Environmentally Associated Variation in Dispersal Distance Affects Inbreeding Risk in a Stream Salamander

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ABSTRACT: Avoiding inbreeding is considered a key driver of dispersal evolution, and dispersal distances should be especially important in mediating inbreeding risk because the likelihood of mating with relatives decreases with dispersal distance. However, a lack of direct data on dispersal distances has limited empirical tests of this prediction, particularly in the context of the multiple selective forces that can influence dispersal. Using the headwater stream salamander *Gyrinophilus porphyriticus*, we tested whether spatial variation in environmental conditions leads to differences in dispersal distances, resulting in spatial variation in the effect of dispersal on inbreeding risk. Using capture-recapture and population genomic data from five streams, we found that dispersal distances were greater in downstream reaches than upstream reaches. Inbreeding risk trended lower for dispersers than nondispersers in downstream reaches but not in upstream reaches. Furthermore, stream reaches did not differ in spatial patterns of individual relatedness, indicating that variation in inbreeding risk was in fact due to differences in dispersal distances. These results demonstrate that environmentally associated variation in dispersal distances can cause the inbreeding consequences of dispersal to vary at fine spatial scales. They also show that selective pressures other than inbreeding avoidance maintain phenotypic variation in dispersal, underscoring the importance of addressing alternative hypotheses in dispersal research.

Keywords: dispersal distance, environmental variation, inbreeding, plethodontid salamanders.

Introduction

Dispersal influences the genetic structure of populations by facilitating gene flow and affecting the spatial distribution of individuals (Clobert et al. 2001; Lowe and Allendorf 2010). Immigrants are an important source of outbred mates, lowering the risk of inbreeding in small populations (Spielman and Frankham 1992; Fitzpatrick et al. 2016). Avoiding the harmful effects of inbreeding (i.e., inbreeding depression) has been identified as one of three main drivers of dispersal evolution (Bengtsson 1978), along with avoiding kin competition (Poethke et al. 2007) and maximizing fitness in the face of spatiotemporal variation in environmental conditions (McPeek and Holt 1992). However, theory has far outpaced empirical tests of these putative drivers, leaving researchers with little understanding of their relative importance in natural populations where dispersal may be shaped by multiple, potentially conflicting selective forces (Waser et al. 1986; Perrin and Goudet 2001). Understanding how inbreeding avoidance interacts with other selective pressures to influence dispersal distances, in particular, will become increasingly important as habitat fragmentation and climate change cause populations to become smaller and more isolated (Haddad et al. 2015), increasing the risk of inbreeding depression.

Knowledge of the spatial structure of genetic differentiation is crucial for evaluating the role of dispersal in inbreeding avoidance because it dictates the scale of dispersal required to reduce the risk of inbreeding. Dispersal separates kin in space, and the likelihood of mating with relatives decreases with increasing dispersal distances (Szulkin and Sheldon 2008). Consequently, the minimum dispersal distance needed to reduce the risk of inbreeding depends on the spatial scale over which individuals are related (Daniels and Walters 2000). Within populations, limited dispersal can create a pattern of isolation by distance, where individuals in close geographic proximity are more genetically similar than individuals that are farther apart (Wright 1943). When these conditions lead to increased mating among close relatives, inbreeding depression is expected because of increased homozygosity and unmasking of deleterious recessive alleles or reduced heterozygosity at loci with heterozygous advantage (Charlesworth and Willis 2009), although the severity of inbreeding

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depression is highly variable in wild populations (Keller and Waller 2002). If inbreeding avoidance is a strong selective pressure, dispersal distances should evolve to exceed the scale of spatial clustering of relatives. This pattern should be evident if both sexes disperse to avoid inbreeding (Nelson-Flower et al. 2012; Díaz-Muñoz and Ribeiro 2014), but this expectation may not hold under scenarios of sex-biased dispersal (i.e., where one sex disperses so that opposite-sex relatives are spatially separated; Greenwood 1980) because same-sex individuals remaining in close proximity would not affect inbreeding. Although there is growing recognition of the influence of dispersal distances on ecological and evolutionary processes (Baguette 2003; Berthouly-Salazar et al. 2013), studies explicitly quantifying dispersal distances and the spatial structure of genetic differentiation are extremely rare, leaving us with little direct empirical insight on the efficacy of dispersal for reducing inbreeding in natural populations.

Theory suggests that inbreeding avoidance is unlikely to explain the evolution of dispersal alone (Perrin and Goudet 2001), and we know from empirical studies that dispersal is often based on multiple factors (Bowler and Benton 2005; Bitume et al. 2013; Baines et al. 2014). Nevertheless, the prediction that dispersal should reduce an individual's spatial proximity to relatives offers a straightforward framework for testing the importance of inbreeding relative to other factors influencing dispersal. For example, female red cockaded woodpeckers did not disperse far enough to avoid mating with close relatives despite evidence of inbreeding depression (Daniels and Walters 2000). The authors posited that acquiring breeding territories was a stronger selective pressure than avoiding inbreeding and that remaining in the natal territory increased an individual's competitive advantage. More generally, this and other studies suggest that the degree to which dispersal functions to reduce inbreeding is mediated by other environmental factors, such as predation (Cronin et al. 2004), competition (Baines et al. 2014), and habitat quality (Bitume et al. 2013), although few studies have explicitly tested how environmentally mediated variation in dispersal distances affects inbreeding risk.

Our goal was to test whether dispersal distance predicts inbreeding risk in the headwater stream salamander *Gyrinophilus porphyriticus* and whether this relationship changes under different environmental conditions. Upland headwater streams are characterized by steep gradients in discharge, stream water chemistry, substrate size, and the composition of prey and predator communities, creating a diverse suite of selective pressures that might influence salamander dispersal (Vannote et al. 1980; Schlosser 1982; McGuire et al. 2014). We hypothesized that these environmental gradients may lead to different relationships between dispersal distance and inbreeding risk along headwater streams. For example, predatory brook trout (*Salvelinus fontinalis*) occur throughout the downstream reaches of our study streams, where their fine-scale distribution is highly dynamic (Lowe et al. 2018; W. H. Lowe, unpublished data), but waterfalls prevent brook trout from occupying the upstream reaches (Warren et al. 2008). If *G. porphyriticus* respond to the dynamic distribution of brook trout in downstream reaches by increasing dispersal distances to access low-risk sites and avoid predation (Cronin et al. 2004; Otsuki and Yano 2014), we would expect dispersal distances to be greater in downstream reaches than in upstream reaches, leading to lower inbreeding risk for dispersers in downstream reaches. Gradients in discharge along streams may also affect dispersal in *G. porphyriticus*. Lower base flows and more frequent drying in upstream reaches could result in longer dispersal distances as individuals track water availability (Jensen et al. 2017), leading to lower inbreeding risk for dispersers in upstream reaches than downstream reaches. Alternatively, if inbreeding avoidance is the primary selective pressure shaping dispersal patterns, we would expect dispersal to be effective at reducing inbreeding risk across environmental conditions.

We used five replicate streams in the Hubbard Brook Experimental Forest (New Hampshire) to test whether spatial variation in environmental conditions leads to differences in *G. porphyriticus* dispersal distances, resulting in spatial variation in the effect of dispersal on inbreeding risk. Specifically, we used a combination of demographic (capture-recapture) and population genomic approaches to address three main objectives: (1) test for differences in individual dispersal distances between downstream and upstream reaches, (2) quantify spatial population genetic structure and inbreeding risk within and among the study streams, and (3) test whether the effect of dispersal on inbreeding risk differs between downstream and upstream reaches. Thus, our goal was to assess the relative importance of inbreeding avoidance as a driver of dispersal in this system by testing whether dispersal reduces inbreeding risk under different environmental conditions. We also assessed evidence for sex-biased dispersal, as this has important implications for expected relationships between inbreeding risk and dispersal distances.

Methods

Study Species and Sites

Gyrinophilus porphyriticus belongs to the Plethodontidae, the lungless salamanders. This species lives in headwater streams along the Appalachian uplift in the eastern United States (Petranka 1988). Larvae have external gills and are therefore exclusively aquatic (Bruce 1980). Adults, having

resorbed external gills during metamorphosis, are mainly aquatic but can forage terrestrially (Degraaf and Rudis 1990; Deban and Marks 2002). During the day, when sampling occurs, larvae and adults are found among the cobble in and along the stream channel (Bruce 2003). Recent growth models indicate that the larval period can last up to 7 years, and adults can live up to 20 years (M. M. Cochrane, unpublished data). Both larval and adult *G. porphyriticus* disperse, and there is no difference in dispersal distance distributions of larvae and adults (Lowe 2003; Addis et al. 2019). Larval dispersal is restricted to linear stream corridors, but adults may be found up to 9 m away from streams (Greene et al. 2008). Nevertheless, extensive overland dispersal is unlikely, given the highly aquatic habits of adults (Petranka 1988). Inbreeding depression has not been documented in this system but is known to occur in another member of the Plethodontidae (Liebgold et al. 2018). Our study therefore aims to elucidate the severity of inbreeding depression in northeastern populations of *G. porphyriticus* by evaluating its influence on the evolution of dispersal distances.

This work was conducted in five hydrologically independent first-order streams (Bear, Canyon, Cascade, Paradise, Zigzag) in the Hubbard Brook Experimental Forest, located in the White Mountains of central New Hampshire (43°56′N, 71°45′W; fig. 1). All five streams flow into the main stem of Hubbard Brook (fig. 1), a tributary of the Pemigewasset River. Brook trout (*Salvenlinus fontinalis*) occur in the main stem of Hubbard Brook and in downstream reaches of Bear, Canyon, Paradise, and Zigzag but have not been detected in Cascade (Warren et al. 2008). Brook trout distributions in these streams are not static, changing within and between years and creating a dynamic and heterogeneous distribution of competition and predation pressure (Lowe et al. 2018; W. H. Lowe, unpublished data). Hubbard Brook streams experience high spring discharge because of melting snow as well as high discharge events throughout the year associated with isolated storms, with base flow conditions occurring in August and September. Hubbard Brook streams are high gradient, and stream substrates include cobble, boulder, and bedrock.

Capture-Recapture Surveys

To test for environmentally mediated differences in the effect of dispersal on inbreeding risk, we divided each stream into two 500-m reaches: downstream reaches began at the confluence with Hubbard Brook, and upstream reaches ended at weirs where long-term stream data are collected and above which sampling is restricted (Bormann

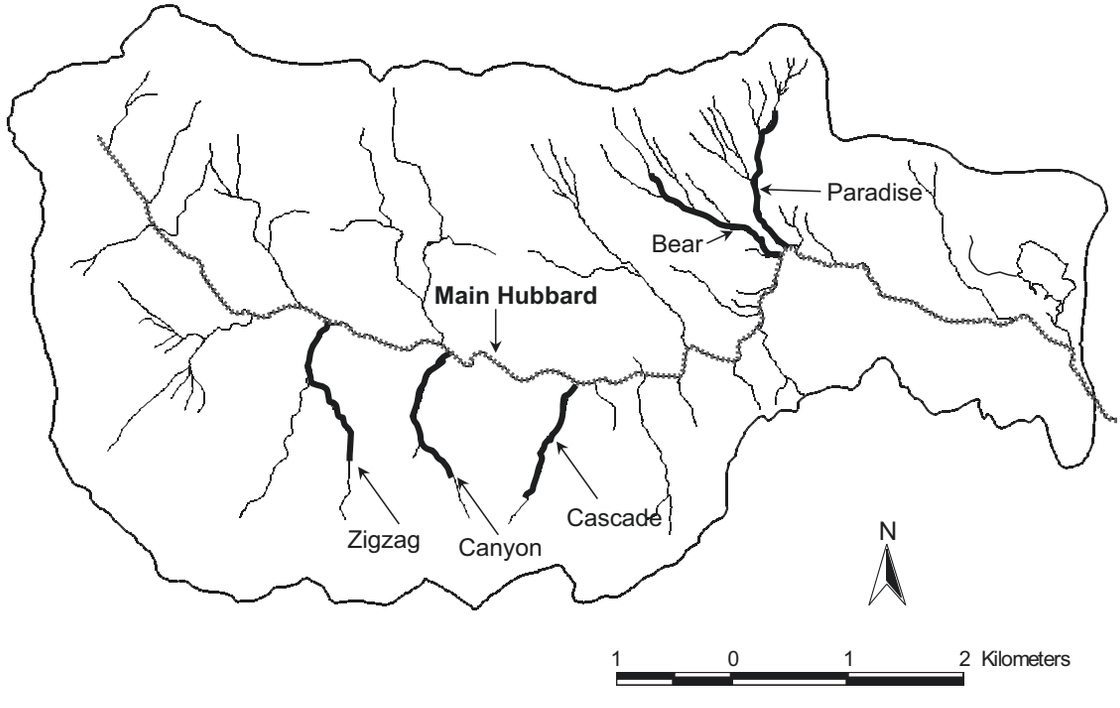

Figure 1: Map of five study streams in Hubbard Brook Experimental Forest in central New Hampshire. Bear, Canyon, Cascade, Paradise, and Zigzag Brooks are hydrologically independent and flow into the Main Hubbard (dashed gray line). Thick black lines along stream channels indicate approximate extent of surveys.

and Likens 1979). Together, these two 500-m reaches encompassed the majority of the perennial portion of each stream, with interreach distances ranging from 0 to 500 m. The upstream extent of brook trout is variable among streams but does not extend into our designated upstream study reaches in any stream (Lowe et al. 2018). All reaches were surveyed nine times throughout the summer (June–August), resulting in 36 total surveys from 2012–2015 in Bear, Paradise, and Zigzag and 27 total surveys from 2012–2014 in Canyon and Cascade. A constant search effort was maintained by turning one cover object per meter of stream; thus, surveys provided spatially explicit information about the capture locations of individual salamanders. Salamanders were uniquely marked with visible implant elastomer (Northwest Marine Technologies, Washington). Tail clips were collected from newly captured individuals and stored in 70% ethanol for genomic analyses. There is no obvious sexual dimorphism in *G. porphyriticus*, but adult males in larger size classes can be distinguished from adult females by the presence of cloacal papillae (Bruce 1972). We sexed adults when possible to test for sex-biased dispersal (see below).

Quantifying Dispersal Distance

We quantified dispersal distances in recaptured individuals as the net distance moved (distance between last and initial locations of capture, measured in meters along the stream channel) from 2012–2015 in Bear, Paradise, and Zigzag and from 2012–2014 in Canyon and Cascade (Turchin 1998). We considered dispersers to be individuals that moved ≥10 m from their initial location over the duration of the study. Previous studies of *G. porphyriticus* used a lower cutoff of 3 m to distinguish dispersers from nondispersers because a majority of individuals moved <3 m along the stream channel (Lowe and McPeek 2012). We used 10 m here to ensure a clear distinction between dispersers and nondispersers and because we expected a priori that the scale over which individuals are related would be significantly larger than the scale of the majority of movements, so that longer dispersal distances are needed to influence an individual's exposure to relatives. Among dispersers (i.e., individuals that moved ≥10 m), we tested for a difference in mean dispersal distance between downstream and upstream reaches using a *t*-test. Absolute dispersal distances were log transformed to increase normality, although *t*-tests are generally robust to deviations from normality when samples sizes are not small (Lumley et al. 2002). To achieve sufficient sample sizes—because the majority of *G. porphyriticus* do not disperse (Lowe 2003)—we pooled dispersal data across streams to test for differences in dispersal distance between downstream and upstream reaches. We also tested for differences between males and females in the proportion of individuals dispersing and mean dispersal distances.

Genomic Library Preparation, DNA Sequencing, and Genotyping

To characterize the spatial structure of genetic differentiation in *G. porphyriticus*, we prepared genomic libraries for 432 individuals across the five study streams. We originally intended to use these genomic libraries to test for genetic variation underlying dispersal phenotypes; therefore, we preferentially sequenced individuals that were recaptured during the study and thus had associated dispersal distances. This caused sample sizes to be uneven among streams, ranging from 25 to 167 individuals per stream. Briefly, genomic libraries were prepared using a modified version of the ddRADSeq method (Peterson et al. 2012), including the addition of a random eight-base pair sequence in the P2 adapter to enable detection of polymerase chain reaction (PCR) duplicates (Schweyen et al. 2014). We used Stacks version 2.1 (Catchen et al. 2011) to demultiplex reads after sequencing and to remove PCR clones. We used the dDocent 1.0 pipeline (Puritz et al. 2014) to remove low-quality bases (Phred quality score <20), construct a de novo assembly of putative RAD tags, and call single nucleotide polymorphisms (SNPs). We employed several bioinformatic filters (table S1) to remove SNPs likely to be the result of sequencing error or paralogs, the latter representing a particular challenge for salamanders with gigantic genomes because of the proliferation of transposable elements (Sun et al. 2012). Details are in the supplemental PDF.

Assessing Genetic Differentiation

We used population genetic analyses to quantify genetic variation within and among the five study streams. Genetic variation within streams was calculated as observed heterozygosity (H_O) and expected heterozygosity (H_E) in Genodive version 2.0b23 (Meirmans and Tienderen 2004). Discrepancies between observed and expected heterozygosity were quantified using F_{IS} (Weir and Cockerham 1984). We tested for isolation by distance within streams using a simple Mantel test between pairwise matrices of Euclidean distances and pairwise genetic distances in the ecodist package in R (Goslee and Urban 2007). We created a Mantel correlogram to visualize isolation by distance patterns across different distance classes in the ecodist R package. We used a lag of 100 m and ran all correlograms for 999 permutations, and we generated 95% confidence intervals with 500 iterations of 90% bootstrapping. Genetic variation among streams was assessed using pairwise F_{ST}. We also estimated pairwise F_{ST} between upstream and

downstream reaches within each stream to test for genetic substructure within streams. Between-stream and between-reach F_{ST} values were calculated in Genodive, and significance was assessed using 10,000 permutations.

Quantifying Inbreeding Risk

We quantified inbreeding risk as an individual's proximity to relatives, calculated as the proportion of genotyped individuals within 50 m of a focal individual that were relatives (i.e., in either upstream or downstream directions along the channel, amounting to 100 m of stream length). We used the last known locations of individuals for this calculation, allowing us to assess postdispersal inbreeding risk. We set the 50-m cutoff a priori on the basis of data from 287 individuals recaptured over 6 years in a stream in northern New Hampshire (Lowe et al. 2006), where mean dispersal distance was 47 m. This suggested that a 50-m cutoff would encompass the majority of potential mates over the remainder of a focal individual's lifetime, accounting for future movements of the focal individual and those potential mates. For example, an individual that dispersed 15 m upstream could then potentially mate with individuals at its initial location (i.e., <50 m downstream of its last known location) but also with new individuals <50 m upstream of its last known location (i.e., individuals 50–65 m upstream of its initial location).

We used the program related (Pew et al. 2015), an R implementation of the program coancestry (Wang 2011), to estimate pairwise coefficients of relatedness (r) between individuals using ddRADSeq derived SNPs. Seven relatedness estimators are available in coancestry, including five moment estimators and two likelihood methods. We conducted simulations to select the best estimator for our data set because the performance of these estimators is known to depend on many population-specific factors (Blouin 2003; Csilléry et al. 2006). We used empirical allele frequencies from our study populations to simulate 100 dyads of each of the following relationship categories: parent-offspring ($r = 0.50$), full siblings ($r = 0.50$), half siblings ($r = 0.25$), and unrelated ($r = 0.0$). Estimator performance was assessed by calculating Pearson's correlation coefficient for relatedness estimates produced by each estimator and true relatedness. The triadic likelihood method (TrioML; Wang 2007) produced relatedness estimates that were most closely correlated with true relatedness (Pearson's $r = 0.972$) and was therefore used for subsequent analyses. We performed 100 bootstrap replicates over loci to calculate 95% confidence intervals for each point estimate of relatedness.

Simulations revealed some imprecision in relatedness estimates for individuals in known relationship categories (fig. S1), so we took a conservative approach and classified individuals as related or unrelated for subsequent analyses rather than using point estimates of relatedness coefficients. We considered related individuals to be pairs with a relatedness coefficient >0.132, the lower 95% confidence limit of the simulation of half siblings with the TrioML estimator. Therefore, related pairs included parent-offspring dyads, full siblings, and half siblings. All other individuals were considered unrelated because we did not have the power to distinguish more distant relationships from unrelated individuals (e.g., first cousins [$r = 0.125$], second cousins [$r = 0.01325$]) because the upper 95% confidence limit of unrelated individuals from simulations was 0.123.

Testing for Effects of Dispersal on Inbreeding Risk

We used a generalized linear mixed model (GLMM) to test for effects of dispersal and stream reach on inbreeding risk. This approach allowed us to pool data across streams by including stream as a random effect, thereby accounting for variation in relatedness among streams. We fit a logistic (binomial) regression model using the lme4 R package (Bates et al. 2015) with the proportion of relatives within 50 m as our response variable. We treated dispersal status (yes, no) and stream reach (downstream, upstream) as fixed effects. We included the dispersal × reach interaction as a fixed effect to test explicitly whether the effect of dispersal on inbreeding risk differed between downstream and upstream reaches. On the basis of the results of the model, we conducted post hoc pairwise comparisons to identify specific differences in mean inbreeding risk between dispersal × reach groups using Tukey's test in the R package emmeans (Lenth 2018). Comparing the sum of squared Pearson residuals to the residual degrees of freedom indicated no evidence of model overdispersion ($\chi^2 = 334.323$, $P = .857$). In this analysis, we treated dispersal as a categorical variable because the rarity of long-distance dispersal makes it challenging to achieve the power to assess effects of continuous variation in dispersal distance; however, we conducted a second GLMM where dispersal status (yes, no) was replaced with individual dispersal distances to visualize this relationship. Larvae and adults were pooled for all analyses because the two life-history stages are not independent; that is, dispersal during the larval stage affects spatial proximity to relatives as an adult.

The focus of our analysis is the effect of inbreeding on dispersal, but we also capitalized on the opportunity to evaluate whether intraspecific competition, through density effects, influences salamander dispersal (Bitume et al. 2013; Baines et al. 2014). To do this, we tallied the total number of individuals (genotyped + ungenotyped) within 50 m of a focal individual and used a linear mixed model to test for effects of dispersal on density. We again used the last

known locations of individuals, so this analysis allowed us to assess whether dispersal reduced competition for resources (i.e., whether dispersers were in proximity to fewer total individuals than nondispersers). The number of conspecifics within 50 m was our response variable, and we included dispersal (yes, no), stream reach (upstream, downstream), and the dispersal × reach interaction as fixed effects. Stream was included as a random effect.

Results

Dispersal Distance

We captured 2,985 salamanders across the five study streams. Information on recapture rates and other sampling parameters are in table 1. All but two of the recaptured individuals stayed within the same reach during the study; one individual in Bear and one individual in Zigzag moved from the upstream reach to the downstream reach. Within-reach dispersal distances in recaptured individuals ranged from 0 to 404 m, and mean dispersal distance in dispersers (i.e., individuals that moved ≥10 m) was greater in downstream reaches than upstream reaches (downstream mean [SE], 80.07 m [13.69]; upstream mean [SE], 47.02 m [8.41]; log-transformed distances: $t = 2.200$, $P = .031$; fig. 2). A nonparametric Wilcoxon rank sum test also indicated greater dispersal distances in downstream reaches than upstream reaches ($P = .047$). Of the individuals that could be sexed ($n = 125$), males ($n = 45$) and females ($n = 80$) did not differ in the proportion dispersing ≥10 m (two-proportion z-test: $\chi^2 = 0.125$, $P = .724$) or mean dispersal distances (males mean [SE], 12.62 m [3.71]; females mean [SE], 17 m [6.94]; log-transformed distances: $t = 1.26$, $P = .21$). Additionally, the proportion of males and females dispersing did not differ between downstream and upstream reaches (males: $\chi^2 < 0.001$, $P = .999$; females: $\chi^2 = 0.818$, $P = .366$). Thus, we do not find evidence of sex-biased dispersal in *Gyrinophilus porphyriticus*.

Genetic Differentiation

The dDocent 1.0 pipeline identified 62,777 variant sites, but our stringent SNP filtering allowed us to retain 297 SNPs for population genetic analyses (table S1). Individuals that were missing data at >40% of sites ($n = 36$) were removed from the data set prior to filtering, resulting in 382 genotyped individuals for subsequent analyses. Mean expected heterozygosity was similar in the five study streams and ranged from 0.334 to 0.343 (table 1). F_{IS} values were not significant for any stream ($P \geq .05$), but estimates were slightly positive for all streams except Cascade, indicating a deficit of heterozygotes (table 1). Mantel tests for isolation by distance were significant in Bear ($r = 0.084$, $P = .001$) and Paradise ($r = 0.075$, $P = .004$; table 1). The lack of a signal of isolation by distance in Cascade, Canyon, and Zigzag was likely due to small sample sizes (table 1). In Bear and Paradise, Mantel correlograms indicated autocorrelation of genetic distances at <300 m and little to no correlation at distances >300 m (fig. 3).

All pairwise F_{ST} values between streams were significant, ranging from 0.007 to 0.022 (table 2). Bear and Paradise, the streams closest together on the landscape (fig. 1), were the least differentiated, and Cascade was the most differentiated from all other streams. Pairwise F_{ST} values between reaches within streams were low (0.001–0.008; table 2) and significant for only Bear and Paradise, indicating

Table 1: Sample sizes and genetic information for *Gyrinophilus porphyriticus* in five headwater streams in Hubbard Brook Experimental Forest

Stream	Total captured (down/up)	Recaptured (down/up)	Genotyped (down/up)	H_E	F_{IS}	Mantel R
Bear	931 (399/532)	246 (89/157)	150 (65/85)	.341	.002	.084*
Canyon	390 (246/144)	50 (32/18)	36 (25/11)	.334	.017	.036
Cascade	239 (153/86)	37 (22/15)	22 (16/6)	.341	−.017	.140
Paradise	868 (349/519)	212 (73/139)	112 (45/67)	.343	.001	.075*
Zigzag	557 (135/422)	118 (11/107)	62 (5/57)	.343	.001	.009
Total	2,985 (1,282/1,703)	663 (227/436)	382 (156/226)			

Note: Numbers in parentheses indicate downstream/upstream (down/up). H_E = expected heterozygosity; $F_{IS} = 1 - (H_O/H_E)$.

* $P < .05$.

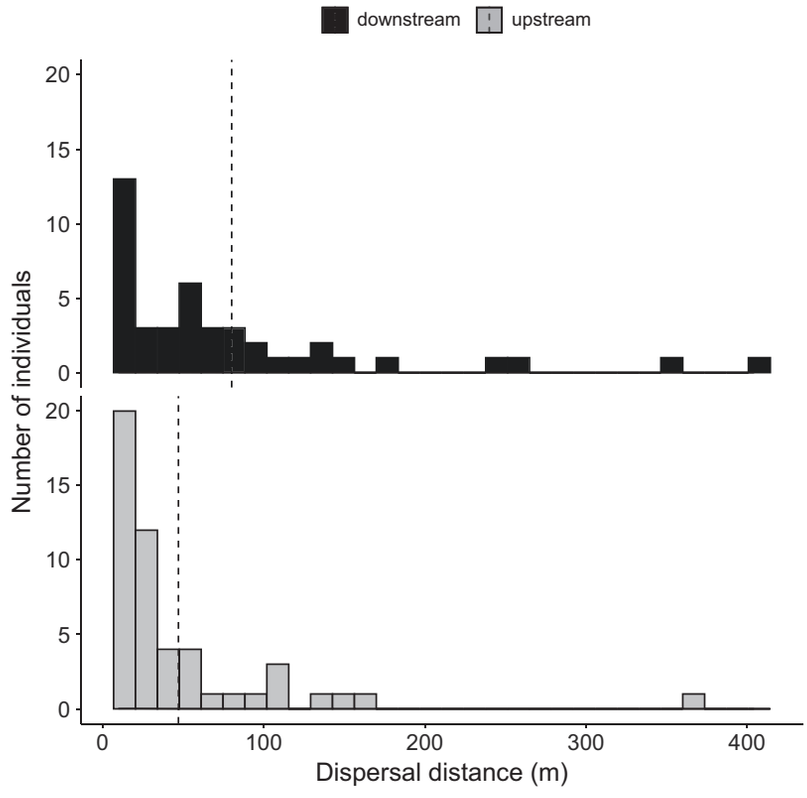

Figure 2: Distribution of dispersal distances of recaptured *Gyrinophilus porphyriticus* individuals in downstream reaches (dark gray, $n = 43$; *top*) and upstream reaches (light gray, $n = 50$; *bottom*) of five streams in Hubbard Brook Experimental Forest. Only dispersers are shown—individuals that moved ≥10 m from their initial location. Dashed lines indicate mean dispersal distances (downstream mean [SE], 80.070 m [13.69]; upstream mean [SE], 47.020 m [8.41]).

weak differentiation between upstream and downstream reaches (table 2).

Effects of Dispersal on Inbreeding Risk

We quantified inbreeding risk as the proportion of relatives ($r > 0.132$) within 50 m of nondispersers and dispersers. Individuals that were not within 50 m from any other genotyped individuals were excluded from subsequent analyses ($n = 12$). The number of genotyped individuals within 50 m of a focal individual ranged from 1 to 30, and the proportion of these that were relatives ranged from 0 to 1. The median proportion of relatives within 50 m was 0.111. Importantly, there was no correlation between the number of individuals within 50 m and proportion of relatives ($r = -0.06$, $P = .29$), indicating that our measure of inbreeding risk was not biased by variation in salamander density along streams.

Results of the GLMM showed a significant main effect of dispersal on the proportion of relatives within 50 m ($\beta = -0.751$, SE = 0.285, $P = .009$), indicating that inbreeding risk was lower for dispersers than for nondispersers. However, the near significance of the dispersal × reach interaction term ($\beta = 0.610$, SE = 0.323, $P = .059$) suggests that the effect of dispersal on inbreeding risk was dependent on stream reach (fig. 4*a*). Post hoc Tukey tests showed that dispersers in downstream reaches were within 50 m of a lower proportion of relatives than nondispersers ($z = 2.632$, $P = .042$), but there was no difference in the proportion of relatives within 50 m of dispersers and nondispersers in upstream reaches ($z = 0.938$, $P = .785$; fig. 4*a*). The pattern was also evident in our GLMM, where dispersal was continuous rather than categorical, but the dispersal × reach interaction was again not significant at the traditional 0.05 threshold ($\beta = 0.008$, SE = 0.004, $P = .068$; fig. 4*b*), likely because of the reduced power of this test. In both models, the main effect of stream reach on proportion of relatives within 50 m of all individuals (dispersers and nondispersers) was not significant ($\beta < 0.161$, SE = 0.110, $P > .140$), indicating that stream reaches did not differ in overall level of inbreeding risk.

We used the number of conspecifics within 50 m of nondispersers and dispersers as an index of intraspecific

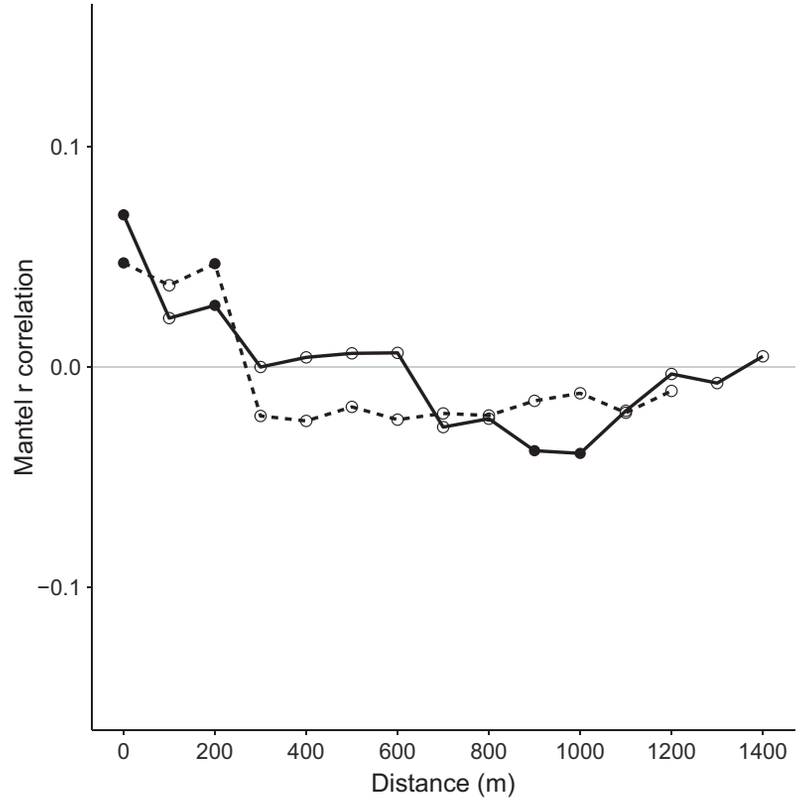

Figure 3: Mantel correlograms for *Gyrinophilus porphyriticus* in Bear (solid line) and Paradise (dashed line) in Hubbard Brook Experimental Forest. Filled circles are statistically significant, and open circles are not statistically significant. Each distance class is 100 m. Correlograms indicate autocorrelation of genetic distances at <300 m and little to no correlation at distances >300 m.

competition. This tally included all individuals captured during surveys (genotyped + ungenotyped individuals). The number of conspecifics within 50 m of a focal individual ranged from 6 to 221, and the median was 89. Our linear mixed model did not show an effect of dispersal on the number of individuals within 50 m ($\beta = -5.692$, SE = 4.216, $P = .177$), indicating that dispersal did not function to reduce an individual's proximity to other salamanders and therefore intraspecific competition for resources (fig. 4*c*). In summary, these results indicate that dispersal does not affect intraspecific competition but is effective for reducing inbreeding risk. The near-significant dispersal × reach interaction suggests that the effect of dispersal on inbreeding risk was stronger in downstream reaches, where dispersal distances were longer.

Discussion

Our results show that dispersal can reduce inbreeding risk in *Gyrinophilus porphyriticus* and also suggest that environmentally associated variation in dispersal distances leads to variation in the effects of dispersal on inbreeding risk. Specifically, we found that in the downstream reaches of our study streams, where dispersal distances were greater (fig. 2), dispersal significantly lowered inbreeding risk (fig. 4*a*). The absence of a trend in upstream reaches suggests that dispersal does not reliably lower inbreeding risk in these reaches, where dispersal distances were lower. These results indicate that selective pressures influencing dispersal distances may vary at fine spatial scales, with resulting consequences for inbreeding risk. Likewise, these results suggest that inbreeding avoidance is not the sole evolutionary driver of dispersal distances in our study system, given that inbreeding risk has not led to increased dispersal distances in upstream reaches.

Our key finding that dispersal distances predict inbreeding risk was due in part to the spatial structure of genetic differentiation in *G. porphyriticus* as well as the absence of sex-biased dispersal. Dispersal is rare in *G. porphyriticus*, creating a pattern of genetic isolation by distance along streams, where relatives are locally clustered, and suggesting that dispersal occurs primarily in or along stream channels (Steele et al. 2009). This pattern

Table 2: Pairwise F_{ST} values for *Gyrinophilus porphyriticus* in five headwater streams in Hubbard Brook Experimental Forest

	Bear	Canyon	Cascade	Paradise	Zigzag
Bear	.008*				
Canyon	.015*	.001			
Cascade	.02*	.017*	.003		
Paradise	.007*	.014*	.022*	.006*	
Zigzag	.012*	.013*	.022*	.014*	.006

Note: Values in the diagonal are pairwise F_{ST} between downstream and upstream reaches. Sample sizes and other population parameters are in table 1.
* $P < .05$.

was statistically significant in Bear and Paradise (fig. 3), and we believe that small sample sizes prevented us from detecting isolation by distance in the other streams (table 1). This fine-scale clustering of relatives created conditions under which dispersal by both sexes effectively lowered inbreeding risk in downstream reaches. Our results show that the same pattern of clustering occurred in upstream reaches: the main effect of reach was not significant in our mixed effects model, indicating that the proportion of relatives within 50 m of a focal individual—our index of inbreeding risk—did not differ between downstream and upstream reaches. However, we observed trends indicating that dispersal did not lower inbreeding risk in upstream reaches, suggesting that the different effects of dispersal on inbreeding risk in downstream and upstream reaches were due to differences in dispersal distances rather than differences in spatial patterns of genetic relatedness. The rarity of long-distance dispersal limited our ability to detect a significant relationship between continuous dispersal distances and inbreeding risk (fig. 4*b*), though the same trends are evident: inbreeding risk declines as dispersal distances increase in downstream reaches only. Given the many factors known to influence dispersal, often simultaneously (Bowler and Benton 2005; Behr et al. 2020), the variability that we observed in these relationships is expected.

Despite evidence of isolation by distance (fig. 3) and trends of reduced inbreeding risk in dispersers in downstream reaches (figs. 2, 4), our results do not support the conclusion that inbreeding avoidance is the only selective pressure influencing dispersal distance in *G. porphyriticus*. If this were the case, we would expect dispersal distances to be greater in upstream reaches (fig. 2), likely leading to reduced inbreeding risk in dispersers (fig. 4*a*, 4*b*). The population divergence metric F_{ST} can be used to assess a population's susceptibility to the deleterious effects of its genetic load (Keller and Waller 2002) and hence the expected strength of inbreeding depression. We report low F_{ST} values between streams ranging from 0.007 to 0.022, indicating that our study streams receive approximately

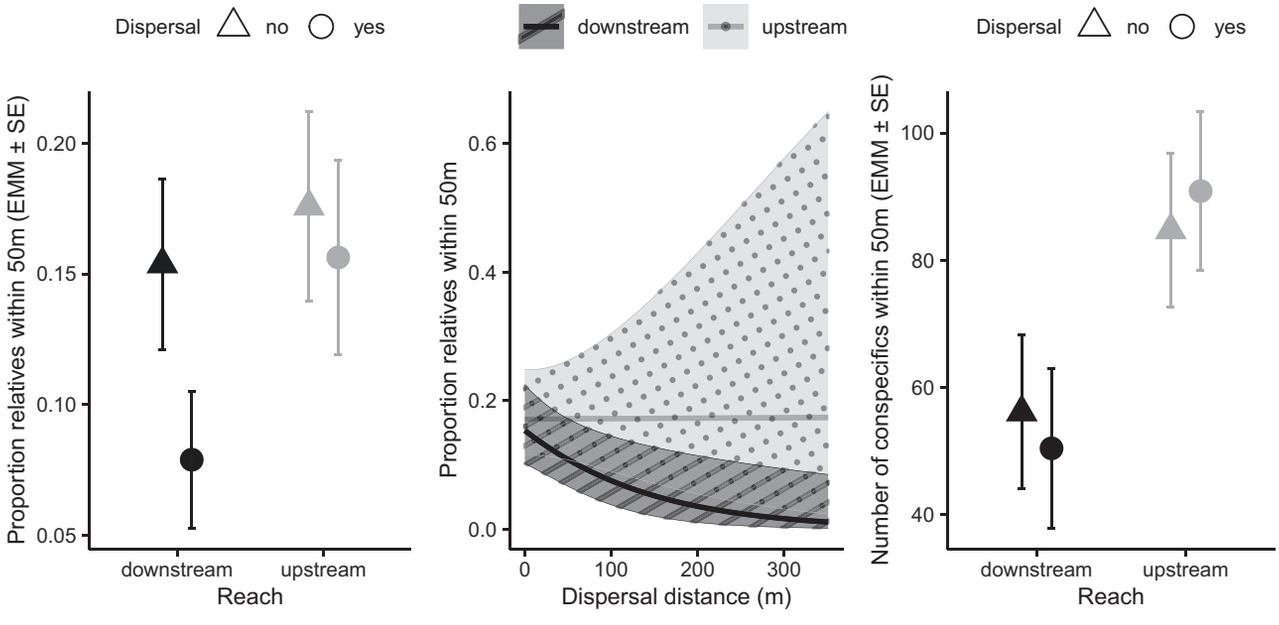

Figure 4: Dispersal reduces inbreeding risk (measured as proportion of relatives within 50 m; *A*, *B*) for *Gryinophilus porphyriticus* in downstream (black) but not upstream (gray) reaches of five streams in Hubbard Brook Experimental Forest. Dispersal has no effect on intraspecific competition (measured as number of conspecifics within 50 m; *C*). Triangles are nondispersers, and circles are dispersers (i.e., individuals that moved ≥10 m from their initial location). Data for *A* and *C* are estimated marginal means (EMMs) and standard errors (SEs) from Tukey contrasts of dispersal × reach groups.

16 migrants per generation, assuming migration-drift equilibrium (Wright 1969). This number of migrants exceeds that which is generally needed to reduce the harmful effects of inbreeding at the population level (Mills and Allendorf 1996); thus, we do not expect inbreeding depression to be severe in our populations. Though we have not explicitly tested for effects of inbreeding on individual fitness, isolation by distance along streams does suggest that inbreeding is a risk within streams, and any negative consequences of inbred matings could therefore influence selection on dispersal distances. It is difficult to distinguish between inbreeding avoidance and reducing kin competition as ultimate drivers of dispersal because dispersing away from relatives should reduce the risk of both. Our results show that dispersal did not affect an individual's proximity to conspecifics (fig. 4*c*), suggesting that intraspecific competition for resources—including competition with kin and nonkin—is not a strong driver of dispersal in this system. Nevertheless, movements to reduce inbreeding will also reduce competition with kin, and we acknowledge that our data do not allow us to differentiate between these two drivers.

There are several ecological differences between downstream and upstream reaches that may explain the observed variation in dispersal distances (fig. 2) and associated effects on inbreeding risk (fig. 4). Previous research at Hubbard Brook has shown that survival in *G. porphyriticus* is generally lower in downstream reaches (Lowe et al. 2018), suggesting that increased dispersal distances in these reaches is a response to increased mortality risk, possibly resulting from co-occurrence with brook trout. Brook trout prey on *G. porphyriticus* larvae and reduce growth rates of larger size classes through interference competition for shared prey (Resetarits 1995; Lowe et al. 2004). *Gyrinophilus porphyriticus* do not reduce activity or seek refuge in the presence of brook trout (Resetarits 1991, 1995), suggesting that active dispersal may be more effective to avoid negative interspecific interactions. However, many other factors also differ along our study streams that could lead to different dispersal distances, including discharge, prey availability, and refuge availability (Schlosser 1982; McGuire et al. 2014; Jensen et al. 2017). Additionally, previous work has shown morphological specialization in *G. porphyriticus* for riffle and pool microhabitats within streams, indicating strong selection for phenotype-environment matching (Lowe and Addis 2019). The benefits of remaining in the habitat to which individuals are matched may therefore outweigh the risk of inbreeding and ultimately select against dispersal. Nevertheless, even if inbreeding avoidance is not the only selective pressure influencing dispersal distances in downstream reaches, the positive fitness effects of reduced matings with relatives may still help to maintain longer dispersal distances there (fig. 2; Perrin and Goudet 2001).

A strength of our study is the use of direct dispersal data rather than inferences from genetic data or other indirect indices of dispersal. Directly measuring dispersal allowed us to test for effects of relatively short-distance movements on inbreeding risk (i.e., shorter than the spatial scale of genetic differentiation; fig. 3). This analysis would not have been possible using indirect genetic methods that require genetic divergence among subpopulations to detect immigrants (i.e., assignment tests; Manel et al. 2005; Hall et al. 2009). Additionally, our approach allowed us to quantify the effects of both larval and adult dispersal on inbreeding risk. Parentage analyses are commonly used to estimate dispersal distances on the basis of the distance between parent-offspring dyads (Waser and Hadfield 2011), but this approach does not account for adult dispersal because it assumes that offspring were born at the location where the parents were sampled (Blouin 2003). This assumption is certainly valid for species with highly philopatric adults (Dobson 1982), but dispersal by reproductive adults is also well documented (Bonte et al. 2008), including in *G. porphyriticus* (Lowe 2003).

This study provides rare empirical support for the fundamental prediction that inbreeding risk decreases with increasing dispersal distances. But our results also underscore the importance of accounting for spatial patterns of genetic relatedness and environmental variation to disentangle the competing selective pressures influencing dispersal distances. Others have hypothesized that inbreeding, kin competition, and environmental variation—the three putative drivers of dispersal evolution—may each require different dispersal distances to reduce associated fitness costs (Bowler and Benton 2005; Duputié and Massol 2013), and our results support this hypothesis by showing that different dispersal distances lead to different effects on inbreeding risk (figs. 2, 4*a*, 4*b*). More broadly, we hope this work shows the value of directly quantifying dispersal distances to understand the relative importance of these alternative selective pressures in shaping dispersal strategies in natural populations.

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Statement of Authorship

B.R.A. and W.H.L. conceived the ideas and designed methodology. B.R.A. carried out fieldwork and molecular lab work, led data analysis, and drafted the manuscript. W.H.L. participated in data analysis and critically revised the manuscript. All authors gave final approval for publication.

Data and Code Availability

Data supporting the results of this study have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.cvdncjt46; Addis and Lowe 2022). Raw sequence reads have been deposited in the National Center for Biotechnology Information Sequence Read Archive (accession no. PRJNA854602). The R script used for these analyses is deposited in Zenodo (https://doi.org/10.5281/zenodo.5787408).

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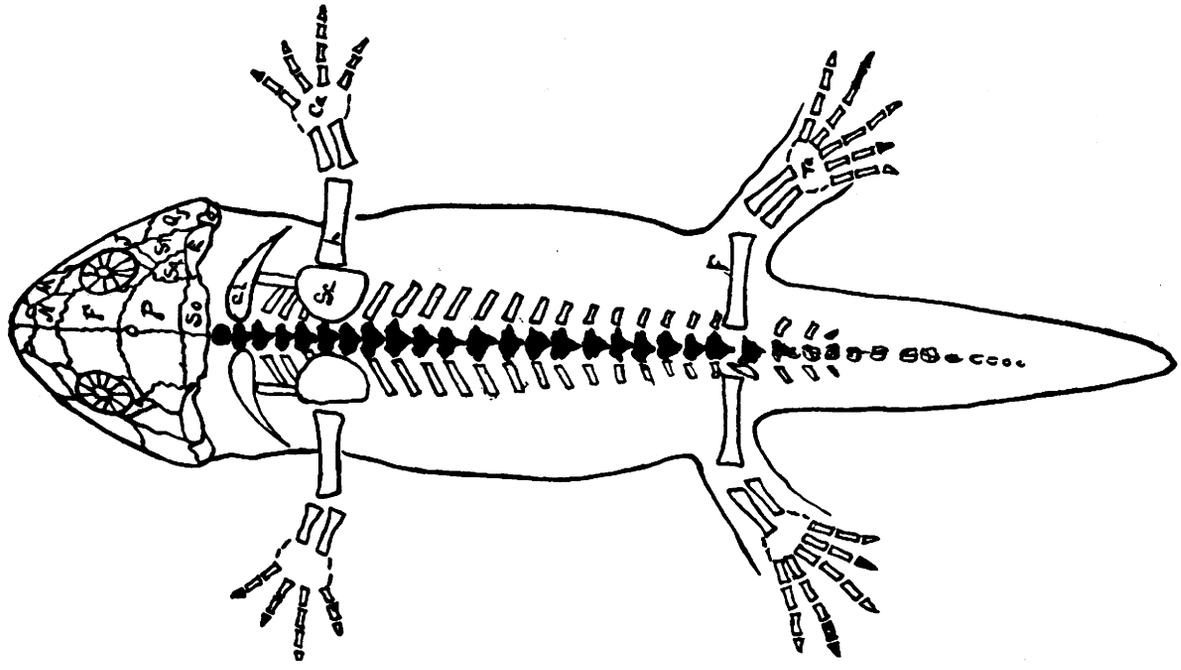

"The form of the body in the Branchiosauria is strikingly salamandrine. . . . In Micrerpeton the tail is quite long and almost equals the length of the presacral region. In *Branchiosaurus fayoli* Thevenin [figured], the tail is shorter but the form is much the same." From "The Ancestry of the Caudate Amphibia" by Roy L. Moodie (*The American Naturalist*, 1908, 42:361–373).