

# Complex landscape topography can facilitate local adaptation during a range shift

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Warming climates provide many species the opportunity to colonise newly-suitable regions at higher latitudes and elevations. Despite becoming warmer, higher latitudes and elevations nevertheless offer novel climatic challenges, such as greater thermal variability and altered frequency of weather events, and these challenges exert selection on expanding populations. However, high gene flow and genetic drift during the expansion phase may limit the degree to which species can adapt to novel climatic conditions at the range front. Here we examine how landscape topographic complexity influences the opportunity for local adaptation to novel conditions during a range shift. Using RAD-seq data, we investigated whether elevation, latitude, climatic niche differentiation, and gene flow across a complex landscape were associated with signatures of adaptation during recent range expansion of the damselfly *Ischnura elegans* in Northeast Scotland. Our data revealed two distinct routes of colonisation, with admixture between these routes resulting in increased heterozygosity and population density. Expansion rates, assessed as directional rates of gene flow, were greater between more climatically similar sites than between climatically divergent sites. Significant genetic structure and allelic turnover was found to emerge near the range front at sites characterised by high elevation, low directional gene flow, and high spatial differentiation in climate regimes. This predictive combination of factors suggests that landscape complexity may be a prerequisite for promoting differentiation of populations, and providing opportunities for local adaptation, during rapid or contemporary range shifts.

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## Abstract

Warming climates provide many species the opportunity to colonise newly-suitable regions at higher latitudes and elevations. Despite becoming warmer, higher latitudes and elevations nevertheless offer novel climatic challenges, such as greater thermal variability and altered frequency of weather events, and these challenges exert selection on expanding populations. However, high gene flow and genetic drift during the expansion phase may limit the degree to which species can adapt to novel climatic conditions at the range front. Here we examine how landscape topographic complexity influences the opportunity for local adaptation to novel conditions during a range shift. Using RAD-seq data, we investigated whether elevation, latitude, climatic niche differentiation, and gene flow across a complex landscape were associated with signatures of adaptation during recent range expansion of the damselfly *Ischnura elegans* in Northeast Scotland. Our data revealed two distinct routes of colonisation, with admixture between these routes resulting in increased heterozygosity and population density. Expansion rates,

assessed as directional rates of gene flow, were greater between more climatically similar sites than between climatically divergent sites. Significant genetic structure and allelic turnover was found to emerge near the range front at sites characterised by high elevation, low directional gene flow, and high spatial differentiation in climate regimes. This predictive combination of factors suggests that landscape complexity may be a prerequisite for promoting differentiation of populations, and providing opportunities for local adaptation, during rapid or contemporary range shifts.

## Introduction

Many species are responding to climate change by shifting their ranges to higher latitudes and elevations (Parmesan & Yohe, 2003; Hickling et al., 2006; Chen et al., 2011b). These range shifts are often accompanied by evolutionary changes in expanding lineages, including local adaptation, admixture, and drift (Dytham, 2009; Krehenwinkel & Tautz, 2013; Swaegers et al., 2015a; Dudaniec et al., 2018). For instance, a stepping-stone process of colonisation may be expected to result in increased evolutionary effects of drift and inbreeding at the poleward range expansion front (Henn & Feldman, 2012; Swaegers et al., 2015a). Alternatively, range expansions can increase genetic diversity at the expansion front by promoting the admixture of previously isolated populations from different parts of the core range, increasing genetic diversity and fodder for future adaptation as the range shift progresses (Krehenwinkel, Rödder & Tautz, 2015). Furthermore, demographic expansion during range expansions may generate and propagate neutral or deleterious gene frequencies in the new part of the range (Peischl et al., 2013; Stapley, Santure & Dennis, 2015), all of which may influence future adaptive trajectories. Each of these non-adaptive evolutionary processes accompanying range shifts may have an important role in enabling or

restricting the ability of species to adapt and persist or thrive through future anthropogenic environmental changes.

Evidence is also accumulating that adaptive evolution, and in particular, patterns of local adaptation, can emerge during poleward or elevational range shifts. This occurs when colonising populations encounter and respond to novel conditions in the new portion of their geographic range (Krehenwinkel & Tautz, 2013; Lancaster et al., 2015; Swaegers et al., 2015b; Dudaniec et al., 2018). Such novel selection pressures encountered during range shifts may derive from climates not encountered in a species historical range (Krehenwinkel & Tautz, 2013; Lancaster et al., 2015; Swaegers et al., 2015b; Dudaniec et al., 2018), novel competitive regimes arisen from new species interactions (Bocedi et al., 2013; Fitt & Lancaster, 2017), or novel resources (Janz & Nylin, 2005), which can produce locally-adapted traits in expanding populations (Swaegers et al., 2015a; Krehenwinkel, Rödder & Tautz, 2015; Lancaster, 2016). However, fine-scale genetic evidence for adaptive evolutionary change during climate-induced range shifts is scant, particularly at the spatial scales relevant to recent range shift processes. For example, records indicate that contemporary range shifts have typically moved populations to higher latitudes and elevations at a rate of ca. 10-20 km per decade (Chen et al., 2011b), suggesting that range expansions in response to anthropogenic climate change have likely typically taken place across gradients of 150km or less. In contrast, most published studies investigating the genomics of range shifts encompass broader spatial-temporal scales, including post-glacial as well as contemporary climate-mediated range shift processes (Wellenreuther et al., 2011; Kremer et al., 2012), or invasions driven by factors other than climate change, such as introduction of exotic species (Colautti & Lau, 2015). Thus little is currently known about how and why patterns of local adaptation may emerge at the

expanding range front, in the face of competing non-adaptive evolutionary processes that are so prevalent during the expansion phase.

The potential for adaptive evolution to occur over such short spatial distances and time scales, such as involved in contemporary range shifts, may be strongly influenced by associated patterns of gene flow (Hill et al., 2001). When gene flow is high between core and expanding populations, the potential for local adaptation to conditions at the leading edge of the range is limited by the input of maladapted genes from the range core (gene swamping; Lenormand, 2002a). Alternatively, when gene flow is restricted towards the range margin, for example if colonisation fronts are highly fragmented, range limit populations may have insufficient genetic diversity for local adaption to occur (Whitlock, 2000). Thus we anticipate that the opportunity for gene flow during expansion will have strong influences on the capacity for adaptation to novel conditions in the new part of the range; moreover we anticipate that the topography over which an expansion occurs will to a large part dictate patterns of gene flow and thus opportunities for selection (Möbius, Murray & Nelson, 2015).

Complex landscapes such as mountainous regions may increase landscape resistance, and thus act to restrict gene flow between core and range front populations. Therefore, when contemporary range shifts take place over fragmented or topographically complex habitats, local adaptation may be less likely to be opposed by gene swamping during the expansion phase than when gene flow is less impeded by terrain (Keyghobadi, Roland & Strobeck, 2005; Herrera & Bazaga, 2008; Perez-Espona et al., 2008). Furthermore, topographically complex environments often provide a wider variety of niches to which populations can adapt, and such variety in niche opportunity may

favourably impact the likelihood that adaptive evolution will occur (Guarnizo & Cannatella, 2013). Alternatively, it has been suggested that topographically, and particularly elevationally, complex habitats may limit the opportunity for adaptation at expanding range limits, as the distances required to track suitable climates in an upslope direction are often shorter and thus insufficient to limit gene flow between populations experiencing differing selection regimes (Hill, Griffiths & Thomas, 2011). Moreover, drift and inbreeding may oppose local adaptation in overly-fragmented expansion fronts (Lenormand, 2002b; Grueber, Wallis & Jamieson, 2013).

To evaluate the influence of landscape complexity on the capacity for local adaptation during contemporary range shifts, we implement a population-genomic study of the blue tailed damselfly, *Ischnura elegans* (Vander Linden 1820), a small species of dispersal limited coenagrionid damselfly which has recently expanded its range dramatically to both higher latitudes and elevations in the topographically rugged, high-latitude Cairngorm mountains of North East Scotland. This species has moved northward by 143 km in the past 20 years within this region and is also reported to have recently colonised higher elevation sites near the latitudinal range front (Hickling et al., 2005; Maclean, 2010). Using high-density RAD-seq SNP data, we inferred patterns of local adaption, population structure, and routes of colonisation across this complex landscape, comprising a 400m elevational gradient and a 130km latitudinal gradient. We specifically asked whether topographically complex landscapes enhanced or diminished genetic diversity and differentiation, or the potential for local adaptation during a range expansion, via the effects of gene flow, fragmentation, and steep environmental gradients in this landscape type. When compared to similar studies undertaken over less complex terrain (e.g., Dudaniec et al.,

2018), the results of this study can contribute to understanding how features of the landscape contribute to evolutionary change during contemporary range expansions.

## Materials & Methods

### The study area

The Cairngorm mountains are situated in the middle of *I. elegans*' current colonisation activity, and are among the most remote, climatically variable, and rugged environments in the UK (Scottish Natural Heritage). The Cairngorms represent a massif with several peaks exceeding 1200m, connected by cliffed troughs and corries shaped first by glacial erosion 10,000 – 18,000 years ago, and subsequently reworked by rivers and storms (Brazier et al., 1996). This rugged landscape is likely to invoke unique patterns of colonisation as species shift their ranges along both elevational and latitudinal gradients. At present, populations of *I. elegans* are observed on all sides and into the lower interiors of the Cairngorms, and many of these sites reflect very recent colonisation events (Fitt & Lancaster; Maclean, 2010). We sampled n = 12 sites (hereafter "populations") at a variety of latitudinal and elevational positions in this study region. Each site consisted of well-vegetated, shallow ponds representing the most suitable habitat for *I. elegans* (Cham et al., 2014).

### Genetic material collection and sequencing

Each of our 12 study sites was visited 3 times during the summer adult flying season in 2014, when damselflies were captured from pond edges using butterfly nets in timed catching bouts during periods of good weather. Population densities of adult *I. elegans* at each site were roughly

estimated as the total number of damselflies captured divided by total catching time, averaged over the number of visits. From each population, where possible, 5 male and 5 females were collected and individually stored whole at 4° C in 1.5ml of 100% etOH. However, due to limited numbers of damselflies at population 12, equal numbers of male and females were not possible and 4 males were substituted for females. In preparation for DNA extraction, individuals were manually pulverised using disposable micropestles. DNA was extracted from the resulting tissue using the Qiagen DNeasy blood & tissue kit, following the spin-column protocol (Qiagen DNeasy Blood & Tissue Handbook 2006). Extracted DNA was measured for quantity and quality using a Qubit fluorimeter and nanodrop (Thermofisher). Following quality assessment, the 118 individuals with the highest quality DNA were selected for sequencing, leaving 10 individuals from all populations, except populations 12 and 4, which were each represented by 9 individuals. RAD sequencing was conducted by NBAF Edinburgh, using 5 multiplexed libraries and the PstI enzyme to generate high density RAD markers. Sequencing was performed on an Illumina HiSeq v3, producing 300+300 million of 100 bp paired-end reads. NBAF returned quality-controlled, base-called reads to us in fastq format.

# Bioinformatics

Raw RAD-seq reads were analysed using STACKs v 1.07 (Catchen et al., 2013). Data was demultiplexed and the raw data was coarsely cleaned to remove low quality reads using process radtags in Stacks. Consistent with the recommended implementation of STACKs, a PHRED score cut off of 10 was used in the initial quality control cutoff (Catchen et al., 2011), with further filtering of RAD loci implemented later in the STACKs work flow using the populations function



(see below). This approach minimises the inclusion of SNP's which have arisen due to erroneous base calls, as allelic polymorphisms must be consistently observed multiple times across populations to remain in the data set. This approach prevents the over-conservative discard of high quality data caused by setting more stringent PHRED thresholds. One individual had consistently low quality reads and was dropped from further analysis. Following this, Ustacks was used to align reads with a minimum depth of coverage of 5 reads and maximum distance between stacks of 5 reads. Catalogues of loci were assembled using Cstacks, with the number of mismatches allowed between sample tags when generating the catalogue set to 2. Samples were matched against the catalogue using Sstacks with default settings. Variant sites (i.e., specific SNPs) that were successfully reconstructed from at least 75% of the individuals under study and present in a minimum of 6 populations were selected using the Populations module of Stacks, leaving 117 individuals from 12 populations, with 16982 SNPs over 8491 loci. PLINK format data and pairwise Fst values were then exported using Populations. Data was reformatted for Bayescan (Foll & Gaggiotti, 2008) and Genepop (Raymond & Rousset, 1995) format using PGDspider v2.0.9.2 (Lischer & Excoffier, 2012).

# Environmental variables

Climatic variables were extracted for each population's location from the Bioclim climate layers with a resolution of 30 arcsecond (Hijmans et al., 2005) and elevation from OS terrain 50 (Ordnance Survey, 2017). Climatic values at each site were checked for collinearity, using cor() function in base R (R Core development Team, 2012) with a cutoff of 0.8. After omitting collinear variables, elevation, latitude, temperature annual range (bio7), mean temperature of wettest quarter

(bio 8), mean temperature of driest quarter (bio 9), mean temperature of warmest quarter (bio10), annual precipitation (bio12) and precipitation of wettest month (bio13) were selected for further analysis based on their biological relevance. Geographical variables retained were chosen to represent the dual axes over which *I. elegans* are range shifting (elevation and latitude), and we retained climatic variables which summarise both temperature and rainfall. Elevation was strongly negatively correlated with mean annual temperature ( $r = 0.93$ ), and latitude was positively correlated with mean diurnal temperature range ( $r = 0.85$ ) across our sites, so in analyses of latitudinal and elevational effects on population genetic parameters, mean annual temperature and diurnal temperature range were omitted from those models. Spatial distances between sites was calculated using `distGeo()`, which accounts for the curvature of the earth and projection of latitude and longitude, using the `geosphere` package in R (Hijmans, 2017), and correlated against pairwise  $F_{st}$  using Mantel tests in the `ade4` package (Dray & Dufour, 2007) to generate estimates of isolation by distance.

### Colonisation dynamics and population genomics

Routes of colonisation were estimated using methods presented in (Peter & Slatkin, 2013) using the R package `rangeExpansion` (Peter), which was used to generate pairwise  $\Phi F_{st}$  (the directional measure of geneflow between populations). Pairwise  $\Phi F_{st}$  was compared with distance between sites and the pairwise difference in environmental variables (described above) using Mantel tests and, in the case of environmental variables, partial Mantel tests which account for geographic distance, using the `ecodist` package for R (Goslee & Urban, 2007).

Population structure was assessed using detrended correspondence analysis (DAPC) from the Adegenet package in R (Jombart, 2008). This was conducted using the `dapc()` function, with 11 principal components and 5 discriminant functions retained for analysis. Dapc was optimised by running multiple models with varying principal components and discriminant functions, with the best model chosen using the `a-score()` function. The first two principal components, representing how genetically similar individuals are by population, were extracted from the DAPC analysis to reduce the dimensionality of the data. PC1 accounted for 18.23% of the variation in genetic structure, and PC1 and 2 combined accounted for 27.81% of the total variation. These were plotted to identify how populations varied in genetic structure across the major axes of variation (Fig. 2 & 3). We also assessed correlations between PC1 or PC2 and site and population characteristics to explore how genetic structure might orient to underlying environmental or population processes (Table 2).

Population heterozygosity was calculated using the `BasicStats()` function in the `r` package `DiveRsity` (Keenan et al., 2013). To identify genes under selection, four SNP outlier methods were applied: `OutFLANK` (Whitlock & Lotterhos, 2015b), `Latent Factor Mixed Models (LFMM)` (Frichot et al., 2013), `pcadapt` (Luu, Bazin & Blum, 2017) and `Bayescan` (Foll & Gaggiotti, 2008). `OutFLANK` was performed using the R package `outflank` (Whitlock & Lotterhos, 2015a), using a  $q$  threshold of 0.1 and a left and right trim fraction set to 0.15. `Pcadapt` was performed in R with a  $K$  value set to 20. The `LEA` package (Frichot & Franc, 2015) in R was used to run LFMM analysis, with models including environmental variables described above, a  $K$  value of 1, and 5 repeats. `Bayescan` was run in the stand-alone platform, with 1000 iterations and thinning set to 10. These models each generated very few significant SNP outliers or SNP-environment correlations, with

no agreement among methods regarding loci putatively under selection. Therefore, we did not run further outlier-based tests for local adaptation. To further to test for patterns of genetic variation corresponding to environmental gradients or population densities, random forests analysis was using the gradientForest package in R (Ellis, Smith & Pitcher, 2012). Gradient forests were conducted using 500 trees, with 201 bins and a correlation threshold 0.5, with response variables set to population density of *I. elegans*, latitude, elevation, temperature annual range (bio7), mean temperature of wettest quarter (bio 8), mean temperature of driest quarter (bio 9), mean temperature of warmest quarter (bio10), annual precipitation (bio12) and precipitation of wettest month (bio13).

## Results

Our routes of colonisation analysis indicated that directional gene flow was generally strongest along the eastern side of the Cairngorms (Fig. 1). Moreover, gene flow is generally in a northern direction, consistent with the poleward range expansion in this region, although some gene flow back to the range core is also apparent (Fig. 1). Absolute values of  $\phi_{fst}$  ranged between 0.0008 and 0.07 for all sampling sites (Table S1).

Differences in Pairwise  $F_{st}$  by distance indicate that there is marginally significant differentiation by distance across our populations ( $z=2.43$ ,  $p=0.10$ ; Table S2, Figure S1). Our directional gene flow estimates  $\phi_{fst}$  correlated positively with among-population differences in population density of *I. elegans* (highest population densities were found at sites with high rates of incoming gene flow;  $z = 0.89$ ,  $p=0.04$ , Fig. 4a) and was negatively associated with difference in mean temperature in the warmest quarter (directional gene flow is strongest between sites most similar in their

summer temperatures;  $z = 14.86$ ,  $p=0.02$ , Fig 4b, indicating a colonisation bias towards climatically similar sites). No other significant correlations were found between directional gene flow and any of the other environmental variables included in this study.

Detrended correspondence analysis (DAPC) results demonstrated that three populations, 7, 8 and 12, demonstrated significant genetic differences from each other and from the other populations (Figs. 2, 3). Genetic variation along PC1 is moderately correlated with temperature and precipitation (correlation between PC1 and precipitation in the wettest month,  $R^2 = 0.26$ , correlation with mean annual precipitation,  $R^2 = 0.20$ , and correlation with mean temperature of the driest quarter,  $R^2 = 0.12$ ). Population 12 differentiates from the others along this axis (Table 1, Fig. 3). Among environmental variables, PC2 is best explained by thermal variability (effect of mean diurnal temperature range on PC2:  $R^2 = 0.23$ ). Populations 7 and 8 differentiate from the other populations, in opposite directions, along this axis (Fig. 3). Populations 8 and 12 also exhibit lower heterozygosity than the other populations (Table 1), in addition to being compositionally differentiated from each other and from other sites.

OutFlank found no SNPs to be outliers, nor did LFMM. However, when the p value cut off was relaxed using LFMM to 0.15, 186 genes were suggested as putatively under selection. Similarly, pcadapt found 186 genes under selection, however when compared candidate genes between the two methods, there was only 3.76% overlap. Moreover, Bayescan analysis resulted in only one outlier SNP which was not found to be under selection as identified by the other three methods. Low concurrence between SNP identification methods suggests that those SNP's identified as outliers may represent false positives.

288

289 The gradient forest analysis revealed that population density of *I. elegans* has the most predictive  
290 power for allelic turnover among populations. Density of *I. elegans* always had the highest  
291 predictive power in the model, followed by latitude. Mean temperature in the wettest quarter, mean  
292 temperature in the warmest quarter, and annual temperature range all had approximately equal  
293 importance and offered the highest predictive power of the climatic variables. Overall, climatic  
294 variables relating to temperature were more important than variables which related to rainfall.

295

## 296 Discussion

297

298 We identify two distinct colonisation routes into northeast Scotland, with the possibility of a third  
299 expansion wave entering from the south (Fig. 1). While the data indicate a primarily northward  
300 movement of species, there are also some cases of gene flow back towards the range core. We also  
301 found suggestive evidence of weak isolation-by-distance among our study sites, consistent with  
302 genetic changes associated with the range expansion. However, high gene flow associated with the  
303 range shift resulted in generally low population structure: DAPC analysis indicated that most of  
304 our sites across the region were well mixed genetically, and did not fall into distinct structured  
305 populations, indicating a strong role for gene swamping limiting local adaptation. Consistent with  
306 this, populations which exhibited the cosmopolitan genotype also experienced the strongest gene  
307 flow from surrounding populations (fig 1). However, three populations (7, 8, and 12) did  
308 distinguish themselves strongly from the background genotypes. Such shifts in genetic structure  
309 corresponded to sites with reduced connectivity, high elevation, and divergent climates. Moreover,  
310 we identify patterns of allelic turnover across the expansion front which correspond to gradients  
311 in climatic, demographic, and spatial variables, indicating complex processes and opportunities  
312 for adaptation during range shifts.

# Colonisation routes

Our routes of colonisation data indicated two distinct paths of colonisation into the Scottish highlands: an eastern route, skirting around the eastern extent of the Cairngorms into central Aberdeenshire, and a westerly route moving northwards around the western extent of the Cairngorms, before moving in a south-easterly direction from the north. The two expansion routes meeting in central Aberdeenshire (Fig 1). Similar patterns of colonisation in this region have been reported in the UK butterfly, *Pararge aegeria*, suggesting that non-linear and circuitous poleward expansion routes may be common among dispersal-limited ectotherms, especially in mountainous regions (Hill et al., 2001). While most directions of colonisation are towards the north (Fig 1), there is also evidence of gene flow back towards the core of the range core, particularly from sites 6 and 11 (fig 1). Colonisation of Northeast Scotland by *I. elegans* damselflies appears to have occurred primarily through the eastern route, indicated by the thickness of the arrows in fig 4, and complementarily, the genetic structure of sites 3 and 9 (putative origin of the eastern expansion route) are highly similar to most of the populations in the region. In contrast, site 12 (putative origin of western route) is genetically distinct from the other sites in NE Scotland (Figs. 2 & 3) and also exhibits lower heterozygosity than other sites (table 1). Whether the lower expansion rates along the western Cairngorms are associated with the low diversity at a putative source population will require more data from populations further south.

# Genetic structure and the capacity for local adaptation

Overall genetic structure is weak across the region. However, we identified three genetically distinct populations within the region (represented by populations 7, 8, and 12). One of these (population 12) appears to reflect either the ancestral gene pool (reflecting southern genotypes ancestral to the Scottish colonisers) or possibly a new, recurrent colonisation wave into the area from further south or west. These genotypes may be adapted to warmer, wetter conditions than other North East Scottish genotypes, as indicated by correlations between overall genetic differentiation at this site and these environmental variables (Fig. 2), as well as gradient forest analysis suggesting genome-wide allelic turnover along gradients of temperatures in the warm and wet season (Fig. 5 & 6). The other two genetically distinct populations (7 and 8) are relatively isolated, high elevation populations, and only weakly connected to other populations through directional gene flow (Fig 1). Despite population 7 and 8 both being similar as high elevation sites, climate regimes are quite divergent between them, and in comparison to most of the other sites, with the western side of the Cairngorm mountain range (population 7) exhibiting low variability in temperature, contrasting to extremely variable temperatures experienced to the southeast of the Cairngorms (population 8). This divergence in climate across the massif reflects the rainshadow and Foehn wind effects that occur on the east coast of the Cairngorms (Birse & Dry, 1970). The pattern of genetic differentiation between each of these populations and from the other sites in our study area correspond moderately to variation in climatic thermal variability (diurnal temperature range; Fig. 2), suggesting that isolation at high elevations may allow these populations to at least partially adapt to their divergent climates. Similarly, gradient forest analysis indicated that allelic turnover across the expansion front is well-predicted by thermal variability, in the form of annual temperature range, in comparison to the predictive ability of most other climatic variables (Fig. 5). These patterns suggest that fragmentation and topographic complexity can increase the potential



for adaptation during range expansions. The alternative explanation, that genetic differentiation at sites 7 and 8 may reflect drift in isolated subpopulations, is not as readily borne out by the data. Three out of 10 individuals from site 7 and two out of 10 individuals from site 8 expressed more cosmopolitan genotypes, indicating that gene flow is sufficiently high to swamp differentiated genotypes unless opposed by selection (figure 2). Variation in genetic differentiation among sites also corresponded to temperature and precipitation (PC1), but variation along this axis was primarily driven by site 12, a site of origin of the western range expansion route.

It is noteworthy also that these signatures of genetic structure and climate do not appear to reflect measurable selection at individual SNPs, at least as could be robustly detected in the data, and this suggests a highly polygenic signature of divergence. Nonetheless, genome-wide divergence associated with environmental variables may reflect patterns of local adaptation if selected traits are highly polygenic (Pritchard & Rienzo, 2010), patterns of genotype-phenotype matching are complex (Goldstein et al., 2010), or if environmental selection at the population level acts on gene frequencies rather than simple allelic substitution within individuals (e.g., (Lancaster et al., 2017)). Accordingly, gradient forest analysis revealed that genome-wide variation in allele frequencies across the study were nearly linearly correlated with temperature during warm and rainy seasons, as well as annual thermal variability (Fig. 6). However, latitude (i.e., distance from the range core) and population density (associated with levels of admixture) also predicted allele frequency changes, indicating that, as expected during an active range expansion, that gradients in allele frequency are likely driven by both adaptive and neutral processes.

#### Determinants of directional gene flow and admixture

382

383 We found evidence for admixture from different colonisation routes, and further evidence that this  
384 admixture provided a fitness benefit in the colonised region, with sites receiving the most migrants  
385 having both high heterozygosity (Table 1) and high population density (Fig. 5, Table 2). Note  
386 particularly that sites with highest heterozygosity and population density (sites 1, 2, and 4) are also  
387 situated at the confluence of the eastern and western colonisation routes (Figure 1). A fitness  
388 benefit from admixture has previously been observed during range expansions and invasions  
389 (Keller et al., 2014), and our results suggest that admixture is likely an important process driving  
390 population growth rates during native range expansions. The gradient forest analysis further  
391 suggests that allelic turnover is correlated with population density (Fig. 5), which implies that  
392 admixture between lineages from multiple colonisation routes may be involved in driving changes  
393 in both allele frequencies and population densities at the range limit, and that admixed populations,  
394 while exhibiting low structure, may contain novel allelic combinations in support of further  
395 adaptation as the range shifts continues to progress northward.

396

397 We detected significantly higher rates of directional gene flow between sites that are more similar  
398 in thermal regimes during the warmest quarter (Fig 4b), after accounting for effects of geographic  
399 distance between sites. This suggests that local adaptation or acclimation to climatic conditions at  
400 an individual's natal site has a strong effect on its colonisation success of a new site. Despite the  
401 ubiquity of model predictions which assume that colonisation events follow climate isoclines,  
402 empirical evidence that climatic similarity from the source population affects colonisation success  
403 is equivocal (Maron, 2006). Our results suggest that, despite the widespread and rapid movement  
404 of *I. elegans* into regions characterised by cooler and dryer climates, and with changes in patterns

of thermal variability, that expansion rates are generally greatest among more climatically matched sites. Climatic differences among even closely-adjacent colonisation sites may present a significant barrier to gene flow, allowing local adaptation to occur. This result provides some additional, albeit indirect support for the hypothesis that the diverse ecological niches provided by rugged terrain offer greater opportunities for local adaptation within the expansion zone.

## Conclusions

Results of this study highlight the complex interaction between colonisation dynamics, gene flow and adaptation during climate change-mediated range shifts. We find some evidence that local adaptation may be occurring within recently colonised populations of the range shifting species *I. elegans*, and this is facilitated by topographic complexity in the region over which the ongoing latitudinal and elevational range shift is occurring. Topographic complexity drives high spatial heterogeneity of local thermal variability regimes, imposing particularly strong pressure for local adaptation. Simultaneously, complex terrain allows for sufficient reductions in directional gene flow for selection to oppose it, at least in relatively isolated populations (Fig. 4). Thus our combined data suggests that mountainous regions can support the opportunity for adaptation during range shifts. Although spatial distances across elevational expansions are often shorter than for latitudinal expansions, the current results suggest that steep climatic gradients and rugged topography in mountainous regions can be effective drivers of both divergent selection regimes and reduced gene flow. As most contemporary latitudinal range shifts are occurring over relatively short geographic distances (Chen et al., 2011a), our results suggest that in the absence of topographic or other forms of landscape complexity, latitudinal expansion routes may not necessarily provide sufficient barriers to gene flow to allow for local adaption during the expansion.

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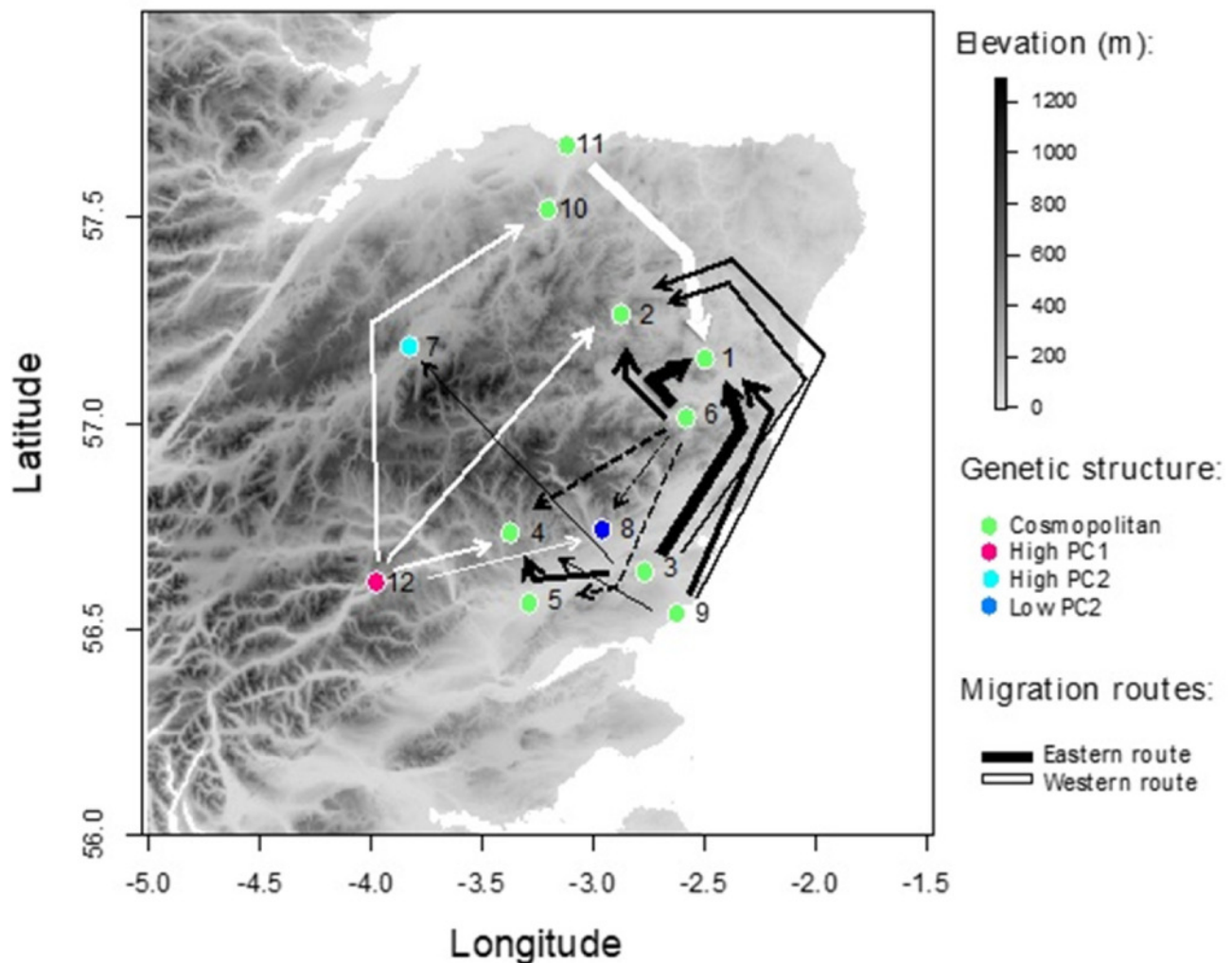
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# Figure 1

Location of each damselfly population, with topography of the study area (Northeast Scotland) depicted.

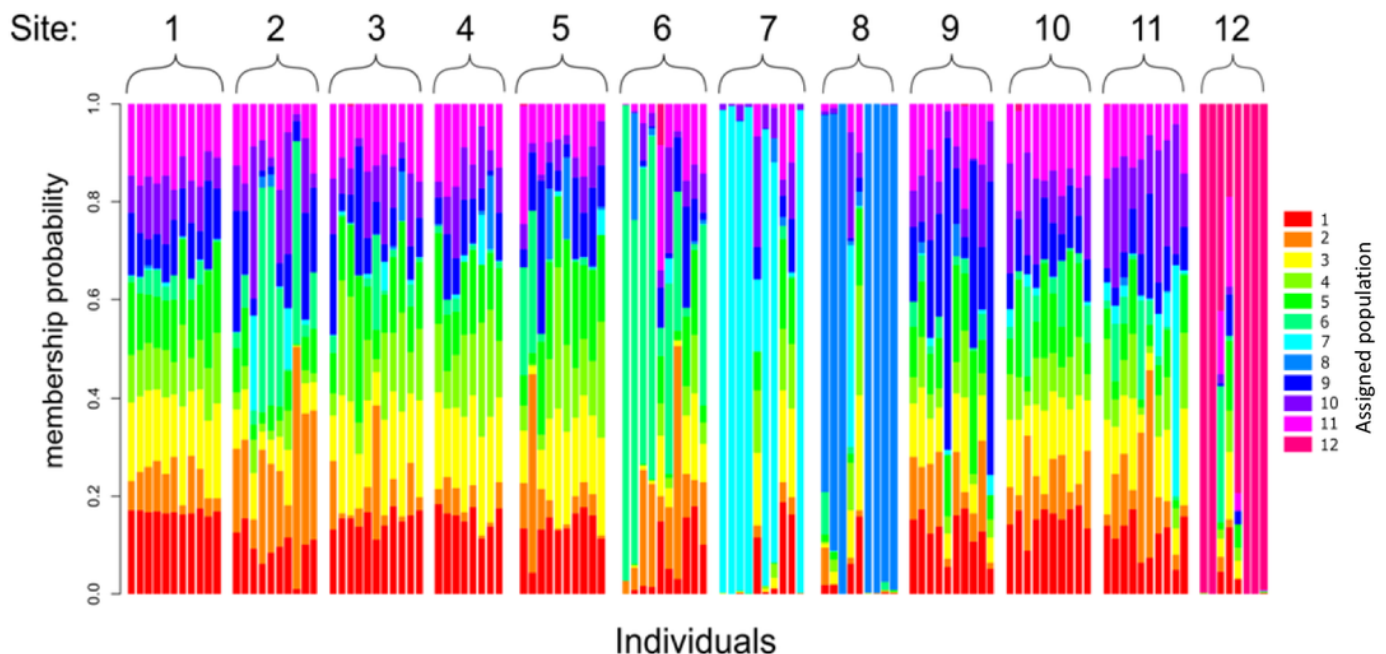
Arrow thicknesses indicate the incidence and direction of significant gene flow ( $\Phi_{Fst}$ ) between populations, where the strength of  $\Phi_{Fst}$  varies between 0.0008 and 0.07 (corresponding to thickness of each arrow).



# Figure 2

Figure 2: Membership probability to each of the 12-predefined populations for each genotyped individual.

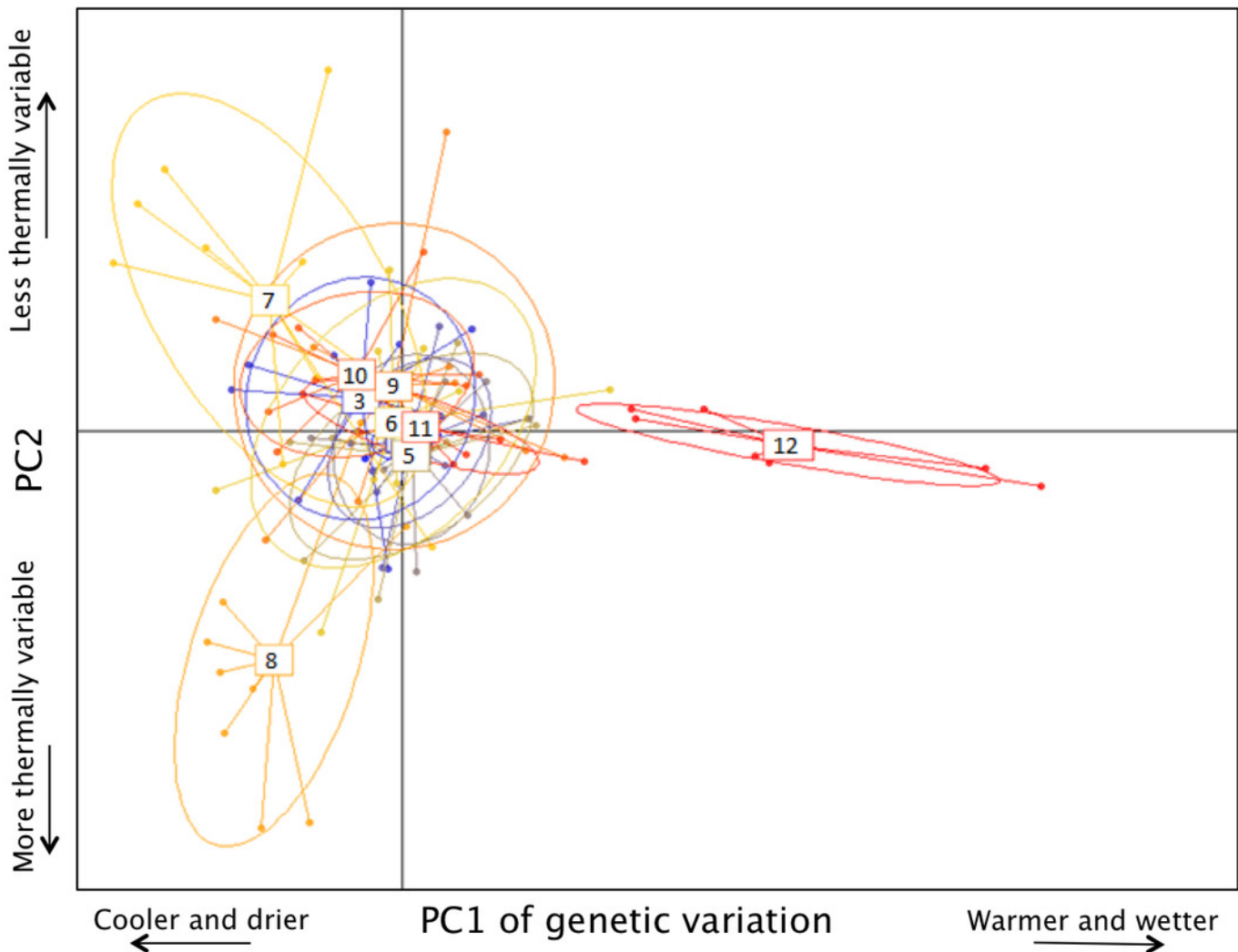
Most individuals exhibit equal probability of membership across the sites, but approximately 3/4 of the sampled individuals from each of populations 7, 8, and 12 show signals of site-specific genomic differentiation.



# Figure 3

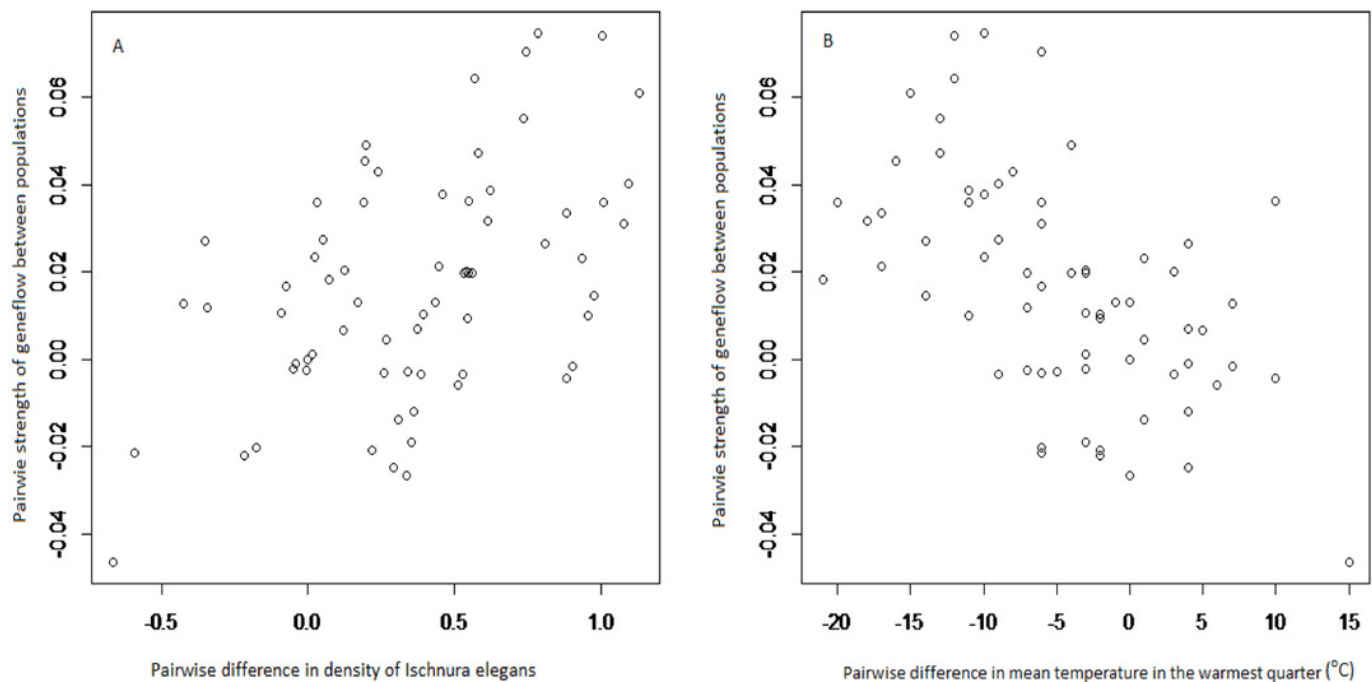
Figure 3: Principal components from DAPC analysis, depicting clustering of individual genotypes, where the X axis depicts PC1, while the Y axis depicts PC2.

Coloured rings indicate populations, with each population identified by its number label.



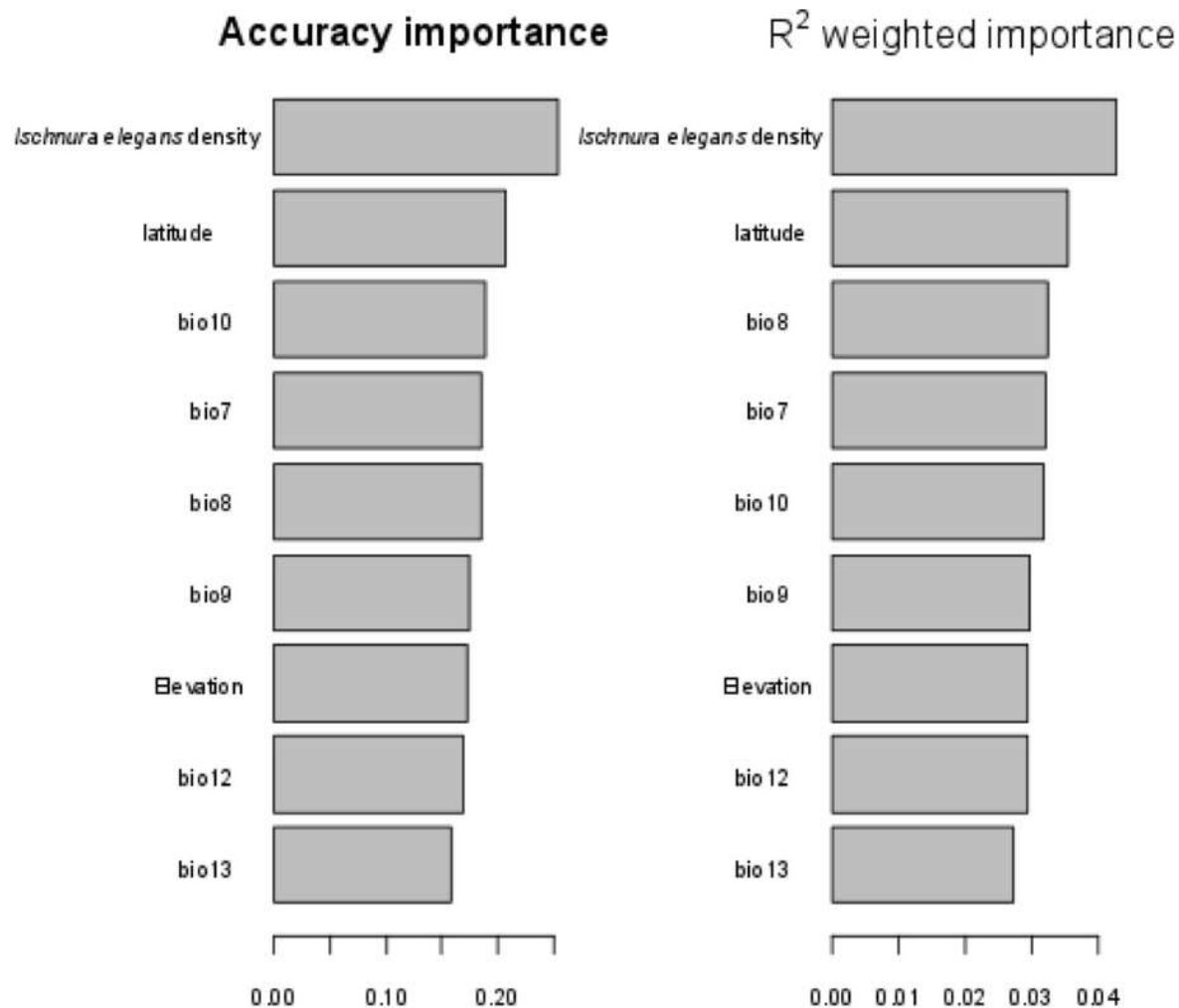
# Figure 4

Figure 4: Pairwise difference in gene flow ( $F_{ST}$ ) are plotted against A) the pairwise difference in density of *Ischnura elegans* between populations, and B) pairwise difference in mean temperature in the warmest quarter (Bio 7) between populations.



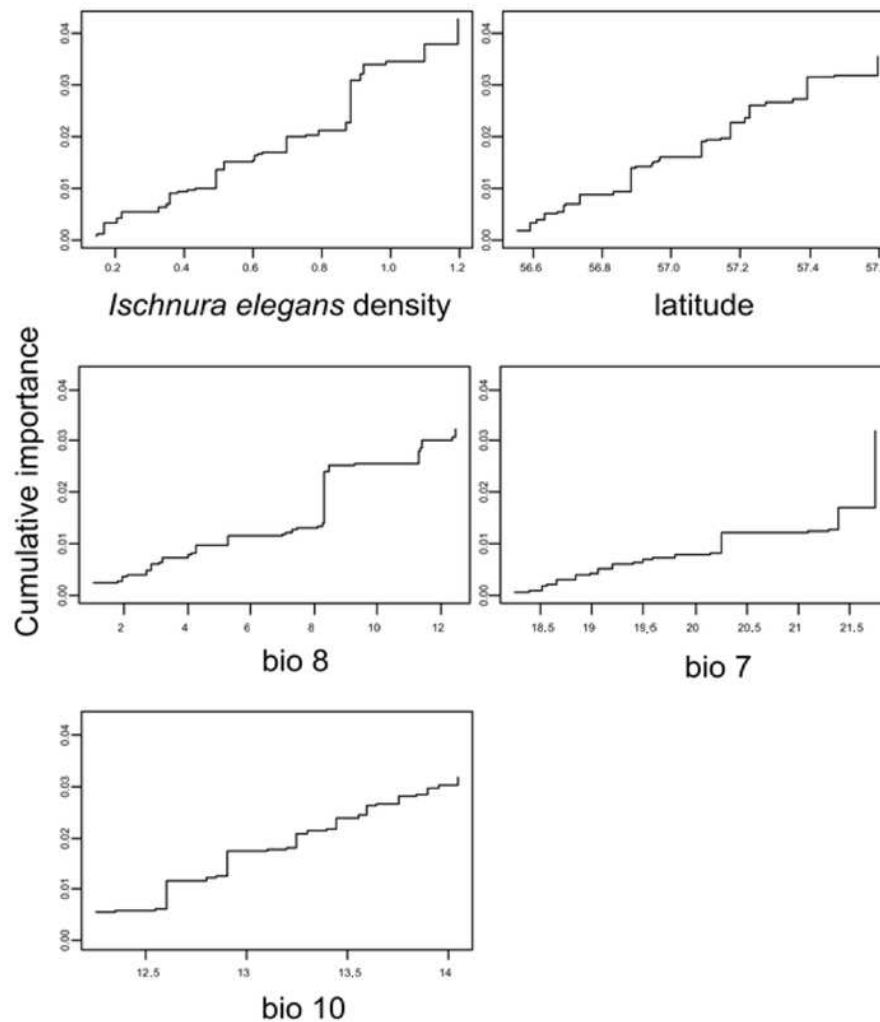
# Figure 5

Figure 5: Accuracy and  $R^2$  importance of environmental parameters in determining gene frequency turnover from gradient forest analysis.



## Figure 6

Figure 6: Mean increase in cumulative importance of the top 5 most important predictor variables of allelic turnover from the gradient forest models.



# **Table 1**(on next page)

Table: Geographic position, elevation, density of *Ischnura elegans*, and population heterozygosity of SNP data at our study sites.

1 Table1

2 Geographic position, elevation, density of *Ischnura elegans*, and population heterozygosity of  
3 SNP data at our study sites.

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Site	Latitude	Elevation (m)	<i>Ischnura elegans Density</i>	Heterozygosity
1	57.15618	188	1.255	0.563
2	57.26532	203	0.708	0.233
3	56.648	84	0.468	0.323
4	56.73116	403	1.133	0.299
5	56.56895	50	1.06	0.289
6	57.01947	93	0.511	0.217
7	57.18623	253	0.687	0.251
8	56.74228	249	0.175	0.189
9	56.54172	9	0.518	0.277
10	57.52185	66	0.158	0.308
11	57.67603	3	0.25	0.445
12	56.6119	119	0.125	0.162

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## Table 2 (on next page)

Correlations between the principal components of the detrended correspondence analysis and the environmental variables, highlighting the correlation between genetic differentiation between populations and the environment.

Correlations with values of  $R^2$  above 0.10 are highlighted in bold.

1 Table 2

2 Correlations between the principal components of the detrended correspondence analysis and the  
3 environmental variables, highlighting the correlation between genetic differentiation between  
4 populations and the environment. Correlations with values of  $R^2$  above 0.10 are highlighted in  
5 bold.

	PC2		PC1	
	Effect	$R^2$	Effect	$R^2$
<i>Ischnura elegans</i> Density	0.4412	-0.0656	-0.7974	-0.05063
Annual Mean Temperature	-0.01568	-0.08344	0.03164	-0.07038
Mean Diurnal Range	-0.4348	<b>0.233</b>	-0.07967	-0.09509
Min Temperature of Coldest Month	-0.01829	-0.05175	0.01508	-0.08559
Temperature Annual Range	0.02303	0.002732	0.003596	-0.0989
Mean Temperature of Driest Quarter	0.01418	3.297e-05	0.03141	<b>0.1157</b>
BIO10 = Mean Temperature of Warmest Quarter	0.02661	-0.06667	0.06600	-0.009929
BIO12 = Annual Precipitation	0.0009961	-0.07967	0.005807	<b>0.2035</b>
Precipitation of Wettest Month	0.006161	-0.08598	0.04710	<b>0.2601</b>

