Ecological character displacement in *Plethodon*: Biomechanical differences found from a geometric morphometric study

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Ecological character displacement describes a pattern where morphological differences between sympatric species are enhanced through interspecific competition. Although widely considered a pervasive force in evolutionary ecology, few clear-cut examples have been documented. Here we report a case of ecological character displacement between two salamander species, *Plethodon cinereus* and *Plethodon hoffmani*. Morphology was quantified by using linear measurements and landmark-based geometric morphometric methods for specimens from allopatric and sympatric populations from two geographic transects in south-central Pennsylvania, and stomach contents were assayed to quantify food resource use. Morphological variation was also assessed in 13 additional allopatric populations. In both transects, we found significant morphological differentiation between sympatric populations that was associated with a reduction in prey consumption in sympatry and a segregation of prey according to prey size. No trophic morphological or resource use differences were found between allopatric populations, and comparisons of sympatric populations with randomly paired allopatric populations revealed that the observed sympatric morphological differentiation was greater than expected by chance. The major trophic anatomical differences between sympatric populations relates to functional and biomechanical differences in jaw closure: sympatric *P. hoffmani* have a faster closing jaw, whereas sympatric *P. cinereus* have a slower, stronger jaw. Because salamanders immobilize prey of different sizes in different ways, and because the observed sympatric biomechanical differences in jaw closure are associated with the differences in prey consumption, the observed character displacement has a functional ecological correlate, and we can link changes in form with changes in function in this apparent example of character displacement.

When two closely related species come into contact, competitive interactions sometimes lead to morphological differentiation that is not apparent when the two species are found separately. Termed character displacement (1), this sympatric differentiation presumably occurs to reduce competition between the two forms, allowing them to coexist (2–6). Although many examples of character displacement have been reported, surprisingly few have held up under scrutiny, and many of the ''classic'' cases of character displacement can be readily explained by alternative hypotheses (2, 3, 7). This has led to the generation of a set of criteria that must be satisfied before the hypothesis of character displacement may be invoked as an explanation of sympatric divergence (2, 3, 6). Surprisingly, of those studies where alternative explanations have been ruled out, few have been able to assign a causal mechanism to explain the morphological divergence (5, 8). Here we report an apparent case of character displacement between two salamander species from the genus *Plethodon*, address the criteria of character displacement by using a combination of traditional and newly developed geometric morphometric techniques, and propose a functional explanation of the morphological divergence by using a simple biomechanical model.

Species of the genus *Plethodon* are terrestrial salamanders that live in moist woodland habitats and have no aquatic larval stage. One of the most wide-ranging species, *Plethodon cinereus*, is found in eastern North America, from the Atlantic seaboard west to the Mississippi River, and from North Carolina through southern Canada (9, 10). It is an effective competitor, and when sympatric with other congeners (e.g., *Plethodon shenandoah*), *P. cinereus* sometimes restricts the range of the congeners to less desirable habitats through competition, and occasionally drives local populations to extinction (11–13). In the Valley and Ridge physiographic province of central Pennsylvania, however, *P. cinereus* is notably absent, and another species, *Plethodon hoffmani*, is found (9, 10). A narrow band of sympatry is found along the contiguously allopatric ranges of these two species, and it is presumed that competition with *P. hoffmani* has resulted in this geographic distribution pattern (9). Because this hypothesis has not been rigorously tested, we studied the contact zone of *P. cinereus* and *P. hoffmani* in more detail to determine whether competition for resources could explain current species distributions.

Materials and Methods

We collected animals from three localities in each of two east-west geographic transects in south-central Pennsylvania that spanned the eastern (Fulton County) and western (Bedford and Somerset Counties) contact zones of the two species (for locality information see ref. 14). Each transect contained an allopatric locality for *P. cinereus*, an allopatric locality for *P. hoffmani*, and a sympatric locality. In addition, samples from 13 additional allopatric populations from the two species were obtained from the National Museum of Natural History and were used in several comparisons. We first performed a preliminary morphometric study by using conventional linear distance measurements. From each salamander, six head measurements (head length, head width, gape width, jaw length, head depth, and eye-nostril distance) and body size [snout-vent length (SVL)] were recorded, and stomach contents were assayed to ascertain food resource use (15). Prey consumed by each salamander were classified into one of 16 prey categories, and the length and width of each prey item was measured and used to estimate prey volume.

For each transect, morphological differences among populations were quantified for each head character individually, as well as for a set of size-adjusted head shape variables. As many methods for size-adjustment exist (16, 17), we chose to compare results from four of the commonly used methods: the set of ratios of each head character on SVL, the set of residuals from regressions of each head character on SVL, the set of principal component scores from an analysis of all head characters and

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Abbreviation: SVL, snout–vent length.

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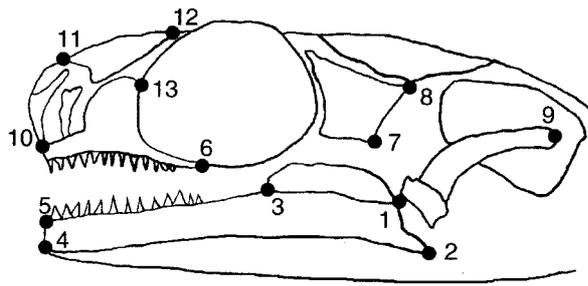
Fig. 1. Positions of 13 landmarks used in this study. All landmarks were digitized from the left-lateral view of the skull (modified from Adams, ref. 14).

SVL for all principal component axes except PC1 (which is generally considered to be size), and the set of Burnaby's (18) size-independent shape variables using an isometric size vector. In addition, we assessed the significance of the sympatric differentiation by using a protocol described by Schluter and McPhail (6), where the observed sympatric morphological differentiation is compared with the morphological differences between randomly paired allopatric populations from across south-central Pennsylvania. Specimens from five additional allopatric populations of *P. hoffmani* and eight additional allopatric populations of *P. cinereus* on both the eastern and western side of the range of *P. hoffmani* (four per side) were obtained from the National Museum of Natural History (Washington, DC), and the morphological measurements described above were quantified. We then paired each allopatric population of *P. hoffmani* to the allopatric populations of *P. cinereus* from both the eastern and western sides of the range and calculated the distance between population means, yielding 30 randomly paired allopatric distances for each transect. These were then compared with the sympatric morphological differentiation for that transect.

Unfortunately, it is difficult to integrate the results of analyses of linear measurements into an accurate visualization of shape differences between populations because the geometric relationships among the different measurements are not included in the data. We therefore performed additional morphological comparisons with shape variables generated by using geometric morphometric methods (19–21). Geometric morphometrics is an approach for studying shape variation and its covariation with other variables (19, 22, 23). We used shape variables generated from the *x,y* coordinates of biologically homologous landmarks, with the effects of any differences in translation, rotation, and scale mathematically held constant (24). These variables provide a rigorous quantification of overall shape that can be used for statistical analyses and to generate graphical representations of mean forms and to study trends in shape variation (21, 25, 26) and their covariation with other variables.

For the geometric morphometric analyses, we cleared and stained all specimens from Fulton County and digitized the locations of 13 biologically homologous landmarks from the skull and mandible of each (Fig. 1). We then used a newly developed method (separate subset method; ref. 27) that allowed for the analysis of articulated structures so that landmarks on both the skull and the mandible could be used to generate overall head shape variables for the multivariate analyses. With this method, shape variables for both the skull and mandible are generated separately by using standard techniques (22–24) and then combined to form a data set that represents the shape of the entire head (which includes a variable describing the ratio of size of the two subsets of landmarks; ref. 27). Extensive computer simulations have revealed that this method has superior statistical

power as compared with other proposed methods for analyzing landmarks from articulated structures (14).

We also analyzed food resource use among populations, which was compared to the resource base at each population as determined by invertebrate diversity surveys made at each locality. The degree of association between morphology and resource use was determined by using a two-block partial least-squares analysis. This technique models the covariation between the two sets of variables being studied as a product of linear combinations of each of the two sets of variables. The linear combinations are computed by using a singular-value decomposition of the matrix of covariances between the shape variables and the trophic variables. Because the variables are in different units, all variables (except those from geometric morphometrics) were standardized. Introductions and examples of this technique are given in refs. 28 and 29. Tests for significant associations between the two sets of variables are carried out by using permutation tests.

Results

Using ANOVA, we found significant morphological divergence in sympatry for all characters except head depth (Bedford County) and eye-nostril distance (Fulton County), with no significant differences among allopatric populations (Fig. 2*a*). Multivariate analyses of size-adjusted head shape revealed significant sympatric differentiation in head shape, regardless of which method of size-adjustment was used (Fig. 2*b*). For both transects, the observed sympatric morphological differentiation was greater than that found in all 30 randomly paired allopatric populations (Fig. 2*c*), implying that the observed difference was greater than expected by chance. In sympatry, *P. cinereus* attained smaller values for all characters, and *P. hoffmani* attained larger values for all characters in both geographic transects. All prey categories were found to be present at all localities, implying that the resource bases for each population were qualitatively similar. However, there were significant differences in prey consumption among populations. Both species consumed fewer prey per salamander when populations were sympatric rather than allopatric, and in sympatry *P. cinereus* specialized on smaller prey and *P. hoffmani* on larger prey (Fig. 2*d*). Randomization tests revealed that this sympatric prey segregation was greater than expected by chance (14, 15). Finally, a significant multivariate association between morphology and resource use was found by using two-block partial least-squares analysis. Smaller salamanders consumed smaller prey, and larger salamanders consumed larger prey.

Using multivariate ANOVA on shape variables from landmark-based geometric morphometric methods, we found significant sympatric divergence of head shape among populations (Fig. 3 *a* and *b*). A two-block partial least-squares analysis revealed that head shape was significantly associated with food resource use in this system (Fig. 3*c*). Salamanders with head morphology of sympatric *P. cinereus* were found to consume small prey, and salamanders with head morphology of sympatric *P. hoffmani* were found to consume large prey. The most pronounced anatomical difference between sympatric populations was in the size of the squamosal relative to the dentary (Figs. 3, *a* and *b*, and 4*a*). An ANOVA of this ratio revealed a significant difference among sympatric populations ($F = 15.478$, $P = 7.76 \times 10^{-9}$). This value is statistically significant, even after taking into account that this is an unplanned comparison and that the α level should be adjusted by using a Bonferroni method to reflect the number of possible landmark combinations that included the articulation point and one landmark each on the skull and mandible ($\alpha = 0.05/32 = 0.0015625$). Finally, it was observed that sympatric *P. hoffmani* attained a significantly smaller ratio and that sympatric *P. cinereus* attained a larger ratio
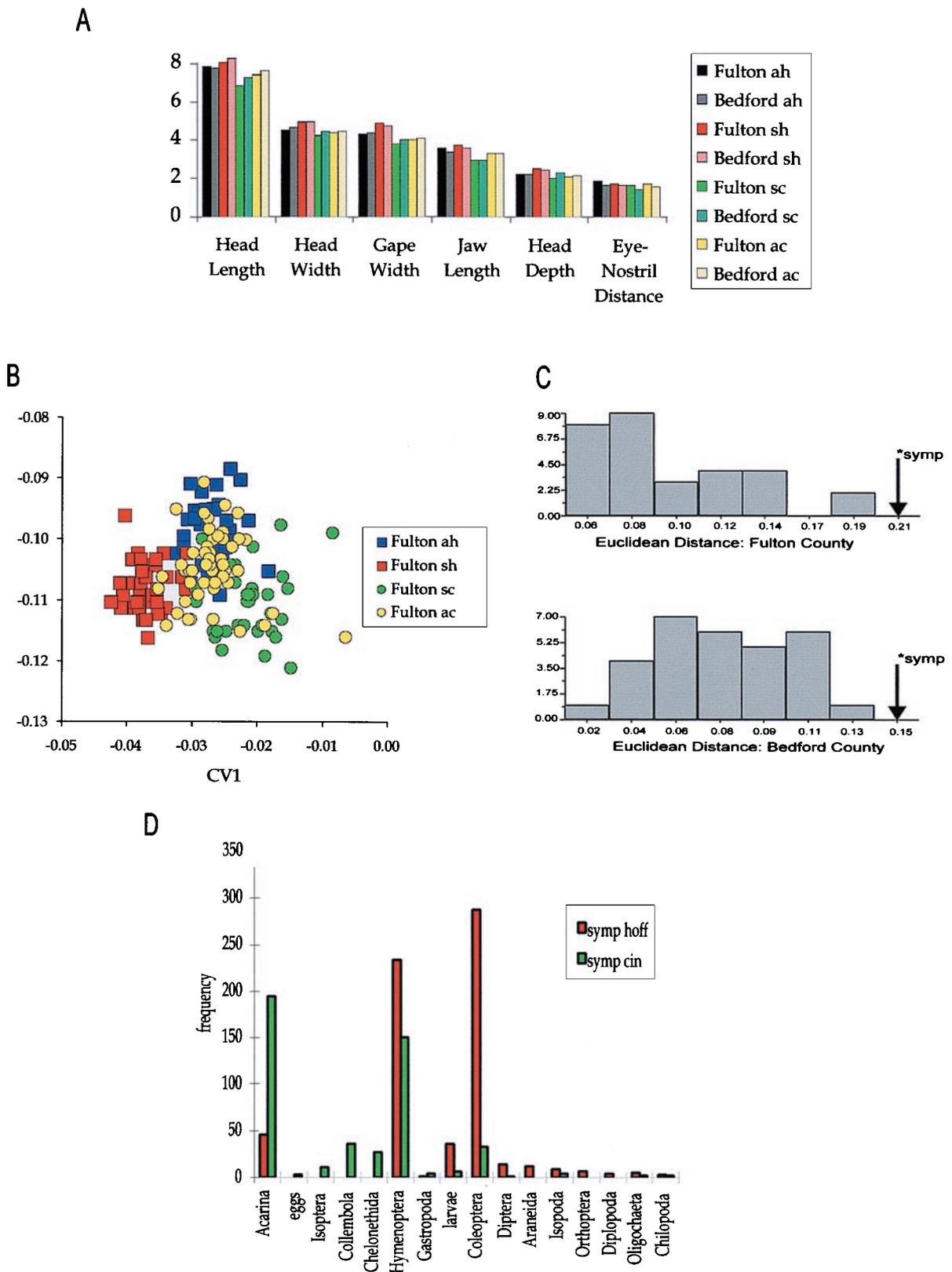
Fig. 2. (*A*) Mean values of morphological characters for *P. cinereus* and *P. hoffmani* in sympatry and allopatry for both the eastern geographic transect (Fulton County, PA) and the western geographic transect (Bedford and Somerset Counties, PA). Population labels are as follows: ah, allopatric *P. hoffmani;* sh, sympatric *P. hoffmani;* sc, sympatric *P. cinereus;* and ac, allopatric *P. cinereus.* (*B*) Plot of the first two axes from a canonical variates analysis of head shape (using ratios on SVL), showing the significant sympatric differentiation of size-adjusted head shape (Wilks' $\Lambda = 0.184$, $F = 18.91$, $P = 1.8 \times 10^{-43}$). (*C*) Histograms showing the distributions of morphological differences between randomly paired allopatric populations relative to the observed sympatric differentiation for both the eastern (Fulton County) and western (Bedford and Somerset Counties) transects. (*D*) Profiles of resource use for sympatric populations. The 16 prey categories are sorted according to size and show the significant segregation of resources (Fulton County data from Adams, ref. 15).
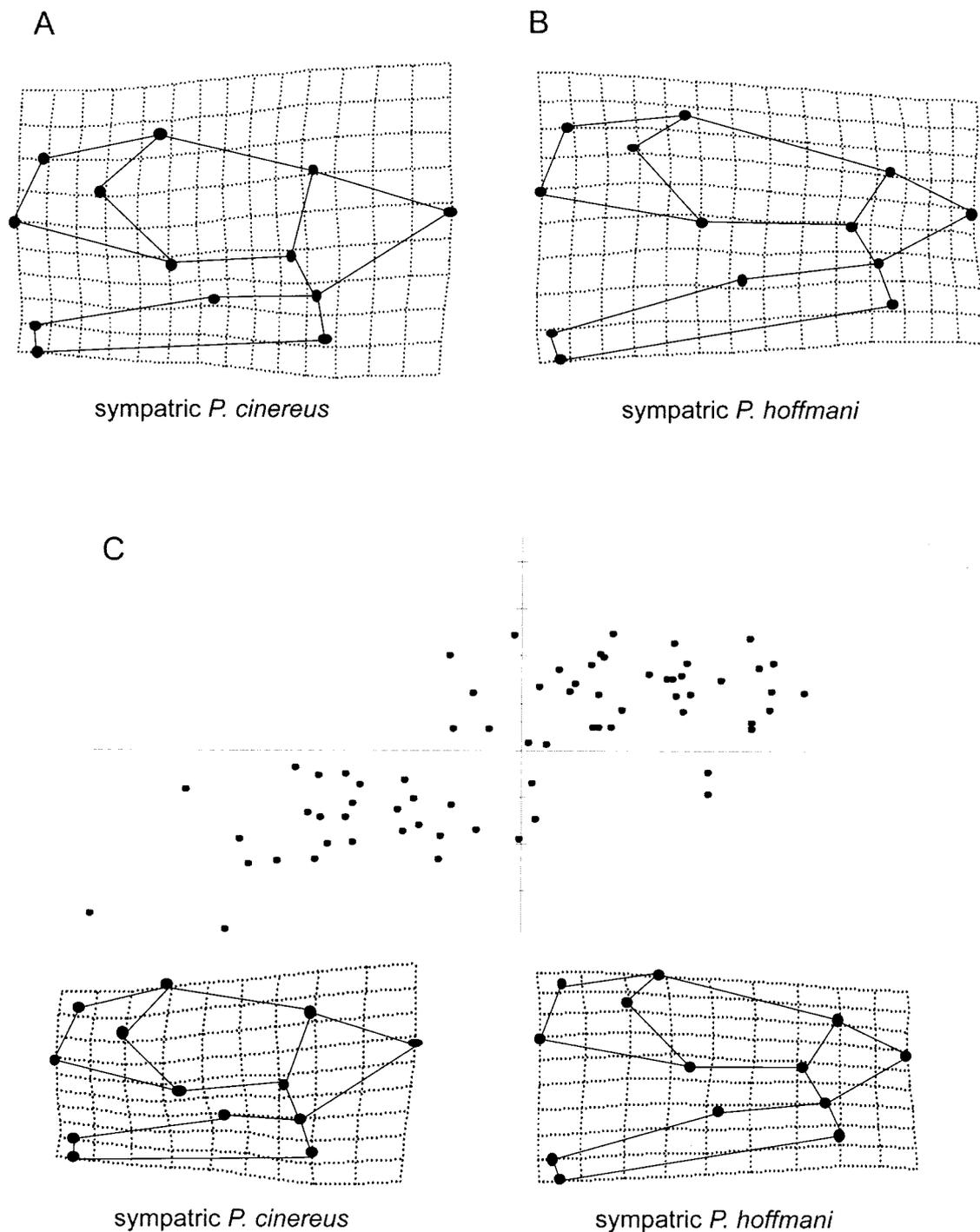
A

sympatric *P. cinereus*

B

sympatric *P. hoffmani*

C

sympatric *P. cinereus*

sympatric *P. hoffmani*

Fig. 3. (*A* and *B*) Thin-plate spline representations of the average specimen from sympatric *P. cinereus* and sympatric *P. hoffmani,* allowing visualization of the significant differences in head shape (Wilks' $\Lambda = 0.0513$, $F = 10.68$, $P = 2.99 \times 10^{-54}$), where the sympatric divergence (Mahalanobis generalized distance) was statistically greater than the allopatric divergence (randomization test; $P_{rand} = 0.02$). The observed sympatric differences have been exaggerated by a factor of 2. (*C*) Multivariate association for sympatric specimens of trophic morphology as represented by geometric shape variables and food resource use (square root of the number of prey consumed per prey category) from a two-block partial least-squares analysis ($r = 0.7591$, $P_{rand} = 0.001$). The *x* axis represents morphology (extremes illustrated by using a thin-plate spline), and the *y* axis represents food resource use (positive values = large prey; negative values = small prey).

(Fig. 4*b*). There were no significant differences between allopatric populations.

Discussion

In this study we found significant sympatric divergence of trophic morphology across two geographic regions spanning the contact zone between *P. cinereus* and *P. hoffmani*, and comparisons to randomly paired allopatric populations revealed that this morphological shift in sympatry was greater than expected by chance. Although the resource bases of each population were qualitatively similar, there was a shift in sympatry, where prey were segregated between *Plethodon* species according to size,
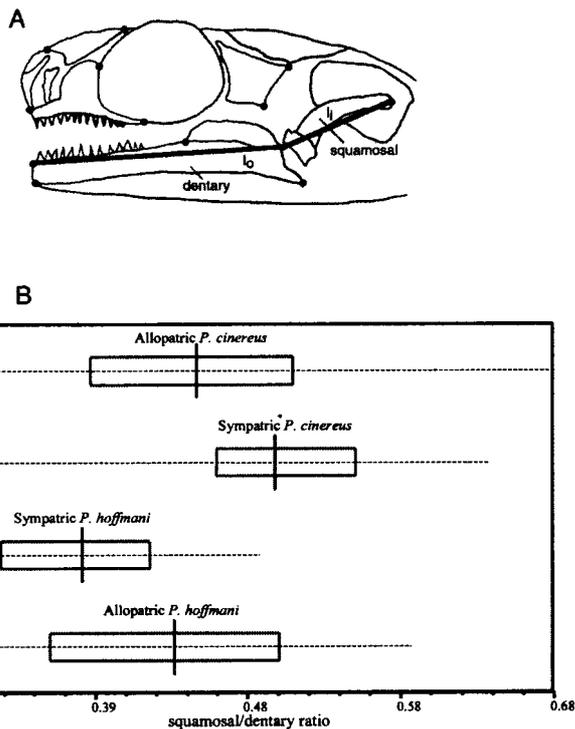
Fig. 4. (A) Biomechanical representation of jaw closing mechanism. The squamosal is represented as the in-lever (l_i), and the dentary is represented as the out-lever (l_o). (B) Plot of ratios of squamosal length to dentary length.

and this segregation of food resource use was statistically associated with trophic morphology. There was also a reduction of prey consumption in sympatry, implying competition for food resources. These data provide strong evidence that character displacement may be proposed as a causal mechanism to explain current morphological differences between sympatric populations of these species. Further, we have addressed aspects of five of the six criteria for character displacement (6), including the following: (*i*) chance can be ruled out as an explanatory factor; (*ii*) other explanatory variables (such as body size or geographic clines) can be eliminated; (*iii*) an association between morphology and resource use exists; (*iv*) there is evidence of competition for resources; and (*v*) the resource bases among populations are qualitatively similar (for a complete discussion see ref. 14).

One result of this study is that the major anatomical difference between sympatric populations, the relative lengths of the squamosal and dentary, has a biomechanical interpretation. Because the major jaw-closing muscle (adductor mandibulae) originates on the squamosal and inserts on the dentary in these salamanders (30–32), differences in this ratio result in changes in forces involved in jaw closure, and a simple biomechanical model may be used to predict these functional changes. In this model (33), the length of the

dentary and squamosal are l_o and l_i (Fig. 4*a*), the contraction force and velocity of the adductor mandibulae are F_i and v_i, and the closing force and velocity of the jaw are F_o and v_o. At equilibrium, there is no motion in this system, so: $F_i l_i = F_o l_o$ and $v_o l_i = v_i l_o$. Solving for the closing force and closing velocity of the jaw yields the following: $F_o = F_i l_i / l_o$ and $v_o = v_i l_o / l_i$. Assuming a constant contraction force and contraction velocity, a decrease in the ratio of squamosal length to dentary length decreases closing force but increases closing velocity, and an increase in ratio of squamosal length to dentary length increases closing force but decreases closing velocity. Interpreting the anatomical differences between sympatric populations in terms of this biomechanical model leads to the inference that sympatric *P. hoffmani* has evolved a faster, but weaker, jaw, and sympatric *P. cinereus* has evolved a slower, more powerful jaw.

Interestingly, the observed change in jaw closure found in sympatry is associated with a change in the prey consumption: sympatric *P. cinereus* has a slower, more powerful jaw and eats smaller prey, whereas sympatric *P. hoffmani* has a faster, weaker jaw and eats larger prey. This association can be explained from a functional morphological perspective. Although during prey capture most prey are initially contacted with the tongue, prey size is known to affect feeding mechanics in salamanders (34, 35), and in *Plethodon* in particular (36, 37). Large prey items are usually immobilized through rapid closure of the jaws, whereas small prey items continue to the rear of the oral cavity, where they are immobilized through pressure between the tongue and the palatal teeth (33, 35). Thus, the faster closing jaw of *P. hoffmani* corresponds to the mode of prey immobilization required for larger prey, and the stronger jaw of *P. cinereus* corresponds to the mode of prey immobilization required for smaller prey.

In this study we have used traditional and modern morphometric techniques to identify a possible case of character displacement in the genus *Plethodon*. Further, by using geometric morphometric techniques, we identified a functionally important anatomical change in sympatry and proposed a simple biomechanical explanation based on the feeding modes of salamanders. Thus, by using these powerful new methods, we were able to link observed character displacement to functional and biomechanical changes based on jaw closure and prey immobilization. Although such findings can be identified by using traditional morphometric methods, one must know *a priori* which ratios or angles to measure. A strength of geometric morphometrics is that all aspects of shape variation among a set of landmarks are captured, so specific anatomical changes (such as squamosal length/dentary length) can be detected without having to be specified and explicitly measured *a priori*. This work serves as an example of how geometric morphometrics may be used to identify such anatomical differences among populations.

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