JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12347

Unravelling the effects of contemporary and historical range expansion on the distribution of genetic diversity in the damselfly *Coenagrion scitulum*

J. SWAEGERS*, J. MERGEAY*†, L. THERRY*, D. BONTE‡, M. H. D. LARMUSEAU§¶ & R. STOKS*

*Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Leuven, Belgium
†Research Institute for Nature and Forest, Geraardsbergen, Belgium
‡Terrestrial Ecology Unit, Ghent University, Ghent, Belgium
§Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Leuven, Belgium
¶Laboratory of Forensic Genetics and Molecular Archaeology, University of Leuven, Leuven, Belgium

Keywords:

Coenagrion scitulum;
contemporary range expansion;
damselfly;
genetic diversity;
genetic structure;
global change;
phylogeography;
post-glacial range expansion.

Abstract

Although genetic diversity provides the basic substrate for evolution, there are a limited number of studies that assess the impact of recent climate change on intraspecific genetic variation. This study aims to unravel the degree to which historical and contemporary factors shape genetic diversity and structure across a large part of the range of the range-expanding damselfly *Coenagrion scitulum* (Rambur, 1842). A total of 525 individuals from 31 populations were genotyped at nine microsatellites, and a subset was sequenced at two mitochondrial genes. We inferred the importance of geography, environmental factors, and recent range expansion on genetic diversity and structure. Genetic diversity decreased going westwards, suggesting a signature of historical post-glacial expansion from east to west and the presence of eastern refugia. Although genetic differentiation decreased going northwards, it increased in the northern edge populations, suggesting a role of contemporary range expansion on the genetic make-up of populations. The phylogeographical context was proven to be essential in understanding and identifying the genetic signatures of local contemporary processes. Within this framework, our results highlight that recent range expansion of a good disperser can decrease genetic diversity and increase genetic differentiation which should be considered when devising suitable conservation strategies.

Introduction

One of the most important human influences on the environment today is climate change, inducing range expansion to formerly unsuitable regions (Davis & Shaw, 2001). Such contemporary range expansions may leave a strong genetic signature (Hill *et al.*, 2011; Watts *et al.*, 2010; Garroway *et al.*, 2011; Pauls *et al.*, 2013). Opposing patterns of genetic diversity have been detected in recently established populations: (i) reduced genetic diversity and significant population structuring compared with core populations and (ii) the persistence of genetic polymorphisms and the lack of differentiation in edge populations (Song *et al.*, 2013). For example, a decrease in allelic richness has been detected in currently range-expanding flying squirrels (Garroway *et al.*, 2011) and damselflies (Watts *et al.*, 2010). The integration of evolutionary processes into conservation policies is crucial as the current fast anthropogenic environmental changes can induce evolutionary pressures that deplete the genetic variation needed for future evolutionary responses (Mergeay & Santamaria, 2012; Santamaría & Méndez, 2012).

The genetic diversity and structure displayed by a species is dynamic and shaped by processes acting on widely different spatial and temporal scales (Foll &

Correspondence: Jane Swaegers, Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Deberiotstraat 32, 3000 Leuven, Belgium.
Tel.: +32 16 32 37 89; fax: +32 16 32 45 75;
e-mail: Janne.Swaegers@bio.kuleuven.be

Gaggiotti, 2006). Genetic signatures of major historical processes are typically studied at large spatial scales covering large part of the range of the species (Huck *et al.*, 2009; Theissinger *et al.*, 2013). In contrast, genetic effects of contemporary processes are mostly studied on more limited spatial scales (Epps *et al.*, 2005; Goossens *et al.*, 2006; Vandergast *et al.*, 2007; Zellmer & Knowles, 2009). As processes at different scales can result in similar genetic signatures (Eckert *et al.*, 2008; Zellmer & Knowles, 2009; Guo, 2012), it is important to jointly investigate historical and contemporary processes as drivers of current genetic patterns (Rönkä *et al.*, 2012; Bray *et al.*, 2013). Studies that span large parts of a species' range can reveal patterns of genetic variation that might otherwise go undetected; moreover, they can provide insights into the relative roles of historical and contemporary processes in shaping genetic diversity and structure (Hoban *et al.*, 2010; Zarza *et al.*, 2011; Cheang *et al.*, 2012; Hasselman *et al.*, 2013). Molecular information can thereby inform on species historical dynamics and contemporary demography necessary to advance species modelling paradigms that seek to integrate climatic and demographic drivers (Scoble & Lowe, 2010).

Despite the increasing interest in understanding the genetic effects of contemporary range expansions, and the gained insight that contemporary processes are best studied in a phylogeographical context, this has rarely been done. Yet, phylogeographical studies investigating the historical processes that generate patterns of genetic diversity and structure may be especially relevant in this context. Indeed, species currently showing range expansions most likely underwent historical post-glacial range expansions along the same axis of expansion which independently impacted the genetic signatures along this axis (Hewitt, 1999). For example, many European species show a latitudinal decrease in genetic diversity due to post-glacial expansion from southern refugia and are currently expanding to the north (Hewitt, 2000; Davis & Shaw, 2001).

In this study, we investigate how historical and contemporary range expansions shape genetic diversity and structure across a large part of the species' range of *Coenagrion scitulum*, a southern European damselfly species currently expanding its range in an eastward, northward and westward direction. Damselflies are suitable study species for climate change research as their geographical distribution has been well recorded through the past decades. Their relatively high dispersal potential is expected to result in signatures of rapid historical range expansion. In order to investigate the phylogeography and distribution of genetic diversity in *C. scitulum,* we analysed the genetic diversity and structure of 31 populations across the species range. We genotyped individuals at nine microsatellite loci and sequenced a subset for two mitochondrial genes. This enabled us to disentangle signatures of historical range expansion as well as the impact of a very recent range expansion on the genetic diversity and structure within the reconstructed phylogeographical framework. In addition, we assessed the effect of contemporary climatic factors as these can also influence the genetic diversity and structure. These data will not only be relevant to identify areas of conservation interest but also as important input for species distribution modelling, particularly in the field of climate adaptation (Scoble & Lowe, 2010).

Materials and methods

Study species and sample collection

The damselfly *C. scitulum* has a Mediterranean distribution (Dijkstra & Lewington, 2006). Its range extends from Western to Eastern Europe and North Africa. In the early nineties, the north-western edge of the range was situated in northern France (Dommanget *et al.*, 1994). However, the species has recently expanded from this historical range and founded edge populations in an eastward (including Swiss), northward (including the Netherlands and Germany) and westward (including Jersey, UK) direction (Fig. 1, references within Swaegers *et al.*, 2013).

We collected individuals from 14 core populations (total of 163 samples) spanning the entire geographical range of *C. scitulum* (Table 1) and seven edge populations ($n = 78$ individuals). Importantly, core populations are defined as those populations within the historical range of the species (recordings before 1990), whereas edge populations are defined as populations that were located outside the historical continuous distribution of the species and were not recorded before 1990. The distinction allows directly assessing the influence of recent range expansion on the distribution of genetic diversity, rather than the influence of 'edge effects' (reviewed in Eckert *et al.*, 2008). All samples were collected during the period 2010–2012. Sample sizes per population varied between nine and 32. For two nearby locations (< 35 km apart) in Algeria and in Bulgaria, we pooled the individuals. As rarefaction curves showed that for all loci the expected heterozygosity stabilizes from at least ten individuals (result given in Appendix S1), these sample sizes are deemed to be sufficient for reliable genetic analyses. Moreover, it has been shown that sampling a large amount of individuals per population does not necessarily improve estimates of, for example, population differentiation (Björklund & Bergek, 2009). We combined these newly obtained genotype data with genotypes from 284 individuals from five core and five edge populations from Swaegers *et al.* (2013). These were from a limited part of the species' range, collected in 2010 and analysed at the same loci. This resulted in a total of 316 samples

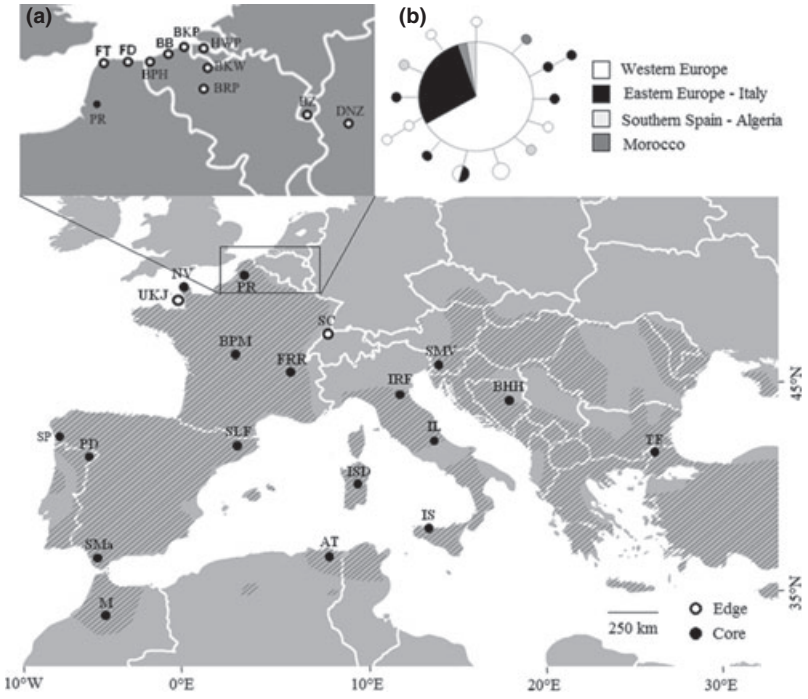

Fig. 1 (a) Map of the study populations. The shaded area represents the historical range based on Dommanget *et al.* (1994), Dijkstra & Lewington (2006) and Boudot (2013, unpublished data). (b).TCS network of CO1 and CO2 concatenated sequences. Circle size is proportional to the number of individuals with a given mtDNA haplotype.

Table 1 Summary statistics of the redundancy analysis model with inclusion of the four phylogroups (encoded by three dummy variables), twelve MEM and the edge–core dummy variable. Only the variables that were significant or marginally significant are reported.

	F	P	R^2	R^2_{adj}
Full model	5.672	0.0001	0.866	0.713
Forward selection model	12.035	0.0001	0.786	0.713
Phylogroup S	26.267	0.0017	0.475	0.431
Phylogroup E	9.137	0.0001	0.129	0.117
Phylogroup W	5.594	0.0263	0.068	0.062
MEM5	3.052	0.0164	0.034	0.031
MEM11	2.722	0.031	0.029	0.026
MEM12	3.402	0.0063	0.033	0.03
Population status	1.845	0.0533	0.017	0.015

from 19 core populations and 209 samples from 12 edge populations.

Sequencing analysis

Samples were stored in absolute ethanol. DNA was isolated from legs using the Nucleospin extraction kit (Macherey Nagel), with the exception of the SLF population, for which DNA was extracted using a Chelex method (see Johansson *et al.*, 2011). To gain insight into the phylogeography of the species, we sequenced a subset of 56 individuals from 32 localities for COI and COII, yielding a total sequence length of 1001 bp (Appendix S1).

Microsatellite analysis

We genotyped 241 damselflies, 125 at eleven microsatellite markers (cosci_05, cosci_07, cosci_27, cosci_38, cosci_01, cosci_08, cosci_15, cosci_25, cosci_34, cosci_37, cosci_46, C2_1050) described in Johansson *et al.* (2011) and Swaegers *et al.* (2012). As cosci_34 and cosci_37 resulted in electropherograms with low signal in populations AT and SMa, we excluded these loci from further data analysis, so that ultimately all populations were genotyped at nine loci. The genotyping protocols together with an assessment of the genotyping error rate (0.7%) have been presented in Swaegers *et al.* (2012). In a preliminary step, all populations were tested for Hardy–Weinberg equilibrium using Genepop (Rousset, 2008) and Microchecker (van Oosterhout *et al.*, 2004) and for deviations from linkage equilibrium using Genepop.

Analysis of mitochondrial DNA haplotypes

We assessed the relationships among the resulting mtDNA haplotypes by creating a haplotype network using TCS (Clement *et al.*, 2000) and calculated two genetic diversity indices: haplotype diversity (h) and nucleotide diversity (p). The Tajima's D statistic (Tajima, 1989) was computed in Arlequin 3.11 (Excoffier & Schneider, 2005). Although originally a test for selective neutrality, a negative D indicates that the population studied has gone through a demographic expansion process in selectively neutral genes (Rogers & Harpending, 1992). We pooled all data and estimated

the time since historical post-glacial expansion using the equation $\tau = 2ut$ (Rogers & Harpending, 1992), where t is the time since expansion (expressed as number of generations) and u is the mutation rate per generation for the sequence under study (with $u = Qk$, where k is the length of the sequence and Q the divergence rate). We used two commonly used divergence rates for insect mitochondrial DNA (Brower, 1994; $\mu = 2.3\%$ Myr-1, Papadopoulou *et al.*, 2010;3.54% Myr-1) and a divergence rate specifically for an Odonate species (Kiyoshi & Sota, 2006; 3.1 % Myr-1). A generation time of one year was set (Cayrou & Céréghino, 2005).

Genetic diversity and differentiation

We calculated the genetic diversity, the standardized number of alleles and private alleles using GENALEX (Peakall & Smouse, 2006) and ADZE (Szpiech *et al.*, 2008) and assessed population-specific F_{ST}s using GESTE 2 (Foll & Gaggiotti, 2006). Pairwise F_{ST}s were calculated to estimate standardized genetic variance between every population pair.

Assignment method

We analysed the large-scale genetic structure in our sampled populations reflecting historical processes and identified genetic clusters, hereafter called phylogroups (Avise & Walker, 1998). We used the program STRUCTURE (Pritchard *et al.*, 2000), which assigns individuals to clusters based on their multilocus genotypes. We firstly analysed individuals from the core populations to infer the genetic constitution of the different regions in the historical distribution (Hutchison & Templeton, 1999; Appendix S1). To detect the geographical origin of the range expansion, we evaluated whether edge populations clustered with core populations by running the above analysis with the edge populations included. Next, we grouped the core populations based on the STRUCTURE results and their geography, and assigned each edge individual to the core groups using GENECLASS 2 (Piry *et al.*, 2004). In addition, we used POPTREE (Takezaki *et al.*, 2010) to construct a neighbour-joining tree of the pooled edge populations and the groups defined according to the STRUCTURE results. We used Nei's genetic distance (D_A, Nei *et al.*, 1983) and performed 1000 bootstraps. Inferring the genetic origin of the edge populations is needed to unambiguously assess the effect of recent range expansion.

Detection of local and regional bottlenecks

Genetic diversity is affected by demographic processes such as expansions and reductions in the effective population size. Here, we analyse whether edge populations on a local scale have experienced recent bottlenecks and additionally test whether any historical demographic changes in the phylogroups on a regional scale can be detected. The presence of local bottlenecks was evaluated using the infinite allele model (IAM) using the software BOTTLENECK (Cornuet & Luikart, 1996). The populations for which 30 individuals were available were deemed to have enough power to detect a bottleneck (Cornuet & Luikart, 1996). For the detection of historical changes, we pooled the populations from each phylogroup and used the two-phase mutation model (70% stepwise mutations, TPM, for further information see Appendix S1).

Geographical and environmental effects on genetic diversity and structure

First, we calculated pairwise Rogers's (1972) genetic distances among populations. This matrix of Euclidean distances was used to perform a principal coordinates analysis (PCoA). To assess genetic structure among populations, we performed a distance-based redundancy analysis (RDA) on the resulting positive axes, constrained by geographical variables calculated with distance-based Moran's eigenvector maps (db-MEM, Appendix S1). We also included a dummy variable representing the population status (core vs. edge populations) and dummy variables for the phylogroups. Using a forward selection procedure, we determined the relative contribution to the spatial structure by (i) the broad-scale phylogeographical history (correcting for dependence of populations to a shared evolutionary ancestry), (ii) the remaining spatial structure within phylogroups as explained by the MEM and (iii) the spatial structure explained by the recent range expansion.

A decrease in genetic diversity indices in any particular direction would be indicative of a post-glacial expansion towards this direction, as a result of serial founder events (Hewitt, 1996, Hewitt, 1999; Ramachandran *et al.*, 2005). To detect any genetic clines related to post-glacial colonization processes, we investigated the effects of longitude, latitude and altitude on genetic diversity and structure. Altitude was extracted from the WorldClim climate database (http://www.worldclim.org/bioclim) using QUANTUM GIS (Quantum GIS Development Team, 2012, http://qgis.osgeo.org). Besides historical post-glacial demographic processes, contemporary environmental variables could also affect the genetic diversity and structure across a species' range indirectly through recent effects on N_e and m (Kittlein & Gaggiotti, 2008). We therefore extracted four bioclimatic variables from the WorldClim climate database: annual mean temperature, annual precipitation, maximum temperature of the warmest month and maximum precipitation of the driest month. The two latter variables were included to investigate the role of drought on the genetic diversity and structure. As desiccation is a primary cause of egg mortality in

Odonates (Corbet, 1999), drought might play a role in extinction and recolonization dynamics. As the degree of isolation could also influence the genetic diversity and structure of populations, we included the number of populations within a radius of either 100 km or 20 km of every sampled population in the analysis to approximate regional population density. For this, we used the most recent European distribution data of *C. scitulum* (J.P. Boudot, unpublished data). For the two cases where two populations were pooled, the midpoint of both locations was used. The distances were chosen based on observed colonization capacities given by on the one hand their capacity to colonize the island of Jersey (close to the French coast, Perchard & Long, 2009) and mainland UK (Parr, 2011) and on the other hand their capacity to colonize 100 km in less than a decade (Wasscher & Goudsmits, 2010). To mitigate the problem of multicollinearity among the listed predictor variables, we performed a principal components analysis (PCA) on the correlation matrix of the nine continuous variables. A varimax normalized rotation was performed, and the factor scores of the PCA axes that showed an eigenvalue greater than one were retained for further analysis. We fitted general linear models for all the three genetic diversity indices, with the factor scores representing the continuous predictor. We added the phylogroup factor that distinguishes the regional groups based on the STRUCTURE analysis (see Results) and the population status factor. By including these factors, we could resolve the effect of the potential historical factors that had an effect on the effective population size (phylogeographical patterns), the effect of the current range expansion and in addition the effects of the regional climate variables. The best model was selected using stepwise selection by means of Akaike information criterion. We calculated the square root of the private number of alleles to achieve normality.

As the pairwise genetic differentiation (F_{ST}) values are not mutually independent, we performed a Bayesian analysis using GESTE 2 (Foll & Gaggiotti, 2006) with the same variables as above to calculate population-specific F_{ST} values, which represent the contribution of each population to the global F_{ST}. This way the relation between population-specific F_{ST} values and geography and contemporary environmental variables can be assessed. The posterior probabilities of all possible models were calculated to identify the model that best explained the observed genetic structure.

Results

Analysis of mitochondrial haplotypes

Of the sixteen mtDNA haplotypes detected, one haplotype was widespread and present in 70% of the individuals (Fig. 1b). Haplotype diversity was 0.52, and nucleotide diversity was 0.0009 (SE = 0.0007). The negative Tajima D of −2.48 ($P < 0.00001$) supports a scenario of demographic expansion. The time since expansion was estimated to be 15 033 years before the present, ybp (95% CI: 2413–24 336 ybp), 9767 ybp (95% CI: 1568–15 812) and 11 153 ybp (1790–18 056) for the divergence rate of 2.3%, 3.54% and 3.1%, respectively. All these timings overlap and coincide with the onset of the Holocene period.

Genetic diversity and pairwise differentiation using microsatellite markers

As none of the loci showed Hardy–Weinberg equilibrium deviations at more than two populations, we retained all loci in the analyses. Genotyping error was lower than 1%. The genetic diversity indices and population-specific F_{ST} values for each population are given in Appendix S2. Mean pairwise genetic differentiation was 0.16 (SE = 0.26, Appendix S3).

Genetic structure and bottleneck analysis

Using the delta K method in STRUCTURE (Evanno *et al.*, 2005), three genetic clusters were detected. Individuals from the South Mediterranean populations SMa (southern Spain) and AT (Algeria) are all ascribed with a high probability to one cluster (black cluster, Fig. 2). Individuals from populations from Italy and Eastern Europe have a high probability of being a member of the second cluster (dark grey, Fig. 2), whereas individuals from Western Europe (France, northern Spain and Portugal) are a mixture of a third cluster (light grey, Fig. 2) and the second cluster. Individuals from the Moroccan population M are here described as a mixture of the three clusters. When the analysis was performed with the edge populations included, the same three clusters were obtained (Fig. 2). GENECLASS 2 assigned all edge individuals to the Western group with probabilities ranging between 99.8% and 100%. The neighbour-joining tree also showed that edge populations cluster with the Western populations (Fig. 2b).

Based on the STRUCTURE results, we defined four phylogroups for further analyses: a group including the eastern and Italian populations (Group E), the Western populations including the edge populations (Group W), the group including the southern Spanish and Algerian populations (Group S) and the Moroccan population (Group M). The PCoA plot shows the relative high genetic difference between the southern Spanish and Algerian populations and the other populations (Fig. S4, Appendix S4).

No local recent bottlenecks where detected assuming the IAM model ($P_{adjusted} > 0.05$). When analysing the pooled STRUCTURE groups on a regional scale, assuming the TPM model, heterozygote deficiency and hence a signature of historical expansion was found in the

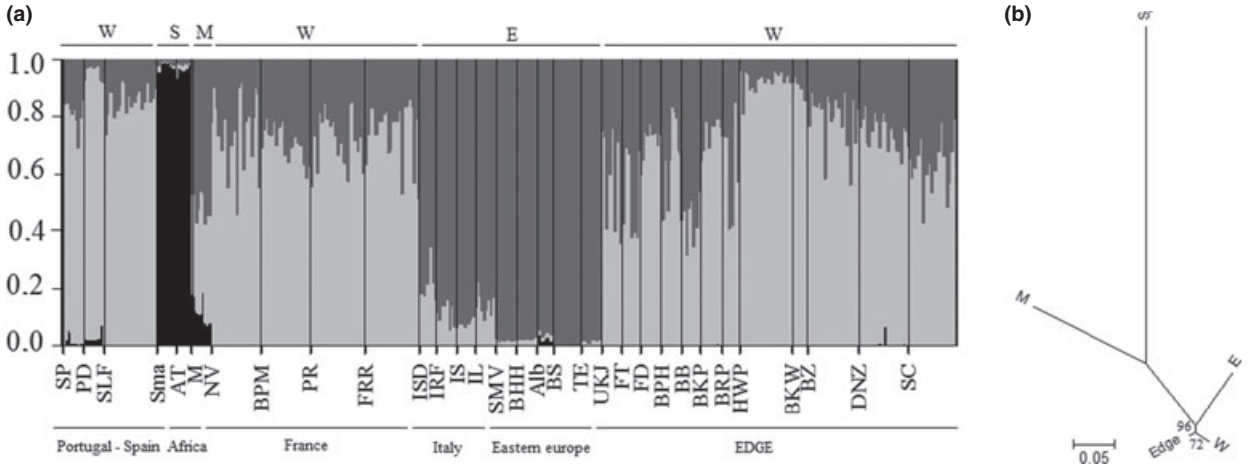

Fig. 2 (a) Results of the structure analysis that identified three clusters. Each vertical line represents one individual and comprises the individual Bayesian assignment probability segments to belong to either one of the three detected genetic clusters. For visualization, populations are grouped geographically. The edge populations are presented at the right. (b) Neighbourhood tree (using D_A distances) showing the relationships between the three structure groups and the group of edge populations. Group W: Western Europe, Group E: Eastern Europe, Group S: Southern Spain and Algeria, Group M: Morocco.

Eastern group and the Western group ($P_{adjusted} < 0.05$) but not in the Southern group, nor in Morocco ($P_{adjusted} > 0.05$).

Effects of geography and climatic variables on genetic diversity and structure

The MEM analysis yielded twelve spatial variables. The RDA model constrained by these variables explained 34% of the variance ($F = 2.291$, $P = 0.0166$). After forward selection, only MEM4 to MEM7 were retained ($F = 3.954$, $P = 0.0036$, $R^2_{adj.} = 0.282$), indicating genetic structure on a large to medium spatial scale. A RDA model including the four phylogroup dummy variables on top of the 12 MEM gave an R^{2s}_{adj} value of 0.713 ($F = 5.672$, $P = 0.0001$,). After forward selection, MEM5, MEM11, MEM12 and the core–edge population status were retained, after inclusion of the phylogroup variables (Table 2), indicating that genetic structure was now also detected on a smaller spatial scale. Jointly, this RDA model explained again 71.3 % of the variance. The phylogroups alone explained 61% of the genetic variance. The remaining MEM accounted for 8.7 %, and the edge–core comparison for 1.5% of the observed genetic variance (Table 2).

The PCA of the continuous predictor variables resulted in four PC axes that had an eigenvalue greater than one. The first PC axis corresponded to latitude effects with positive values characterizing more southern sites with higher annual temperature and maximum temperature of the month but lower precipitation in the driest month. The second and third PC axes were positively related to annual precipitation and longitude, respectively. The fourth PC axis was negatively related to altitude (Table 3).

Table 2 Factor loadings of the continuous geographical and environmental predictor variables in the principal components. Prec-Dry-M: precipitation of the driest month; Max-T-Warm-M: maximum temperature of the warmest month; Dens20: number of populations within a radius of 20 km; Dens100: number of populations within a radius of 100 km.

	PC1	PC2	PC3	PC4
Geography				
Latitude	−0.77	−0.16	−0.11	0.58
Longitude	0.01	0.02	0.97	−0.03
Altitude	0.13	0.11	0.02	−0.97
Climate				
Ann. Precipitation	−0.07	0.84	−0.27	0.08
Ann. Temperature	0.94	0.16	−0.03	0.25
Prec-Dry-M	−0.79	0.27	0.15	0.32
Max-T-Warm-M	0.79	0.11	0.31	−0.35
Regional population density				
Dens20	−0.06	−0.66	−0.22	0.17
Dens100	−0.29	−0.62	−0.37	0.48
Explained Variation	2.84	1.68	1.33	1.82
Proportion Total	0.32	0.19	0.15	0.2

The third PC axis (longitude) had a positive effect on all three genetic indices, meaning that genetic diversity increased going eastwards (Fig. 3a–c, Table 3). The phylogroup had an effect on allelic richness and heterozygosity. Group S displayed the lowest genetic diversity and Group W the highest genetic diversity. The population status had an effect on heterozygosity: edge populations were less heterozygous than core populations.

Table 3 Results of general linear model analysis of the effects of PC scores based on geographical and environmental variables on the three studied genetic diversity indices. Significant *P* values (< 0.05) are shown in bold. Only the results of the selected factors after model selection are shown.

	Allelic Richness				Private alleles				Heterozygosity			
	MS	d.f.	*F*	*P*	MS	d.f.	*F*	*P*	MS	d.f.	*F*	*P*
PC1									0.002	1	2.567	0.122
PC3	2.13	1	6.328	**0.019**	0.091	1	6.508	**0.016**	0.008	1	8.247	**0.008**
PC4	0.57	1	1.689	0.206								
Status	0.96	1	2.847	0.104	0.051	1	3.674	0.066	0.006	1	6.097	**0.021**
Phylogroup	4.83	3	14.373	**< 0.001**					0.027	3	28.73	**< 0.001**
Error	0.34	24			0.014	28			0.001	24		

The same trend ($P = 0.10$, Table 3) was observed for the number of private alleles.

The Bayesian analysis in GESTE 2 indicated a role of geography and environmental variables in the genetic structuring of the populations. Strongest support was found for the model containing the factors PC1 and population status (posterior probability $P = 0.187$). Population-specific F_{ST} values increased with PC1 (coefficient: 0.505; 95% HPDI: [−0.175; 0.875]), meaning that population-specific F_{ST} values decreased going northwards and with precipitation of the driest month, whereas they increased with annual temperature and maximum temperature of the warmest month (Fig. 3d). Within this model, edge populations showed higher population-specific F_{ST} values than core populations (coefficient: 0.391; 95% HPDI: [0.0481; 0.735], Fig. 3d).

Discussion

Historical signatures of post-glacial range expansion

The low diversity in the mitochondrial sequences and the significant negative value of theta suggest a rapid post-glacial expansion from a very limited mitochondrial gene pool, assuming that the variation among the COI and COII sequences is selectively neutral (Avise,

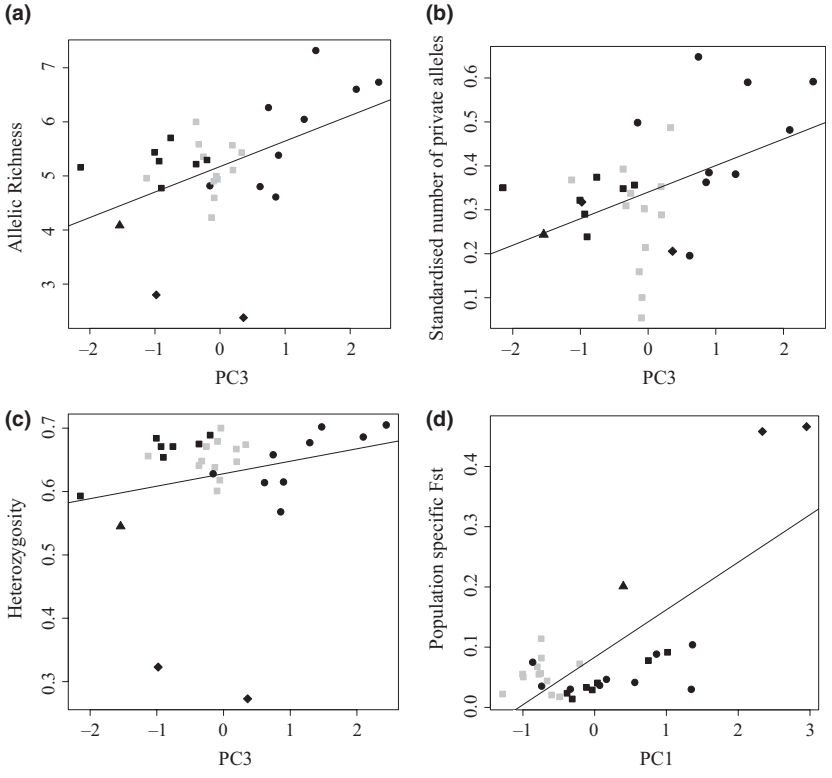

Fig. 3 (a–c). Relationships between the three genetic diversity indices and PC3 (longitude). (d). Relationship between population-specific F_{st} values and PC1 (latitude, annual temperature, precipitation driest month, maximum temperature warmest month).The symbols denote the four phylogroups (■: Western European core population ●: Eastern Europe, ♦: Southern Spain and Algeria, ▲: Morocco) and the Western European edge populations (■).

2000). The time since expansion using the mitochondrial sequences was estimated to be younger than 25 000 ybp based on the three different divergence rates used and most likely occurred between 10 000 and 15 000 ybp. Although these are estimates derived from only one gene region and not specifically estimated for *C. scitulum*, they could give an idea about the time frame of the expansion. The results suggest that this expansion occurred after the last glacial maximum and might have been related to the increased availability of suitable habitat at that time.

In contrast with the low resolution at the mitochondrial markers, the microsatellite markers allowed unravelling of the genetic structure across the historical core range, thereby suggesting eastern refugia. Both the STRUCTURE results and the PCoA showed that the two South Mediterranean populations (Group S) are highly differentiated relative to the other populations. Additionally, we had to exclude two loci from the analysis as they resulted in null alleles in these two populations, further highlighting the differentiation of these two populations from the remaining populations. However, mtDNA haplotypes of these sites were either the same as the common *C. scitulum* haplotype or differed with one mutation, consistent with expectations for populations sampled from within the same species. As microsatellite markers are generally known to mutate faster than mitochondrial DNA (Selkoe & Toonen, 2006), this suggests that the detected differentiation using microsatellites occurred after the last glacial maximum. The analyses show a significant effect of longitude on allelic richness, heterozygosity as well as the number of private alleles, with genetic diversity increasing when going eastwards. Although latitudinal clines in genetic diversity due to post-glacial expansion from southern refugia have been widely observed (Hewitt, 2000), only more recently have longitudinal patterns in genetic diversity gained more attention (Atkinson *et al.*, 2007; Stewart *et al.*, 2010; Conord *et al.*, 2012). Longitudinal clines can result either from post-glacial recolonization from eastern refugia or from associated clines in climate severity during the last glacial cycle in the Mediterranean region (Conord *et al.*, 2012). The recent expansion signal found in the mitochondrial data and the known fast mutation rates of microsatellite markers (Selkoe & Toonen, 2006) suggest it is more likely that the detected longitudinal cline is a consequence of post-glacial expansion from the east to the west. In this case, the successive founding events could have led to a gradual decrease in genetic diversity (Austerlitz *et al.*, 1997). In a study on the damselfly *I. elegans*, allelic richness also decreased with longitude, and a similar trend was found for heterozygosity (Wellenreuther *et al.*, 2011). These combined results suggest the presence of eastern refugia for both damselfly species during the last glacial maximum and subsequently a westward expansion. This might be plausible as the Balkan region is one of the main potential refugia identified by a number of phylogeographical studies (Taberlet *et al.*, 1998; Hewitt, 1999; Stewart *et al.*, 2010). At the phylogroup level, the bottleneck analysis showed that both the Western and Eastern phylogroups were not in mutation-drift equilibrium, suggesting a genetic signature of demographic expansion in the Eastern group and the Western group given our stated assumptions. After the establishment of populations in these regions, a demographic expansion might have occurred. Although it should be considered that this signal could also have been caused by population structure not accounted for if gene flow between the populations within the phylogroups has been sufficiently low (Peter *et al.*, 2010). When dividing the Eastern phylogroup into two geographical subsets (Italy and Eastern Europe), Italy shows a signature of expansion, whereas this signal remains undetected in Eastern Europe (result not given), as would be expected when populations in the latter area would have served as refugia during the last glacial maximum. It shows that pooling populations in this analysis might confound true signals of expansion.

Our genetic data do not suggest the presence of southern refugia, but instead might suggest that the southern populations were founded last after the last glacial maximum. Although one could argue that a latitudinal cline in genetic diversity may not have been detected due to the smaller geographical sampled area in the south–north direction compared with the east–west direction, we did find a latitudinal effect on the population-specific F_{ST} values. This effect was in the opposite direction than that expected based on a scenario of southern refugia: F_{ST} increased towards the south. Latitudinal effects on allelic frequency indices are frequently observed as a consequence of post-glacial expansion from southern refugia (Hewitt, 2000; Hill *et al.*, 2011). However, in these cases, genetic differentiation increases towards the north, that is, the opposite direction compared with our study. Aside from the influence of correlated contemporary abiotic factors (see further), we suggest that this result might be the consequence of a post-glacial western expansion from eastern refugia, followed by the establishment of populations in southern direction. In this regard, the high F_{ST} and low genetic diversity of the southern populations might be due to a fast post-glacial expansion, where the establishment of populations in southern Spain and the North African region has only occurred in the latest stage of the expansion process and hence from already diminished genetic sources. However, we cannot rule out the presence of genetically uniform southern refugia that did not contribute to the repopulation of Europe.

The effect of contemporary environmental variables

The positive effect of the first PC axis on population-specific F_{ST}s values indicates that higher temperatures

and drought increase the population-specific F_{ST} values. These variables are a proxy for the suitability of the habitat, where higher temperatures and less precipitation lead to more extreme conditions and therefore potentially stronger extinction recolonization dynamics, hereby increasing the genetic structure (Wade & McCauley, 1988). Alternatively, isolation by environment could also have led to genetic differentiation in environmentally extreme populations (Sexton *et al.*, 2013).

The low degree of pairwise genetic differentiation and absence of genetic bottlenecks might indicate that populations are well connected and therefore well buffered against strong genetic drift associated with small population sizes. Damselflies have high dispersal capacities, which can maintain gene flow among sites (De Block *et al.*, 2005) and hence can explain the low genetic differentiation among populations, thereby alleviating rapidly the effects of eventual population bottlenecks (Crow & Aoki, 1984). Nevertheless, correcting for phylogenetic relatedness among the identified phylogroups, a significant portion of the genetic variation was explained by small-scale geographical structure, indicating that even on a more local-scale geography can still play a role in the structuring of relatively high dispersive species.

Contemporary range expansion embedded in a phylogeographical framework

Our results highlight several reasons for considering a phylogeographical framework when studying the genetic signature of a contemporary range expansion. First, only the large-scale phylogeographical approach allowed a solid identification of the regional source of the edge populations. The analyses indicated that the current expansion front has its origins in the genetic cluster comprised of the French and Spanish populations.

Secondly, the phylogeographical approach indicated the importance of considering latitudinal and longitudinal genetic patterns in the core range, hence the signature of the historical post-glacial range expansion, to correctly identify the genetic signatures of the recent range expansion in the edge populations. Our results suggest this recent expansion was accompanied by a decrease in heterozygosity and an increase in F_{ST}. Although F_{ST} decreased towards more northern populations, when controlling for latitude, edge populations were more differentiated than core populations, indicating the importance of both historical as well as current spatial processes on the genetic structure of a species. The elevated genetic differentiation may not have been detected in a sampling scheme that only included more southerly core populations, highlighting the importance of a well-designed sampling scheme. Further, when the effect of the contemporary range expansion on the number of private alleles was analysed in a *t*-test, the number of private alleles decreased significantly in the edge populations (result given in Appendix S5). Nonetheless, this effect was less profound when controlling for longitude. This further highlights the importance of incorporating a phylogeographical framework when studying contemporary processes (Knowles, 2009; Eckert *et al.*, 2008). Here, the number of private alleles of edge populations was probably lower because they originated from Western populations that were already diminished in genetic diversity due to their historical past. This suggests that overestimating the effect of contemporary range expansion is possible when the influence of historical range expansion is not corrected for.

Thirdly, the inclusion of a phylogeographical framework also allowed disentangling and identification of the relative importance of ancient, large-scale patterns vs. more recent to very recent small-scale patterns. The RDA analysis without the phylogeographical prior suggested that there is no important or significant small-scale (c. 500 km) genetic structure nor an effect of the recent range expansion. The RDA with the phylogeographical prior showed that most of the genetic variance is caused by ancient phylogeographical processes; however, a small but marginally significant spatial signal of the recent range expansion could also be detected.

Conclusions

This study shows that the genetic structure of *C. scitulum* is likely shaped by both historical (rapid post-glacial range expansion) and contemporary processes (environmental factors, current range expansion due to climate change). Moreover, it confirms recent findings that longitude can be a strong structural element of genetic diversity in the Mediterranean region (Conord *et al.*, 2012). This study shows that with the information gained on genetic diversity and structure across a large part of the range of this species, historical influences can be assessed so that contemporary genetic influences on the remaining genetic structure can be evaluated (Eckert *et al.*, 2008). The phylogeographical context, particularly the knowledge about historical colonization processes, proved to be essential in understanding and identifying the genetic signatures of local contemporary processes. Within this phylogeographical framework, our results highlight that even recent range expansion of a good disperser can influence the distribution of genetic diversity which could potentially alter their evolutionary potential and further colonization capacity. This implies that conservation policies should take action fast enough to ensure the maintenance of genetic diversity a species requires to be able to respond to future climate change (Santamaría & Méndez, 2012). The molecular data obtained will be directly relevant to advance species modelling paradigms that seek to

integrate climatic and demographic drivers, and enable conservation managers to plan for the protection of areas of evolutionary potential (Scoble & Lowe, 2010).

Acknowledgments

We kindly thank the people that have helped collecting the samples: Jean-Pierre Boudot, Gianmaria Carchini, Adolfo Cordero, Geert De Knijf, Roberto Fabbri, Sonia Ferreira, Josep Garcia-Moreno, Nurten Hacet, Frank Johansson, Rassim Khelifa, Dejan Kluijer, Bern Kunz, Yordan Kutsarov, Calogero Muscarella, Viktor Nilsson, Ali Salamun and Audrey St-Martin. Kay Boulton and Julia Davison improved the English. We thank Mike Ritchie, Brent Emerson and an anonymous reviewer for insightful comments on an earlier version of this manuscript. MHD Larmuseau received a postdoctoral fellowship of FWO-Vlaanderen. This study was funded by the KU Leuven Centre of Excellence Financing on 'Eco- and socio-evolutionary dynamics' (Project Number PF/2010/07) and a research grant from the Fund for Scientific Research (FWO) Flanders.

References

Atkinson, R.J., Rokas, A. & Stone, G.N. 2007. Longitudinal patterns in species richness and genetic diversity in European oaks and oak gall wasps. In: *Phylogeography of Southern European Refugia* (S. Weiss & N. Ferrand, eds), pp. 127–151. Springer, New York.

Austerlitz, F., Jung-Muller, B., Godelle, B. & Gouyon, P.-H. 1997. Evolution of coalescence times, genetic diversity and structure during colonization. *Theor. Popul. Biol.* **51**: 148–164.

Avise, J.C. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA, USA.

Avise, J.C. & Walker, D. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. *Proc. R. Soc. B.* **265**: 457–463.

Björklund, M. & Bergek, S. 2009. On the relationship between population differentiation and sampling effort: is more always better? *Oikos* **118**: 1127–1129.

Bray, S.C.E., Austin, J.J., Metcalf, J.L., Østbye, K., Østbye, E., Lauritzen, S.-E. *et al.* 2013. Ancient DNA identifies post-glacial recolonisation, not recent bottlenecks, as the primary driver of contemporary mtDNA phylogeography and diversity in Scandinavian brown bears. *Divers. Distrib.* **19**: 245–256.

Brower, A. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. R. Soc. B.* **91**: 6491–6495.

Cayrou, J. & Céréghino, R. 2005. Life-cycle phenology of some aquatic insects: implications for pond conservation. *Aquatic Conserv: Mar. Freshw. Ecosyst.* **15**: 559–571.

Cheang, C.C., Tsang, L.M., Ng, W.C., Williams, G.A., Chu, K.H. & Chan, B.K.K. 2012. Phylogeography of the cold--water barnacle *Chthamalus challengeri* in the north-western Pacific: effect of past population expansion and contemporary gene flow. *J. Biogeogr.* **39**: 1819–1835.

Clement, M., Posada, D. & Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657–1659.

Conord, C., Gurevitch, J. & Fady, B. 2012. Large-scale longitudinal gradients of genetic diversity: a meta-analysis across six phyla in the Mediterranean basin. *Ecol. Evol.* **2**: 2600–2614.

Corbet, P.S. 1999. *Dragonflies: Behaviour and Ecology of Odonata*. Harley Books, Colchester.

Cornuet, J.M. & Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.

Crow, J.F. & Aoki, K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *PNAS* **81**: 6073–6077.

Davis, M.B. & Shaw, R.G. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* **292**: 673–679.

De Block, M., Geenen, S., Jordaens, K., Backeljau, T. & Stoks, R. 2005. Spatiotemporal allozyme variation in the damselfly, *Lestes viridis* (*Odonata*: *Zygoptera*): gene flow among permanent and temporary ponds. *Genetica* **124**: 137–144.

Dijkstra, K.D.B. & Lewington, R. 2006. *Field Guide to the Dragonflies of Britain and Europe*. British Wildlife Publishing, Oxford, UK.

Dommanget, J.L., Procida, G., Brulard, J.F., Da Costa, H. & Haffner, P. 1994. Atlas préliminaire des Odonates de France. *Secretariat de la Faune et de la Flore (Hrsg.): Collection Patrimoines naturels (Paris)* **16**: 1–92.

Eckert, C.G., Samis, K.E. & Lougheed, S.C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol. Ecol.* **17**: 1170–1188.

Epps, C.W., Palsbøll, P.J., Wehausen, J.D., Roderick, G.K., Ramey, R.R. & McCullough, D.R. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecol. Lett.* **8**: 1029–1038.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Excoffier, L.G. & Schneider, S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform Online* **1**: 47–50.

Foll, M. & Gaggiotti, O. 2006. Identifying the environmental factors that determine the genetic structure of populations. *Genetics* **174**: 875–891.

Garroway, C.J., Bowman, J., Holloway, G.L., Malcolm, J.R. & Wilson, P.J. 2011. The genetic signature of rapid range expansion by flying squirrels in response to contemporary climate warming. *Glob. Change Biol.* **17**: 1760–1769.

Goossens, B., Chikhi, L., Ancrenaz, M., Lackman-Ancrenaz, I., Andau, P. & Bruford, M.W. 2006. Genetic signature of anthropogenic population collapse in Orang-utans. *PLoS Biol.* **4**: e25.

Guo, Q. 2012. Incorporating latitudinal and central-marginal trends in assessing genetic variation across species ranges. *Mol. Ecol.* **21**: 5396–5403.

Hasselman, D.J., Ricard, D. & Bentzen, P. 2013. Genetic diversity and differentiation in a wide ranging anadromous fish, American shad (*Alosa sapidissima*), is correlated with latitude. *Mol. Ecol.* **22**: 1558–1573.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linnean Soc.* **58**: 247–276.

Hewitt, G.M. 1999. Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.* **68**: 87–112.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Hill, J.K., Griffiths, H.M. & Thomas, C.D. 2011. Climate change and evolutionary adaptations at species' range margins. *Annu. Rev. Entomol.* **56**: 143–159.

Hoban, S.M., Borkowski, D.S., Brosi, S.L., McCleary, T.S., Thompson, L.M., McLachlan, J.S. *et al.* 2010. Range-wide distribution of genetic diversity in the North American tree *Juglans cinerea*: a product of range shifts, not ecological marginality or recent population decline. *Mol. Ecol.* **19**: 4876–4891.

Huck, S., Büdel, B., Kadereit, J.W. & Printzen, C. 2009. Range-wide phylogeography of the European temperate-montane herbaceous plant *Meum athamanticum* Jacq.: evidence for periglacial persistence. *J. Biogeogr.* **36**: 1588–1599.

Hutchison, D. & Templeton, A. 1999. Correlation of pairwise genetic and geographic distance measure: inferring the relative influences of gene flow and drift on distribution of genetic variability. *Evolution* **53**: 1898–1914.

Johansson, H., Ingvarsson, P.K. & Johansson, F. 2011. Cross-species amplification and development of microsatellites for six species of European Coenagrionid damselflies. *Conserv. Genet. Resour.* **4**: 191–196.

Kittlein, M.J. & Gaggiotti, O.E. 2008. Interactions between environmental factors can hide isolation by distance patterns: a case study of *Ctenomys rionegrensis* in Uruguay. *Proc. R. Soc. B.* **275**: 2633–2638.

Kiyoshi, T. & Sota, T. 2006. Differentiation of the dragonfly genus *Davidius* (*Odonata*: *Gomphidae*) in Japan inferred from mitochondrial and nuclear gene genealogies. *Zoolog. Sci.* **23**: 1–8.

Knowles, L.L. 2009. Statistical Phylogeography. *Annu. Rev. Ecol. Evol. Syst.* **40**: 593–612.

Mergeay, J. & Santamaria, L. 2012. Evolution and biodiversity: the evolutionary basis of biodiversity and its potential for adaptation to global change. *Evol. Appl.* **5**: 103–106.

Nei, M., Tajima, F. & Tateno, Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* **19**: 153–170.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**: 535–538.

Papadopoulou, A., Anastasiou, I. & Vogler, A.P. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Mol. Biol. Evol.* **27**: 1659–1672.

Parr, A. 2011. Migrant and dispersive dragonflies in Britain during 2010. *J. British Dragonfly Soc.* **27**: 69–79.

Pauls, S.U., Nowak, C., Bálint, M. & Pfenninger, M. 2013. The impact of global climate change on genetic diversity within populations and species. *Mol. Ecol.* **22**: 925–946.

Peakall, R. & Smouse, P.E. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295.

Perchard, R. & Long, R. 2009. The rediscovery of Dainty Damselfly *Coenagrion scitulum* (Ramb.) in Jersey. *Atropos* **38**: 3–5.

Peter, B.M., Wegmann, D. & Excoffier, L. 2010. Distinguishing between population bottleneck and population subdivision by a Bayesian model choice procedure. *Mol. Ecol.* **19**: 4648–4660.

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L. & Estoup, A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J. Hered.* **95**: 536–539.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Ramachandran, S., Deshpande, O., Roseman, C.C., Rosenberg, N.A., Feldman, M.W. & Cavalli-Sforza, L.L. 2005. Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. *PNAS* **102**: 15942–15947.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. *Studies in Genetics VII. Univ. of Texas Publ* **7213**: 145–153.

Rogers, A. & Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* **9**: 552–569.

Rönkä, N., Kvist, L., Pakanen, V.-M., Rönkä, A., Degtyaryev, V., Tomkovich, P. *et al.* 2012. Phylogeography of the Temminck's Stint (*Calidris temminckii*): historical vicariance but little present genetic structure in a regionally endangered Palearctic wader. *Divers. Distrib.* **18**: 704–716.

Rousset, F. 2008. GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **8**: 103–106.

Santamaría, L. & Méndez, P.F. 2012. Evolution in biodiversity policy - current gaps and future needs. *Evol. Appl.* **5**: 202–218.

Scoble, J. & Lowe, A.J. 2010. A case for incorporating phylogeography and landscape genetics into species distribution modelling approaches to improve climate adaptation and conservation planning. *Divers. Distrib.* **16**: 343–353.

Selkoe, K.A. & Toonen, R.J. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.* **9**: 615–629.

Sexton, J.P., Hangartner, S.B. & Hoffmann, A.A. 2013. Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* **68**: 1–15.

Song, G., Yu, L., Gao, B., Zhang, R., Qu, Y., Lambert, D.M. *et al.* 2013. Gene flow maintains genetic diversity and colonization potential in recently range-expanded populations of an Oriental bird, the Light-vented Bulbul (*Pycnonotus sinensis*, Aves: *Pycnonotidae*). *Divers. Distrib.* **119**: 1248–1262.

Stewart, J.R., Lister, A.M., Barnes, I. & Dalén, L. 2010. Refugia revisited: individualistic responses of species in space and time. *Proc. R. Soc. B.* **277**: 661–671.

Swaegers, J., Mergeay, J., Maes, G.E., Van Houdt, J.K.J., Larmuseau, M.H.D. & Stoks, R. 2012. Microsatellite marker development and putative SNP detection for a northward expanding damselfly species using next generation sequencing. *Conserv. Genet. Resour.* **4**: 1079–1084.

Swaegers, J., Mergeay, J., Therry, L., Larmuseau, M., Bonte, D. & Stoks, R. 2013. Rapid range expansion increases genetic differentiation while hardly reducing genetic diversity in a damselfly. *Heredity* **111**: 422–429.

Szpiech, Z.A., Jakobsson, M. & Rosenberg, N.A. 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics* **24**: 2498–2504.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.* **7**: 453–464.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Takezaki, N., Nei, M. & Tamura, K. 2010. POPTREE2: software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Mol. Biol. Evol.* **27**: 747–752.

Theissinger, K., Bálint, M., Feldheim, K.A., Haase, P., Johannesen, J., Laube, I. *et al.* 2013. Glacial survival and post-glacial recolonization of an arctic-alpine freshwater insect (*Arcynopteryx dichroa*, *Plecoptera*, *Perlodidae*) in Europe. *J. Biogeogr.* **40**: 236–248.

Vandergast, A.G., Bohonak, A.J., Weissman, D.B. & Fisher, R.N. 2007. Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (*Orthoptera*: *Stenopelmatidae*: *Stenopelmatus*). *Mol. Ecol.* **16**: 977–992.

Wade, M.J. & McCauley, D.E. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* **42**: 995–1005.

Wasscher, M. & Goudsmits, K. 2010. De gaffelwaterjuffer (*Coenagrion scitulum*), terug van weggeweest in Noordwest-Europa. *Brachytron* **13**: 19–25.

Watts, P.C., Keat, S. & Thompson, D.J. 2010. Patterns of spatial genetic structure and diversity at the onset of a rapid range expansion: colonisation of the UK by the small red-eyed damselfly Erythromma viridulum. *Biol. Invasions.* **12**: 3887–3903.

Wellenreuther, M., Sánchez-Guillén, R.A., Cordero-Rivera, A., Svensson, E.I. & Hansson, B. 2011. Environmental and climatic determinants of molecular diversity and genetic population structure in a coenagrionid damselfly. *PLoS ONE* **6**: e20440. Public Library of Science.

Zarza, E., Reynoso, V.H. & Emerson, B.C. 2011. Discordant patterns of geographic variation between mitochondrial and microsatellite markers in the Mexican black iguana (*Ctenosaura pectinata*) in a contact zone. *J. Biogeogr.* **38**: 1394–1405.

Zellmer, A.J. & Knowles, L.L. 2009. Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. *Mol. Ecol.* **18**: 3593–3602.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Detailed methods.

Appendix S2 Pairwise F_{ST} table.

Appendix S3 Overview studied populations.

Appendix S4 PCoA plot.

Appendix S5 Difference in uncorrected private alleles between core and edge populations.

Data deposited at Dryad: doi:10.5061/dryad.rk13d

Received 16 October 2013; revised 23 January 2014; accepted 23 January 2014