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Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: validation of an intrinsic determinant of macroecological and phylogeographic structure

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Global warming is now recognized as the dominant threat to biodiversity because even protected populations and habitats are susceptible. Nonetheless, current criteria for evaluating species' relative endangerment remain purely ecological, and the accepted conservation strategies of habitat preservation and population management assume that species can mount ecological responses if afforded protection. The insidious threat from climate change is that it will attenuate or preclude ecological responses by species that are physiologically constrained; yet, quantitative, objective criteria for assessing relative susceptibility of diverse taxa to warming-induced stress are wanting. We explored the utility of using interspecies physiological variation for this purpose by relating species' physiological phenotypes to landscape patterns of ecological and genetic exchange. Using a salamander model system in which ecological, genetic and physiological diversity are well characterized, we found strong quantitative relationships of basal metabolic rates (BMRs) to both macroecological and phylogeographic patterns, with decreasing BMR leading to dispersal limitation (small contemporary ranges with marked phylogeographic structure). Measures of intrinsic physiological tolerance, which vary systematically with macroecological and phylogeographic patterns, afford objective criteria for assessing endangerment across a wide range of species and should be incorporated into conservation assessment criteria that currently rely exclusively upon ecological predictors.

1. INTRODUCTION

Anthropogenic warming is now the dominant threat to global biodiversity, but objective, operational criteria for assessing species' relative endangerment from climate change remain wanting (IUCN 2001; Willott & Thomas 2001; Thomas et al. 2004). Present criteria are exclusively ecological (e.g. population size, range size), underlain by the implicit assumption that protected species can mount biological responses to climate change if afforded protection. This conservation scheme ignores species differences in physiological optima and tolerances that determine their ecological potential during climate change (Bernardo & Spotila 2006). There is a pressing need in conservation biology and climate change research for cross-species, quantitative, objective criteria for assessing the susceptibility of species to climate change-induced extinction.

One attempt to address this shortcoming is phenomenological, using climatic descriptions of contemporary ranges coupled with expected temperatures to predict future ranges. These bioclimatic models are best case scenarios because, while they reflect existing interspecific differences in projected ranges under global warming, they also implicitly assume that all species have similar potential to access and exploit predicted climate space.

Here we explore an alternative, mechanistic approach that uses intrinsic properties of species to predict their responses to climate change. The first step of this approach (examined here) is to assess whether physiological differences among species are quantitatively related to population ecological and genetic dynamics. If so, such functional relationships could be used to make explicit quantitative predictions about not only potential ranges, but also species' ecological and population genetic fates as warming ensues. Ecologists have long appreciated that physiological diversity must relate to interspecific differences in habitat occupancy and use, but there are surprisingly few quantitative data demonstrating such a relationship. As Gaston (2003) observed: 'impressions as to such differences abound, but seem little more developed than that.' Compared with widely studied extrinsic regulators of dispersal and establishment (e.g. interspecific interactions, climate), the impact of interspecific physiological differences on macroecological patterns is underappreciated (Brown 1995; Kirkpatrick & Barton 1997; Clarke 2003). Similarly, while ecological variables are being incorporated into comparative phylogeographic analyses, the effects of interspecific physiological variation are not studied. Thus, demonstrations of quantitative connections between physiology and macroecological patterns or, especially, phylogeographic patterns, are generally lacking.

We studied physiological variation among *Desmognathus* salamanders endemic to the Appalachian Mountains of eastern North America. This

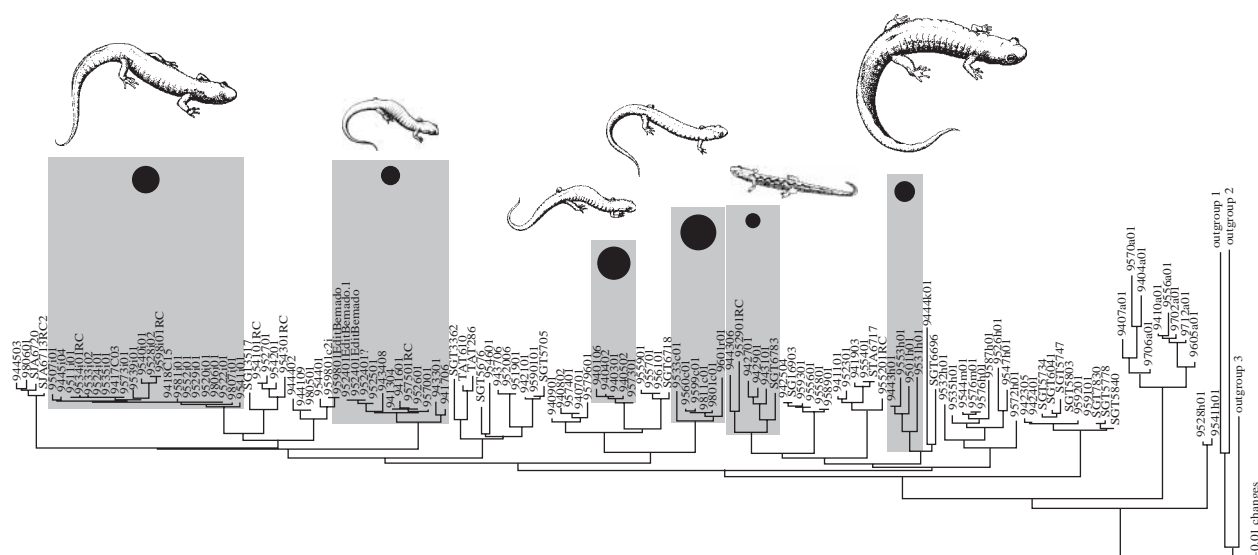This journal is © 2007 The Royal Society

Figure 1. *Desmognathus* phylogeny (nj cytB) illustrating interspecific variation in phylogeographic structure among study species. Grey boxes encompass population samples of each species. Branch lengths are proportional to change (see scale in image). Black circles within rectangles depict the least-squares means (LSM) BMR (table 1; see §2) of each species, scaled to the maximum rate (100% = *Desmognathus fuscus*).

radiation comprises 27 nominal and undescribed species (J. Bernardo, S. G. Tilley, M. M. Yoke & K. A. Crandall 2007) that exhibit sevenfold variation in adult size, which is closely associated with patterns of microhabitat use (Tilley & Bernardo 1993) but, surprisingly, not range size. Both large and small species are narrowly and widely distributed, suggesting that factors other than body size affect range occupation.

We previously reported (Bernardo & Spotila 2006) that two montane species with restricted ranges (*Desmognathus ocoee* and *Desmognathus carolinensis*) exhibit marked physiological depression at moderate environmental temperatures, which was inversely correlated with elevation. We hypothesized that these forms are physiologically specialized to cool montane habitats, apparently at the expense of lowland dispersal, a physiological trade-off analogous to the one first articulated by Janzen (1967) for tropical organisms. This paper tests those ideas.

2. MATERIAL AND METHODS

(a) *Species' characteristics and metabolic rate data*
We studied six species for which comparable basal metabolic rate (BMR) data (VO_2 max from trials at 5, 10, 15 and 20°C) were available (figure 1; for detailed descriptions of species, ranges and habits, see electronic supplementary material, appendix 1). Fortuitously, these species vary considerably in body size, ecological niche and range size, and both widely and narrowly distributed groups contain large- and small-bodied species and highly aquatic and highly terrestrial forms. Importantly, these species also capture much of the phylogenetic diversity in this genus (figure 1). Units were standardized (ml h^{-1}) and data were ln-transformed to meet linearity assumptions of GLM.

(b) *Quantitative estimates of range size*
We computed the range of Cowee *D. ocoee* as the area of the Cowee Mountains above 900 m, its lower elevational limit (Bernardo & Spotila 2006). Range sizes of other species were calculated from range maps (Petranka 1998) that were scanned, pixelated, scaled to square kilometre and calibrated by scans of known area.

(c) *Phylogeographic structure parameter*
For each pair of conspecific populations, we computed the ratio of sequence divergence (%SD) estimated from a 540 bp region of

cytochrome *b*, to great circle distance (km) between samples. These estimates (%SD/km) were then averaged for each species.

(d) *Statistical analyses: unbiased estimates of species-specific BMR*
We used analysis of covariance (ANCOVA) carrying body size of subjects (mass, g) as a covariate (Packard & Boardman 1999) and species and temperature as factors to evaluate whether there were significant interspecific differences in BMR to justify it as a predictor variable (least-squares means (LSMs) of BMR, i.e. unbiased species-specific estimates, independent of allometric effects and experimental temperatures) for the hypothesis testing analyses of range size and phylogeographic structure.

(e) *Statistical analyses: hypothesis tests*
To test for a relationship between BMR and range size, we computed ANCOVAs using LSM BMR (§2d) and species' characteristic body size (maximum or average adult size (mm), snout-to-vent length in separate analyses) as factors. To test for a relationship between BMR and phylogeographic structure, we regressed mean %SD/km against LSM BMR using nonlinear regression (figure 2b).

3. RESULTS
We found significant interspecific differences in BMR (figure 1) after accounting for the effects of subject size and trial temperature (ANCOVA: $F_{10,44} = 167.879$, $p < 0.001$; $R^2 = 0.9745$; table 1), thus justifying interspecies physiological differences as a predictor variable in the analyses of landscape-level patterns.

Ranges of the study species varied by four orders of magnitude, from 1715 km^2 in Cowee Mountains *D. ocoee* to 6 857 949 km^2 in *Desmognathus fuscus*. Yet, species-specific BMR closely determined range size; this effect was distinctly not attributable to species' characteristic body sizes (adult size, ANCOVA, $F_{2,5} = 43.0090$, $p = 0.0029$; $R^2 = 0.9663$; table 2; electronic supplementary material). Species with the highest BMRs had the largest ranges, whereas species with low BMRs had far smaller ranges restricted to the montane Southern Appalachian Highlands. This pattern was not explained by body size: both large- and small-bodied species had broad and restricted ranges (figure 2a), and neither body size estimate explained a significant proportion of
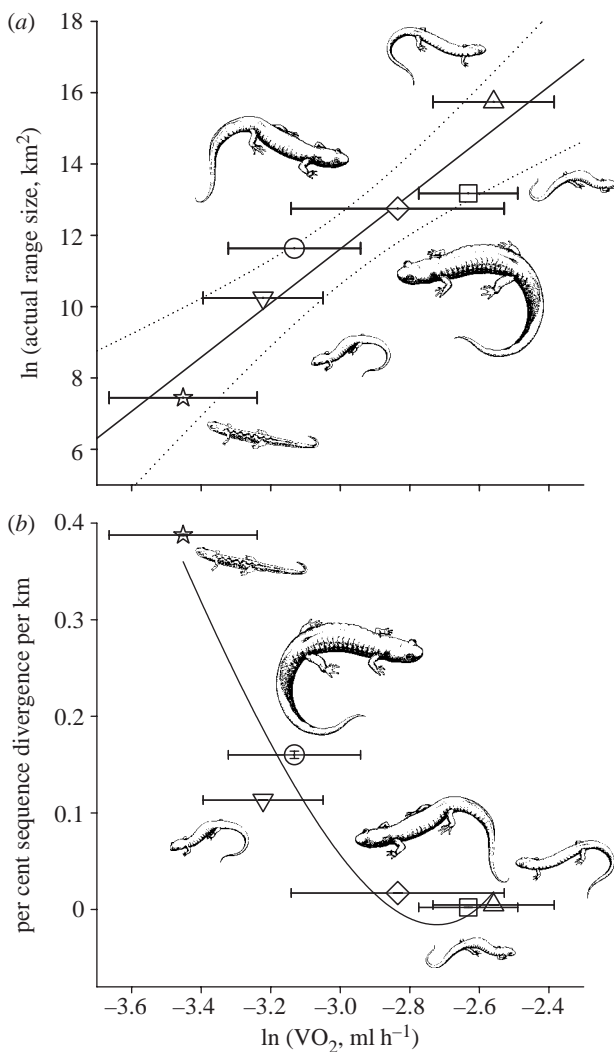
Figure 2. (*a*) Relationship of range size (ln (km^2)) to BMR (ln (VO$_2$, ml h^{-1})) in six species of *Desmognathus*. Species' characteristic body size is indicated by scaled depictions of adult males. Symbols depict LSM BMR$\pm$s.e., which account for the effects of body size and temperature (§2*d*; table 1). Hypothesis tests are based on ANCOVA and are reported in the text; the linear regression (for illustrative purposes) and 95% CI use only mean species' values. (*b*) Relationship of phylogeographic structure (%SD/km) to basal metabolic rate (ln (VO$_2$, ml h^{-1})). Scaled depictions of adult males are as shown in figure 1. Symbols depict LSM BMR$\pm$s.e., which account for the effects of body size and temperature (§2*d*). Error bars for phylogeographic divergence are $\pm$s.e. of %SD/km, but these are too small to be seen for most points. The nonlinear regression (see text) and 95% CI use only mean species' values of each parameter. Star, Cowee *D. ocoee*; upside down triangle, *D. carolinensis*; circle, *D. quadramaculatus*; diamond, *D. monticola*; square, *D. ochrophaeus*; triangle, *D. fuscus*.

interspecific variance in range size (table 2; electronic supplementary material).

We reasoned that if physiological properties influenced vagility, we could also detect dispersal limitation using genetic data. Indeed, there was a striking, non-linear association between BMR and genetic exchange (figure 2*b*) and BMR explained a high proportion of interspecific variance in genetic structure (%SD/km$=$ $(8.39+(45.77/\text{LSM VO}_2)+(62.29/(\text{LSM VO}_2)^2))$, $F_{3,3}=23.2308$, $p<0.025$; adjusted $R^2=0.9176$).

Table 1. Analysis of covariance of ln-transformed basal metabolic rate (ln (VO$_2$)) of six species of *Desmognathus* salamanders as a function of body mass (covariate), temperature and species. (Model, $F_{10,54}=274.051$, $p<0.0001$; SS, sum of squares. Least-squares means from this analysis are used as a predictor variable in the analysis of range size and are illustrated in figure 1.)

source of variation	d.f.	type III SS	*F*-ratio	*p*>*F*
ln (mass)	1	40.0683	245.4518	<0.0001
temperature	4	12.7417	8.3199	<0.0001
species	5	5.2574	6.4412	<0.0001
error	44	7.1827		
corrected total	54	281.2336		

Table 2. Analysis of covariance of ln-transformed range sizes of six species of *Desmognathus* salamanders using maximum adult body size as an estimate of species' characteristic body size. (See text for details and electronic supplementary material for comparable analysis using average adult size. Model, $F_{2,5}=38.374$, $p<0.0062$; SS, sum of squares.)

source	d.f.	type III SS	*F*-ratio	*p*>*F*
metabolic rate	1	36.3543	81.4900	<0.0029
maximum adult body size	1	2.4853	5.5709	<0.0994 (n.s.)
error	3	1.3384		
corrected total	5	39.7127		

Eurythermal generalists (*Desmognathus ochrophaeus*, *D. fuscus* and *Desmognathus monticola*) exhibited little to no phylogeographic structure, whereas montane specialists showed moderate to extreme levels of genetic differentiation over very small spatial scales (figure 2*b*).

4. DISCUSSION

We hypothesize that the strong quantitative associations between species' intrinsic physiological characteristics and their landscape-scale ecological and genetic structure are driven by trade-offs between specialist (stenothermal) and generalist (eurythermal) physiological phenotypes (Huey & Kingsolver 1993; Gilchrist 1995) influencing dispersal. This interpretation accords with mechanistic data, showing that the two montane specialists suffer pronounced metabolic depression in low-elevation populations living near their tolerance limits (Bernardo & Spotila 2006). Hence, we infer that metabolic stress arising from low intrinsic BMR of montane stenotherms impedes their dispersal, leading to restricted ranges and inhibiting genetic exchange. In contrast, the high BMR of widespread eurytherms permits exploitation of a wider range of thermal environments, conferring higher vagility and facilitating range expansion, broader range occupancy and substantial genetic exchange.

Although our data are correlative, we conclude that intrinsic BMR phenotypes are the mechanistic driver of the contrasting landscape patterns, because the capacity to tolerate warmer environments must have largely preceded their widespread colonization and occupation (Janzen 1967; Huey & Kingsolver 1993; Gilchrist 1995). This mechanism unites the

ecological process of dispersal with the population genetic outcome of gene flow, a long recognized relationship whose underlying causes remain poorly understood. The strong quantitative relationship between BMR and genetic exchange deserves careful scrutiny among diverse organisms.

Clearly, correlations between low-level mechanism (BMR) and high-level patterns (figure 2) are transduced through intermediate mechanisms: first, through individual performance and fitness (Arnold 1983) and, then, through population processes. Previous work on plethodontid salamanders (Spotila 1972; Feder & Londos 1984) indicates that physiological challenges cause performance limitation. Research exploring these mechanistic linkages is ongoing.

These findings have several distinct implications for conservation biology, some of which have not previously been recognized. First, our data highlight two insidious paths of biodiversity loss via climate change. Although biologists have suspected that cold-adapted stenotherms are especially susceptible to climate change-induced extinction, our data provide a mechanistic basis for understanding and quantitatively predicting this elevated vulnerability. We conclude that the implicit assumption underlying the dominant conservation paradigm that threatened species will respond ecologically given suitable habitat is overly optimistic.

Less widely recognized is the likelihood of increased genetic erosion in montane stenotherms. Range contraction by these species, characterized by substantial phylogeographic structure, will cause loss of distinct population segments and reduction in effective population sizes.

The last distinct implication for conservation is that the practice of using properties of closely related species to develop conservation schemes for others, though seemingly reasonable, is unreliable. Biologists recognize that closely related species typically vary in body size, niche and macroecological features such as range size, but our data show that physiological attributes of closely related forms may also be highly divergent. Because thermal physiology is expected to directly influence species' responses to climate change, this finding demands caution in assuming conserved physiology in the absence of comparative physiological data.

Prediction of endangerment using various criteria and approaches remains a major activity in conservation biology. Given the paucity of quantitative connections between physiology and macroecological patterns discussed earlier, and the dearth of examples relating physiology to genetic exchange, it is not surprising that there has been little attempt to relate intrinsic physiological features to the more applied problem of assessing species' relative endangerment due to global warming. Our approach, in which contemporary patterns in ecological and genetic exchange can be related to interspecific physiological predictors, validates the use of comparative physiological data as an objective criterion in conservation.

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NOTICE OF CORRECTION

The affiliations for Keith A. Crandall is now correct.

3 September 2007