Complex landscape topography can facilitate local adaptation during a range shift

Robert Nicholas Fitt[1,2], Lesley Therese Lancaster[1]

[1] School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom
[2] Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom

Corresponding Author:
Robert Fitt[1]
Institute of Integrative Biology, Biosciences Building, University of Liverpool, Crown Street, Liverpool, L69 7ZB, United Kingdom
Email address: robfitt@live.co.uk

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.

Key Words: Range shifts, Climate change, Odonata, Adaptation

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

<u>The study area</u>

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 site 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 Pstl 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 (Ordinance 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 Fst 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 $^{\varphi}$Fst (the directional measure of geneflow between populations). Pairwise $^{\varphi}$Fst 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 preformed 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 $^{\varphi}$fst ranged between 0.0008 and 0.07 for all sampling sites (Table S1).

Differences in Pairwise Fst 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 $^{\varphi}$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.

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

Discussion

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

<u>Colonisation routes</u>

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 than 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.

<u>Genetic structure and the capacity for local adaptation</u>

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

We found evidence for admixture from different colonisation routes, and further evidence that this admixture provided a fitness benefit in the colonised region, with sites receiving the most migrants having both high heterozygosity (Table 1) and high population density (Fig. 5, Table 2). Note particularly that sites with highest heterozygosity and population density (sites 1, 2, and

4) are also situated at the confluence of the eastern and western colonisation routes (Figure 1). A fitness benefit from admixture has previously been observed during range expansions and invasions (Keller et al., 2014), and our results suggest that admixture is likely an important process driving population growth rates during native range expansions. The gradient forest analysis further suggests that allelic turnover is correlated with population density (Fig. 5), which implies that admixture between lineages from multiple colonisation routes may be involved in driving changes in both allele frequencies and population densities at the range limit, and that admixed populations, while exhibiting low structure, may contain novel allelic combinations in support of further adaptation as the range shifts continues to progress northward.

We detected significantly higher rates of directional gene flow between sites that are more similar in thermal regimes during the warmest quarter (Fig 4b), after accounting for effects of geographic distance between sites. This suggests that local adaptation or acclimation to climatic conditions at an individual's natal site has a strong effect on its colonisation success of a new site. Despite the ubiquity of model predictions which assume that colonisation events follow climate isoclines, empirical evidence that climatic similarity from the source population affects colonisation success is equivocal (Maron, 2006). Our results suggest that, despite the widespread and rapid movement 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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Data Accessibility Statement

All the data used in this manuscript will be made freely available before publication, the genomic data used will be made available via genbank, and density data for *Ischnura elegans* is currently available on dryad (doi.org/10.5061/dryad.kp89j).

References

Birse EL, Dry FT. 1970. Assessment of Climatic Conditions in Scotland. 1.Based on Accumulated Temperature and Potential Water Deficit. In: The Macaulay Institute for Soil Research, Aberdeen,.

Bocedi G, Atkins KE, Liao J, Henry RC, Travis JMJ, Hellmann JJ. 2013. Effects of local adaptation and interspecific competition on species' responses to climate change. *Annals of the New York Academy of Sciences* 1297:83–97. DOI: 10.1111/nyas.12211.

Brazier V, Gordon JE, Hubbard A, Sugden DE. 1996. The Geomorphological Evolution of a Dynamic Landscape: the Cairngorm Mountains, Scotland. *Botanical Journal of Scotland* 48:13–30. DOI: 10.1080/03746609609480371.

Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. 2011. Stacks: building and genotyping Loci de novo from short-read sequences. *G3 (Bethesda, Md.)* 1:171–82. DOI: 10.1534/g3.111.000240.

Catchen J, Hohenlohe PA, Bassham S, Amores A. 2013. Stacks : an analysis tool set for population genomics. *Molecular Ecology* 22:3124–3140. DOI: 10.1111/mec.12354.

Cham S, Nelson B, Parr A, Prentice S, Smallshire D, Taylor P. 2014. *Atlas of Dragonflies in Britain and Ireland*. the Field Studies Council for the Biological Records Centre, Centre for Ecology & Hydrology, with the British Dragonfly Society.

Chen I-C, Hill JK, Ohlemüller R, Roy DB, Thomas CD. 2011a. Rapid range shifts of species associated with high levels of climate warming. *Science (New York, N.Y.)* 333:1024–1026. DOI: 10.1126/science.1206432.

Chen I-C, Hill JK, Shiu H-J, Holloway JD, Benedick S, Chey VK, Barlow HS, Thomas CD. 2011b. Asymmetric boundary shifts of tropical montane Lepidoptera over four decades of climate warming. *Global Ecology and Biogeography* 20:34–45. DOI: 10.1111/j.1466-8238.2010.00594.x.

Colautti robert I, Lau JA. 2015. Contemporary evolution during invasion : evidence for differentiation , natural selection , and local adaptation. *Molecular Ecology* 24:1999–2017. DOI: 10.1111/mec.13162.

Dray S, Dufour A-B. 2007. The **ade4** Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software* 22:1–20. DOI: 10.18637/jss.v022.i04.

Dudaniec RY, Yong CJ, Lancaster LT, Svensson EI, Hansson B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (Ischnura elegans). *Molecular Ecology* 27:2576–2593.

Dytham C. 2009. Evolved dispersal strategies at range margins. *Proceedings. Biological sciences / The Royal Society* 276:1407–13. DOI: 10.1098/rspb.2008.1535.

Ellis N, Smith SJ, Pitcher RC. 2012. Gradient forests : calculating importance gradients on physical predictors. *Ecology* 93:156–168.

Fitt RN, Lancaster LT. Range shifting species reduce phylogenetic diversity in high latitude damselfly assemblages via competition.

Fitt RNL, Lancaster LT. 2017. Range shifting species reduce phylogenetic diversity in high latitude communities via competition. *Journal of Animal Ecology* 86:543–555. DOI: 10.1111/1365-2656.12655.

Foll M, Gaggiotti O. 2008. A Genome-Scan Method to Identify Selected Loci Appropriate for Both Dominant and Codominant Markers: A Bayesian Perspective. 993:977–993. DOI: 10.1534/genetics.108.092221.

Frichot E, Franc O. 2015. APPLICATION LEA : An R package for landscape and ecological association studies. *Methods in Ecology and Evolution* 6:925–929. DOI: 10.1111/2041-210X.12382.

Frichot E, Schoville SD, Bouchard G, François O. 2013. Testing for Associations between Loci and Environmental Gradients Using Latent Factor Mixed Models. *Molecular Biology and Evolution* 30:1687–1699. DOI: 10.1093/molbev/mst063.

Goldstein BA, Hubbard AE, Cutler A, Barcellos LF. 2010. An application of Random Forests to a genome-wide association dataset : Methodological considerations & new findings. *BMC Genetics* 11:1:13.

Goslee SC, Urban DL. 2007. The ecodist Package for Dissimilarity-based Analysis of Ecological Data. *Journal of Statistical Software* 22:1–19. DOI: 10.18637/jss.v022.i07.

Grueber CE, Wallis GP, Jamieson IG. 2013. Genetic drift outweighs natural selection at toll-like receptor (*TLR*) immunity loci in a re-introduced population of a threatened species. *Molecular Ecology* 22:4470–4482. DOI: 10.1111/mec.12404.

Guarnizo CE, Cannatella DC. 2013. Genetic divergence within frog species is greater in topographically more complex regions. *Journal of Zoological Systematics and Evolutionary Research* 51:n/a-n/a. DOI: 10.1111/jzs.12027.

Henn BM, Feldman MW. 2012. The great human expansion. *Proceedings of the National Academy of Sciences* 109:17758–17764. DOI: 10.1073/pnas.1212380109.

Herrera CM, Bazaga P. 2008. Adding a third dimension to the edge of a species ' range : altitude and genetic structuring in mountainous landscapes. *Heredity* 100:275–285. DOI: 10.1038/sj.hdy.6801072.

Hickling R, Roy DB, Hill JK, Fox R, Thomas CD. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology* 12:450–455. DOI: 10.1111/j.1365-2486.2006.01116.x.

Hickling R, Roy DB, Hill JK, Thomas CD. 2005. A northward shift of range margins in British

Odonata. *Global Change Biology* 11:502–506. DOI: 10.1111/j.1365-2486.2005.00904.x.
Hijmans RJ. 2017. geosphere: Sherical Trigonometry.
Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965–1978. DOI: 10.1002/joc.1276.
Hill JK, Griffiths HM, Thomas CD. 2011. Climate change and evolutionary adaptations at species' range margins. *Annual review of entomology* 56:143–59. DOI: 10.1146/annurev-ento-120709-144746.
Hill JK, Yvonne C, Thomas CD, Blakeley DS, Fox R, Moss D, Huntley B. 2001. Impacts of landscape structure on butterfly range expansion. *Ecology letters* 4:313–321.
Janz N, Nylin S. 2005. The Oscillation Hypothesis of Host-Plant Range and Speciation. :203–215.
Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403–1405. DOI: 10.1093/bioinformatics/btn129.
Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA. 2013. diveRsity : An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4:782–788. DOI: 10.1111/2041-210X.12067.
Keller SR, Fields PD, Berardi AE, Taylor DR. 2014. Recent admixture generates heterozygosity – fitness correlations during the range expansion of an invading species. *Journal of evolutionary biology* 27:616–627. DOI: 10.1111/jeb.12330.
Keyghobadi N, Roland J, Strobeck C. 2005. Genetic differentiation and gene flow among populations of the alpine butterfly , Parnassius smintheus , vary with landscape connectivity. *Molecular Ecology* 14:1897–1909. DOI: 10.1111/j.1365-294X.2005.02563.x.
Krehenwinkel H, Rödder D, Tautz D. 2015. Eco-Genomic analysis of the poleward range expansion of the wasp spider Argiope bruennichi shows rapid adaptation and genomic admixture. *Global change biology*. DOI: 10.1111/gcb.13042.
Krehenwinkel H, Tautz D. 2013. Northern range expansion of European populations of the wasp spider Argiope bruennichi is associated with global warming-correlated genetic admixture and population-specific temperature adaptations. *Molecular ecology* 22:2232–48. DOI: 10.1111/mec.12223.
Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, Bridle JR, Gomulkiewicz R, Klein EK, Ritland K, Kuparinen A, Gerber S, Schueler S. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology letters*:378–392. DOI: 10.1111/j.1461-0248.2012.01746.x.
Lancaster LT. 2016. Widespread, ongoing range expansions shape latitudinal variation in insect thermal limits. *Nature Climate change*:1–5. DOI: 10.1038/nclimate2945.
Lancaster LT, Dudaniec RY, Hansson B, Svensson EI. 2015. Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion. *Journal of Biogeography* 42:1953–1963. DOI: 10.1111/jbi.12553.
Lancaster LT, Dudaniec RY, Hansson B, Svensson EI. 2017. Do group dynamics affect colour morph clines during a range shift? *Journal of Evolutionary Biology* 30:728–737. DOI: 10.1111/jeb.13037.
Lenormand T. 2002a. Gene flow and the limits to natural. *Trends in Ecology and Evolution* 17:183–189.
Lenormand T. 2002b. Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* 17:183–189. DOI: 10.1016/S0169-5347(02)02497-7.

Lischer HEL, Excoffier L. 2012. PGDSpider : an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics* 28:298–299. DOI: 10.1093/bioinformatics/btr642.

Luu K, Bazin E, Blum MGB. 2017. *pcadapt* : an R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources* 17:67–77. DOI: 10.1111/1755-0998.12592.

Maclean N. 2010. *Silent summer : the state of wildlife in Britain and Ireland*. Cambridge University Press.

Maron JL. 2006. The relative importance of latitude matching and propagule pressure in the colonization success of an invasive forb. *Ecography* 29:819–826.

Möbius W, Murray AW, Nelson DR. 2015. How Obstacles Perturb Population Fronts and Alter Their Genetic Structure. *PLOS Computational Biology* 11:e1004615. DOI: 10.1371/journal.pcbi.1004615.

Ordinance Survey. 2017.OS Terrain 50. *Available at https://www.ordnancesurvey.co.uk/business-and-government/products/terrain-50.html*

Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42. DOI: 10.1038/nature01286.

Peischl S, Dupanloup I, Kirkpatric M, Excoffier L. 2013. On the accumulation of deleterious mutations during range expansions. *Molecular Ecology* 22:5972–5982. DOI: 10.1111/mec.12524.

Perez-Espona S, Perez-Baraeris FJ, Mcleod JE, Jiggins CD, Gordon IJ. 2008. Landscape features affect gene flow of Scottish Highland red deer (Cervus elaphus). *Molecular Ecology* 17:981–996. DOI: 10.1111/j.1365-294X.2007.03629.x.

Peter B. rangeExpansion

Peter BM, Slatkin M. 2013. Detecting range expansions from genetic data. *Evolution* 67:3274–3289. DOI: 10.1111/evo.12202.

Pritchard JK, Rienzo A Di. 2010. Adaptation – not by sweeps alone. *Nature reviews: Genetics* 11:665–667. DOI: 10.1038/nrg2880.

R Core development Team. 2012. R: A language and environment for statistical computing.

Raymond M, Rousset F. 1995. GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *Journal of Heredity* 86:248–249. DOI: 10.1093/oxfordjournals.jhered.a111573.

Scottish Natural Heritage.Wild Land Area descriptions. *Available at https://www.nature.scot/wild-land-area-descriptions* (accessed June 19, 2019).

Stapley J, Santure AW, Dennis SR. 2015. Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. *Molecular ecology* 24:2241–52. DOI: 10.1111/mec.13089.

Swaegers J, Mergeay J, Geystelen AVAN, Therry L. 2015a. Neutral and adaptive genomic signatures of rapid poleward range expansion. *Molecular Ecology* 24:6163–6176. DOI: 10.1111/mec.13462.

Swaegers J, Mergeay J, Van Geystelen A, Therry L, Larmuseau MHD, Stoks R. 2015b. Neutral and adaptive genomic signatures of rapid poleward range expansion. *Molecular Ecology* 24:6163–6176. DOI: 10.1111/mec.13462.

Wellenreuther M, Sánchez-Guillén RA, Cordero-Rivera A, Svensson EI, Hansson B. 2011. Environmental and Climatic Determinants of Molecular Diversity and Genetic Population Structure in a Coenagrionid Damselfly. *PLoS ONE* 6:e20440. DOI:

10.1371/journal.pone.0020440.
Whitlock MC. 2000. Heterosis increases the effective migration rate Pa. *Proceedings of the Royal Society B: Biological Sciences* 267:1321–1326. DOI: 10.1098/rspb.2000.1145.
Whitlock MC, Lotterhos KE. 2015a. Reliable Detection of Loci Responsible for Local Adaptation : Inference of a Null Model through Trimming the Distribution of F ST *. *The American naturalist* 186:S25–S35. DOI: 10.1086/682949.
Whitlock MC, Lotterhos KE. 2015b. Reliable Detection of Loci Responsible for Local Adaptation: Inference of a Null Model through Trimming the Distribution of F_{ST}. *The American Naturalist* 186:S24–S36. DOI: 10.1086/682949.

Figure 1: Location of each damselfly population, with topography of the study area (NortheastScotland) depicted. Arrow thicknesses indicate the incidence and direction of significant gene flow ($^{\varphi}$Fst)between populations, where the strength of $^{\varphi}$Fst varies between 0.0008 and 0.07(corresponding to thickness of each arrow).

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: 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: Pairwise difference in gene low ($^{\varphi}$Fst) 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: Accuracy and R2 importance of environmental parameters in determining gene frequency turnover from gradient forest analysis.

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

Figure 1

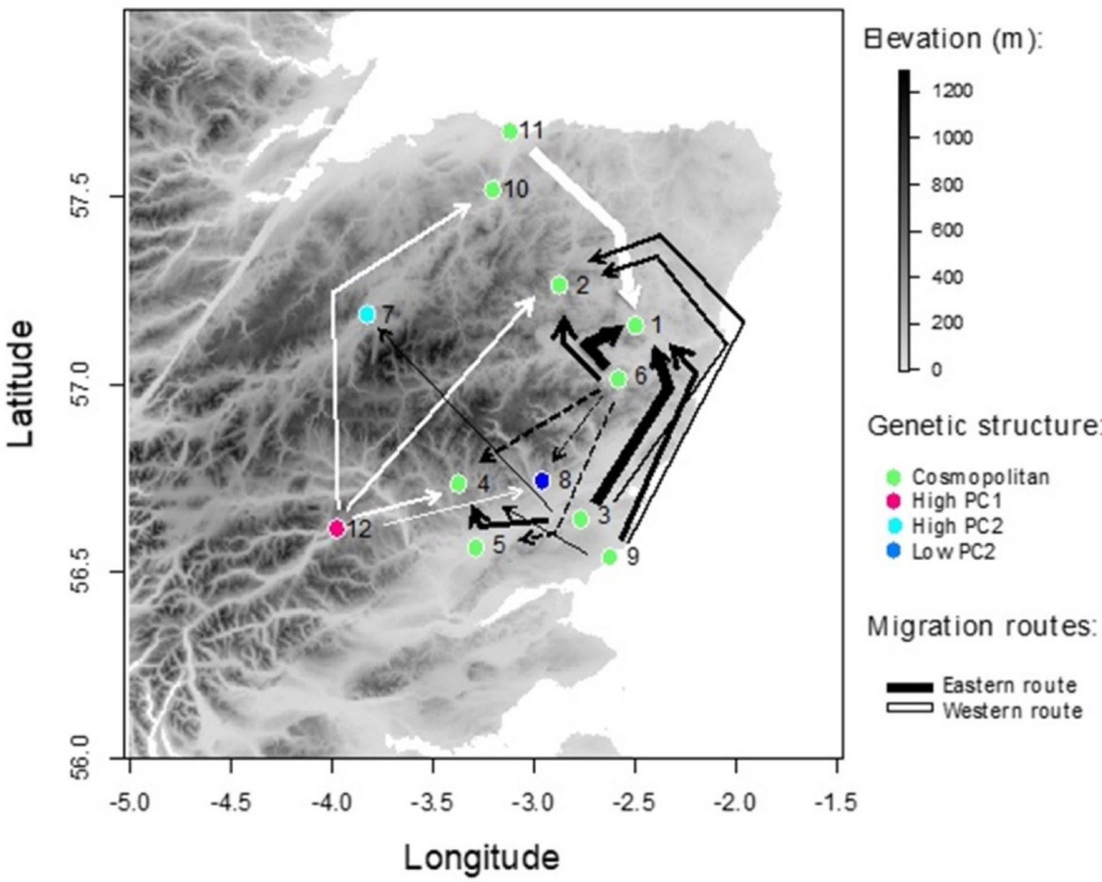

Figure 2

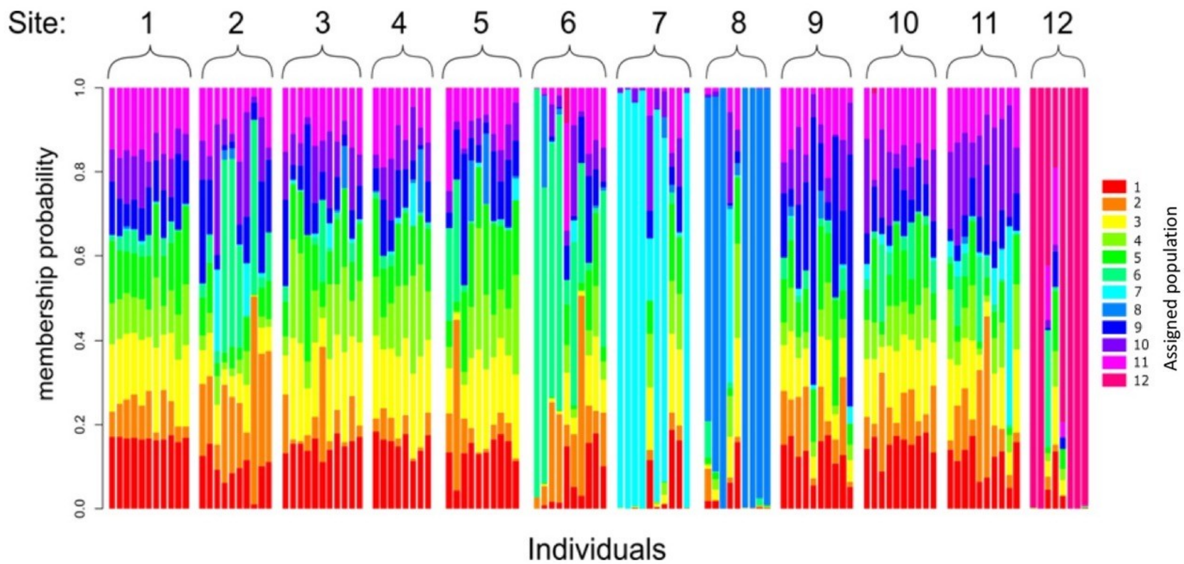
Site:
1
2
3
4
5
6
7
8
9
10
11
12
membership probability
0.0
0.2
0.4
0.6
0.8
1.0
Individuals
1
2
3
4
5
6
7
8
9
10
11
12
Assigned population

Figure 3

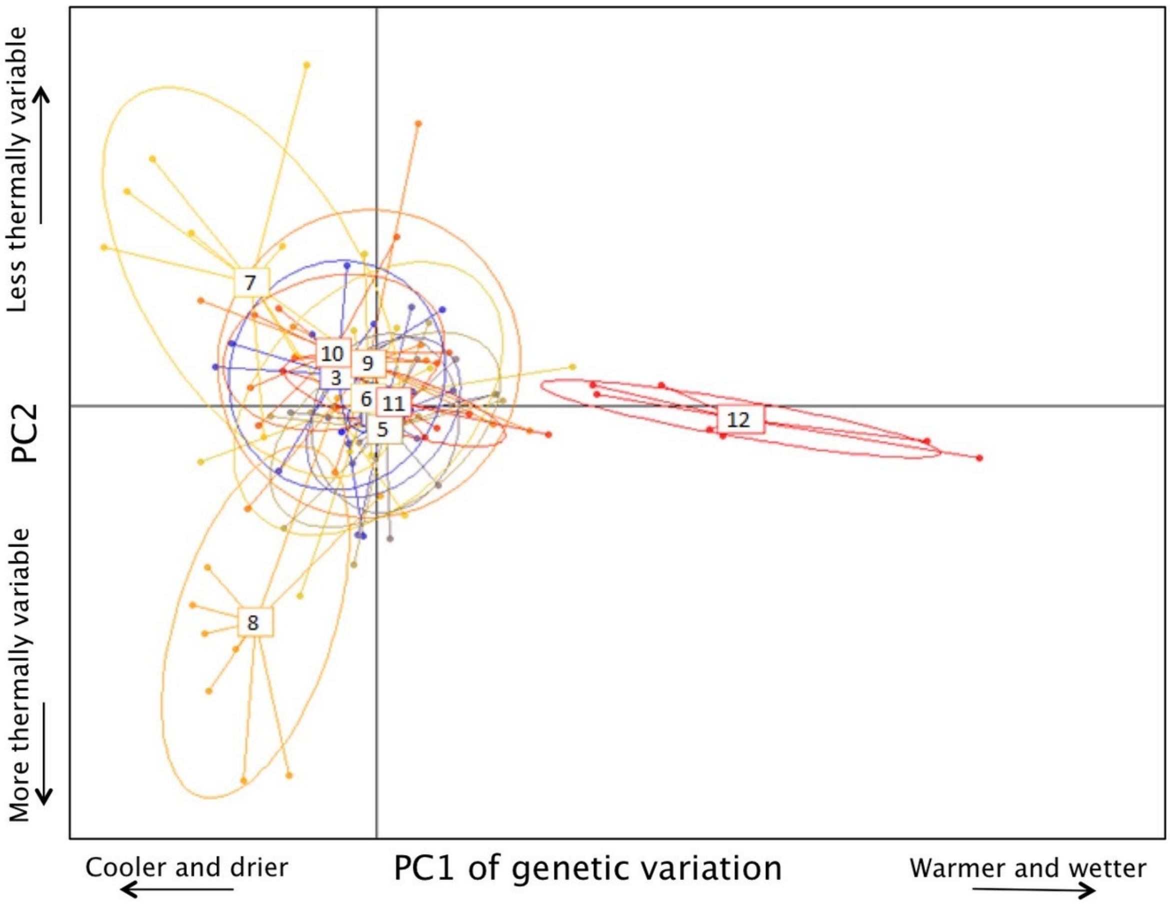
Less thermally variable
More thermally variable
PC2
PC1 of genetic variation
Cooler and drier
Warmer and wetter
7
10
9
3
6
11
5
12
8

Figure 4

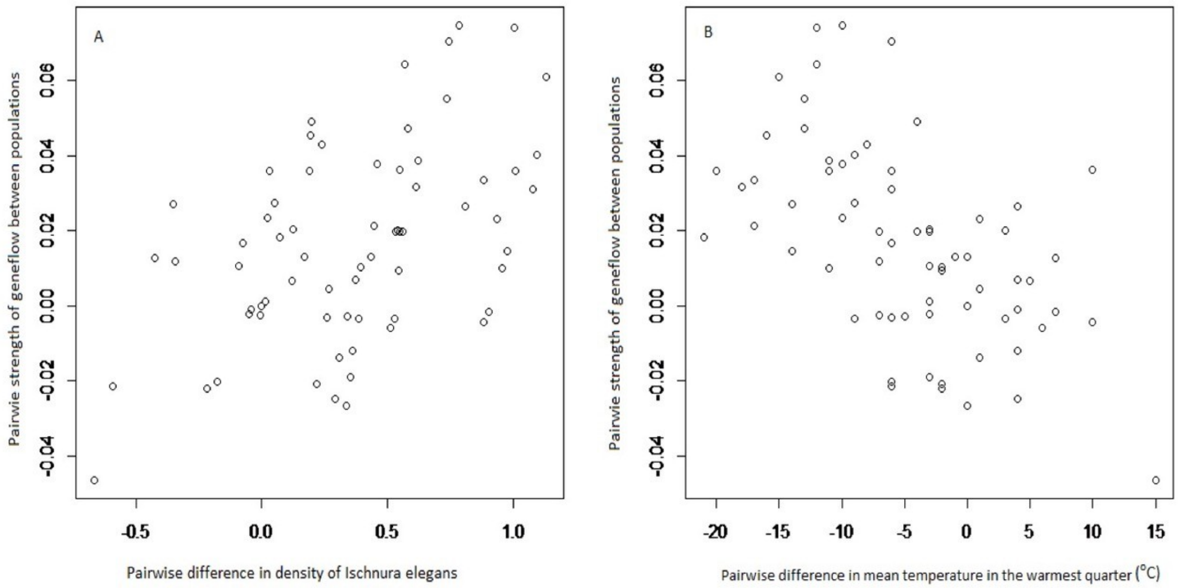
A
Pairwie strength of geneflow between populations
Pairwise difference in density of Ischnura elegans
-0.04
-0.02
0.00
0.02
0.04
0.06
-0.5
0.0
0.5
1.0
B
Pairwie strength of geneflow between populations
Pairwise difference in mean temperature in the warmest quarter (°C)
-20
-15
-10
-5
0
5
10
15

Figure 5

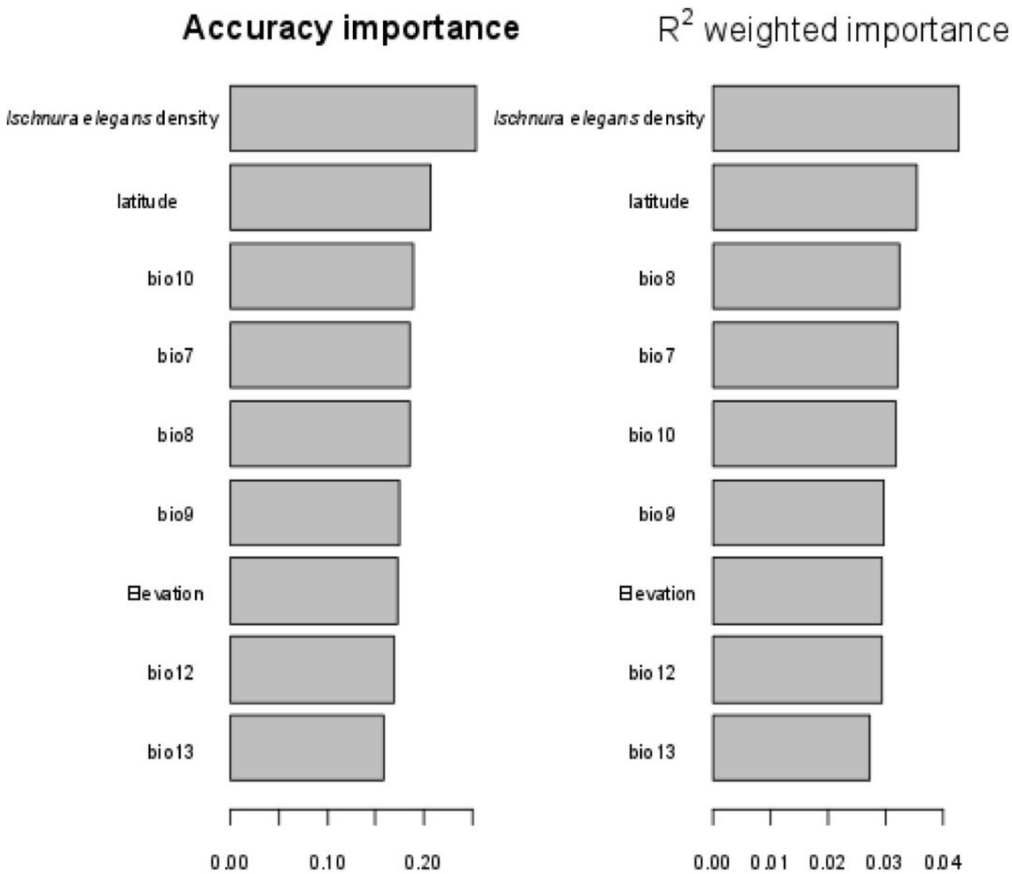
Accuracy importance
R2 weighted importance
Ischnura elegans density
latitude
bio10
bio7
bio8
bio9
Elevation
bio12
bio13
0.00
0.10
0.20
Ischnura elegans density
latitude
bio8
bio7
bio10
bio9
Elevation
bio12
bio13
0.00
0.01
0.02
0.03
0.04

Figure 6

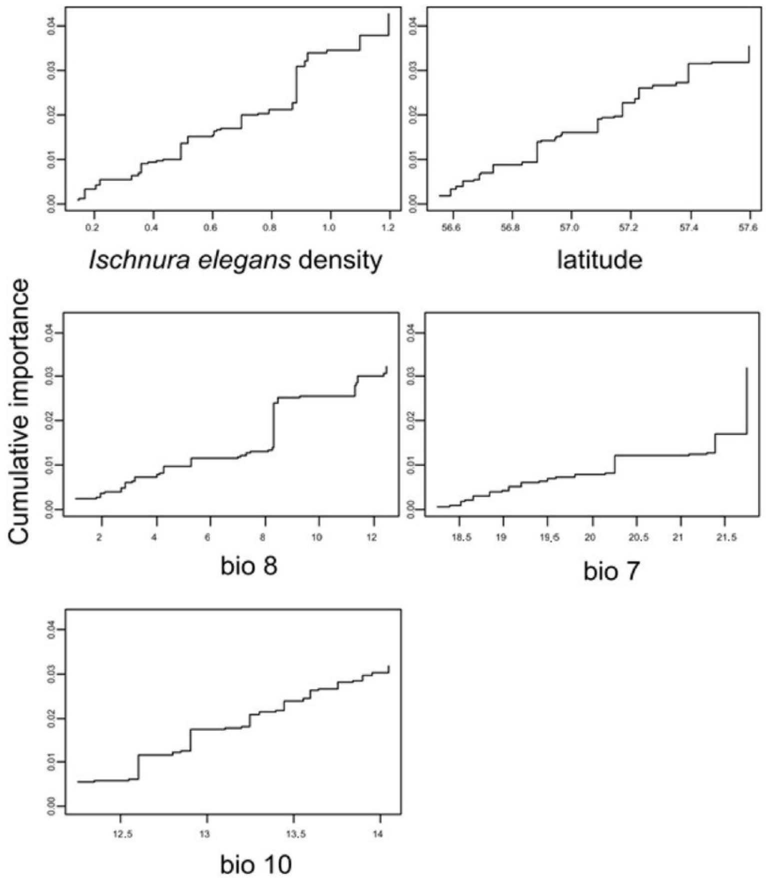

Table1

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

Site	Latitude	Elevation (m)	*Ischnura elegans Density*	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

Table 2

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.

	PC2 Effect	R^2	PC1 Effect	R^2
Ischnura elegans 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	**0.233**	-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	**0.1157**
BIO10 = Mean Temperature of Warmest Quarter	0.02661	-0.06667	0.06600	-0.009929
BIO12 = Annual Precipitation	0.0009961	-0.07967	0.005807	**0.2035**
Precipitation of Wettest Month	0.006161	-0.08598	0.04710	**0.2601**