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 sympathy 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 contiguous 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

*Abbreviation: SVL, snout-vent length.

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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 measures 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 morphometric methods (19–21). Geometric morphometrics is comparisons with shape variables generated by using geometric data. We therefore performed additional morphological com-
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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).
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 sympathy was greater than expected by chance. Although the resource bases of each population were qualitatively similar, there was a shift in sympathy, where prey were segregated between *Plethodon* species according to size.
and the 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 cline) 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), the major jaw-closing muscle (adductor mandibulae) originates on the squamosal and dentary, has a biomechanical interpretation. Because the squamosal is represented as the in-lever (4i), and the dentary is represented as the out-lever (4o). 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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Fig. 4. (A) Biomechanical representation of jaw closing mechanism. The squamosal is represented as the in-lever (4i), and the dentary is represented as the out-lever (4o). (B) Plot of ratios of squamosal length to dentary length.