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# Linking movement behaviour to dispersal and divergence in plethodontid salamanders

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**Abstract**

**To better understand the evolutionary and ecological effects of dispersal, there is growing emphasis on the need to integrate direct data on movement behaviour into landscape-scale analyses. However, little is known about the general link between movement behaviour and large-scale patterns of dispersal and gene flow. Likewise, although recent studies suggest that nonrandom, directionally biased movement and dispersal can promote evolutionary divergence, the generality of this mechanism is unknown. We test the hypothesis that directionally biased movement and dispersal by plethodontid salamanders interact with the topography of headwater areas to affect genetic and phenotypic divergence. Movements by *Gyrinophilus porphyriticus* and *Eurycea bislineata* show contrasting directional biases: upstream bias in *G. porphyriticus* and downstream bias in *E. bislineata*. Consistent with predictions of how these biases interact with slope to affect dispersal and gene flow, genetic distance increased with slope in *G. porphyriticus* and decreased with slope in *E. bislineata* over a standardized distance of 1 km along six headwater streams. In both species, phenotypic divergence in relative trunk length was positively related to genetic divergence. These results indicate that landscape-scale patterns of dispersal and gene flow are closely related to movement behaviour in *G. porphyriticus* and *E. bislineata*, and underscore the value of information on movement behaviour for predicting and interpreting patterns of dispersal and gene flow in complex landscapes. This study also provides new evidence that directionally biased movement and dispersal can be important sources of intra- and interspecific variation in population divergence, and highlights the value of explicit, *a priori* predictions in landscape genetic studies.**

*Keywords*: *Eurycea bislineata*, gene flow, *Gyrinophilus porphyriticus*, headwater streams, Hubbard Brook, morphology

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## Introduction

Dispersal is widely perceived to be a fundamental process in evolution, demography and community assembly (Holt & Gomulkiewicz 1997; Nichols *et al*. 2000; Holyoak *et al*. 2005), and can be important to the persistence of species in human-impacted landscapes (Mills & Allendorf 1996; Hanski & Gilpin 1997). Because measuring dispersal directly at scales that are relevant to evolutionary, demographic and ecological processes is difficult, empirical research on dispersal often relies on indirect inference from spatial and temporal variation in genetic markers and local abundance (Clobert *et al*. 2001; Bullock *et al*. 2002; Nathan 2005). These indirect indices yield valuable insight, but can be imprecise, masking details that are critical to understanding the causes of their variability, including dispersal rates (Bossart & Prowell 1998; Whitlock & McCauley 1999), directional biases (Kawecki & Holt 2002; Runge *et al*. 2006), and traits of individual dispersers (Hanski *et al*. 2004; Kokko & Lopez-Sepulcre 2006). While the precision of indirect measures of dispersal has improved with recent analytical advances, such as assignment tests and other methods for detecting asymmetrical gene flow (Beerli &

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Felsenstein 2001; Berry *et al*. 2004), a significant gap remains between the widespread perception of the evolutionary and ecological importance of dispersal and empirical resolution of its effects.

To close this gap, there is growing emphasis on the need to integrate direct data on movement behaviour into landscape-scale analyses of dispersal and gene flow (e.g. Bélisle 2005; Fahrig 2007). Arguments for this integrated approach assume that proximal attributes of movement behaviour are related to landscape-scale patterns of dispersal and gene flow, as represented by indirect measures. However, it is equally possible that direct measures of movement behaviour are unrelated to large-scale patterns of dispersal and gene flow, either because movement behaviour itself varies over space or time (Morales & Ellner 2002), or because its contribution is overshadowed by extrinsic controls on dispersal (Nathan *et al*. 2003). To evaluate the benefit of integrating direct movement data into studies of dispersal and gene flow, and more fundamentally, to assess the role of movement behaviour as a source of variation in population divergence, studies must explicitly test the link between movement behaviour and landscape-scale patterns of gene flow and divergence.

Resolving how proximal attributes of movement behaviour are related to large-scale dispersal and gene flow is crucial to advancing understanding of how dispersal affects evolutionary divergence. Dispersal, and the associated gene flow, is traditionally viewed as a homogenizing evolutionary force that impedes population differentiation (Wright 1951; Slatkin 1987). This view is based on the assumption that dispersal is a diffusive process that, over evolutionary time, results in the random exchange of individuals among subpopulations. However, there is growing evidence that individual movement behaviour can lead to nonrandom, directionally biased dispersal (Peterson & Fausch 2003; Pe'er *et al*. 2004; Macneale *et al*. 2005) and that nonrandom dispersal can promote evolutionary divergence in the absence of strong spatial variation in selection (Garant *et al*. 2005; Hare *et al*. 2005; Postma & van Noordwijk 2005). Systems where empirical data on movement behaviour are available for multiple species, and where these data show directional bias, represent valuable resources for assessing the general contribution of directionally biased dispersal to divergence.

Plethodontid salamanders are a model for studies of evolutionary divergence and diversification (e.g. Wake & Larson 1987; Highton 1995; Adams & Rohlf 2000; Wiens *et al*. 2007), but a lack of data on dispersal in plethodontids has limited understanding of its contribution to these processes. Plethodontids have their greatest species diversity in upland, headwater areas of North and Central America (Wake & Lynch 1976; Highton 1995; Tilley 1997; Campbell 1999). Indirect evidence points to several potential causes of this elevational trend in diversity, which other groups show as well (e.g. small mammals and woody plants; McCain 2005; Oommen & Shanker 2005), including climatic and time-since-colonization effects on species accumulation (McCain 2004; Wiens *et al*. 2007), and topographic effects on habitat heterogeneity and gene flow (Rahbek & Graves 2001; Doebeli & Dieckmann 2003). While species accumulation rates are difficult to assess directly because they hinge on speciation and extinction events occurring over millions of years, contemporary relationships among dispersal, topography, and divergence may elucidate how these proximal factors have influenced plethodontid diversification.

Here we explore the hypothesis that directionally biased movement and dispersal by two plethodontid salamanders interact with the topography of upland, headwater areas to affect intraspecific genetic and phenotypic divergence. The spring salamander, *Gyrinophilus porphyriticus*, is a large plethodontid [up to 110 mm snout-vent length (SVL)] found in and along headwater streams of the Appalachian Mountains, from Alabama to southern Québec (Brandon 1966; Petranka 1998). Capture–recapture studies in 16 streams throughout New Hampshire, USA, have consistently documented upstream-biased movement by *G. porphyriticus* (Lowe 2003; Lowe *et al*. 2006a; Cosentino *et al*. in press). This directional bias and movement distance were unrelated to individual size and life-history stage (i.e. larva and adult), and the bias was observed in streams that varied in chemistry, physical structure, and abundance of fish predators and invertebrate prey. Because gravity dictates that the energy required for upstream dispersal increases with slope, we predicted that dispersal and gene flow would decrease with change in elevation over a standardized distance along streams, causing genetic and phenotypic divergence to increase with slope. This relationship is likely to have the strongest effect on divergence when downstream movement occurs infrequently or at small spatial scales (Bunn & Hughes 1997). In larvae and adults of *G. porphyriticus*, downstream movement occurs significantly less frequently and over shorter distances than upstream movement (Lowe 2003; Lowe *et al*. 2006a; Cosentino *et al*. in press).

*Eurycea bislineata*, the two-lined salamander, a smaller plethodontid (up to 40 mm SVL), is found in and along the same streams as *G. porphyriticus* throughout eastern North America (Petranka 1998). Unlike *G. porphyriticus*, *E. bislineata* larvae are highly prone to downstream movement (Johnson & Goldberg 1975; Stoneburner 1978; Bruce 1986), while adults show no directional bias (Ashton & Ashton 1978; Bruce 1986). In *E. bislineata*, we predicted that as stream slope increases, gravity should interact with the strong downstream bias in movement to increase the frequency and distance of downstream dispersal and gene flow, causing genetic and phenotypic divergence to decrease. Like in *G. porphyriticus*, the strength of the downstream bias in *E.*

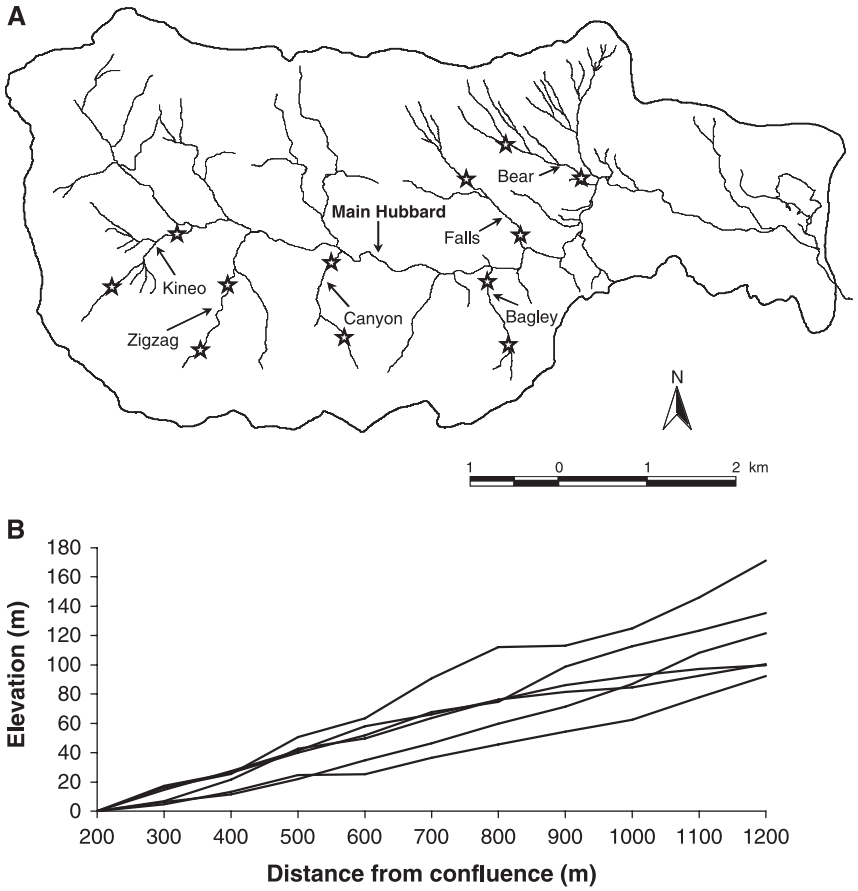


**Fig. 1** (A) Map of the Hubbard Brook Experimental Forest, New Hampshire, USA, which comprises the majority of the Hubbard Brook Watershed. Study streams and the main Hubbard Brook are labelled. Stars indicate the locations of sites where *Gyrinophilus porphyriticus* and *Eurycea bislineata* tissue samples and morphological data were collected. Sampling sites within each stream were 1 km apart, measured along the stream. (B) Standardized elevation profiles between downstream and upstream sampling sites in the six study streams based on elevation measurements taken every 100 m of stream length. The profiles correspond to the following streams, in order of increasing change in elevation between downstream and upstream sampling sites: Zigzag, Bagley, Falls, Kineo, Bear, Canyon.

*bislineata* movement suggests that compensatory effects of slope on upstream dispersal and gene flow are unlikely (Bruce 1986).

## Materials and methods

### *Study sites and sampling protocol*

The 31.6 km$^2$ Hubbard Brook Experimental Forest (HBEF), located in the White Mountains of central New Hampshire, USA (43°56′N, 71°45′W), comprises all but a small portion of the Hubbard Brook Watershed (Fig. 1A). This study was conducted in six hydrologically independent streams on both south- and north-facing aspects of the Hubbard Brook Watershed that were selected to represent a range of drainage slopes (Fig. 1B). Typical of headwater streams in New Hampshire, the study streams have low conductivity (12–15 μS), slight acidity (pH of 5–6), high dissolved oxygen content (80–90% saturation), and moderate midday summer temperatures (13–17 °C) (Likens & Buso 2006). The dominant tree species in forests surrounding these streams were *Acer saccharum*, *Fagus grandifolia*, *Betula alleghaniensis*, *Picea rubens*, *Abies balsamea*, *B. papyrifera*.

Sampling occurred at downstream and upstream sites along the primary, perennial channel of each stream (Fig. 1A). Based on distance along the stream, the downstream site was the reach between 100 and 200 m from the downstream confluence with a higher-order stream, and the upstream site was the reach between 1200 and 1300 m from this downstream confluence. Consequently, downstream and upstream sites were separated by a standard distance of 1 km along the stream. Adjusting for the steep topography in the Hubbard Brook Watershed, overland distances (i.e. Euclidean) and stream distances between sites in different streams were all greater than 1 km. To quantify slope between paired sites in each stream, we calculated the elevational difference (metres above sea level) between the upper end of the downstream site and the lower end of the upstream site (Fig. 1B). Distances and elevations were obtained using a Global Positioning System receiver (Garmin Ltd) and Terrain Navigator software (MAPTECH) with an enhanced digital elevation model of the Hubbard Brook Watershed.

For *Gyrinophilus porphyriticus*, tissue samples were collected at all sites in June, July, and August of 2003 ($n$ = 10 individuals per site), and morphological data were collected

during the same period in 2005 ($n$ = 9 to 15 individuals per site). For *Eurycea bislineata*, tissue samples were collected at all sites in June, July, and August of 2005 ($n$ = 9 to 12 individuals per site), and morphological data were collected during the same period in 2006 ($n$ = 8 to 11 individuals per site). Larval and adult *G. porphyriticus* were sampled for morphological analysis, and there were no differences among streams or between downstream and upstream sites in the ratio of larvae to adults sampled [analysis of variance (ANOVA): stream effect: $F = 2.46$, d.f. = 5, 5, $P = 0.17$; site effect: $F = 0.03$, d.f. = 1, 5, $P = 0.88$]. All *E. bislineata* sampled for morphological analysis were post-metamorphic adults. Individuals of both species were sampled from throughout the 100-m long study sites.

Tissue samples were obtained nonlethally by removing a small piece from the tip of the animal's tail, which subsequently regenerates. Tissue was placed in 90% ethanol and stored at –80 °C. To collect morphological data, animals were taken to a laboratory facility at the HBEF, measured, and then returned to the location of capture. We measured two variables for each individual, snout-vent length (SVL) and trunk length, and body mass of *G. porphyriticus* individuals was measured to the nearest 10 mg on a Pesola scale (Pesola AG). Trunk length is the distance from the posterior insertion point of the forelimbs to the anterior insertion point of the hind limbs. Trunk length has been shown to be informative in studies of morphological divergence in plethodontids and amphibians in general (Carroll *et al*. 1999). Trunk length varies at many taxonomic levels in plethodontids (Petranka 1998; Parra-Olea & Wake 2001), including in the genera *Gyrinophilus* (Brandon 1966) and *Eurycea* (Tumlison *et al*. 1990), and has been shown to vary genetically and intraspecifically in *Batrachoseps* (Jockusch 1997). The ecological implications of trunk length are likely to vary among plethodontid taxa, but may include effects on locomotion and refuge use. Snout-vent length and trunk length were measured to the nearest 0.01 mm with digital calipers.

### *AFLP amplification, scoring, and analyses*

Total DNA was extracted from tissue samples using standard phenol extraction methods. Amplified fragment length polymorphism (AFLP) loci were developed using the manufacturer's instructions with the AFLP Plant Mapping Kit [Applied Biosystems (ABI)]. Loci from the *Eco*RI-ACA primer labelled with the FAM fluorochrome paired with the *Mse*I-CAC primer were selectively amplified. Amplified products were run on an ABI 3100 sequencer, and AFLP peaks were categorized and scored using the ABI Genotyper version 3.0 software. We identified a total of 92 polymorphic loci among the 120 *G. porphyriticus* individuals sampled, and 90 polymorphic loci among the 124 *E. bislineata* individuals sampled. Peak profiles were highly repeatable in estimated sizes and fluorescence intensity.

Because AFLPs are dominant markers, Hardy–Weinberg equilibrium is often assumed in analyses of genetic diversity and genetic structure using these markers, raising concerns about their suitability for population genetic studies relative to codominant markers such as microsatellites (Holsinger *et al*. 2002; Holsinger & Wallace 2004). We used analytical techniques that do not rely on this assumption, but do assume that deviations from Hardy–Weinberg equilibrium and from linkage equilibrium are similar across sites. To increase confidence in our results, we also used two different metrics of genetic distance: $\Phi_{ST}$ and $\theta^B$ (Excoffier *et al*. 1992; Holsinger *et al*. 2002). Both metrics are analogous to $F_{ST}$ at the molecular level (Wright 1951), describing the level of genetic divergence between sites within streams. More generally, we chose to use AFLPs in this study because of their high variability and genome-wide coverage, which provide well-resolved estimates of genetic divergence over relatively small spatial scales (Freeland 2005).

Pairwise $\Phi_{ST}$ values for *G. porphyriticus* and *E. bislineata* at downstream and upstream sites within each stream were estimated using the analysis of molecular variation (AMOVA) framework implemented in WINAMOVA version 1.55 (Excoffier *et al*. 1992). This procedure uses pairwise Euclidian distances among AFLP marker profiles for analyses, and does not require indirect estimates of allele frequencies. The distance matrix and other input files needed for AMOVA were produced using AMOVA-PREP version 1.1 (Miller 1997a). We used the Bayesian method developed by Holsinger *et al*. (2002) to estimate pairwise $\theta^{B.}$ statistics for downstream and upstream sites. These analyses were performed using Hickory version 1.0.3 (Holsinger & Lewis 2003). Average heterozygosity and percentage of polymorphic loci at each site were obtained using Tools for Population Genetic Analysis (TFPGA) version 1.3 (Miller 1997b). These genetic data for *G. porphyriticus* were previously published in Lowe *et al*. (2006b).

Assuming a stepping-stone model at drift/migration equilibrium, a positive relationship between genetic distance and change in elevation would indicate that gene flow decreases with increasing slope, and a negative relationship would indicate that gene flow increases with increasing slope (Kimura & Weiss 1964). Linear regression analysis was used to test the predictions that genetic distance ($\Phi_{ST}$ and $\theta^B$) increases in *G. porphyriticus* and decreases in *E. bislineata* with change in elevation between downstream and upstream sites. To increase normality, change in elevation was square-root transformed. To assess within-stream variation in genetic diversity in *G. porphyriticus* and *E. bislineata*, we used nested ANOVA (site nested within stream) to test for differences between downstream and upstream sites in average heterozygosity and percent polymorphic loci.

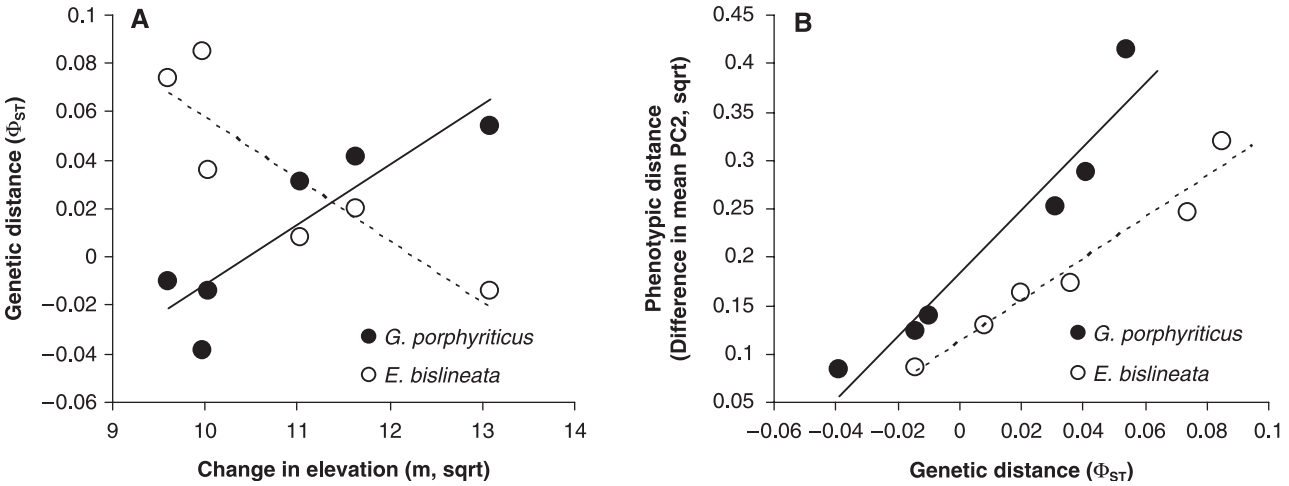


**Fig. 2** (A) The relationship of genetic distance, represented by $\Phi_{ST}$, to change in elevation (m, square-root transformed) between downstream and upstream sites in six streams where *Gyrinophilus porphyriticus* and *Eurycea bislineata* tissue samples were collected. (B) The relationship of phenotypic distance, represented by differences in mean PC2 scores, to genetic distance ($\Phi_{ST}$) between downstream and upstream sites in the same six streams. Downstream and upstream sites were separated by 1 km of distance along the streams (Fig. 1). Least-squares regression lines are plotted.

### *Morphological analyses*

For morphological analyses, we generated size-adjusted shape variables using principal component analysis (PCA) of log-transformed SVL and trunk length measurements. This analysis corrects for the expected positive correlation between all measures based on variation in overall body size (Bookstein 1989; Jungers *et al*. 1995; Adams & Beachy 2001), thus ensuring that morphological differences between sites do not simply represent differences in body size. We extracted two principal components from the covariance matrix including all populations of each species. The first principal component (PC1) was expected to represent generalized size because SVL and trunk length were strongly and positively correlated. The second principal component (PC2) was expected to be a size-adjusted morphological character. Phenotypic distance between downstream and upstream sites was calculated as the Euclidean distance between mean PC2 scores (i.e. multivariate group means; Adams 2004). Linear regression analysis was used to test the prediction that phenotypic distance (square root-transformed) increased with genetic distance between downstream and upstream sites ($\Phi_{ST}$ and $\theta^B$).

To test for consistent differences between downstream and upstream sites in PC1 and PC2 of both species, and in size-corrected mass of *G. porphyriticus*, we used nested ANOVA models (site nested within stream). Log-transformed SVL and mass measurements were used to calculate size-corrected mass (log mg) (following recommendations in Green 2001), an index of body condition that is positively related to growth rate and reproductive potential in *G. porphyriticus* (Lowe 2003; Lowe *et al*. 2006a). Mass measurements were not collected for *E. bislineata*. We also tested for a difference between *G. porphyriticus* larvae and adults in PC2 scores to assess whether the use of both stages influenced morphological analyses.

## Results

In *Gyrinophilus porphyriticus*, $\Phi_{ST}$ and $\theta^B$ were positively related to change in elevation between downstream and upstream sites in the six study streams ($\Phi_{ST}$: $F = 14.85$, d.f. = 1, 4, $P = 0.02$, $r^2 = 0.79$; $\theta^B$: $F = 31.29$, d.f. = 1, 4, $P = 0.005$, $r^2 = 0.89$; Fig. 2A). In *Eurycea bislineata*, $\Phi_{ST}$ and $\theta^B$ were negatively related to change in elevation between downstream and upstream sites in the six study streams ($\Phi_{ST}$: $F = 12.22$, d.f. = 1, 4, $P = 0.03$, $r^2 = 0.75$; $\theta^B$: $F = 11.44$, d.f. = 1, 4, $P = 0.03$, $r^2 = 0.74$; Fig. 2A). Because sampling sites in each stream were 1 km apart, measured along the stream, these relationships between change in elevation and genetic divergence are independent of stream distance. *G. porphyriticus* and *E. bislineata* did not differ between downstream and upstream sites in average heterozygosity (*G. porphyriticus*: $F = 0.46$, d.f. = 1, 5, $P = 0.53$; *E. bislineata*: $F = 0.05$, d.f. = 1, 5, $P = 0.83$) and percent polymorphic loci (*G. porphyriticus*: $F = 0.14$, d.f. = 1, 5, $P = 0.73$; *E. bislineata*: $F = 0.01$, d.f. = 1, 5, $P = 0.92$; Table 1).

In *G. porphyriticus* and *E. bislineata*, genetic distances between downstream and upstream sites were positively related to phenotypic distances, both when genetic distance was represented by $\Phi_{ST}$ (*G. porphyriticus*: $F = 46.63$, d.f. = 1, 4, $P = 0.002$, $r^2 = 0.92$; *E. bislineata*: $F = 89.66$, d.f. = 1, 4, $P = 0.0007$, $r^2 = 0.96$; Fig. 2B) and by $\theta^B$. (*G. porphyriticus*: $F = 28.60$, d.f. = 1, 4, $P = 0.006$, $r^2 = 0.88$; *E. bislineata*:

**Table 1** Descriptive statistics for amplified fragment length polymorphism variation at 12 sites in the Hubbard Brook Watershed, New Hampshire, USA, where *Gyrinophilus porphyriticus* and *Eurycea bislineata* tissue samples were collected. Downstream and upstream sites were separated by 1 km, measured along each stream. Genetic data for *G. porphyriticus* were previously published in Lowe *et al.* (2006b)

| | | *G. porphyriticus* | | | | *E. bislineata* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Genetic differentiation | | | | Genetic differentiation | |
| Stream | Site | Average heterozygosity | % polymorphic loci (95% criterion) | $\Phi_{ST}$ | $\theta^B$ (± 1 SD) | Average heterozygosity | % polymorphic loci (95% criterion) | $\Phi_{ST}$ | $\theta^B$ (± 1 SD) |
| Bagley | Downstream | 0.18 | 39.81 | –0.04 | 0.01 ± 0.010 | 0.17 | 35.56 | 0.09 | 0.08 ± 0.033 |
| | Upstream | 0.17 | 38.83 | | | 0.12 | 30.00 | | |
| Bear | Downstream | 0.20 | 48.54 | 0.04 | 0.03 ± 0.018 | 0.21 | 51.11 | 0.02 | 0.02 ± 0.016 |
| | Upstream | 0.22 | 49.51 | | | 0.29 | 72.22 | | |
| Canyon | Downstream | 0.12 | 31.07 | 0.05 | 0.06 ± 0.036 | 0.36 | 82.22 | –0.01 | 0.01 ± 0.008 |
| | Upstream | 0.09 | 20.39 | | | 0.31 | 75.55 | | |
| Falls | Downstream | 0.16 | 36.89 | –0.01 | 0.01 ± 0.010 | 0.16 | 36.67 | 0.04 | 0.04 ± 0.029 |
| | Upstream | 0.27 | 58.25 | | | 0.14 | 31.11 | | |
| Kineo | Downstream | 0.16 | 37.86 | 0.03 | 0.02 ± 0.017 | 0.15 | 41.11 | 0.01 | 0.03 ± 0.021 |
| | Upstream | 0.17 | 39.81 | | | 0.12 | 30.00 | | |
| Zigzag | Downstream | 0.14 | 34.95 | –0.01 | 0.01 ± 0.010 | 0.12 | 25.56 | 0.07 | 0.06 ± 0.033 |
| | Upstream | 0.13 | 32.04 | | | 0.16 | 36.67 | | |

$F = 65.24$, d.f. = 1, 4, $P = 0.001$, $r^2 = 0.94$). In *a posteriori* analyses of covariance (ANCOVA) of the effects of species, genetic distance ($\Phi_{ST}$ or $\theta^B$), and their interaction on phenotypic distance, we found no significant interactive effect ($P > 0.05$), indicating that the slopes of the least-squares regression lines in Fig. 2B did not differ.

In both species, PC1 scores were positively correlated with trunk length and SVL, and thus we interpret PC1 as an overall measure of body size. Genetic distances between downstream and upstream sites ($\Phi_{ST}$) were unrelated to distances between mean PC1 scores (i.e. differences in generalized body size; *G. porphyriticus*: $F = 2.28$, d.f. = 1, 4, $P = 0.21$, $r^2 = 0.20$; *E. bislineata*: $F = 2.77$, d.f. = 1, 4, $P = 0.17$, $r^2 = 0.26$). Also, downstream and upstream sites did not differ in PC1 scores (*G. porphyriticus*: $F = 0.34$, d.f. = 6, 112, $P = 0.91$; *E. bislineata*: $F = 0.39$, d.f. = 6, 103, $P = 0.89$).

Scores of PC2 were positively correlated with trunk length and negatively correlated with SVL. PC2 thus represents a contrast between trunk length and SVL, where low scores indicate a relatively short trunk and long SVL and high scores indicate a relatively long trunk and short SVL. Mean PC2 scores of *G. porphyriticus* were higher at the upstream sites of all six streams (Fig. 3A). In *E. bislineata*, mean PC2 scores were higher at the upstream sites of four streams, and higher at the downstream sites of two streams (Fig. 3B). Consequently, a marginally significant effect of site was evident in PC2 scores for *G. porphyriticus* ($F = 2.00$, d.f. = 6, 112, $P = 0.07$), but not for *E. bislineata* ($F = 0.48$, d.f. = 6, 103, $P = 0.82$). Standard error bars are presented in Fig. 3 to show within-group variation, but group means were used to calculate phenotypic distances (Fig. 2B), consistent with current approaches (Adams & Rohlf 2000; Adams 2004). Size-corrected mass of *G. porphyriticus* did not differ between downstream and upstream sites ($F = 1.19$, d.f. = 6, 112, $P = 0.32$), and PC2 scores did not differ between *G. porphyriticus* larvae and adults ($F = 2.10$, d.f. = 1, 122, $P = 0.15$).

## Discussion

Our results indicate that landscape-scale patterns of dispersal and gene flow are closely related to movement behaviour in *Gyrinophilus porphyriticus* and *Eurycea bislineata*, and thus underscore the value of information on movement behaviour for predicting and interpreting patterns of dispersal and gene flow in complex landscapes. In both species and across six hydrologically independent streams, we found that patterns of genetic and phenotypic divergence were consistent with predictions based on the expected interaction between movement behaviour and stream slope (Fig. 2). Like all empirical studies, ours is limited in taxonomic and spatial extent. However, by incorporating replicate species and replicate sites in the study design, we greatly increase support for integrating direct data on movement behaviour in landscape-scale research on dispersal and gene flow (Bélisle 2005; Fahrig 2007; Clark *et al*. 2008).

Consistent with predictions based on empirical studies of movement behaviour (Ashton & Ashton 1978; Stoneburner 1978; Bruce 1986; Lowe 2003), genetic divergence increased with slope in *G. porphyriticus* and, in the same streams, decreased with slope in *E. bislineata* (Fig. 2A). In both species, phenotypic divergence between downstream and upstream sites was closely and positively related to

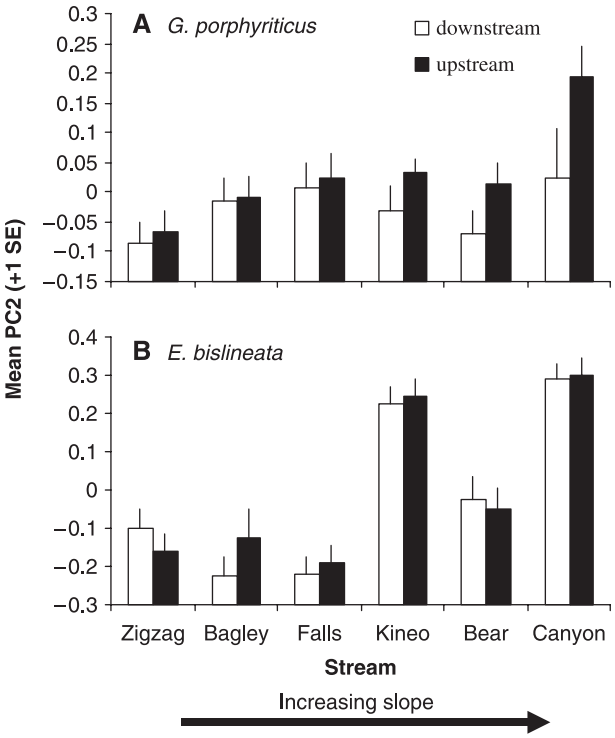


**Fig. 3** Mean PC2 scores of *Gyrinophilus porphyriticus* (A) and *Eurycea bislineata* (B) at downstream and upstream sampling sites in the six study streams (Fig. 1). In both species, PC2 was positively correlated with trunk length and negatively correlated with SVL. Downstream and upstream sites were separated by a standard distance of 1 km along all streams. Standard error bars are presented to illustrate within-group variation, but group means were used to calculate phenotypic distances (Fig. 2B), consistent with current approaches (Adams & Rohlf 2000; Adams 2004).

genetic divergence (Fig. 2B). Although Fig. 3 shows within-site variation in morphology unaccounted for in standard methods for estimating phenotypic divergence (Adams & Rohlf 2000; Adams & Collyer 2007), the validity of these methods is supported by the strong positive relationships between phenotypic and genetic divergence in both species. These strong relationships also suggest that patterns of phenotypic divergence in both species were genetically based, as opposed to plastic responses to local environmental conditions. This interpretation is consistent with the findings of Jockusch (1997), who showed a significant genetic component to intraspecific variation in trunk length in the plethodontid genus *Batrachoseps*. These results add to evidence of fine-scale divergence in plethodontids (e.g. Maerz *et al*. 2006; Cabe *et al*. 2007; Marsh *et al*. 2007) and of the effects of topography on gene flow in amphibians (Funk *et al*. 2005; Giordano *et al*. 2007), but are especially striking given that downstream and upstream sites were separated by just 1 km along the stream channel (Fig. 1).

In addition to the support for our *a priori* predictions, two observations suggest that patterns of divergence in the focal species were caused primarily by the interaction of directionally biased dispersal and stream slope, as opposed to spatial variation in selection. First, in *a posteriori* analyses, both genetic and phenotypic distances were uncorrelated with elevational variation in 17 aquatic conditions measured every 100 m along the study streams (Likens & Buso 2006), several of which are known to affect stream salamanders (e.g. water temperature, acidity, nitrogen concentration; Barr & Babbitt 2002; Green 2006; Johnson *et al*. 2006). Second, if variation in selection were the cause of divergence, then we would expect divergence to increase with slope in both species due to the general decrease in the spatial scale of environmental variation with slope (Vannote *et al*. 1980; Lomolino 2001). This was not the case (Fig. 2A).

Average heterozygosity and percent polymorphic loci did not differ between downstream and upstream sites in *G. porphyriticus* or *E. bislineata* (Table 1). This suggests that histories for local demographic processes that could influence genetic diversity at these sites (e.g. population bottlenecks and demographic expansions) have been similar over the recent past (Hartl & Clark 1997), and that selection may maintain local genetic diversity when gene flow is low (but see cautions in Hedrick 1999). The similarity of genetic diversity at downstream and upstream sites also supports the assumption that gene flow occurs primarily along the main, perennial channel of each study stream, and not between the main channel and ephemeral tributaries (Fig. 1A). We would expect to see lower genetic diversity at upstream sites in *G. porphyriticus* and higher genetic diversity at downstream sites in *E. bislineata* if these ephemeral tributaries were important sinks or sources, respectively, for gene flow (e.g. Hughes *et al*. 1995; Gornall *et al*. 1998).

The consistent difference between downstream and upstream sites in the morphology of *G. porphyriticus* (mean $PC2_{downstream}$ < mean $PC2_{upstream}$; Fig. 3A) points to some regularity in selection gradients acting on this species along streams, or if our interpretation that these differences in morphology are genetically based is incorrect, in plastic responses to local environmental conditions. However, no drivers of this pattern of morphological divergence were evident in *a posteriori* analyses of aquatic conditions, and other potential drivers are known not to differ along streams. All sites were above barriers to brook trout and below elevations where forest conditions change significantly (Schwarz *et al*. 2001; Warren *et al*. in press), and the invertebrate prey base varied little along streams (Greene *et al*. in press). Morphological divergence in *E. bislineata* did not show this same consistency (Fig. 3B), suggesting that patterns of morphological divergence in that species were driven by relatively small differences in selection or environmental conditions, with a random component influencing these patterns as well (Storz 2002).

The contribution of the directionally biased dispersal × slope interaction to species-level diversification in plethodontids depends largely on how divergence along streams is related to reproductive isolation (Muller 1942; Gleason & Ritchie 1998; Fitzpatrick 2002). Variation in courtship behaviour and mate-recognition systems is commonly associated with reproductive isolation in plethodontids (Tilley *et al*. 1990; Arnold *et al*. 1996; Mead & Verrell 2002), including *G. porphyriticus* (Beachy 1996) and *E. bislineata* (Kozak 2003). If courtship success between individuals from downstream and upstream sites is reduced by morphological differences (Fig. 3; e.g. Richmond & Jockusch 2007), or by correlated divergence in other phenotypic traits, then the interaction of directionally biased dispersal and slope may ultimately lead to reproductive isolation and speciation. This mechanism of reproductive isolation would likely be reinforced by environmental conditions promoting phenotypic divergence (Hendry *et al*. 2000).

In light of a recent study by Wiens *et al*. (2007), our results suggest that the predominant controls on plethodontid diversification may vary regionally as a function of variation in life history and ecology among the major plethodontid clades. Phylogenetic data on tropical bolitoglossine plethodontids showed no relationship between rates of diversification and elevational distribution (Wiens *et al*. 2007). However, all members of the clade Bolitoglossini, distributed in western North America, Central, and South America, are terrestrial, with direct-developing larvae (Wake 1966; Wake & Hanken 1996). Like *G. porphyriticus* and *E. bislineata*, many of the plethodontids of eastern North America are stream-associated, with aquatic larvae and adults that remain in the riparian corridor (Petranka 1998; Chippindale *et al*. 2004; Crawford & Semlitsch 2007; Greene *et al*. in press). This association with linear networks of stream and riparian habitat is likely to promote dispersal-mediated divergence by restricting large-scale movement to a single dimension and limiting pathways for gene flow relative to two-dimensional, terrestrial habitats (Bunn & Hughes 1997; Rissler *et al*. 2004; Kozak *et al*. 2006; Grant *et al*. 2007).

The opposing effects of topography on divergence in *G. porphyriticus* and *E. bislineata* (Fig. 2A) show that variation in movement behaviour between ecologically similar species can produce very different patterns of divergence at the same spatial scale. These results also indicate that directionally biased movement can be an important mechanism of intraspecific variation in population divergence, one that should be examined in other species known to exhibit directionally biased movement (e.g. Conradt *et al*. 2000; Peterson & Fausch 2003; Pe'er *et al*. 2004; Macneale *et al*. 2005). More broadly, our study underscores the value of explicit, *a priori* predictions in landscape genetic studies. The increasing accessibility of population genetic and GIS data has allowed for rapid advances in the field of landscape genetics. However, the large number of potential predictors of divergence that can be derived from GIS data, and a lack of explicit hypotheses that justify including these predictors in analytical models can reduce the scientific rigour and mechanistic insight of landscape genetic studies. By collecting direct data on movement behaviour and using those data to develop a priori predictions of divergence based on intrinsic behaviour and extrinsic environmental conditions, we can ensure that advances in landscape genetics are grounded in scientific method and mechanistic understanding.

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## References

Adams DC (2004) Character displacement via aggressive interference in Appalachian salamanders. *Ecology*, **85**, 2664–2670.

Adams DC, Beachy CK (2001) Historical explanations of phenotypic variation in the plethodontid salamander *Gyrinophilus porphyriticus*. *Herpetologica*, **57**, 353–364.

Adams DC, Collyer ML (2007) Analysis of character divergence along environmental gradients and other covariates. *Evolution*, **61**, 510–515.

Adams DC, Rohlf FJ (2000) Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences, USA*, **97**, 4106–4111.

Arnold SJ, Verrell PA, Tilley SG (1996) The evolution of asymmetry in sexual isolation: a model and a test case. *Evolution*, **50**, 1024–1033.

Ashton RE, Ashton PS (1978) Movements and winter behavior of *Eurycea bislineata* (Amphibia, Urodela, Plethodontidae). *Journal of Herpetology*, **12**, 295–298.

Barr GE, Babbitt KJ (2002) Effects of biotic and abiotic factors on the distribution and abundance of larval two-lined salamanders (*Eurycea bislineata*) across spatial scales. *Oecologia*, **133**, 176–185.

Beachy CK (1996) Reduced courtship success between parapatric populations of the plethodontid salamander *Gyrinophilus porphyriticus*. *Copeia*, **1996**, 199–203.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences, USA*, **98**, 4563–4568.

Bélisle M (2005) Measuring landscape connectivity: the challenge of behavioral landscape ecology. *Ecology*, **86**, 1988–1995.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.

Bookstein FL (1989) 'Size and shape': a comment on semantics. *Systematic Zoology*, **38**, 173–180.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution*, **13**, 202–206.

Brandon RA (1966) *Systematics of the Salamander Genus Gyrinophilus*. The University of Illinois Press, Urbana, Illinois.

Bruce RC (1986) Upstream and downstream movements of *Eurycea bislineata* and other salamanders in a southern Appalachian stream. *Herpetologica*, **42**, 149–155.

Bullock JM, Kenward RE, Hails RS eds. (2002) *Dispersal Ecology: the 42nd Symposium of the British Ecological Society*. Blackwell, Malden, Massachusetts.

Bunn SE, Hughes JM (1997) Dispersal and recruitment in streams: evidence from genetic studies. *Journal of the North American Benthological Society*, **16**, 338–346.

Cabe PR, Page RB, Hanlon TJ *et al.* (2007) Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. *Heredity*, **98**, 53–60.

Campbell JA (1999) Distribution patterns of amphibians in Middle America. In: *Patterns of Distribution of Amphibians: A Global Perspective* (ed. Duellman WE), pp. 111–210. Johns Hopkins University Press, Baltimore, Maryland.

Carroll RL, Kuntz A, Albright K (1999) Vertebral development and amphibian evolution. *Evolution and Development*, **1**, 36–48.

Chippindale PT, Bonett RM, Baldwin AS, Wiens JJ (2004) Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution*, **58**, 2809–2822.

Clark RW, Brown WS, Stechert R, Zamudio KR (2008) Integrating individual behaviour and landscape genetics: the population structure of timber rattlesnake hibernacula. *Molecular Ecology*, **17**, 719–730.

Clobert J, Danchin E, Dhondt AA, Nichols JD eds. (2001) *Dispersal*. Oxford University Press, Oxford, UK.

Conradt L, Bodsworth EJ, Roper TJ, Thomas CD (2000) Non-random dispersal in the butterfly *Maniola jurtina*: implications for metapopulation models. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267**, 1505–1510.

Cosentino BJ, Lowe WH, Likens GE (in press) Population biology and movement of the northern spring salamander, *Gyrinophilus porphyriticus*, in four New Hampshire headwater streams. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*.

Crawford JA, Semlitsch RD (2007) Estimation of core terrestrial habitat for stream-breeding salamanders and delineation of riparian buffers for protection of biodiversity. *Conservation Biology*, **21**, 152–158.

Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. *Nature*, **421**, 259–264.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fahrig L (2007) Non-optimal animal movement in human-altered landscapes. *Functional Ecology*, **21**, 1003–1015.

Fitzpatrick BM (2002) Molecular correlates of reproductive isolation. *Evolution*, **56**, 191–198.

Freeland JR (2005) *Molecular Ecology*. John R. Wiley & Sons, West Sussex, UK.

Funk WC, Blouin MS, Corn PS *et al.* (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, **14**, 483–496.

Garant D, Kruuk LEB, Wilkin TA, McCleery RH, Sheldon BC (2005) Evolution driven by differential dispersal within a wild bird population. *Nature*, **433**, 60–65.

Giordano AR, Ridenhour BJ, Storfer A (2007) The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactulym*). *Molecular Ecology*, **16**, 1625–1637.

Gleason JM, Ritchie MG (1998) Evolution of courtship song and reproductive isolation in the *Drosophila willistoni* species complex: Do sexual signals diverge the most quickly? *Evolution*, **52**, 1493–1500.

Gornall RJ, Hollingsworth PM, Preston CD (1998) Evidence for spatial structure and directional gene flow in a population of an aquatic plant, *Potamogeton coloratus*. *Heredity*, **80**, 414–421.

Grant EHC, Lowe WH, Fagan WF (2007) Living in the branches: population dynamics and ecological processes in dendritic networks. *Ecology Letters*, **10**, 165–175.

Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? *Ecology*, **82**, 1473–1483.

Green LE (2006) *The Ecology of Plethodontid Salamanders in Acidic Headwater Streams in Virginia*. PhD Dissertation. University of Virginia, Charlottesville, Virginia.

Greene BT, Lowe WH, Likens GE (in press) Forest succession and prey availability influence the strength and scale of terrestrial-aquatic linkages in a headwater salamander system. *Freshwater Biology*.

Hanski I, Gilpin ME eds. (1997) *Metapopulation Biology: Ecology, Genetics, and Evolution*. Academic Press, San Diego, California.

Hanski I, Eralahti C, Kankare M, Ovaskainen O, Siren H (2004) Variation in migration propensity among individuals maintained by landscape structure. *Ecology Letters*, **7**, 958–966.

Hare MP, Guenther C, Fagan WF (2005) Nonrandom larval dispersal can steepen marine clines. *Evolution*, **59**, 2509–2517.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*. Sinauer Associates, Sunderland, Massachusetts.

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**, 516–518.

Highton R (1995) Speciation in eastern North American salamanders of the genus *Plethodon*. *Annual Review of Ecology and Systematics*, **26**, 579–600.

Holsinger KE, Lewis PO (2003) *Hickory: A Program for Analysis of Population Genetic Data*, version 1.0.3. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut.

Holsinger KE, Wallace LE (2004) Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). *Molecular Ecology*, **13**, 887–894.

Holsinger KE, Lewis PO, Dey DK (2002) A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology*, **11**, 1157–1164.

Holt RD, Gomulkiewicz R (1997) How does immigration influence local adaptation? A reexamination of a familiar paradigm. *The American Naturalist*, **149**, 563–572.

Holyoak M, Leibold MA, Holt RD eds. (2005) *Metacommunities: Spatial Dynamics and Ecological Communities*. University of Chicago Press, Chicago, Illinois.

Hughes JM, Bunn SE, Kingston DM, Hurwood DA (1995) Genetic differentiation and dispersal among populations of *Paratya australiensis* (Atyidae) in rain-forest streams in southeast Queensland, Australia. *Journal of the North American Benthological Society*, **14**, 158–173.

Jockusch EL (1997) Geographic variation and phenotypic plasticity of number of trunk vertebrae in slender salamanders, *Batrachoseps* (Caudata: Plethodontidae). *Evolution*, **51**, 1966–1982.

Johnson JE, Goldberg AS (1975) Movement of larval two-lined salamanders (*Eurycea bislineata*) in the Mill River, Massachusetts. *Copeia*, **1975**, 588–589.

Johnson BR, Wallace JB, Rosemond AD, Cross WF (2006) Larval salamander growth responds to enrichment of a nutrient poor headwater stream. *Hydrobiologia*, **573**, 227–232.

Jungers WL, Falsetti AB, Wall CE (1995) Shape, relative size, and size-adjustments in morphometrics. *Yearbook of Physical Anthropology*, **38**, 137–161.

Kawecki TJ, Holt RD (2002) Evolutionary consequences of asymmetric dispersal rates. *The American Naturalist*, **160**, 333–347.

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.

Kokko H, Lopez-Sepulcre A (2006) From individual dispersal to species ranges: perspectives for a changing world. *Science*, **313**, 789–791.

Kozak KH (2003) Sexual isolation and courtship behavior in salamanders of the *Eurycea bislineata* species complex, with comments on the evolution of the mental gland and pheromone delivery behavior in the plethodontidae. *Southeastern Naturalist*, **2**, 281–292.

Kozak KH, Blaine RA, Larson A (2006) Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Molecular Ecology*, **15**, 191–207.

Likens GE, Buso DC (2006) Variation in streamwater chemistry throughout the Hubbard Brook Valley. *Biogeochemistry*, **78**, 1–30.

Lomolino MV (2001) Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, **10**, 3–13.

Lowe WH (2003) Linking dispersal to local population dynamics: a case study using a headwater salamander system. *Ecology*, **84**, 2145–2154.

Lowe WH, Likens GE, Cosentino BJ (2006a) Self-organisation in streams: the relationship between movement behaviour and body condition in a headwater salamander. *Freshwater Biology*, **51**, 2052–2062.

Lowe WH, Likens GE, McPeek MA, Buso DC (2006b) Linking direct and indirect data on dispersal: isolation by slope in a headwater stream salamander. *Ecology*, **87**, 334–339.

Macneale KH, Peckarsky BL, Likens GE (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology*, **50**, 1117–1130.

Maerz JC, Myers EM, Adams DC (2006) Trophic polymorphism in a terrestrial salamander. *Evolutionary Ecology Research*, **8**, 23–35.

Marsh DM, Page RB, Hanlon TJ *et al*. (2007) Ecological and genetic evidence that low-order streams inhibit dispersal by red-backed salamanders (*Plethodon cinereus*). *Canadian Journal of Zoology*, **85**, 319–327.

McCain CM (2004) The mid-domain effect applied to elevational gradients: species richness of small mammals in Costa Rica. *Journal of Biogeography*, **31**, 19–31.

McCain CM (2005) Elevational gradients in diversity of small mammals. *Ecology*, **86**, 366–372.

Mead LS, Verrell PA (2002) Evolution of courtship behaviour patterns and reproductive isolation in the *Desmognathus ochrophaeus* complex. *Ethology*, **108**, 403–427.

Miller MP (1997a) *AMOVA-PREP: A Program for the Preparation of Input Files for Use with WINAMOVA*, version 1.1. Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona.

Miller MP (1997b) *Tools for Population Genetic Analysis (TFPGA): A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data*, version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509–1518.

Morales JM, Ellner SP (2002) Scaling up animal movements in heterogeneous landscapes: the importance of behavior. *Ecology*, **83**, 2240–2247.

Muller HJ (1942) Isolating mechanisms, evolution and temperature. *Biological Symposium*, **6**, 71–125.

Nathan R (2005) Long-distance dispersal research: building a network of yellow brick roads. *Diversity and Distributions*, **11**, 125–130.

Nathan R, Perry G, Cronin JT, Strand AE, Cain ML (2003) Methods for estimating long-distance dispersal. *Oikos*, **103**, 261–273.

Nichols JD, Hines JE, Lebreton JD, Pradel R (2000) Estimation of contributions to population growth: a reverse-time capture–recapture approach. *Ecology*, **81**, 3362–3376.

Oommen MA, Shanker K (2005) Elevational species richness patterns emerge from multiple local mechanisms in Himalayan woody plants. *Ecology*, **86**, 3039–3047.

Parra-Olea G, Wake DB (2001) Extreme morphological and ecological homoplasy in tropical salamanders. *Proceedings of the National Academy of Sciences, USA*, **98**, 7888–7891.

Pe'er G, Saltz D, Thulke HH, Motro U (2004) Response to topography in a hilltopping butterfly and implications for modelling nonrandom dispersal. *Animal Behaviour*, **68**, 825–839.

Peterson DP, Fausch KD (2003) Upstream movement by nonnative brook trout (*Salvelinus fontinalis*) promotes invasion of native cutthroat trout (*Oncorhynchus clarki*) habitat. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 1502–1516.

Petranka JW (1998) *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, D.C.

Postma E, van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature*, **433**, 65–68.

Rahbek C, Graves GR (2001) Multiscale assessment of patterns of avian species richness. *Proceedings of the National Academy of Sciences, USA*, **98**, 4534–4539.

Richmond JQ, Jockusch EL (2007) Body size evolution simultaneously creates and collapses species boundaries in a clade of scincid lizards. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1701–1708.

Rissler LJ, Wilbur HM, Taylor DR (2004) The influence of ecology and genetics on behavioral variation in salamander populations across the Eastern Continental Divide. *The American Naturalist*, **164**, 201–213.

Runge JP, Runge MC, Nichols JD (2006) The role of local populations within a landscape context: defining and classifying sources and sinks. *The American Naturalist*, **167**, 925–938.

Schwarz PA, Fahey TJ, Martin CW, Siccama TG, Bailey A (2001) Structure and composition of three northern hardwood-conifer forests with differing disturbance histories. *Forest Ecology and Management*, **144**, 201–212.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Stoneburner DL (1978) Salamander drift; observations on the two-lined salamander (*Eurycea bislineata*). *Freshwater Biology*, **8**, 291–293.

Storz JF (2002) Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. *Molecular Ecology*, **11**, 2537–2551.

Tilley SG (1997) Patterns of genetic differentiation in Appalachian desmognathine salamanders. *Journal of Heredity*, **88**, 305–315.

Tilley SG, Verrell PA, Arnold SJ (1990) Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochrophaeus*. *Proceedings of the National Academy of Sciences, USA*, **87**, 2715–2719.

Tumlison R, Cline GR, Zwank P (1990) Morphological discrimination between the Oklahoma salamander (*Eurycea tynerensis*) and the graybelly salamander (*Eurcyea multiplicata griseogaster*). *Copeia*, **1990**, 242–246.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing SE (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Science*, **37**, 130–137.

Wake D, Larson A (1987) Multidimensional analysis of an evolving lineage. *Science*, **238**, 42–48.

Wake DB (1966) Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Memoirs of the Southern California Academy of Science*, **4**, 1–111.

Wake DB, Hanken J (1996) Direct development in the lungless salamanders: What are the consequences for developmental biology, evolution and phylogenesis? *International Journal of Developmental Biology*, **40**, 859–869.

Wake DB, Lynch JF (1976) The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. *Scientific Bulletin of the Natural History Museum of Los Angeles County*, **25**, 1–65.

Warren DR, Likens GE, Buso DC, Kraft CE (in press) Status and distribution of fish in an acid-impacted watershed of the northeastern United States (Hubbard Brook, NH). *Northeastern Naturalist*.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82**, 117–125.

Wiens JJ, Parra-Olea G, Garcia-Paris M, Wake DB (2007) Phylogenetic history underlies elevational biodiversity patterns in tropical salamanders. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 919–928.

Wright S (1951) The genetical structure of natural populations. *Annals of Eugenics*, **15**, 323–354.

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