

Bioclimatic modelling, morphology, and behaviour reveal alternative mechanisms regulating the distributions of two parapatric salamander species

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ABSTRACT

Question: What ecological and evolutionary processes are important in maintaining parapatric distributions of sibling species?

Hypotheses: Previous research has suggested that competition is a likely factor limiting species distributions and maintaining parapatric distributions. Alternatively, abiotic restrictions such as climatic factors and local adaptation can also limit species' ranges.

Organisms: *Plethodon hubrichti*, a mountaintop endemic salamander, has an extremely limited geographic range that is entirely surrounded by the widespread *Plethodon cinereus*. Previous studies have suggested that *P. hubrichti* may be geographically restricted as a result of interspecific competition with *P. cinereus*.

Methods: We studied the relationship between trophic morphology and diet in allopatric and sympatric populations to examine the effect of exploitative competition on geographic distribution. Behavioural experiments were conducted in the laboratory to determine the effects of interference competition. We complemented these analyses with bioclimatic modelling of species' ranges and climate–phenotype correlations to examine the abiotic impact.

Results: Our results suggest that aggressive interactions with *P. hubrichti* restrict the distribution of *P. cinereus* and not the converse, whereas *P. hubrichti* appears to be limited by abiotic climatic factors.

Keywords: aggression, geometric morphometrics, interspecific competition, *Plethodon*, species' distributions.

INTRODUCTION

Theoretical and empirical studies indicate that species' distributions result from interacting ecological and evolutionary factors (Kirkpatrick and Barton, 1997; Case and Taper, 2000; Cicero, 2004; Case *et al.*, 2005), including, but not limited to, abiotic restrictions, interspecific competition, dispersal

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limitation, and local adaptation. Studies that examine the boundaries between parapatric sibling species can offer valuable insight into which of these processes are more prevalent in shaping species' distributions (e.g. Cicero, 2004).

The terrestrial salamanders of the genus *Plethodon* exhibit a geographic pattern that makes them ideal for studying distributional limits. Throughout northeastern North America, the wide-ranging redback salamander (*P. cinereus*) completely surrounds the ranges of several closely related species. Previous studies have indicated that the distributional pattern of these salamanders is likely a result of intensive biotic interactions, such as competition among species (e.g. Highton, 1972; Hairston, 1980; Griffiths and Jaeger, 1998). One species, the Peaks of Otter salamander (*P. hubrichti*), is geographically restricted to an area less than 120 km² in the Blue Ridge Mountains of west-central Virginia (Pague and Mitchell, 1990) and has a narrow sympatric zone with *P. cinereus*. Both *P. cinereus* and *P. hubrichti* appear to be territorial [*P. cinereus* (reviewed in Jaeger, 1986), *P. hubrichti* (Thurow, 1976; Wicknick, 1995)]. Based on similar patterns in other *Plethodon*, Highton (1972) and Jaeger (1974) suggested that *P. hubrichti* is geographically restricted as a result of interspecific competition with *P. cinereus*.

We tested ecological hypotheses of range restriction in these two woodland salamander species. Specifically, we tested the competitive interaction hypothesis using a combination of morphological, behavioural, and food-use data. We then used bioclimatic modelling and climate–morphology correlations to determine the importance of environmental gradients and local adaptation. We combined results from our analyses to illuminate the processes that maintain the parapatric distributions and narrow sympatric zones of these two salamander species.

MATERIALS AND METHODS

Collection sites

We obtained 571 adult specimens (251 *P. cinereus* and 320 *P. hubrichti*) from 18 localities (sites) across the range of *P. hubrichti* and the surrounding habitat occupied by *P. cinereus*. Specimens were obtained from the National Museum of Natural History (Washington, DC), the Carnegie Museum of Natural History (Pittsburgh, PA), and from our own personal holdings collected in multiple years. Two localities represented sympatric sites for the two species, while the remaining 16 localities represented single-species allopatric sites (8 localities per species).

Food use

We quantified food use from stomach contents of 87 preserved specimens collected in 2004 (DCA & SA collection) to determine whether food-use niche-partitioning was evident. The specimens were obtained from a north–south transect comprising an allopatric *P. cinereus* site (23 specimens), a sympatric site (23 *P. hubrichti* and 15 *P. cinereus*), and an allopatric *P. hubrichti* site (26 specimens). All specimens were collected on the same day between 09.00 and 15.00 h and formalin fixed the same evening. Entire stomachs were excised from specimens and stomach contents were removed and suspended in 90% ethanol. Prey consumed was classified to the level of order (e.g. Adams and Rohlf, 2000). To determine whether interspecific competition resulted in food-use niche-partitioning, we used a *G*-test of

independence that compared resource-use profiles of sympatric and allopatric populations of both species.

Morphometrics

We quantified head shape morphology for all specimens using landmark-based geometric morphometrics methods (Rohlf and Marcus, 1993; Adams *et al.*, 2004). First, we obtained digital images of the left-lateral side of each head using a Nikon DXM-1200 digital camera mounted on a Nikon SMZ1500 stereomicroscope. We then digitized the location of 11 biologically homologous landmarks from the skull and jaw (Fig. 1) using TPSDIG (Rohlf, 2004). Variation in the position of the mandible relative to the skull was standardized by rotating the mandible of all specimens to a fixed angle relative to the skull (Adams, 1999). Non-shape variation was then removed using a generalized Procrustes analysis [GPA (Rohlf and Slice, 1990)], which superimposes specimens to a common coordinate system after accounting for differences in position, orientation, and scale. From the aligned specimens we generated shape variables as partial warp scores from the thin-plate spline (Bookstein, 1991) and the standard uniform components (Rohlf and Bookstein, 2003). Head shape was then represented by the set of principal component scores from all principal component axes that expressed variation.

To test for sympatric character divergence, we assessed morphological differentiation between species and between sites (allopatry vs. sympatry) using a two-way multivariate analysis of variance (see Adams *et al.*, 2007). Differences in head shape among populations were visualized using a principal components analysis (PCA). To test for morphological divergence between species in sympatry relative to allopatry, we employed two different permutation procedures (*sensu* Adams, 2004). The first examines whether observed sympatric differences between species (Euclidean distance between group means) are greater than or less than differences between randomly paired allopatric populations. This procedure evaluates between-population divergence relative to that observable by chance alone, while maintaining the within-population morphological covariance. The second compares the observed divergence between allopatric and sympatric populations (D_{obs}) to the divergence between allopatric and sympatric populations where individuals are randomly assigned (4999 permutations) to populations (D_{rand}). This second procedure provides a complementary test of divergence by comparing between-population divergence against patterns of within-population covariation as generated by chance alone.

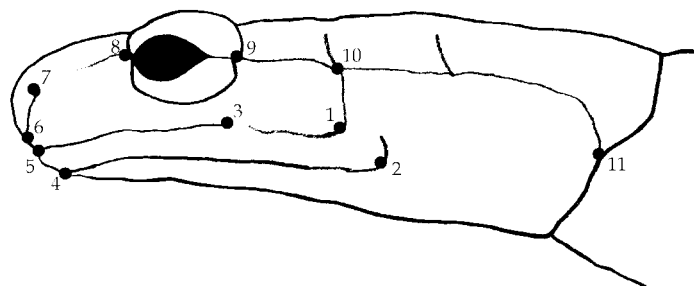


Fig. 1. Positions of 11 landmarks used in this study. All landmarks were digitized from the left-lateral view of the skull (adapted from Adams, 2004).

Behaviour

Experiments were conducted to determine whether the two species potentially competed behaviourally via an interference mechanism. Thirty adult males of each species were collected from two allopatric sites in October 1993 (JAW collection). Specimens were maintained in the laboratory on a 12:12 h light/dark cycle at $16 \pm 1^\circ\text{C}$ and were fed 20–30 *Drosophila virilis* (Diptera) once weekly. The *P. hubrichti* specimens had a snout-to-vent length (SVL) of 44.3–51.7 mm, the *P. cinereus* specimens a snout-to-vent length of 41.3–52.3 mm.

Individuals of each species were allowed 104 days to establish territories in individual $230 \times 230 \times 20$ mm test chambers lined with 7 mm of soil moistened with spring water. We recorded frequencies of aggressive and submissive behaviours from 60 paired interspecific interactions. Since size is important to the outcome of behavioural interactions, animals were paired by size as follows. We grouped animals of each species into three SVL categories – large, medium, and small. Each resident was then randomly paired with a heterospecific intruder from the same size category (maximum size difference 6.1 mm). Each individual was used once as a resident and once as an intruder, in random order. Pairings were not repeated. Seven days elapsed between trials.

Residents and intruding heterospecifics were picked up and then placed under opaque-sided acclimation dishes in the residents' chambers for 5 min. Behavioural data were collected from residents for 15 min. after the first agonistic behaviour (Jaeger, 1984) was performed. Aggressive behaviours (look toward, move toward, all trunk raised, bite) and submissive behaviours (look away, move away, flat) were categorized based on previous studies of *Plethodon* behaviour (Wrobel *et al.*, 1980; Jaeger, 1984). To establish which of the two species was more aggressive, we analysed total frequency of aggressive behaviour and total frequency of submissive behaviour using Mann-Whitney *U*-tests (two-tailed) with $\alpha = 0.05$.

Bioclimatic modelling and climate–morphology association

To test the hypothesis that abiotic factors limit the range of *P. hubrichti*, we generated bioclimatic models and examined correlations among climatic variables and morphology. Nineteen local climatic indices were extracted from the WORLDCLIM data set (Hijmans *et al.*, 2005) for the set of geo-referenced locations of available localities using the software DIVA-GIS (Hijmans *et al.*, 2004). An examination of the correlation matrix of all 19 variables revealed potential redundancy among the majority of the variables (Table 1). We chose average temperature (Bio1) and average precipitation (Bio12) as a proxy for other variables (for $r > 0.9$ between proxy variables and all excluded variables). Variables that could not be excluded with this demarcation ($r < 0.90$ among any set of two variables) included precipitation seasonality (Bio15) and temperature seasonality (Bio4).

To assess correlations among morphology and climate, we used a two-block partial least squares [PLS (Rohlf and Corti, 2000)] to determine the degree of association between the four local environmental variables and the average morphology for each of the geographic localities. Tests of statistical significance were carried out with a permutation procedure in TPSPLS (Rohlf, 2005). Since both data sets may have a spatial component (Lennon, 2000; Legendre *et al.*, 2002), we conducted multiple Mantel tests to explore the association of the variables with geographic distance. If any one of the data sets is spatially autocorrelated, then the *P*-value

Table 1. A listing of the 19 environmental variables found in the WORLDCLIM (Hijmans *et al.*, 2005) data set

Abbreviation	Environmental variable
Bio1	Annual mean temperature
Bio2	Mean diurnal temperature range (mean of monthly maximum temperature minus minimum temperature)
Bio3	Isothermality (Bio2/Bio7 * 100)
Bio4	Temperature seasonality (standard deviation of monthly temperature)
Bio5	Minimum temperature of the coldest month
Bio6	Maximum temperature of the warmest month
Bio7	Temperature range (maximum temperature of the warmest month minus minimum temperature of the coldest month)
Bio8	Mean temperature of wettest quarter (i.e. mean temperature of four consecutive wettest months)
Bio9	Mean temperature of driest quarter
Bio10	Mean temperature of warmest quarter
Bio11	Mean temperature of coldest quarter
Bio12	Annual precipitation
Bio13	Precipitation of wettest month
Bio14	Precipitation of driest month
Bio15	Precipitation seasonality (standard deviation of monthly precipitation)
Bio16	Precipitation of driest quarter
Bio17	Precipitation of wettest quarter
Bio18	Precipitation of warmest quarter
Bio19	Precipitation of coldest quarter

Note: Bio1, Bio4, Bio12, and Bio15 were used to assess the association among climate and morphology. Ecological-niche models were constructed using these 4 and all 19 variables.

for association between morphology and climate is likely to be biased downwards (Legendre *et al.*, 2002). Morphological and climatic distance matrices were constructed from PLS scores, while the geographic distance matrix was calculated from latitude/longitude coordinates. We evaluated the correlation of each variable with geographic distance using two-way Mantel tests. A three-way Mantel test (Legendre and Legendre, 1998) was used to evaluate the relationship between the two variables while holding geographic distance constant. Statistical significance of the partial Mantel correlations was evaluated using 9999 Monte Carlo randomizations. All Mantel tests were conducted using the software PASSAGE (Rosenberg, 2002).

Next, we used bioclimatic modelling to examine whether the distributions of both species in this region are closely approximated by their predicted distribution based on climatic variables (Peterson *et al.*, 1999; Raxworthy *et al.*, 2003). Bioclimatic models were constructed using the four bioclimatic indices over a 1-km² resolution grid of points. We modelled the expected ranges of *P. hubrichti* and *P. cinereus* using 23 and 1744 geo-referenced localities respectively. We then compared the predicted range of *P. cinereus* to the current distribution of *P. hubrichti*. For both models, we used the BIOCLIM (Nix, 1986) algorithm with a true/false criterion and a standard percentile cut-off (95 and 97.5 percentile) in DIVA-GIS.

RESULTS

We found no evidence to support the hypothesis that biotic interactions with *P. cinereus* have restricted the geographic range of *P. hubrichti*. First, the two species did not partition food resources in sympatry as was expected from the exploitative competition hypothesis ($G_{\text{adj}} = 15.87$, d.f. = 15, $P = 0.2559$). In sympatry (Fig. 2) and in allopatry (data not shown for allopatric populations), the dominant prey items for both species were Acarina, Haplotaxida (earthworms), Collembola, and Hymenoptera. All Acarina, Collembola, and Haplotaxida species eaten appeared to be morphologically similar (personal observation). Second, when head shape was examined we found significant phenotypic differentiation between species (Wilks' $\lambda = 0.54$, $F = 16.34$, $P < 0.0001$) and between sites (Wilks' $\lambda = 0.157$, $F = 4.82$, $P < 0.0001$), but sympatric phenotypic divergence between species was not greater than allopatric phenotypic divergence between species ($P_{\text{rand}} = 0.8$). Instead, we found significant phenotypic convergence for both sympatric localities, where the observed sympatric phenotypic divergence was smaller than the divergence for 60 of 66 ($P < 0.0001$) and 43 of 66 ($P = 0.0047$) randomly paired allopatric populations of both species (Fig. 3). Finally, when interference competition was considered, we found that *P. hubrichti* was both significantly more aggressive (*P. hubrichti*: $\bar{X} = 23.6$, *P. cinereus*: $\bar{X} = 11.7$, $N = 30$, $Z = 4.83$, $P < 0.0001$) and significantly less submissive (*P. hubrichti*: $\bar{X} = 0.47$, *P. cinereus*: $\bar{X} = 2.07$, $N = 30$, $Z = 3.88$, $P < 0.0001$) than *P. cinereus* during interspecific encounters in the laboratory (Fig. 4).

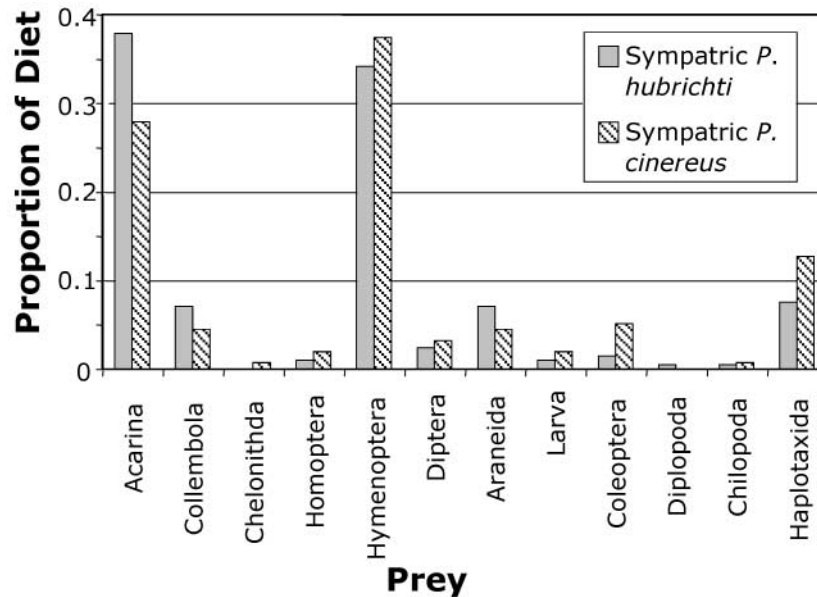


Fig. 2. Profile of stomach contents of sympatric *P. hubrichti* and *P. cinereus*. Data from 15 *P. cinereus* and 23 *P. hubrichti* specimens are shown. Prey types are ordered by relative magnitude of size (no actual size measurements were taken) from small to large. Because of differences in specimen number, prey items eaten are represented as proportion of total diet for ease of interpretation. Total prey items numbered 214 for *P. hubrichti* and 158 for *P. cinereus*.

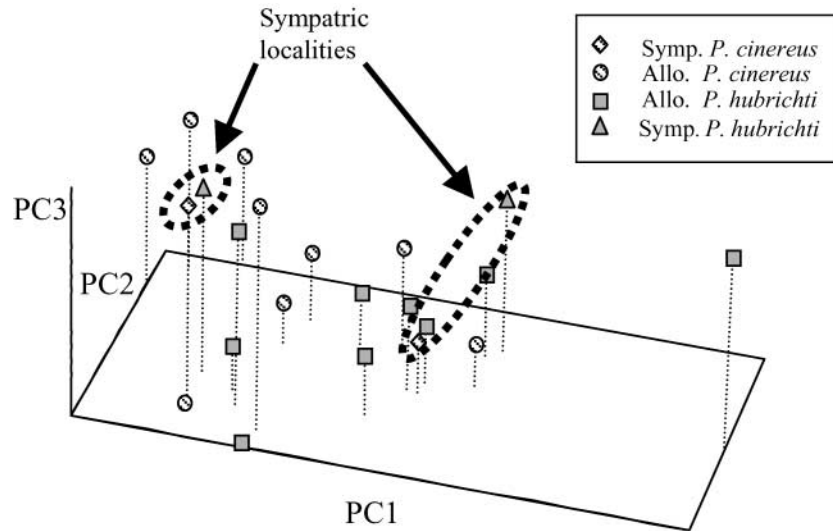


Fig. 3. Principal components plot describing differences in head shape variation among allopatric and sympatric populations. The three principal components axes account for 79% of the variation in the data. Populations from sympatric sites indicated (population averages are shown).

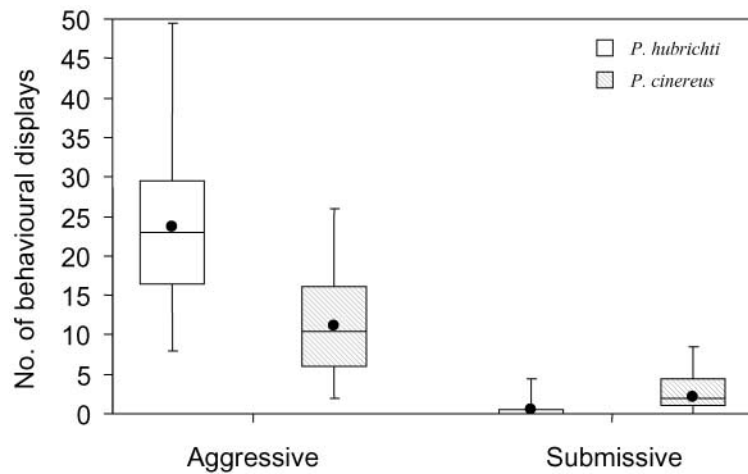


Fig. 4. Box-and-whisker plots of interspecific behaviours exhibited during laboratory trials. Aggressive behaviours include look toward, move toward, all trunk raised, and bite; submissive behaviours include look away, move away, and flat. The figure displays medians (lines), 25% to 75% quartiles (boxes), ranges (whiskers), and means (black circles).

We found evidence to support the hypothesis that the abiotic environment has affected the distribution of *P. hubrichti*. Ecological niche modelling revealed that environmental climatic information was sufficient to predict the range of *P. hubrichti* (Fig. 5A). Models using both 4 and all 19 variables had qualitatively similar results and models based on all 19 variables were retained for further analysis. The expected range of *P. hubrichti* correctly

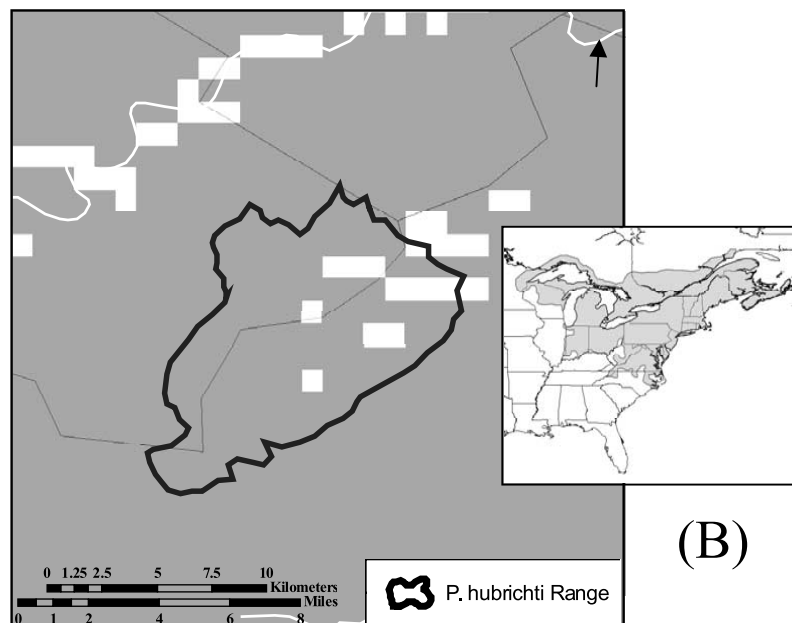
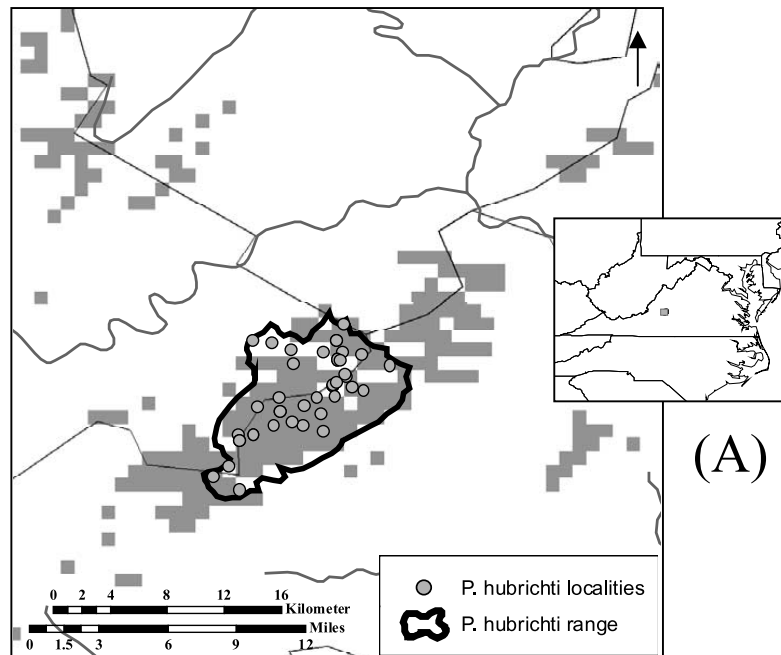


Fig. 5. (A) Ecologically viable range for *P. hubrichti* (grey), as predicted using the BIOCLIM algorithm (true/false criterion with 95 percentile cut-off). The current distribution of *P. hubrichti* is outlined in black. (B) Ecologically viable range for *P. cinereus* (grey) near the range of *P. hubrichti*, as predicted using the BIOCLIM algorithm (true/false criterion with 97.5 percentile cut-off). The current distribution of *P. hubrichti* is outlined in black; *P. cinereus* is nearly absent from this region. For both predictions, 19 bioclimatic variables were used (predictions based upon four bioclimatic indices were nearly identical).

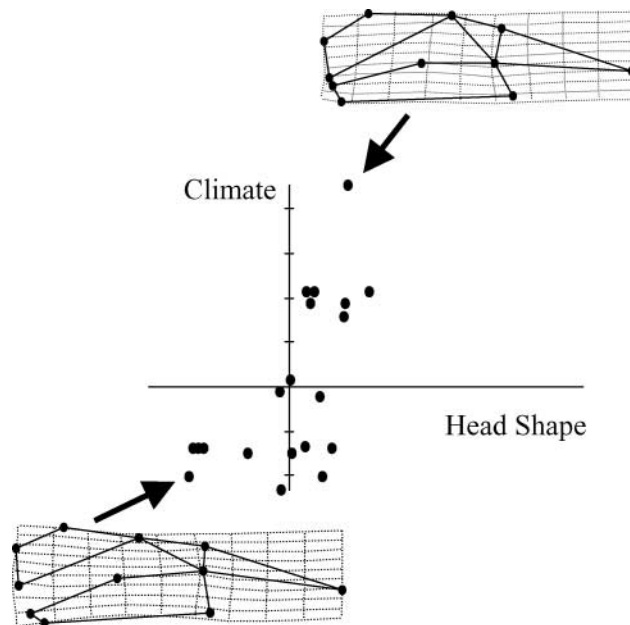


Fig. 6. Multivariate association of head shape and environmental variation for the 20 populations used in this study ($r = 0.61$, $P = 0.05$). The x-axis represents morphology (extremes illustrated by using a thin-plate spline), while the y-axis represents environmental variation (positive values = higher temperatures and more seasonal variation, negative values = higher rainfall).

predicted 80% of the known localities for the species. There is some over-prediction by the model in areas adjacent to the southwestern and northeastern limits of the observed range (Fig. 5A), while other over-predicted areas lie beyond geographical barriers such as river valleys. In contrast, the predicted ecologically viable range of *P. cinereus* encompassed 90% of the distribution of *P. hubrichti* (Fig. 5B), a region where *P. cinereus* is largely absent. Second, there was a significant association between the local environmental variables and average morphology for all 20 populations ($r = 0.61$, $P = 0.05$; Fig. 6). Salamanders from regions with more rainfall had more robust lower jaws relative to the cranium, while salamanders from regions with higher temperatures and more seasonal variation in temperature and rainfall had the opposite phenotype (Fig. 6). Two-way Mantel tests revealed a significant spatial autocorrelation of the climatic variables ($r = 0.7131$, $t = 5.768$, $P = 0.0001$). There was no support for a similar clinal trend in morphology ($r = 0.1321$, $t = 0.9666$, $P = 0.2224$). With geographic distance held constant, the correlation between morphology and climate did not remain statistically significant ($r = 0.1328$, $t = 1.5748$, $P = 0.0624$); however, it is still in proximity to a 0.05 significance level.

DISCUSSION

In this study, we examined the mechanisms maintaining the parapatric distributions of two salamander species. We found no support for the hypothesis that competitive interactions restrict the range of *P. hubrichti*. Lack of niche-partitioning of diet in sympatry (Fig. 2) suggests exploitative competition is unlikely to be an important factor. Behavioural

experiments indicate that interference competition might be important in sympatric communities, but competition would limit the geographic expansion of *P. cinereus*, not *P. hubrichti*, since *P. hubrichti* is more aggressive than *P. cinereus* (Fig. 4). These results are contrary to the expectation that *P. hubrichti* is geographically restricted as a result of interspecific competition with *P. cinereus* (Highton, 1972; Jaeger, 1974). Additionally, sample surveys over an 11-year period show constant proportions of both species at one sympatric site [~60–70% *P. hubrichti* (Wicknick, 1995; Aasen and Reichenbach, 2004; present study)]. This pattern corroborates the hypothesis that the sympatric zones are ecologically static and *P. hubrichti* is dominant over *P. cinereus*.

We did find evidence that the current geographic distribution of *P. hubrichti* is consistent with and predicted by abiotic factors (Fig. 5A). Our models show no such climatic restriction for *P. cinereus* (Fig. 5B). Surveys of the flora around the boundary areas of species' distributions revealed no drastic change in vegetation from the allopatric distributions of one species to that of the other (Wicknick, 1995). Additionally, sympatric morphological convergence (Fig. 3) and an association between climate and morphology (Fig. 6) suggest that local adaptation may also be important in maintaining current distributions, as both patterns are consistent with local adaptation (Mittelbach *et al.*, 1999; Pakkasmaa and Piironen, 2001). Although the correlation between morphology and climate is not statistically significant when spatial autocorrelation is taken into account ($P = 0.0624$), it is marginally significant. As such it is unclear whether *P. hubrichti* is limited due to a physiological abiotic restriction or local adaptation. Nonetheless, recent work (Kozak and Wiens, 2006) suggests that several other *Plethodon* mountaintop endemics may also be range-restricted due to climatic factors.

Some caveats about our results and analyses should be noted. First, the results from our laboratory-based behavioural experiments should be directly tested with field experiments. Unpublished data on transplant experiments in *P. hubrichti* and *P. cinereus* communities show an adverse affect of *P. hubrichti* on *P. cinereus* at the population level (R. Highton, personal communication). These results are congruent with our conclusions based on laboratory experiments. Second, there is no direct evidence for the influence of local adaptation in our system. However, our results provide evidence of sympatric morphological convergence and association of morphology and climate, both of which are consistent with local adaptation. Conclusive evidence for the importance of local adaptation requires more intensive investigation.

Overall, our results indicate that *P. cinereus* appears to be excluded due to competition with *P. hubrichti*, while *P. hubrichti* remains restricted because of climatic factors. Hence, the narrow sympatric zones of the two species are maintained by both abiotic and biotic factors, which restrict one or the other species from expanding its current range. A similar situation is found between *P. ouachitae*, an extremely aggressive mountaintop isolate, and the wide-ranging *P. albagula*. Here the smaller endemic species maintains its geographic range behaviourally, but does not expand its range at the expense of *P. albagula* (Anthony *et al.*, 1997). Future research should determine whether *P. ouachitae* is similarly geographically restricted as a result of local adaptation and/or abiotic restrictions.

ACKNOWLEDGEMENTS

We thank C. Anthony, C. Beachy, M. Collyer, J. Dixon, R. Jaeger, J. Mitchell, D. Moen, D. Ruggiero, S. Smith, N. Valenzuela, J. Wiens, and D. Wake for reading earlier versions of the manuscript, and

P. Rengusang and R. Hijmans for assistance with GIS software. C. Anthony, R. Jaeger, and J. Mitchell provided assistance to J.A.W. for the behavioural study. Specimens were collected under permits Virginia-SCP9348, US Forest Service (File code 2600), and US Department of the Interior (RM-29) (to J.A.W.) and Virginia-023890, US Forest Service (File code 2600), and US Department of the Interior (BLRI-2004-SCI-0019) (to D.C.A.). Finally, we are deeply indebted to A. Wynn, whose patience and continual assistance in providing specimens from the collections of the Smithsonian made our research possible. This project was sponsored in part by National Science Foundation grants DEB-0122281 and DEB-0446758 (to D.C.A.), DEB-9314081 (to R.G. Jaeger), National Geographic Society grant 5108-93 (to R.G. Jaeger), and Society of Sigma Xi and the GSO at University of Louisiana at Lafayette (to J.A.W.), and support from the Blue Ridge Parkway and the Jefferson National Forest (to J.A.W.).

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