

5. Morphometric Divergence and Functional Similarity in *Sciurus vulgaris* and *Sciurus carolinensis*

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Summary

We used geometric morphometric techniques to explore and compare the shapes of dentaries and skulls in four species of tree squirrels: Eurasian red squirrel (*Sciurus vulgaris*), eastern grey squirrel (*S. carolinensis*), eastern fox squirrel (*S. niger*) and western grey squirrel (*S. griseus*). These species were chosen because of current competitive interactions amongst the species, and because of their phylogenetic affinities. A canonical variates analysis of Procrustes shape coordinates revealed significant shape differences between the skulls and dentaries of *S. carolinensis* and *S. vulgaris*. We compared biomechanical properties of the dentaries for the four species, and used discriminant function analysis to discriminate between the species in a jaw-function space. Here, there was extensive functional overlap between *S. carolinensis* and *S. vulgaris*, but not between *S. carolinensis* and *S. niger*. Although the skulls and dentaries of *S. carolinensis* and *S. vulgaris* differ morphologically, they are functionally similar.

Introduction

Both *Sciurus carolinensis* (eastern grey squirrel; Brueummer et al. 2000) and *S. niger* (eastern fox squirrel; UNEP-WCMC 2010) have been introduced from eastern North America to biomes outside their natural ranges, including Europe, Australia, Canada and the western United States of America (USA) (Palmer et al. 2007). The ecological consequences of these introductions and subsequent expansions are varied. In the western USA there was initial concern that introductions of *S. carolinensis* and *S. niger* were associated with declines in populations of the western grey squirrel (*S. griseus*: Muchlinski et al. 2009; Cooper & Muchlinski 2013; Johnston 2013), which is listed as threatened in Washington state (Washington Department of Wildlife 1993). However, the recovery plan for the western grey squirrel (Linders & Stinson 2007) suggests the invasive *S. carolinensis* and *S. niger* are found primarily in degraded or fragmented habitats and are dependent on resources associated with human disturbance. Linders and Stinson (2007) did not show a causal relationship between the presence of the invasive species and declines in populations of *S. griseus*. Both Linders and Stinson (2007) and Johnston (2013) noted the association of *S. carolinensis* with riparian and urban areas while *S. griseus* was able to exploit more xeric habitats.

In contrast, the literature on the effects of introduced populations of *S. carolinensis* on abundance and persistence of *S. vulgaris* (Eurasian red squirrel) is clear (e.g. Gurnell et al. 2004, 2015). In Europe, populations of *S. carolinensis* have increased dramatically, while *S. vulgaris* populations have been substantially reduced or extirpated. The demographic and life history attributes of *S. carolinensis*, as well as its tolerance of fragmented and disturbed habitats, result in competitive advantages of *S. carolinensis* over *S. vulgaris* that it appears not to enjoy over *S. griseus*. Whereas extensive field and modeling studies (Armitage et al. 1997; Skelcher 1997; Gurnell et al. 2015) have elucidated the competitive relationship between *S. carolinensis* and *S. vulgaris*, there has been no exploration of the functional morphological components of this dynamic system.

A fundamental component of any inter-specific interaction is size. Size influences metabolic rate, locomotion, energy demand and expenditure, life span, ecology, and reproduction (van Bergen & Phillips 2005). However, (because of both static and ontogenetic allometric effects) for animals within a narrow range of sizes, shape is perhaps more important in competition between species. Animal shape determines to a large extent how that organism functions (Garland 1985; Oxnard 1991). Certainly, mandibular shape is related to function (Hautier et al. 2011; Renaud et al. 2015; Stefen et al. 2016) and it is likely to determine the variety and types of food resources that can be used and the efficiency with which those resources can be processed. This has some bearing on the potential inclusive fitness of an individual (Boell & Tautz 2011) and may affect the outcome of inter-specific competitive interactions.

In terms of the system studied here, jaw shape has obvious implications for efficiency of food processing, and it may limit which types of foods can be processed. Surface area on the dentary available for insertion of the masseter and temporalis, the effort arms through which the muscles act, and the load arms associated with the muscles will determine the forces that can be applied at the incisors and the molar tooth row. Similarly, shape of the skull and area available for masticatory muscle origins is important as well.

Whereas the shape of a particular object is easy to understand, it is somewhat more difficult to describe shape mathematically, especially to explore shape independently of size. Fortunately, geometric morphometric techniques provide an ideal tool with which to explore shape variation (Bookstein 1991; Swiderski 2003; Klingenberg 2011; Lu et al. 2014). Using homologous landmarks and thin plate splines, it is possible to visualize and describe shape transformations from one form to another. The bending energy associated with these transformations can then be analyzed using traditional statistical techniques.

In this study, we used geometric morphometric techniques to explore and compare the shapes of dentaries and skulls in *S. vulgaris*, *S. carolinensis*, *S. niger* and *S. griseus*. We test the hypothesis that *S. carolinensis* and *S. vulgaris*

are functionally more similar than *S. carolinensis* and *S. griseus*. This hypothesis is based on the premise that *S. carolinensis* has competitively displaced *S. vulgaris*, but not *S. griseus*. We include *S. niger* because it is naturally sympatric with *S. carolinensis*, allowing us to investigate relationships of two species that presumably experienced varying levels of competition as they coevolved. If shape and function reflect a niche axis, it is reasonable to assert these attributes play a role in competitive exclusion of *S. vulgaris* by *S. carolinensis* in Europe.

Methods

Skulls and dentaries of adult *S. vulgaris*, *S. carolinensis*, *S. niger* and *S. griseus* were photographed using a Canon 6D camera and Canon 100mm macro lens mounted on a copy stand. Lighting was provided by two Canon Speedlite strobes (models 580ex II and 420ex) positioned so that light was parallel to the specimen surface in an effort to reveal surface detail. Lens aperture was minimized ($f = 32$) to maximize depth of field and improve resolution. An mm ruler was included in each frame to provide scale information. All specimens were placed on modeling clay and orientated so that either the palate of the skull, or ramus of the dentary was parallel to the plane of the camera sensor. Specimens of *S. carolinensis* and *S. niger* were from the collections of the Virginia Museum of Natural History; those of *S. griseus* and *S. vulgaris* were from collections of the Field Museum of Natural History. Specimens are listed in the Chapter Appendix.

Programme tpsUTIL (<http://life.bio.sunysb.edu/morph/>) was used to create a file of randomly ordered images, and programme tpsDIG2 was used to place landmarks on each specimen. Landmarks chosen for the dentary were identical to those used by Swiderski (2003), and landmarks for the ventral skull generally followed those of Lu et al. (2014). Landmarks are defined in Table 1. Each image in the data set was scaled using the mm ruler in the image, so that measurements between landmarks could be expressed in mm.

All specimens were orientated using Procrustes superimposition. We used programme MorphoJ (Klingenberg 2011) to perform a canonical variates analysis of shape differences among the four species and to describe the shape differences using wire-frame diagrams. For both the skull and dentary, differences amongst the species were assessed using permutation tests with 10,000 replicates. Here, we assumed the within group covariance matrices were equal. To satisfy this assumption we used the pooled within-group covariance matrix. We then compared the Mahalanobis distances among groups.

Biomechanical properties of the dentary were assessed by estimating load arms and effort arms for the masseter and temporalis muscles (Figure 1) and then computing the mechanical advantage of each muscle. We defined the

Table 1. Landmarks for the dentary and ventral skull used in this study. See Figures 2C and 3C for illustrations.

Dentary	Description
1	Dorsal edge of aperture of incisor alveolus
2	Anterior edge of 4 th premolar
3	Posterior edge of 2 nd molar
4	Tip of coronoid process
5	Anterior edge of condyle
6	Posterior edge of condyle
7	Posterolateral corner of angular process
8	Medial corner of angular process
9	Junction of angular process and dental ramus
10	Anterior end of ventral edge of masseteric fossa
11	Ventral edge of aperture of incisor alveolus
Ventral Skull	
1	Medial tip of the premaxilla
2	Anterolateral edge of the incisor alveolus
3	Anterior margin of the incisive foramen
4	Posterior margin of the incisive foramen
5	Dorsolateral suture between the premaxilla and maxilla
6	Posterolateral margin of the rostrum
7	Anterior margin of the tooth row
8	Posterior margin of the tooth row
9	Posterior margin of the maxillary process of the zygomatic arch
10	Posterior margin of the palate
11	Posterior margin of the jugal
12	Point of maximum width on the medial margin of the zygomatic arch
13	Anterior margin of the auditory bulla
14	Medial margin of the auditory bulla
15	Ventral margin of the foramen magnum
16	Medial margin of the occipital condyle
17	Lateral margin of the occipital condyle

effort arm of the masseter as the vector from the mandibular condyle to the anterior margin of the masseteric fossa (distance from the condylar mid-point: average of the coordinates for dentary landmarks 5 and 6, and landmark 10). The effort arm for the temporalis was estimated as the distance between the condylar mid-point and the tip of the coronoid process (landmark 4). The load arm at the incisor was computed as the distance between the condylar mid-point and landmark 1, while the load arm at the molar tooth row was computed as the distance between the condylar mid-point and landmark 3. Mechanical advantage was estimated as the quotient of effort arm over load arm for masseter at the incisor, masseter at the molar tooth row, temporalis at the incisor, and temporalis at the molar tooth row.

We estimated the relative area of the masseter on the dentary as centroid size for landmarks 5 through 10. Centroid size was computed as the square root of the sum of the squared distances between each landmark (5 - 10) from the mean for landmarks 5 through 10. We computed the relative size of the dentary associated with the masseter by dividing centroid size for the masseter by the corresponding centroid size for the entire dentary. These percentage values were then used in a Kruskal-Wallis test for equal medians to test the hypothesis that relative masseter area among the four species was equal. We did not use a more powerful ANOVA because 1) percentage scores are not normally distributed and 2) there was heteroscedasticity among the variances (Table 2). Finally, we used discriminant function analysis within programme PAST (<http://folk.uio.no/ohammer/past/>) to assess the distribution of species within the multivariate biomechanical space, and to identify those biomechanical features that differed amongst species. We used a Type I error rate of .05 for all statistical tests.

Table 2. Mean (upper) and SD (lower) for 11 metrics associated with dentary biomechanics in squirrels. SC = *Sciurus carolinensis*, SG = *S. griseus*, SN = *S. niger* and SV = *S. vulgaris*. N = sample size, TDA = total dentary area, MA = masseter area, MAR = masseter area ratio, MEA = masseter effort arm, TEA = temporalis effort arm, ILA = incisor load arm, TRLA = tooth row load arm, MMAI = masseter mechanical advantage at the incisor, TMAI = temporalis mechanical advantage at incisor, MMAM = masseter mechanical advantage at molar and TMAM = temporalis mechanical advantage at molar.

	N	TDA	MA	MAR	MEA	TEA	ILA	TRLA	MMAI	TMAI	MMAM	TMAM
SC	25	208.5	159.9	0.686	25.9	7.9	34.3	18.0	0.756	0.229	1.4	0.435
		7.139	7.15	0.013	1.03	0.77	1.00	0.60	0.015	0.020	0.03	0.037
SG	18	231.0	175.6	0.677	31.4	9.7	39.6	21.6	0.793	0.245	1.5	0.449
		8.72	6.75	0.013	1.79	0.95	2.07	1.52	0.013	0.017	0.04	0.022
SN	27	242.2	180.8	0.656	31.9	9.5	42.9	21.9	0.743	0.222	1.5	0.436
		9.68	8.63	0.011	1.26	0.68	1.50	0.90	0.013	0.013	0.03	0.028
SV	19	182.6	140.4	0.684	22.7	7.4	30.1	15.6	0.754	0.245	1.4	0.471
		13.94	12.99	0.021	1.31	0.75	1.52	0.91	0.016	0.017	0.04	0.034

Results

A total of 97 skulls and 89 dentaries were used in the analysis (Table 2). These specimens were complete with all landmarks clearly visible and easily defined. Based on total centroid size of the dentaries, *S. niger* was the largest species, followed by *S. griseus* and *S. carolinensis*. *Sciurus vulgaris* was the smallest of the four species (Table 2).

The canonical variates analysis (CVA) revealed significant shape differences among the four species for both the dentary and skull (Figures 2 and 3 and Tables 3 and 4). Within the space defined by three canonical axes for dentary shape, the 95% confidence ellipses for *S. vulgaris* and *S. carolinensis* do not overlap (Figure 2B). For skull shape (Figure 3B), these two species do not overlap along the first canonical axis, but overlap is apparent along the second. These two species also overlap along the third canonical axis (not pictured). With respect to the dentary, *S. griseus* does not overlap with any of the other species along the first canonical axis, whereas *S. carolinensis* and *S. niger* exhibit considerable overlap along both axes. Within the skull shape space, *S. vulgaris* does not overlap the other species along the first canonical axis, whereas *S. carolinensis* overlaps *S. griseus* moderately and *S. niger* minimally along both axes. Differences among the four species are supported by the significant permutation tests for pairwise comparisons of Mahalanobis distances within both the dentary and skull spaces (Table 4). The shape changes associated with these differences are evident from the wire-frame diagrammes in Figures 2 and 3. For the dentary, most of the differences between *S. carolinensis* and *S. vulgaris* occur along canonical axis two. Here, *S. vulgaris* exhibits a less procumbent incisor (Figure 2A; black wire frame), a slightly anterior deflection of the angular process and a more ventral position for the anterior margin of the masseteric fossa relative to *S. carolinensis*. Skull shape differences between *S. carolinensis* and *S. vulgaris* are most pronounced along canonical axis one. Here, *S. vulgaris* exhibits a slightly broader rostrum and broadening of the skull at the posterior portion of the jugal on the zygomatic arch (Figure 3D; black wire frame).

Relative area for insertion of the masseter differed among the four species of *Sciurus*. The Kruskal-Wallis test for equality of group medians was significant ($H_{3, n=89} = 39.67, p < .01$). Results of the a-posteriori MannWhitney pairwise comparison test with a Bonferroni correction is presented in Table 5. Relative area of the masseter did not differ between *S. carolinensis* and either *S. vulgaris* or *S. griseus*, and *S. vulgaris* was not different from *S. griseus*. However, *S. niger* was different from each of the other species. The relative area of the masseter for *S. niger* was smaller than that of the other species (MAR: Table 2).

Figure 1. Dentary of *Sciurus griseus* (FMNH 8943) showing the effort arms for the temporalis (TEA) and masseter (MEA), and the load arms for the molar tooth row (TRLA) and incisor (ILA). Shaded areas along the angular process and margin of the masseteric fossa illustrate the margins for the insertion of the masseter, and shaded regions along the coronoid process show insertion areas for the temporalis.

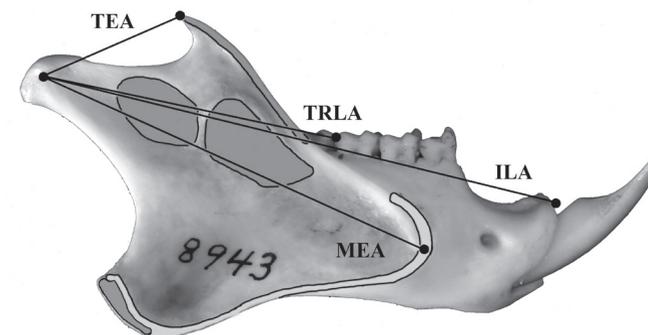


Figure 2. Distribution of four species of *Sciurus* within the dentary shape space defined by the first two canonical axes. A) Wire frame diagramme showing shape change along Canonical Variate 2; black frame illustrates shape at extreme positive end of the axis. B) 95% confidence ellipses. Black = *S. carolinensis* (SC) and *S. vulgaris* (SV), grey = *S. griseus* (SG) and *S. niger* (SN). C) Illustration of the dentary with landmarks (modified from Hall 1981; see Table 1 for descriptions). D) Wire frame diagramme showing shape change along Canonical Variate 1; black frame illustrates shape at extreme positive end of the axis.

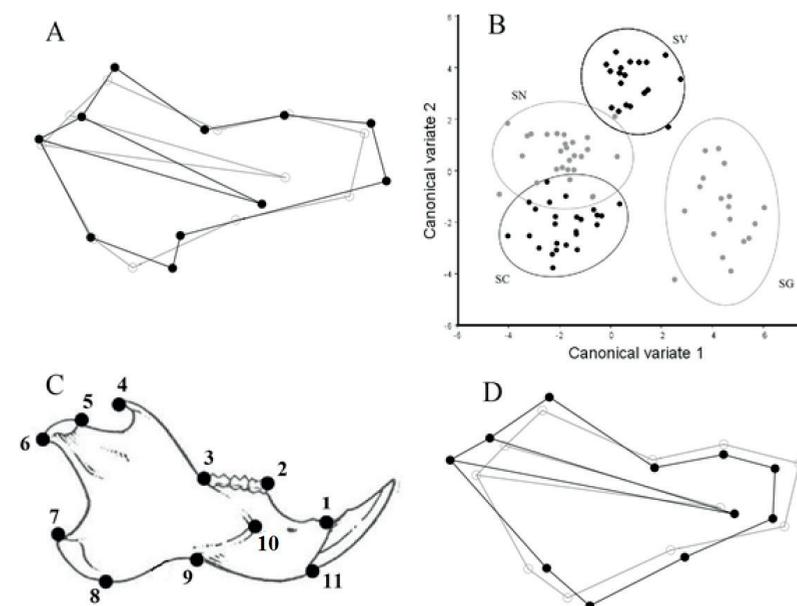


Table 3. Eigenvalues and % variance for the canonical variates analysis of dentary shape (above) and skull shape (below) for four species of *Sciurus*.

Dentary Canonical Axis	Eigenvalue	Variance	Cumulative Variance
1	6.313	50.46	50.46
2	4.669	37.32	87.78
3	1.528	12.22	100.00
Skull Canonical Axis			
1	7.960	48.98	48.98
2	5.282	32.50	81.47
3	3.011	18.53	100.00

Table 4. Mahalanobis distances between pairs of *Sciurus* species within the canonical space, based on 10,000 permutations. All distances are significantly different ($p < 0.01$). SC = *S. carolinensis*, SG = *S. griseus*, SN = *S. niger* and SV = *S. vulgaris*

Skull	SC	SG	SN
SG	5.116		
SN	5.097	6.389	
SV	7.086	8.221	7.571
Dentary			
SG	6.467		
SN	3.871	6.686	
SV	6.219	6.368	4.785

The discriminant function analysis of biomechanical metrics for the dentary produced significant discrimination between the species. The first two discriminant functions accounted for 98% of the variance in the discriminant space (Table 6). The confusion matrix (Table 7) indicated that the discrimination among the four species was almost perfect with respect to the biomechanical variables. Only *S. griseus* and *S. vulgaris* were not perfectly classified. *Sciurus griseus* was classified as *S. carolinensis* in 11% of cases, and *S. vulgaris* was classified as *S. carolinensis* in 5.3% of cases. The variables with the greatest effect on discrimination between the species were incisor load arm, effort arm of the masseter and load arm for the temporalis at the molar tooth row (Table 6). Within the discriminant space, *S. niger* is associated with larger values for these variables, while *S. carolinensis* and *S. vulgaris* are associated with smaller values (Figure 4).

Discussion

The system explored here represents a natural experiment on the ability of invasive tree squirrels to displace endemics. In North America, both *S. carolinensis* and *S. niger* have been introduced to communities in which *S. griseus* is endemic. Anthropogenic introductions of *S. niger* in southern California have resulted in the establishment of local populations in fragmented and disturbed habitats, with subsequent displacement of *S. griseus* (Muchlinski et al. 2009; Cooper and Muchlinski 2015). Similarly, in areas near Tacoma, Washington there is an established population of *S. carolinensis* (Johnston 2013), with the potential for displacement of *S. griseus*. However, in both southern California and Washington, the invasive squirrels are restricted to disturbed or fragmented habitats, and *S. griseus* appears to persist in larger tracts of undisturbed habitat. In Europe, the introduction of *S. carolinensis* has resulted in the competitive exclusion of *S. vulgaris* (Gurnell et al. 2004). Whereas knowledge of the natural history of the species is essential in order to develop strategies for preservation of the endemics, an understanding of the functional morphology of their skulls may inform our understanding of the system as well.

Sciurus vulgaris is the smallest of the four species both with respect to body size (Carraway & Verts 1994; Koprowski 1994a, 1994b; Lurz et al. 2005) and size of the dentary. Because size is related to metabolic processes (van Bergen & Phillips 2005), use of habitat and the physics of both locomotion and food handling, it is relevant to the competitive interaction between *S. carolinensis* and *S. vulgaris*. As noted by Swiderski and Zelditch (2010), allometric scaling is generally expected in the absence of a functional shift. Within the Sciurini, the general pattern is one of isometry, with jaw size being negatively allometric (Swiderski & Zelditch 2010).

Figure 3. Distribution of four species of *Sciurus* within the skull shape space defined by the first two canonical axes. A) Wire frame diagramme showing shape change along Canonical Variate 2; black frame illustrates shape at extreme positive end of the axis. B) The ellipses represent the 95% confidence ellipses. Black = *S. carolinensis* (SC) and *S. vulgaris* (SV), grey = *S. griseus* (SG) and *S. niger* (SN). C) Illustration of the skull with landmarks (modified from Hall 1981; see Table 1 for descriptions). D) Wire frame diagramme showing shape change along Canonical Variate 1; black frame illustrates shape at extreme positive end of the axis.

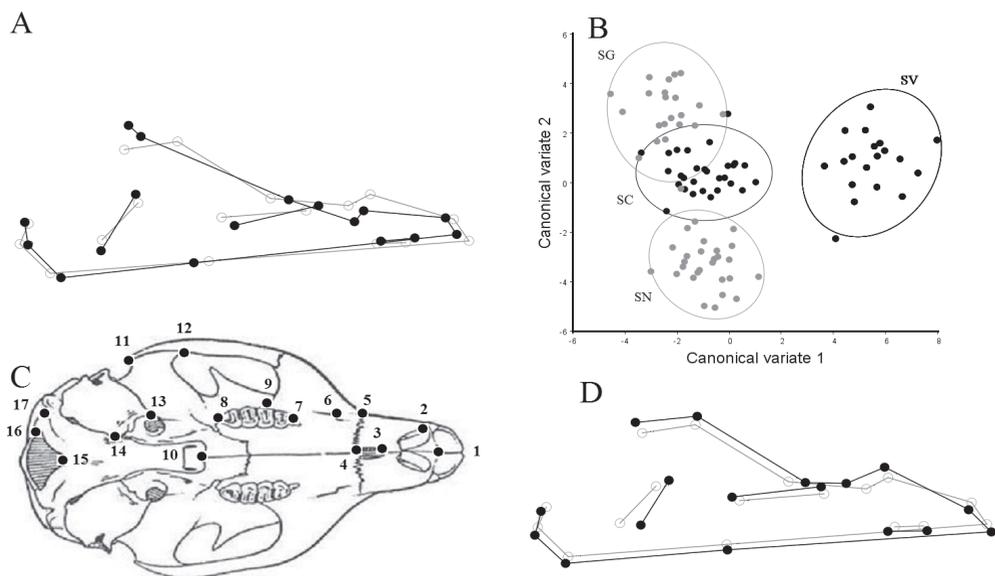


Figure 4. Results of the discriminant function analysis of dentary biomechanical attributes. Observations for each species are enclosed in 95% confidence ellipses, and a bi-plot indicates variables with the largest loadings (ILA = incisor load arm, MEA = masseter effort arm, TRLA = molar load arm and TEA = temporalis effort arm). The confidence ellipses are from left: *Sciurus vulgaris*, *S. carolinensis*, *S. griseus* and *S. niger*. The first discriminant function (DF 1) separates species based on incisor load arm and masseter effort arm, while the second function (DF2) separates species based on these variables as well as molar load arm.

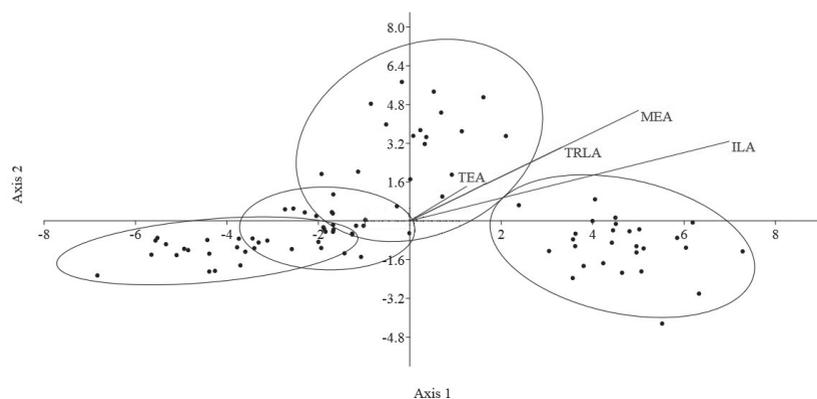


Table 5. Probabilities of the Mann Whitney pairwise comparisons for relative area of the masseter among four species of *Sciurus*. Species with probabilities smaller than 0.05 are significantly different. SC = *S. carolinensis*, SG = *S. griseus*, SN = *S. niger* and SV = *S. vulgaris*.

	SC	SG	SN	SV
SC		0.1993	0.0001	1
SG	0.1993		0.0001	1
SN	0.0001	0.0001		0.0004
SV	1	1	0.0004	

Table 6. Discriminant function analysis loading for the dentary biomechanics data set for four species of *Sciurus*. Important variable loadings are noted in bold.

Variable / Discriminant Function	Axis 1	Axis 2	Axis 3
Relative Area of Masseter	-0.003	0.001	0.007
Masseter Effort Arm	0.989	0.901	-0.249
Temporalis Effort Arm	0.246	0.281	-0.320
Incisor Load Arm	1.382	0.649	-0.187
Tooth Row Load Arm	0.659	0.602	-0.046
Mech. Adv. of Masseter at the Incisor	-0.001	0.010	-0.003
Mech. Adv. of the Temporalis at the Incisor	-0.002	0.003	-0.009
Mech. Adv. of the Masseter at the Molar	0.002	0.002	-0.010
Mech. Adv. of the Temporalis at the Molar	-0.003	0.000	-0.018
Eigenvalue	12.2250	3.0518	0.3106
% Variance	78.43	19.58	1.99

Table 7. Confusion matrix for discriminant function analysis of dentary biomechanics. SC = *Sciurus carolinensis*, SG = *S. griseus*, SN = *S. niger* and SV = *S. vulgaris*. Values along the diagonal (bold) indicate number of specimens correctly classified.

From/To	SC	SG	SN	SV	Total
SC	25	0	0	0	25
SG	2	16	0	0	18
SN	0	0	27	0	27
SV	1	0	0	18	19
Total	28	16	27	18	89

Resolution of phylogenetic relationships within Sciurini is complicated by conservative morphology (Emry & Thorington 1982; Thorington & Darrow 1996; Thorington et al. 1997; Swiderski 2003) and multiple, relatively rapid diversification events that produced at least 28 species in the genus *Sciurus* (Wilson & Reeder 2005). Three species of *Sciurus*, including *S. vulgaris*, are native to Eurasia. Six species, including *S. carolinensis*, *S. griseus*, and *S. niger*, are native to North America. Multilocus analyses of nuclear and mitochondrial genes indicated that the genus *Sciurus* originated in Eurasia and entered North America via Beringia fewer than nine million years ago (Mercer & Roth 2003; Pečnerová & Martínková 2012; Pečnerová et al. 2015). Molecular analyses consistently produced trees that placed *S. vulgaris* in a well-supported basal position relative to all North American species of *Sciurus* (Pečnerová & Martínková 2012; Villalobos & Gutierrez-Espeleta 2014; Pečnerová et al. 2015). Following colonization of North America, the *Sciurus* lineage experienced an adaptive radiation, with divergence of *S. griseus*, *S. niger*, and *S. carolinensis*, in that order (Pečnerová et al. 2015). We note that *S. carolinensis* and *S. vulgaris* are the most distantly related species in our study. Whereas several lineages within the Sciurini exhibit morphological diversification along with phylogenetic diversification (Casanovas-Vilar & van Dam 2013), the genus *Sciurus* does not. Because skull and jaw shape is a consequence of phylogenetic history as well as adaptation to resource use, it is not unreasonable to expect both morphological similarity and similarity in resource use within *Sciurus*.

Morphological similarity (including size) is often associated with ecological similarity (Hutchinson 1959; Harmon et al. 2005) and we expect species with extensive niche overlap to compete for resources when resources are limiting (MacArthur & Levins 1967; Smith 2012). Alternatively, in coevolved systems natural selection should operate to minimize niche overlap. That is, if skull and jaw shape reflect resource use, competition should result in divergence in shape among sympatric taxa that have not diverged along other niche dimensions. In light of the conservative nature of *Sciurus* morphology, it seems unlikely that selection has been sufficiently strong to effect morphological change in the short time that *S. carolinensis* and *S. vulgaris* have been sympatric, or in the short time that *S. carolinensis* and *S. niger* have each been sympatric with *S. griseus*. The successful displacement of *S. vulgaris* by *S. carolinensis*, and the limited displacement of *S. griseus* by *S. carolinensis* are consistent with predictions based on functional morphology.

Based on Mahalanobis distances, the skulls of *S. carolinensis* and *S. vulgaris* are more dissimilar than either *S. carolinensis* and *S. griseus* or *S. niger* and *S. griseus*. With respect to the first two canonical discriminant functions, the confidence ellipses of the North American species do not overlap with *S. vulgaris*. Among the North American species, there is considerable overlap between *S. carolinensis* and *S. griseus*. This result suggests, in terms of food processing, some degree of niche separation between *S. vulgaris* and *S.*

carolinensis. With respect to the dentary, *S. carolinensis* and *S. vulgaris* have non-overlapping distributions in the canonical space, and they are relatively far apart in terms of Mahalanobis distance as well. Within this canonical space there is also considerable separation between *S. griseus* and both *S. carolinensis* and *S. niger*. If skull and jaw shape reflect a niche axis, this axis would appear to be uninformative about possible competitive exclusion between the species. However, both the skull and dentary are complex structures, and evolution within these structures may be constrained. As suggested by Swiderski and Zelditch (2010), this may be a consequence of the ‘demands of arboreality and durophagy.’

The relationship between the evolution of skeletal form and function is complicated by the fact that bone is dynamic tissue and responds via remodeling to the forces applied to the structure. Anderson et al. (2014) demonstrated remodeling of the mandible in mice as a result of a dietary shift. To a large extent, this phenotypic plasticity is not heritable. Dietary shifts may also explain apparent phenotypic plasticity in the North American pine squirrel (*Tamiasciurus hudsonicus*). The expansion of the pine squirrel from coniferous forests in Michigan into hardwood deciduous forests in Indiana, where it became sympatric with *S. carolinensis*, resulted in a change in both cranial size and biomechanical metrics for *T. hudsonicus* (Goheen et al. 2003). One consequence of these changes was an improvement in the ability of *T. hudsonicus* to process hard mast. The relative contributions of phenotypic plasticity and heritable change resulting from natural selection are unknown.

Our *S. carolinensis* specimens were not collected from areas of sympatry with *S. vulgaris* therefore, our assessment of form and function may be incomplete. Because dentary function is determined by its associated musculature, it is worthwhile to explore the dentary from a functional perspective. Among the species investigated here, *S. vulgaris* and *S. carolinensis* have the largest relative areas for insertion of the masseter, but the smallest masseter effort arms. Mechanical advantage for the masseter at the incisor and the molar tooth row is similar in *S. vulgaris* and *S. carolinensis*. Overall, these functional variables reveal similarities between both *S. vulgaris* and *S. carolinensis*, and *S. carolinensis* and *S. griseus*, but differences between *S. carolinensis* and *S. niger*. This pattern is reflected in Figure 4, where the confidence ellipse for *S. carolinensis* overlaps the ellipses of both *S. vulgaris* and *S. griseus*. If mechanical function of the dentary can be interpreted as a niche axis, then *S. carolinensis* overlaps both *S. vulgaris* and *S. griseus*, but not *S. niger* (with which it is naturally sympatric). The fact that *S. carolinensis* displaces *S. vulgaris* but not *S. griseus* warrants additional study.

Although *S. carolinensis* and *S. vulgaris* differ in shape space, they overlap in functional space. From a mechanical perspective, these species may be quite similar in terms of bite forces, and thus the variety of foods they are able to exploit (Steele & Wauters this volume, Chapter 6). A detailed comparative

exploration of the anatomy of the temporalis and masseter muscles in these four species will be necessary to fully understand these patterns. In addition to life history characteristics associated with demography that may enable *S. carolinensis* to displace *S. vulgaris*, locomotor and behavioral attributes may be important as well. For example, locomotor performance and appendicular kinematics (Iniarte-Diaz 2002; Schmidt 2005; Schmidt & Fischer 2011) may influence patterns of habitat use, and flight initiation distance (Dill & Houtman 1989) may determine, to some extent, the degree of habitat fragmentation that a species can tolerate. Surprisingly, little is known about the locomotor kinematics and flight initiation distances of the species explored here (but see Uchida et al. 2015). Whereas the findings reported here do little to prevent the displacement of *S. vulgaris* by *S. carolinensis*, they improve our understanding of why the displacement has been so rapid and so effective.

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Appendix: Specimens Examined

VMNH = Virginia Museum of Natural History. FMNH = Field Museum of Natural History

Sciurus carolinensis (n=29). UNITED STATES. Georgia: Jasper Co.; 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 02 (VMNH 1181, 1185, 1186). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 07 (VMNH 1187). Jones Co.; 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 02 (VMNH 1182, 1191, 1192, 1193, 1195, 1196, 1204). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 03 (VMNH 1206, 1209). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 04 (VMNH 1213, 1216). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 06 (VMNH 1221). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 07 (VMNH 1225). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 08 (VMNH 1228, 1229). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 16 (VMNH 1234, 1235, 1236, 1237, 1240, 1241, 1243). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 25 (VMNH 1246). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 29 (VMNH 1248, 1249).

Sciurus griseus (n=22). UNITED STATES. California: Calaveras Co.; Swiss river (FMNH 1654, 1655). Colusa Co.; Snow Mts (FMNH 1649, 1650, 1651). Humboldt Co. Eureka (FMNH 8943). Marin Co. Nicasio (FMNH 5713). Riverside Co.; Smith Mts (FMNH 4715, 4716). Santa Clara Co.; San Antonio (FMNH 1656, 1657, 1659, 16550). Santa Cruz Co.; Big Basin (FMNH 7433, 7434, 7435). Sonoma Co.; Petaluma (FMNH 8941, 8942). Tulare Co.; Mt. Langley, Inyo National Forest, Jordan Hot Springs, 1968 m (FMNH 12413). Oregon: Curry Co.; Agness (FMNH 8944, 8945). No Location (FMNH 4714).

Sciurus niger (n=28). UNITED STATES. Georgia: Baker Co. (VMNH 358, 360, 361, 363). 0.25 mi S, 15.5 mi W Camilla (in Mitchell Co.) (VMNH 344). 0.5 mi S, 15.5 mi W Camilla (in Mitchell Co.) (VMNH 345). 1.5 mi S, 14.5 mi W Camilla (in Mitchell Co.) (VMNH 347). 2.5 mi S, 13.75 mi W Camilla (in Mitchell Co.) (VMNH 350). 2.75 mi N, 19.75 mi W Camilla (in Mitchell Co.) (VMNH 341). 4.5 mi N, 17 mi W Camilla (in Mitchell Co.) (VMNH 340). 6.5 mi S, 18.25 mi W Camilla (in Mitchell Co.) (VMNH 354). J.W. Jones Ecol. Res. Ctr., Lower Pasture (VMNH 2152). J.W. Jones Ecol. Res. Ctr., Main Dr. (VMNH 2153, 2160). J.W. Jones Ecol. Res. Ctr., Mims Store Rd (VMNH 2155). J.W. Jones Ecol. Res. Ctr., Pole Line Rd (VMNH 2148, 2151). J. W. Jones Ecol. Res. Center at Ichauway, 74548 E, 345382 N (VMNH 356). Jasper Co.; 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 03 (VMNH 1254). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 06 (VMNH 1258). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 07 (VMNH 1260). Jones Co.; 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 02 (VMNH 1269). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 03 (VMNH 1271, 1275). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 04 (VMNH 1285). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 06 (VMNH 1289). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 16 (VMNH 1300). 10 km W Round Oak, Piedmont Nat'l Wildlife Refuge, Comp 30 (VMNH 1306).

Sciurus vulgaris (n=21). AUSTRIA. Nieder-Osterreich (FMNH 65385, 65386, 65387). BAVARIA. Augsburg (FMNH 23538, 23541, 23542, 23546, 23547). CHINA. Mongolia, 45NE Urga (FMNH 25451). 60NE Urga (FMNH 24452). FRANCE. Seine Maritime, Bouvain Court (FMNH199869). GERMANY. Westfalen (FMNH 54651). N. MANCHURIA. Gr. Khingan Mts. (FMNH 46042, 46043). NORWAY. Oslo area (FMNH 191518, 191519, 191520). SWITZERLAND. Brunig (FMNH 6415). Meiringen (FMNH 6416, 6418, 6419).