain (Table 1). Although at many sites the genotyped specimens showed the presence of only a single species, this does not exclude the presence of the other species in low density. Extensive trapping campaigns and genotyping

would be required to test this. Preliminary tests showed no effect of geography on mandible shape in our sample despite the fact that previous studies on *A. sylvaticus* did show such patterns (Renaud & Michaux, 2003).

#### GENOTYPING

DNA was extracted from ethanol-preserved samples using the ‘DNeasy Blood and Tissue’ kit (Qiagen, France) following the manufacturer’s instructions. We used a PCR approach that is based on two sets of specific primers as described previously (Michaux *et al.*, 2001) to determine if our *Apodemus* specimens belong to the species *sylvaticus* or *flavicollis*.

#### DISSECTION

All muscles linking the mandible with the cranium were dissected, and muscle bundles were removed individually and preserved in 70% ethanol (Herrel *et al.*, 2008; see Fig. 1). Twelve muscle bundles were dissected: the digastricus, the superior masseter, the anterior deep masseter, the posterior deep masseter, the anterior zygomaticomandibularis, the posterior zygomaticomandibularis, the infra-orbital zygomaticomandibularis, the lateral temporalis, the medial temporalis, the temporalis pars suprazygomatica, the external pterygoid and the internal pterygoid (Cox & Jeffery, 2011; Baverstock, Jeffery & Cobb, 2013). Note that for some of the analyses muscles bundles were grouped into larger functional groups (masseter, zygomaticomandibularis, temporalis and pterygoid). After dissection, the hemi-mandibles were separated and cleaned for photography.

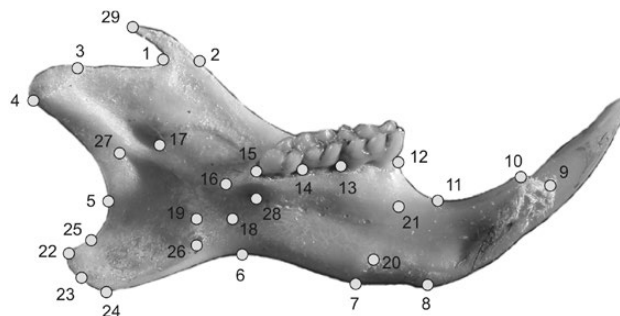
Muscles were blotted dry to remove excess alcohol and weighed using a Mettler AE100 electronic

balance ( $\pm 0.0001$  g). The muscles were then placed in a 30% nitric acid solution (Loeb & Gans, 1986) until the connective tissue was sufficiently dissolved that the fibres would separate easily when gently prodded with a pair of narrow tweezers. At this point, the nitric acid was removed and replaced by a 50% aqueous glycerol solution, and the fibres of each muscle bundle were photographed using a Leica macroscope Z6 ( $\times 0.5$  objective,  $\times 2$  zoom) coupled to a Leica digital camera (6 mega pixels). The lengths of ten to 15 fibres were measured using Image J. The volume of each muscle bundle was then calculated by dividing muscle mass by muscle density ( $1.06$  g/cm<sup>3</sup>; Mendez & Keys, 1960), and the physiological cross-sectional area (PCSA) was calculated by dividing the volume by fibre length.

#### BIOMECHANICAL MODEL

The model used is identical to that previously described (Cleuren, Aerts & De Vree, 1995; Herrel, Aerts & De Vree, 1998a, b; Herrel *et al.*, 2008) and relies on the computation of the static force equilibrium. The input for the model consists of the three-dimensional coordinates of origin and insertion, the PCSA of the jaw muscles and the three-dimensional coordinates of the point of application of the bite force and the centre of rotation (Fig. 1). The centroid area of insertion was used for muscle bundles with relatively broad areas of origin and insertion. The coordinates were determined from lateral, dorsal and ventral drawings made during the dissection using a stereomicroscope with *camera lucida*, and from photographs of skulls and mandibles taken using a Leica macroscope Z6 ( $\times 0.5$  objective, zoom  $\times 2$ ) attached to a Leica digital camera. Separate muscle and bite-point coordinates were taken for one specimen of *A. flavicollis* and *A. sylvaticus* each. Complex pennate muscles were separated into their component parts; therefore, no correction for pennation was included. Cross-sectional areas were scaled using a conservative muscle stress estimate of  $30$  N/cm (Herzog, 1994).

The simulations were run at gape angles of  $0^\circ$ ,  $30^\circ$  and  $60^\circ$  with all jaw closer muscles set as maximally active. We ran the model for each individual of each species using the measured variation in muscle PCSA. Bite forces were calculated at a range of orientations for the food reaction forces, and at two different bite points: the incisor and the first molar. Model output consists of the magnitude of the bite forces and joint forces and the orientation of the joint forces at any given orientation of the food reaction forces. The  $\log_{10}$ -transformed bite and joint forces and the joint force orientations were then used as input for multivariate analyses of variance (MANOVA) with species as fixed



**Figure 2.** The 29 anatomical landmarks taken on each mandible illustrated on a photograph of the medial side of the mandible of an *Apodemus*. The landmark descriptions are provided in Supporting Information, Table S1.

effect to test for differences in the functional output of the jaw system between species.

To validate the output of our model, we measured bite force from 18 individuals of *A. sylvaticus* caught in the field in Southern France using a piezoelectric force transducer (Kistler, type 9203, range 500 N; Kistler, Winterthur, Switzerland) attached to a handheld charge amplifier (Kistler, type 5995). The transducer was mounted between two bite plates as described by Herrel *et al.* (1999). The tips of both upper and lower bite plates were covered with a layer of cloth medical tape to provide a non-skid surface and to protect the teeth of the animals. The distance between the bite plates was adjusted to assure a gape of about  $20^\circ$ . Bite force was measured during molar biting. Five trials were conducted for each animal.

#### MORPHOMETRICS

The medial side of the left hemi-mandibles of each specimen was photographed using a Leica macroscope Z6 ( $\times 0.5$  objective,  $\times 2$  zoom) coupled to a Leica digital camera (6 mega pixels). Twenty-nine anatomical landmarks (Fig. 2; Supporting Information, Table S1) were digitized in this view using TPSDIG2 (Rohlf, 2010). The entire landmark set was used for the large sample ( $N = 119$  specimens). The morphometric analyses were performed in R using a generalized Procrustes analysis (GPA; Rohlf & Slice, 1990). GPA removes the variation among landmark configurations due to the isometric effects of size, position and orientation (Adams, Rohlf & Slice, 2004, 2013). The logarithm of the centroid size was used as an indicator of size, and allometric effects were tested by performing multivariate regressions in MorphoJ (Klingenberg, 2011; Table 2). A MANOVA testing for species differences was performed on 90% of the variability. This involved the first 22 principal components (PCs) of a principal component analyses (PCAs) performed on the Procrustes coordinates in MorphoJ (Klingenberg, 2011). A discriminant

function analysis was run on the morphometric data to test whether species could be separated based on their mandible shape. Covariation between mandibular shape and the muscle data (muscle mass, fibre length and PCSA) was tested using RV tests (Escoufier, 1973) across all individuals to investigate if mandible shape changes are linked to muscle differences.

#### MICROWEAR

This method permits the identification of the general diet and the type of food items ingested by the animal a few days prior to death (Walker, Hoeck & Perez, 1978; Teaford & Oyen, 1989). The protocol used (Merceron *et al.*, 2004, 2005) was one adapted to small mammal teeth (Rodrigues, Merceron & Viriot, 2009; Rodrigues, Marivaux & Vianey-Liaud, 2012). Translucent casts of the occlusal crowns of upper molars were photographed using transmitted light stereomicroscopy at  $\times 100$  magnification. The square sample area ( $0.01 \text{ mm}^2$ ) was centred on the lingual facet of the hypocone, or another homologous facet, as all tooth facets in murids are subject to the same antero-posterior (propalinal) masticatory movements. However, not all the specimens could be analysed due to the small size of dental facets, damage or cast artefacts. Microwear scars were counted and measured using ImageJ coupled to ObjectJ. Three variables were considered: the numbers of fine scratches (Nfs), wide scratches (Nws) and large pits (Nlp) (Grine, 1986; Supporting Information, Fig. S1). These data were compared to a database based on extant rodents (Rodrigues *et al.*, 2012), which were classified in three dietary categories: the animal-dominated feeders (ADF), the fruit-dominated feeders (FDF) and the grass-dominated feeders (GDF). A PCA of the *Apodemus* data and the comparative data set was performed on the three microwear variables to

infer the principal dietary component for each populations. Multiple means comparison tests, the Fischer's Least Significant Difference (LSD) test and the more conservative Tukey's Honestly Significant Difference (HSD) test, were used for rank-transformed variables (Conover & Iman, 1981) to determine the source of variation between species and dietary categories.

#### RESULTS

##### BIOMECHANICS OF BITING

The modelled bite forces for *A. flavicollis* ranged from 5.03 to 7.75 N at the molars at gapes of  $60^\circ$  and  $0^\circ$ , respectively. For *A. sylvaticus*, modelled bite forces were somewhat lower ranged from 4.62 to 6.98 N at the molars at gapes of  $60^\circ$  and  $0^\circ$ , respectively (Table 3). A comparison of biting on the molars at  $30^\circ$  in *A. sylvaticus* with the *in vivo* data shows good correspondence (model:  $6.42 \pm 1.51 \text{ N}$  vs.  $7.6 \pm 1.59 \text{ N}$  *in vivo*) suggesting that the model output gives a reliable estimate of *in vivo* data.

The MANOVA performed on the relative contribution of the different muscles to total bite force shows that there is a significant difference between the two species (Table 4). Univariate analyses of variance (Table 5) showed significant species differences for the anterior deep masseter, the temporalis anterior and temporalis posterior, the temporalis pars suprazygomatica (tps<sub>z</sub>) and the infra-orbital zygomaticomandibularis. Muscles with a higher contribution to the total bite force in *A. sylvaticus* include the temporalis anterior for gapes from  $0^\circ$  to  $60^\circ$ , the tps<sub>z</sub> at gapes from  $30^\circ$  to  $60^\circ$  and the anterior deep masseter, although only at a  $0^\circ$  gape. In *A. flavicollis*, muscles with a higher participation include the temporalis posterior and the infra-orbital part of the zygomaticomandibularis. The

**Table 2.** Regressions of shape (Procrustes coordinates) on centroid size to test for allometric effects

Species	d.f.	SS	MS	$R^2$	$F$	$Z$	$P$
All specimens							
Log(size)	1	0.019	0.019	0.062	7.92	6.45	<b>0.004</b>
Residuals	120	0.29	0.0024				
Total	121	0.31					
<i>Apodemus flavicollis</i> ( $n = 26$ )							
Log(size)	1	0.0030	0.0030	0.052	1.33	1.20	0.18
Residuals	24	0.055	0.0023				
Total	25	0.058					
<i>Apodemus sylvaticus</i> ( $n = 93$ )							
Log(size)	1	0.021	0.021	0.091	9.14	7.21	<b>0.004</b>
Residuals	91	0.21	0.0023				
Total	92	0.23					

Bold values represent significant allometric effects. d.f., degrees of freedom; MS, mean squares; SS, sum of squares.

**Table 3.** Summary of the calculated bite force for both species and at different gape angles

Species	Bite point	Gape	Bite force	Joint force	Angle joint force	<i>In vivo</i> bite force
<i>Apodemus flavicollis</i>	Molar	60	5.03 ± 1.44	3.12 ± 0.83	136.20 ± 4.42	7.6 ± 1.59
		30	7.02 ± 1.93	2.58 ± 0.57	137.12 ± 5.44	
		0	7.75 ± 2.16	2.33 ± 0.47	135.40 ± 3.77	
	Incisor	60	2.96 ± 0.85	4.13 ± 1.09	139.61 ± 3.38	
		30	4.13 ± 1.14	3.98 ± 0.96	131.06 ± 3.59	
		0	4.56 ± 1.27	3.64 ± 0.86	117.35 ± 2.89	
<i>Apodemus sylvaticus</i>	Molar	60	4.62 ± 1.12	3.27 ± 0.72	141.96 ± 2.22	7.6 ± 1.59
		30	6.42 ± 1.51	2.83 ± 0.62	142.96 ± 2.48	
		0	6.98 ± 1.61	2.65 ± 0.58	143.18 ± 4.06	
	Incisor	60	2.72 ± 0.66	4.21 ± 0.94	143.56 ± 1.73	
		30	3.78 ± 0.89	4.08 ± 0.91	135.71 ± 1.73	
		0	4.11 ± 0.95	3.69 ± 0.82	125.03 ± 2.58	

Table entries are means ± SD. *In vivo* bite force in *Apodemus sylvaticus* was measured at a gape angle of 20°.

**Table 4.** Results of MAN(C)OVAs performed on biomechanical and morphometric data testing for differences between species

	Pillai trace	<i>F</i>	d.f.1	d.f.2	<i>P</i>
Biomechanical data by species					
Biomechanical output	0.76	17.78	3	17	< 0.001
Fractional participation (all)	0.60	15.08	4	40	< 0.0001
Fractional participation (gape × species)	1.31	3.79	20	156	< 0.05
Fractional participation (gape 0)	0.66	4.91	4	10	0.019
Fractional participation (gape 30)	0.73	6.68	4	10	< 0.01
Fractional participation (gape 60)	0.70	5.76	4	10	0.011
Morphometric data					
Shape by species (MANCOVA)	0.74	12.00	1	94	< 0.0001
Shape by size (MANCOVA)	0.58	5.91	1	94	< 0.0001
Shape by species × size (MANCOVA)	0.27	1.59	1	94	0.064
Shape (PC) by sex	0.99	1.93	43	1	0.52
Shape (Procrustes) by sex	0.97	1.67	42	2	0.45

Bold values indicate significant differences. As a covariate, the centroid size was used. MANCOVA, multivariate analysis of covariance; PC, principal component.

two temporalis (anterior and posterior) muscles show the greatest difference between species.

A MANOVA on the output of the biomechanical model (Table 4) calculated for a gape angle of 0° showed a significant species effect (Pillai trace = 0.76;  $F_{3,17} = 17.78$ ;  $P < 0.001$ ). However, subsequent univariate ANOVAs showed no significant difference in bite force ( $P = 0.57$ ) and joint force ( $P = 0.91$ ) between the species (Table 5). The angle of the joint force was, however, different between species ( $P < 0.001$ ) and was significantly greater in *A. sylvaticus*. These results were consistent at all gape angles tested (0°–60°; see Table 5) and at both bite points. This indicates that the angle of joint force is orientated more posteriad in *A. sylvaticus* compared to *A. flavicollis*. A regression of joint force on bite force at 0° of gape and for bite point was significant

( $R^2 = 0.96$ ;  $P < 0.001$ ; results for other gape angles and bite points are indicated in Supporting Information, Table S2). An analysis of the residual data showed that the joint force is significantly higher in *A. sylvaticus* for a given bite force ( $F_{1,19} = 22.78$ ;  $P < 0.001$ ), and further analysis shows this is true at all gape angles and bite points (Supporting Information, Table S2).

#### MORPHOMETRICS

An ANOVA on morphometric data indicated significant differences between species in centroid size ( $F_{1,117} = 4.09$ ;  $P = 0.046$ ). However, the size ratio difference between species is less than 1.3 (Hutchinson ratio) for both a linear measurement (body length ratio = 1.04) and a mandible centroid size (1.03). A MANCOVA (Table 4)

**Table 5.** Results of ANOVAs testing for differences between species in the fractional participation ( $\log_{10}$ ) of the individual muscles, fractional participation of the muscle bundles and of the biomechanical output

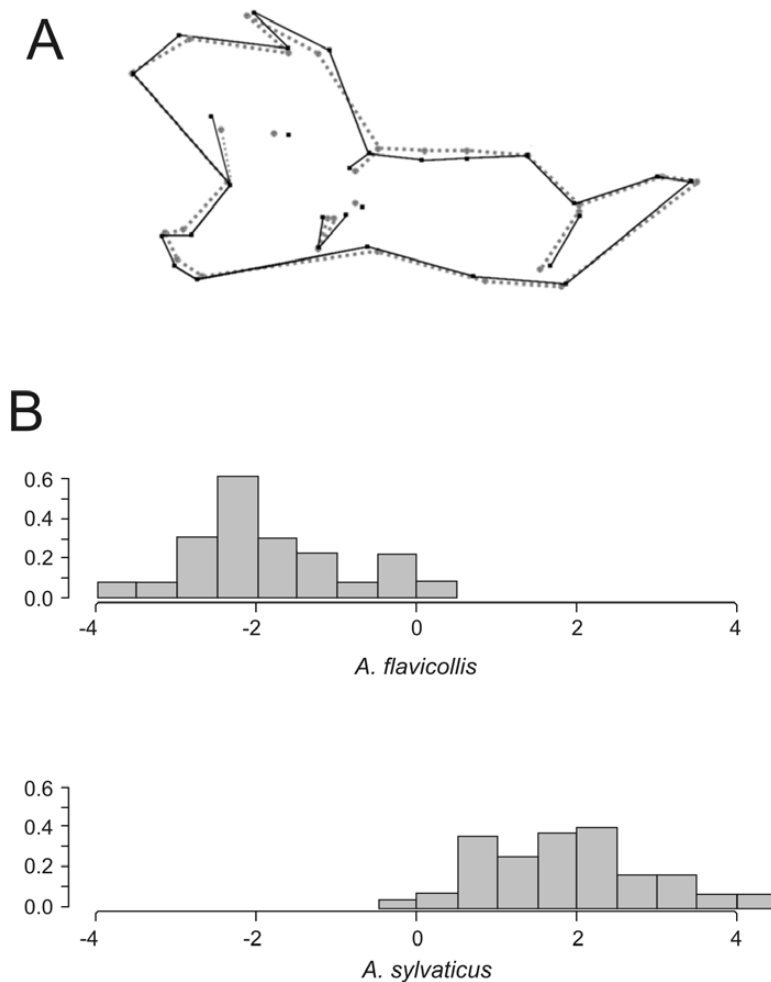
	Gape 0				Gape 30				Gape 60			
	<i>P</i>	<i>F</i>	d.f.1	d.f.2	<i>P</i>	<i>F</i>	d.f.1	d.f.2	<i>P</i>	<i>F</i>	d.f.1	d.f.2
Fractional participation												
Sm	0.43	0.67	1	19	0.49	0.50	1	19	0.30	1.15	1	19
adm	<b>0.011</b>	7.84	1	19	0.052	4.31	1	19	0.30	1.16	1	19
pdm	0.67	0.19	1	19	0.71	0.14	1	19	0.57	0.34	1	19
azm	0.14	2.42	1	19	0.052	4.31	1	19	0.28	1.23	1	19
pzm	0.25	1.40	1	19	0.20	1.80	1	19	0.20	1.78	1	19
iozm	<b>0.041</b>	4.79	1	19	<b>0.045</b>	4.60	1	19	<b>0.045</b>	4.61	1	19
tp	< <b>0.001</b>	87.59	1	19	< <b>0.001</b>	132.2	1	19	< <b>0.001</b>	287.6	1	19
ta	< <b>0.001</b>	73.54	1	19	< <b>0.001</b>	112.2	1	19	< <b>0.001</b>	344.1	1	19
tpsz	<b>0.073</b>	3.61	1	19								
ep	0.72	0.13	1	19	0.54	0.40	1	19	0.25	1.41	1	19
ip	0.19	1.81	1	19	0.98	0.001	1	19	0.46	0.57	1	19
Mass	<b>0.0056</b>	9.75	1	19	<b>0.015</b>	7.24	1	19	0.088	3.24	1	19
Zmand	<b>0.045</b>	4.60	1	19	<b>0.0018</b>	13.11	1	19	0.085	3.31	1	19
Temp	0.72	0.14	1	19	0.56	0.36	1	19	0.65	0.21	1	19
Pter	0.11	2.76	1	19	0.92	0.009	1	19	0.21	1.69	1	19
Biomechanical output												
Bite point 1												
prey rf	0.57	0.012	1	20	0.63	0.24	1	20	0.65	0.21	1	20
b-b jf	0.91	0.012	1	20	0.87	0.028	1	20	0.88	0.022	1	20
a.joint f	< <b>0.001</b>	37.03	1	20	<b>0.001</b>	14.55	1	20	<b>0.003</b>	11.21	1	20
Bite point 2												
prey rf	0.57	0.34	1	20	0.63	0.24	1	20	0.65	0.21	1	20
b-b jf	0.414	0.70	1	20	0.57	0.34	1	20	0.76	0.095	1	20
a.joint f	< <b>0.001</b>	20.23	1	20	<b>0.004</b>	10.73	1	20	<b>0.002</b>	12.45	1	20

Bold values represent significant differences between species. sm, superior masseter; adm, anterior deep masseter; pdm, posterior deep masseter; azm, anterior zygomaticomandibularis; pzm, posterior zygomaticomandibularis; iozm, infra-orbital zygomaticomandibularis; tp, temporalis posterior; ta, temporalis anterior; tps, temporalis pars suprazygomatica; ep, external pterygoid; ip, internal pterygoid; Mass, masseter bundle; Zmand, zygomaticomandibularis bundle; Temp, temporalis bundle; Pter, pterygoid bundle; prey rf, prey reaction force (bite force); b-b jf, bone-on-bone joint force; a.joint f, angle joint force. ANOVA, analysis of variance; d.f., degrees of freedom.

indicated differences between species in shape (Pillai = 0.74;  $F_{1,94} = 11.99$ ;  $P < 0.001$ ). Although significant allometry was detected across all specimens (Pillai = 0.58;  $F_{1,94} = 5.91$ ;  $P < 0.01$ ; see also Table 2), no interaction between size and species was observed (Pillai = 0.27;  $F_{1,94} = 1.59$ ;  $P = 0.064$ ). Interestingly, whereas allometric effects were significant for *A. sylvaticus*, this was not the case for *A. flavicollis* when analysing species separately (Table 2). A discriminant function analysis was able to discriminate the two species based on the first 22 PC axes (Fig. 3). Twenty-three out of 26 *A. flavicollis* were correctly classified; for *A. sylvaticus*, all specimens were correctly classified when using a leave-one-out cross-validation. The mandible of *A. flavicollis* has a more vertical coronoid, and the coronoid process is slanted slightly more anteriorly; there is a slightly higher articular, a slightly longer and narrower angular and a more narrow molar region.

*Apodemus sylvaticus* has a slightly flatter coronoid and articular (= condylar process), a slightly shorter and wider angular and a more robust molar region especially around the m2 and m3 (Fig. 3).

While species differences do exist, they do not account for the majority of variance in the data set. The PCAs show that the mandibles of the two species overlap in shape space (Supporting Information, Fig. S2). The first PC accounts for 16.18% of the variance for the entire sample. The second PC accounts for 9.09% (all sites with coronoid). On the positive side of the first axis, specimens have a lower more concave molar region, coupled with a shorter (but not narrower) angular and a slightly longer articular. On the negative side of PC1 mandibles with a slightly flatter molar alveolar region, a less vertical anterior edge of the coronoid, a shorter articular and a longer angular are found.



**Figure 3.** Wireframes describing average shapes of each species (A) and the results of a discriminant function analysis (B) showing that species can be discriminated based on mandible shape.

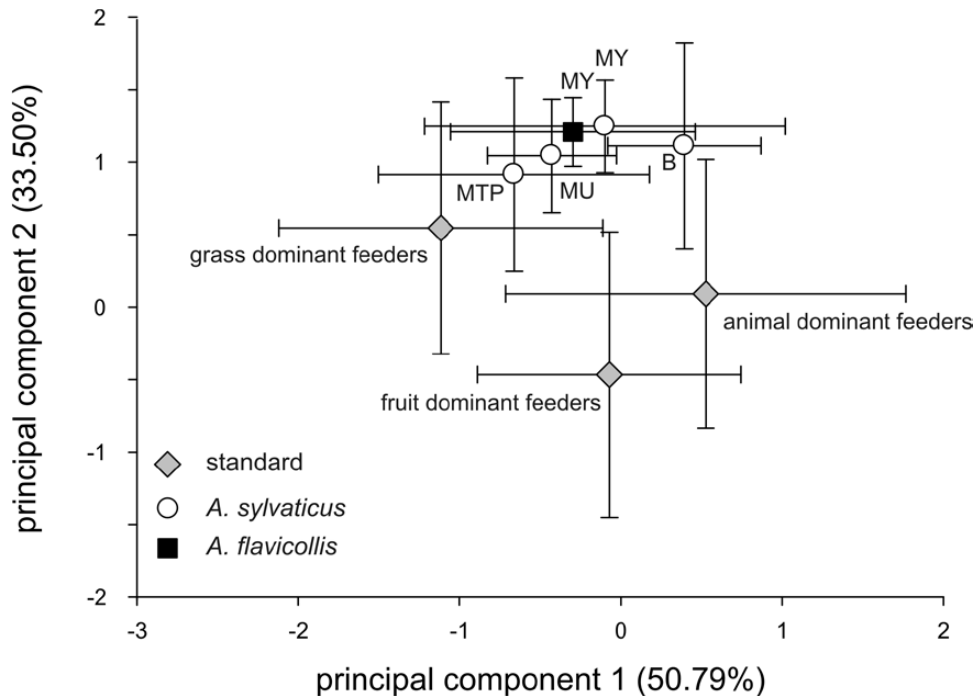
RV tests were performed to investigate relationships between shape and biomechanical properties. There was no significant relationship between shape (PC scores describing 90% of the overall variability) and the output of the biomechanical model or between shape and the PCSA of the muscles ( $P = 0.07$ ). However, there was a significant relationship ( $P = 0.03$ ) between the mass of the muscle bundles and mandible shape (both for PC axes and Procrustes coordinates; Supporting Information, Table S3). As separate muscle bundles, only the temporalis muscle bundle mass was significantly associated with mandible shape ( $P = 0.01$ ; PC coordinates).

#### MICROWEAR

A PCA performed on the microwear data extracted two axes together explaining 84% of the variance in the data set. On the plot of axes one and two, populations

of both species were distributed in a cloud between two of the standards, grass feeding (GDF) and animal feeding (ADF), with no apparent clustering or grouping (Fig. 4). There were significant differences ( $P < 0.05$ ) between each of the populations and the dietary category standards except for the *A. sylvaticus* from Montpellier against GDF (Supporting Information, Table S4). However, none of the populations had an exclusive feeding type. The *A. sylvaticus* from Montpellier appear to be somewhat more prone to grass feeding, while the *A. sylvaticus* from Belgium were more prone to insect feeding. In addition, no significant differences in the microwear were observed between species or between animals from different sites (Supporting Information, Table S4) with the exception of the Belgian population that differed from two of the Southerly populations. However, this difference was only observed when using the LSD test for fine scratches and large pits. This indicates that the





**Figure 4.** Plot of the first two principal components of a principal component analysis performed on the tooth wear data. Data for different populations are plotted together with feeding standards (animal, fruit and grass feeders; diamonds). Note how the populations of both species fall between grass- and animal-dominant feeders suggesting an omnivorous diet. The two species from the same locality (Montseny) cluster closely together. Open circles: *Apodemus sylvaticus*; closed square: *Apodemus flavicollis*. B, Belgium; MTP, Montpellier; MU, Murcia; MY, Montseny.

species and populations are not discriminated by microwear, except for a slight difference between the Belgian population vs. Murcia and Montpellier. However, the populations of the two species from Montseny cluster close together in the microwear space suggesting that they have similar diets in syntopy. Finally, no association between microwear and the mandible shape was observed ( $P = 0.26$ ; RV coefficient: 0.15).

## DISCUSSION

There are significant differences between the two species in muscle cross-sectional areas, the fractional participation of the muscles and mandible shape (Tables 3 and 4). Biting appears more optimized in *A. flavicollis*, with a lower joint force for a given bite force and a smaller joint force angle than in *A. sylvaticus*. Although there are no differences in the maximal bite force calculated, the differences in mandible shape between the species suggest some differences in function that could be related to diet. The more robust molar region in *A. sylvaticus* may indicate greater amount of grinding, as does the reduced angular process and the posteriorly shifted coronoid and the higher contribution of the anterior temporalis (Cox & Jeffery, 2011). Similar

differences are also observed in the species *A. speciosus* in comparison to *Apodemus argenteus* (Renaud & Millien, 2001). The lower articular could also reflect a more habitually closed jaw posture while biting or chewing. The more vertical coronoid in *A. flavicollis* may improve the moment arm of the temporalis, and the longer and narrower angular will affect the areas of insertion of the masseter and the pterygoid muscles. These differences are subtle, however, and probably reflect ecological tendencies rather than exclusive feeding patterns. Moreover, local adaptations at the population level may play an important role (Renaud & Michaux, 2003).

In addition to the differences in mandible shape, biomechanical properties other than maximal bite force may indicate species variation in diet. *Apodemus flavicollis* appears to be more optimized in terms of the efficacy of the production of bite force. The larger angle of joint force in *A. sylvaticus* may indicate that it tends to masticate at narrow gape, therefore exerting less destabilizing forces on the joint. This is moreover corroborated by the higher relative joint force for a given bite force in *A. sylvaticus*. Correspondingly, it may be possible that *A. flavicollis* habitually uses larger bite forces and requires a better optimization of its bite force to reduce the corresponding joint forces.

The greater participation of different muscle groups could also reflect feeding tendencies. There is a greater contribution of the masseter in *A. sylvaticus*, the bundle that controls biting and jaw movement at a narrow gape. There is also a greater participation of the zygomaticomandibularis bundle in *A. flavicollis*. This bundle lies underneath the masseter bundle and passes through the infra-orbital fossa to attach anteriorly on the rostrum. This muscle has been shown to contribute to the stabilization of the mandible (Cox, Kirkham & Herrel, 2013) and may account for the more optimized joint force and joint force angle in *A. flavicollis*.

Allometric variation explains some of the variation in shape across both species and in *A. sylvaticus* (Table 2). However, this is not the case for *A. flavicollis* where a regression of shape on centroid size was not significant (Table 2). Despite these differences in allometry between species, both overlap nearly completely on the first two axes of a PCA. In coexisting Japanese *Apodemus* species, the overall shape variation across the two species is also the same, despite having a slightly higher size ratio difference than the current species, around the Hutchinson value of 1.3 (Renaud & Millien, 2001). This suggests that morphological variation within *Apodemus* is often conserved in closely related species, even if they are more disparate in size than *A. sylvaticus* and *A. flavicollis*, and suggests an important role for allometry in structuring intrapopulation variation (Renaud & Auffray, 2013).

The overall mandible shape across both species is also linked to the mass of the temporalis bundle (Supporting Information, Table 3). It is likely that the temporalis is linked to the entire mandible shape and not only the region of its insertion, as modules do not alter in form independently, as has been observed in other rodents (Zelditch, Wood & Swiderski, 2009). The mass of this muscle bundle appears to be the only muscle property linked to mandible shape in these species, in contrast to what has been observed in other taxa (Fabre *et al.*, 2014; Cornette, Tresset & Herrel, 2015a). This is possibly due to the fact that differences in muscle cross-sectional area do not directly translate into biomechanical differences, as different combinations of individual muscles or muscle bundles can result in the same or similar biomechanical performance. This many-to-one mapping of morphology on function has been observed in the jaw apparatus of many taxa and is especially common in fish that have extremely complex jaw systems with many elements (Alfaro, Bolnick & Wainwright, 2005).

The microwear signature is indistinguishable between the two species and is different from the standards of grass-dominated and animal-dominated feeding, as could be expected for omnivorous species.

Notably, the two species that occur in syntopy at Montseny show very similar microwear signatures suggesting that differences in diet do not contribute to niche differentiation. Overall, our results suggest that the omnivory in these *Apodemus* species tends towards the plant-eating type. The only source of geographic variation in microwear was observed for the Belgian population (*A. sylvaticus*), which was significantly different from the populations of the same species in Montpellier and Murcia. The Montpellier population is also the only population with no significant difference from the grass-feeding standard. This suggests that the Belgian population may tend more towards animal (probably insect) and fruit feeding compared to two other populations. Finally, we observed no correlation between the microwear profile and mandible shape, suggesting that at least the short-term feeding patterns do not impact overall mandible shape as has been suggested previously (Renaud & Michaux, 2003). Feeding differences that may potentially exist between the species are thus not identifiable through dental microwear, which appears limited to detect subtle variations.

It is often considered that character displacement and niche segregation is required for related, similar species to coexist. However, the two *Apodemus* species considered in the present study have a very similar size and show no direct evidence for differences in diet. It is likely that different taxa interact differently in terms of how and what kind of selection pressures are mutually imposed due to sympatry. However, character displacement has been observed in taxa as different as birds, salamanders, lizards and shrews (Adams & Rohlf, 2000; Grant & Grant, 2006; Stuart *et al.*, 2014; Cornette *et al.*, 2015b). However, ecologically similar, related species may readily and sustainably coexist in the same habitat where there is no limitation in the vital shared resources (Den Boer, 1979; Okuzaki *et al.*, 2010). The two species densities will then probably fluctuate due to environmental fluctuations that alternatively favour the slight differences between the species. These scenarios have been demonstrated to be viable based on simple models (Yo Mo, Xu & Urabe, 1996). An example of this is the successful sympatry of two pairs of bird species within the genus *Calidris* permitted by differential resource exploitation through fluctuations in yearly population size, differences in distribution and migratory timing (Holmes & Pitelka, 1968). In analogy, perhaps the differences seen in the *Apodemus* species studied here may be due to the allopatric selection pressures driving phenotypic differences that remained upon secondary contact. The selection pressures that are exerted by the coexistence of

these species do not appear to drive a notable additional divergence in size or morphology (Dayan & Simberloff, 2005). Niche segregation may therefore be more subtle, involving behavioural differences as much if not more than biomechanical or morphological changes (Simberloff & Boecklen, 1981). Differences in spatial use, staggering of breeding patterns and differences in distribution and dispersion also exist between these two *Apodemus* species, and these may be sufficient to allow differential resource exploitation (Montgomery, 1980, 1981, 1989; Marsh *et al.*, 2001; Mitter, Sumasgutner & Gamauf, 2015).

### CONCLUSION

Our data on mandible shape and the biomechanics of biting suggest that *A. sylvaticus* may have a tendency to eat smaller food items that require greater amounts of grinding (e.g. plant leaves, stems and roots). This would account for the more robust molar region, associated changes in shape of the mandibular ramus, the greater development of the masseter bundle and the less optimal joint force to bite force ratio. The anatomy and function of *A. flavicollis*, on the other hand, suggests a greater tendency to eat larger food items that require the application of greater bite forces (nuts, hard-shelled fruits). This would concur with the more optimized bite force and the vertical shape of the coronoid associated with the attachment of the temporalis muscle. The morphological and biomechanical differences that do exist nonetheless do not seem sufficient to create a consistent dietary niche separation. This is suggested by the microwear data, which show no discernable difference between the species, especially for syntopic populations. Further studies with larger sample sizes and focusing in syntopic populations of the two species in addition to an examination other niche dimensions are required in order to better understand the coexistence of these two species.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Definition of the landmarks used for the geometric morphometric analysis.

**Table S2.** Regression of the Log10-transformed bite force vs. the Log10-transformed joint force and the results of an ANOVA testing for differences between species performed on the residual joint force.

**Table S3.** Analyses of covariance between muscle properties and morphology (Principal Components and Procrustes coordinates) of both species using an RV-test. Muscle mass is by muscle bundle.

**Table S4.** ANOVA post-hoc tests on the microwear data, using Fisher's least significance (L) and Tukey's honestly significant test (H). Grey squares indicate a significant difference at  $\alpha = 0.05$ , for number of wide scratches (Nws), number of fine scratches (Nfs) and number of large pits (Nlp). Diet standards are animal dominant feeding (ADF), grass dominant feeding (GDF) and fruit dominant feeding (FDF).

**Figure S1.** Picture taken of a tooth cast illustrating the large pits (Lp), the fine scratches (Fs) and the wide scratches (Ws) that were quantified.

**Figure S2.** Principal components analysis performed on the mandibular shape data. Different symbols represent different populations (circle = Montseny; diamond = Montpellier; hexagon = Murcia; triangle down = Senart; triangle up = Orleans; square = Belgium) and different colours represent different species (black = *A. flavicollis*; white = *A. sylvaticus*). Note how the variability of *A. sylvaticus* largely encompasses the variability of *A. flavicollis* for the syntopic site of Montseny as illustrated by the minimum convex polygons. Across all sites the variability of *A. flavicollis* is nearly completely contained within that of *A. sylvaticus* (dashed lines).