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Genetic consequences of range expansions along several fronts in *Crocothemis erythraea*

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Global warming has altered the ranges of many species, especially those of insects and other ectotherms that are particularly susceptible to rising temperatures. Four decades ago, the dragonfly *Crocothemis erythraea* began to demonstrate northern range expansion in Germany, as well as in Belgium, the Netherlands, Poland and the UK. The rapid range expansion of *C. erythraea* has highlighted the capacity of this dragonfly for dispersal, making this species a good model to investigate the genetic consequences of expansions from several fronts. We predict that the recently established populations of *C. erythraea* in central Europe (Germany & Switzerland) will show only a minimal reduction in genetic diversity (because founders may derive from a broader set of core populations) with respect to populations from core regions of this species, and an increase in the genetic differentiation (given the multiple independent expansion axes along the broad front). To test our hypothesis, we compared genetic variation, in terms of genetic diversity and genetic differentiation using two mitochondrial genes (cytochrome b and NADH dehydrogenase), between central Europe and three core regions (south-west Europe, Italy and Africa). Results were in concordance with our hypothesis: populations from central Europe did not show a significant reduction in the overall genetic diversity but were highly differentiated from Africa, Italy and south-west Europe populations.

Keywords: *Crocothemis erythraea*; range expansion; global warming; genetic diversity; genetic differentiation

Introduction

Evolutionary processes associated with colonization can be affected by several factors such as: phenotypic plasticity (Lee, Remfert, & Chang, 2007), high propagule pressure (Drake, Baggenstos, & Lodge, 2005) or heterosis (Hahn & Rieseberg, 2017). Additionally, the number of founders, strength and direction of selective pressures in the new environment, and time since colonization (which each leave a genetic fingerprint in recently established populations) can also affect the evolutionary potential for colonization (Grapputo, Boman, Lindstrom, Lyytinen, & Mappes, 2005). A rapid colonization by a relatively small population can result in the loss of intra-population genetic diversity because of founder effect and genetic drift, and the genetic fingerprint may persist for several generations (Frankham, 2005; Hewitt, 1999; Roman & Darling, 2007). However, when populations from different origins genetically admix upon secondary

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contact in the newly colonized region, the colonization can also result in an increase in genetic diversity (Kolbe et al., 2004; Lockwood, Cassey, & Blackburn, 2005). Moreover, genetic drift during expansion can increase genetic differentiation of edge populations compared to core populations (Swaegers et al., 2013). Understanding the effects of colonization processes on genetic diversity in invasive species provides essential information for biodiversity management, eradication and restoration (Excoffier, Foll, & Petit, 2009; Rodriguez, 2006).

Geographic ranges of many species have been altered (in the form of expansions and contractions) in response to rising global temperatures (Parmesan, 2006; Parmesan, Ryrholm, Steganescu, & Hill, 1999). Ectotherms, and particularly insects, are strongly influenced by environmental temperatures (Deutsch et al., 2008), due to their short generation times and high reproductive rates (see Hickling, Roy, Hill, & Thomas, 2005; Ott, 2010; Parmesan et al., 1999). In fact, lepidopterans heteropterans, neuropterans, orthopterans and odonates are undergoing range expansions induced by global warming (see Sánchez-Guillén, Córdoba-Aguilar, Hansson, Ott, & Wellenreuther, 2016). Within the last three decades some Mediterranean species, such as *Crocothemis erythraea* (Ott, 2007), and more recently some African odonate species, have expanded their ranges northwards (Ott, 2009, 2010). Although odonates are highly responsive insects to rising temperatures in terms of range expansion (Parmesan et al., 1999), only a handful of molecular studies have investigated genetic diversity patterns in odonates undergoing range expansions. The migratory dragonfly *Anax junius* in North and Central America shows high and contrasting levels of haplotype and nucleotide diversity (Freeland, May, Lodge, & Conrad, 2003), a characteristic pattern of a rapid demographic expansion from a small effective population size (Avise, 2000). The damselflies *Coenagrion scitulum* and *Erythromma viridulum* show contrasting patterns, typically from one single and from several disperser foci, respectively. A decrease in genetic diversity was detected at edge populations of the damselfly *Coenagrion scitulum*, because of its recent range expansion from one single front (Swaegers et al., 2013, 2015). However, the damselfly *Erythromma viridulum*, which has recently colonized the UK from two different foci, showed a lack of genetic divergence at the core of UK populations, but high genetic differentiation between both colonization foci (Watts, Keat, & Thompson, 2010) at regional scales. Another example of a dispersing damselfly is *Ischnura elegans*. Although this damselfly is experiencing range expansion in central-north Spain (Sánchez-Guillén, Wellenreuther, Cordero-Rivera, & Hansson, 2011), the evolutionary potential of its colonization process has been enhanced through introgressive hybridization with the endemic Iberian and North African sister species, *I. graellsii* (Sánchez-Guillén, Van Gossum, & Cordero-Rivera, 2005; Sánchez-Guillén, Wellenreuther, & Cordero-Rivera, 2012). Wellenreuther, Sánchez-Guillén, Cordero-Rivera, Svensson, and Hansson (2011) detected an increased genetic variation (weak population structure and a high degree of genetic variation within populations) in populations of the recently colonized regions of northern Spain.

In this study, we have investigated the genetic consequences of the current and rapid range expansion of the dragonfly *Crocothemis erythraea* in central Europe (Germany and Switzerland). To investigate the genetic fingerprints of its rapid range expansion from several fronts, we measured genetic variation (in terms of genetic diversity and genetic differentiation) using two mitochondrial genes (cytochrome b and NADH dehydrogenase) in core and peripheral populations covering the latitudinal range of *Crocothemis erythraea* from Europe and Africa, with a focus on central Europe. Based on the multiple front expansion of *C. erythraea* in central Europe, we predict that the recently established populations of *C. erythraea* in this region will show an increase in the genetic differentiation given the more independent expansion axes along the broad front, but only weak reduction of the genetic diversity because founders may derive from a broader set of core populations. To test our hypothesis, we compared the genetic variation in between central Europe, which was completely colonized during the last 40 years, and south-western Europe, Italy and Africa.

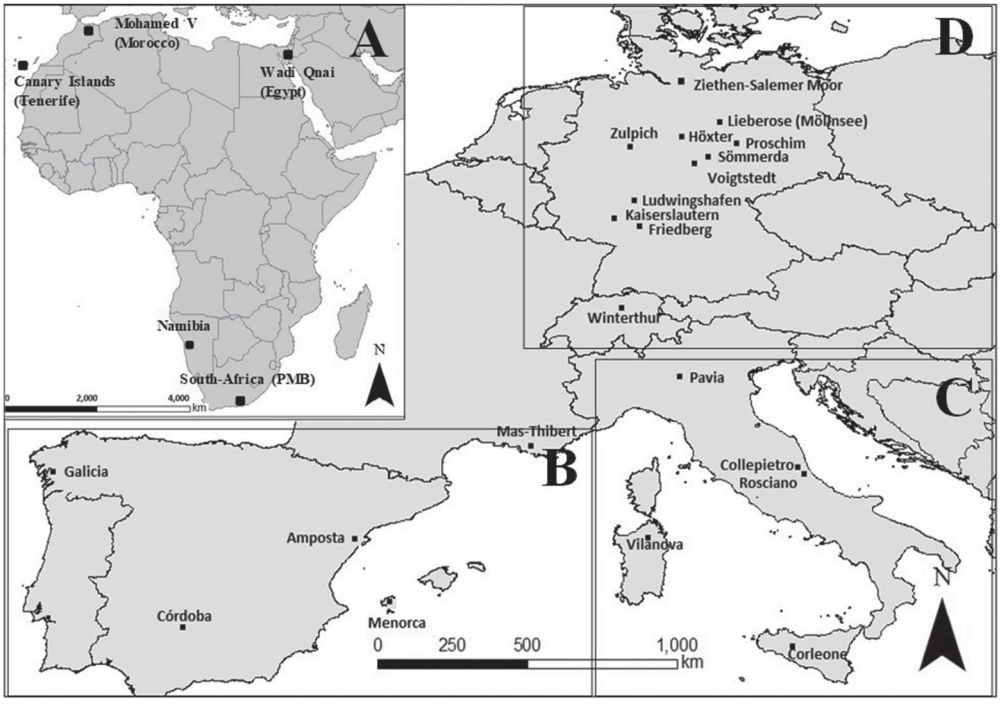

Figure 1. Map of sampling populations of *Crocothemis erythraea* ($n = 26$). Five populations in Africa (A), five in south-west Europe (B), five in Italy (C) and 11 in central Europe (D).

Material and methods

Sample collection

We analysed 51 specimens belonging to 26 localities from Europe and Africa, with a focus on Germany & Switzerland (Table 1 and Figure 1). Sampling locations covered almost the complete distributional range of *C. erythraea*, spanning from 45° N to 20° S, and 34° E to –8° W (Boudot, Kalkman, Azpilicueta-Amorín, Bogdanovic, Dommanget, 2009) (see Table 1 and Figure 1). Most of the sampled individuals were males (to avoid an impact on the reproductive capacity of the population). Sampling was done between 2006 and 2009 using hand nets. Sampled specimens (complete bodies, or a single leg) were preserved in 100% ethanol for later molecular analyses. In order to detect the genetic fingerprint of the different expansion fronts in the recently colonized region (central Europe), the sampled localities were clustered on four regions: (1) central Europe, (2) south-west Europe, (3) Italy and (4) Africa. The first region (central Europe) included 11 localities ($N = 18$ samples): Ludwigshafen, Kaiserslautern, Höxter, Ziethen-Salemer Moor, Proschim, Friedberg, Sömmerda, Zülpich, Lieberose (Möllnsee), Voigtstedt (all in Germany) and Winterthur (Switzerland) (see Figure 1). The second region (south-west Europe) included five localities ($N = 10$ samples): Menorca, Amposta, Córdoba, Galicia and Mas-Thibert. The third region (Italy) included five localities ($N = 11$ samples): Sardinia-Vilanova, Sicily-Corleone, Pavia, Collepietro and Rosciano. The fourth region (Africa) included five localities ($N = 12$ samples): Morocco-Barrage Mohamed V, Namibia-Swakopmund, South-Africa-Pietermaritzburg (PMB), Tenerife-Canary Islands, and Egypt-Wadi Qnai.

Table 1. Region, country and sampling localities.

Regions	Country	Locality	A	*N*	Latitude (°N)	Longitude (°E)	Date
Africa	Morocco	Barrage Mohamed V	MOR	2	34.360728	2.534318	2009
Africa	Namibia	Na	NAM	4	20.185582	17.141136	2007
Africa	South Africa	PMB	SOU	2	29.360803	30.203072	2008
Africa	Canary Islands	Tenerife	TEN	2	28.323357	16.212391	2007
Africa	Egypt	Wadi Qnai	EGY	2	28.270108	34.263795	2008
South-west Europe	Spain	Menorca	MEN	2	39.565547	4.063410	2008
South-west Europe	Spain	Amposta	AMP	1	40.393029	0.401229	2008
South-west Europe	Spain	Córdoba	COR	3	37.525778	4.463931	2008
South-west Europe	Spain	Galicia	GAL	2	42.282959	8.542251	2006
South-west Europe	France	Mas-Thibert	MAS	2	43.315545	4.455858	2009
Central Europe	Germany	Ludwigshafen	LUD	6	49.324164	8.234174	2006
Central Europe	Germany	Kaiserslautern	KAI	3	49.284737	7.405440	2009
Central Europe	Germany	Höxter-Sömmerda-void	HÖX	1	51.452026	9.222913	2009
Central Europe	Germany	Ziethen-Salemer Moor	SAL	1	53.401211	10.482360	2008
Central Europe	Germany	Proschim-Lieberose	PRO	1	51.324061	14.123259	2009
Central Europe	Germany	Friedberg-Zülpich	FRI	1	50.223068	8.513868	2009
Central Europe	Germany	Sömmerda	SOM	1	51.114205	11.081775	2009
Central Europe	Germany	Zülpich	ZÜL	1	50.411033	6.354194	2009
Central Europe	Germany	Lieberose (Möllnsee)	LIE	1	51.580720	14.123700	2009
Central Europe	Germany	Voigtstedt	VOI	1	51.235246	11.200492	2009
Central Europe	Switzerland	Winterthur	WIN	1	47.291676	8.411421	2009
Southern Europe	Italy	Sardinia-Vilanova	SAR	1	40.453625	9.011306	2008
Southern Europe	Italy	Sicily-Corleone	COE	2	37.492777	13.195727	2009
Southern Europe	Italy	Pavia	PAV	2	45.132601	9.022960	2007
Southern Europe	Italy	Collepietro	COL	3	42.122473	13.462248	2007
Southern Europe	Italy	Rosciano	ROS	3	42.181589	14.023073	2007
South America	Cuba*	na		1	na	na	2008
Europe	India*	Mohurti-Tadoba reserve		1	20.111582	79.203228	2008

*****Out-group: *Crocothemis servilia*
Abbreviations: A, abbreviation of population names; *N*, number of samples; "Date" indicates sampling year. "na" denotes information not available.

DNA extraction and mitochondrial DNA sequencing

We used two mitochondrial DNA genes: cytochrome b (CYTB) and NADH dehydrogenase 1 (ND1) because mitochondrial DNA has uniparental inheritance and a relatively high evolutionary rate, which are both useful in analysing intraspecific variation (Avise, 2000), but subject to strong genetic drift (Avise, 2004). Although most of the mitochondrial variation can be lost during a bottleneck, it can be especially informative in the case of multiple sources of invasions (Grapputo et al., 2005). DNA was extracted from the legs, incubated at 40°C for 36 h in a extraction buffer with Tris HCl pH 8 (0.5 M), EDTA pH 8 (0.5 M), NaCl (5 M), DTT (1 M), SDS pH 7.2 10%, 3 μl of RNAse (10 mg μl^{-1}) and 5 µl K proteinase (10 mg μl^{-1}). The extraction followed a standard phenol-chloroform (1:1) procedure and DNA was precipitated with absolute ethanol and NH_4Ac (4.4 M) for 24 h at 4°C. Pellets were re-suspended in TE 1 × and preserved at 4°C. DNA amplification of the fragments of both mtDNA genes was done by polymerase chain reaction (PCR) using the following universal primers: 359 bp of the cytochrome b (CYTB) gene with CB-J-10933 and CB-N-11367 (Simon et al., 1994), and 429 bp of tRNA-Serina and part of the NADH dehydrogenase one (ND1) gene with CB-J-11545 and N1-N-12051(Simon et al., 1994). DNA amplification was done in a total reaction volume of 20 μl. The amplification conditions were as follows: 50 ng of DNA (1 µl), 1 unit (0.2 µl) of Taq DNA polymerase (Ecogen, Madrid, España), 2 µl 10 × of reaction buffer (Ecogen, Madrid, España), 0.5 µl of $MgCl_2$ (50 mM) (Ecogen), 0.5 µL of dNTPs Mix (Sigma, Madrid, Spain) (200 μM), and 1 µl of each primer (10 pmol). All PCR reactions were completed in a GeneAmp PCR system 2700 thermocycler (Applied Biosystems, Madrid, Spain). The PCR program had an initial cycle at 95°C

for 3 min, annealing temperature at 45°C for 1 min, and an elongation period at 72°C for 45 s, followed by 34 cycles at 95°C for 30 s, with annealing for 45 s, and an elongation phase at 72°C for 45 s, and a final extension phase at 72°C for 10 min. Bidirectional sequencing reactions were conducted using Bigdye^{TM} terminator cycle sequencing kit (Applied Biosystems) using automatic sequencer 3730XL. Forward and reverse sequences were edited in Codon Code Aligned (CodonCode, Dedham, MA, USA) and consensus sequences were aligned with Clustal X (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) implemented in MEGA v. 4.0 (Tamura, Dudley, Nei, & Kumar, 2007). Variable positions were revised by eye, and only high-quality sequences were considered.

Genetic diversity and population structure

Molecular diversity in the four studied regions: central Europe ($n = 18$), south-west Europe ($n = 10$), Italy ($n = 11$) and Africa ($n = 12$) were assessed with $D_{NA}SP$ V4.10 (Rozas, Sánchez-DelBarrio, Messeger, & Rozas, 2003), in terms of: (i) number of haplotypes (h); (ii) number of polymorphic informative and non-informative sites (s); (iii) average number of pairwise nucleotide differences (K); (iv) number of mutations; (v) haplotype diversity (H), which describes the number and frequency of different haplotypes; and (vi) nucleotide diversity (π), which illustrates the average number of nucleotide differences per site between two sequences, according to Nei (1987). To explore population differentiation between regions the degree of genetic differentiation was measured as F_{ST} between pairs of regions. Significance was tested by 10,000 permutations of haplotypes among regions with ARLEQUIN v 3.0 (Excoffier, Laval, & Schneider, 2005).

Numerous statistical tests can be used to determine the population growth. Population expansion and/or selective sweeps usually lead to an increase in rare alleles at both synonymous and non-synonymous mutations, while purifying selection eliminates deleterious nonsynonymous mutations (Hahn, Rausher, & Cunningham, 2002). Thus, we used Tajima's D to test alternative hypotheses involving expansion/or selective sweeps from population bottlenecks and population subdivision (Hahn, Rausher, & Cunningham, 2002). Positive Tajima's D values indicate a bias towards intermediate frequency alleles, while negative values indicate a bias towards an excess in the number of rare alleles, which is a signature of recent population expansion.

Haplotype network

To infer the evolutionary processes that could have shaped the observed geographical distribution of the recorded haplotypes, we constructed a haplotype network using statistical parsimony method (Templeton, Crandall, & Sing, 1992) by the software TCS 1.13 (Clement, Posada, & Candall, 2000).

Results

Genetic diversity and population structure

Final alignments for CYTB and ND1 fragments included 359 and 429 bp, respectively. All new sequences were deposited in GenBank (accession numbers: KC430233.1 to KC430282.1, and KC430283.1 to KC430326.1). The CYTB fragment showed 40 polymorphic sites revealing a total of 40 mutations, of which 16 were parsimony informative, with 23 haplotypes. The fragment of ND1 presented 19 haplotypes, defined by 27 polymorphic sites and 27 mutations, of which 10 were parsimony informative.

Table 2. Diversity and neutrality indices calculated from nucleotide sequence of mitochondrial CYTB and ND1.

Region	*n*	S	K	H	Hd ± SD	π	D
			CYTB				
Africa	12	20	4.28	9	0.909 ± 0.079	0.012	–1.5486*
South-west Europe	9	12	2.67	6	0.833 ± 0.127	0.007	–1.8764*
Italy	9	6	1.61	6	0.889 ± 0.091	0.004	–1.1788
Central Europe	15	12	2.40	6	0.743 ± 0.094	0.007	–1.3623
			ND1				
Africa	9	12	2.83	5	0.722 ± 0.159	0.006	–1.6195*
South-west Europe	7	8	2.66	6	0.952 ± 0.07	0.006	–1.3594
Italy	7	1	0.47	2	0.476 ± 0.071	0.001	0.5590
Central Europe	18	11	2.09	10	0.888 ± 0.061	0.005	–1.3426

Statistical significance: *$p < 0.05$.
Abbreviations: *n*, number of sequences; S, number of segregating (polymorphic/variable) sites; K, average number of pairwise nucleotide differences; H, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; D, Tajima's D test statistic.

Table 3. F_{ST} values between the four regions Africa, south-west Europe, Italy and central Europe.

	Africa	South-west Europe	Italy	Central Europe
Africa	—	0.0022	0.0060	0.1348*
South-west Europe	0.0208	—	0.0267	0.1399*
Italy	–0.0041	0.0445	—	0.1562*
Central Europe	0.0162	0.0467	0.0631	—

Above-diagonal F_{ST} values for CYTB and below-diagonal F_{ST} values for ND1. Significance levels are denoted by an asterisk ($p < 0.05$*).

Genetic diversity was similar in both fragments, CYTB and ND1; haplotype diversity (H) was 0.870 ± 0.042 (mean ± SD; $N = 45$) and 0.855 ± 0.049 (mean ± SD; $N = 41$) in CYTB and ND1 and nucleotide diversity (π) was 0.0084 and 0.0052 respectively.

The four regions exhibited a high degree of genetic variation (Table 2). For CYTB, Africa presented the highest values for the numbers of haplotypes, haplotype and nucleotide diversity, while the remaining regions showed similar values. However, for ND1, all regions except for Italy presented similar values for the numbers of haplotypes, haplotype and nucleotide diversity. Conflict among estimates of phylogeny from different mitochondrial regions has been noted, but the basis for this conflict has typically remained unclear (Meiklejohn et al., 2014). We attribute the incongruence between both non-recombinant mtDNA genes to homoplasy or to the lower number of informative characters from the ND1 (see Campos-Soto, Torres-Pérez, & Solari, 2015).

For the CYTB gene, the four regions showed negative Tajima's D values [central Europe ($D = –1.3623$; $p = 0.08$); southern Europe ($D = –1.8764$; $p = 0.01$); Italy (D = –1.1788; $p = 0.14$); and Africa (D = –1.5486; $p = 0.05$)], although only in two regions (Africa and south-west Europe) were Tajima's D values significant (Table 2). Additionally, for the ND1 three regions showed negative Tajima's D values [Italy (D = 0.5590; $p = 0.8$); central Europe (D = –1.3426; $p = 0.08$); southern Europe (D = −1.3594; $p = 0.09$); Africa (D = –1.6195; $p = 0.03$)], although only in Africa were Tajima's D values significant (Table 2).

Pairwise region differentiation for CYTB and ND1 was $F_{ST} = 0$–0.0631 and $F_{ST} = 0.0022$–0.1562, respectively (Table 3). Central Europe presented the highest differentiation, ranging from 0.0162 to 0.0631 (for CYTB) and from 0.1348 to 0.1562 (for ND1). However, these values were only significant for CYTB. The remaining three regions presented (for both genes), similar and non-significant degrees of genetic differentiation (Table 3).

Haplotype network

A map of haplotypes (see Figure 2A–D) shows shared haplotypes along the colonization pathways: the most common haplotype (H1) for both genes (CYTB and ND1) was present in the four studied regions. However, the second most common haplotype (for both genes) was only found in central Europe and consisted of a one-step mutation to the haplotype 1, while the third most common haplotype (for CYTB) was detected in Africa and Italy, and consisted of two-step mutations to the haplotype 1. The haplotype network (Figure 2E, F) identified 23 haplotypes for CYTB and 19 for ND1. The maximum number of mutational steps (between any pair of haplotypes) was nine for CYTB, and six for ND1. Several intermediate haplotypes (for both genes) were absent from the haplotype network, maybe due to the small sample size by locality. The remaining haplotypes (20 for CYTB sequences, and 16 for ND1 sequences), appeared only once. Interestingly, the most divergent haplotypes were found in Africa (see Figure 2E, F).

Discussion

Multiple introductions (of one species into a region), can result in an increase in genetic differentiation because of the genetic admixture upon secondary contact (Swaegers et al., 2013). Therefore, multiple introductions can facilitate the geographic spread of a species into a new region. Here we explored the genetically detectable fingerprints that the rapid range expansion from multiple fronts of *Crocothemis erythraea* have left in the recent colonized regions in central Europe. Our genetic analyses, in line with the predictions, detected both a weak reduction in genetic diversity and high genetic differentiation in central European populations with respect to south European and African populations.

Crocothemis erythraea is a good disperser, and is not strongly associated with specific biotopes (Ott, 2007). In fact, its distributional range covers the whole of Africa, except for the forested Congo basin and the West African lowland forests, from there stretching eastwards via Afghanistan to India (Boudot et al. 2009). When a population colonizes a new geographical area, within this area, the resulting population could be genetically homogeneous due to the founder effect (Templeton, Routman, & Phillipe, 1995). However, in *C. erythraea* expansion has been driven consistently from three independent axes along a broad front: (i) via the central German low mountains region; (ii) via Austria and Hungary; and (iii) through the Czech Republic and Poland (Ott, 2007). We detected high and significant pairwise-genetic differentiation levels between central Europe (Germany and Switzerland) and the three natural distribution regions of *C. erythraea* (Africa, Italy and south-west Europe). However, although pairwise-genetic differentiation levels in between these three natural distribution regions were low and non-significant (see Table 3), we cannot discard that these low levels of pairwise-genetic differentiation could be the result of gene flow preventing geographic differentiation over large areas (see Slatkin, 1987). Additionally, and consistent with founders deriving from a broad set of core populations, as was previously detected in *C. erythraea* dispersion (see Ott, 2007), we detected a weak reduction of the genetic diversity in populations of central Europe.

On the other hand, in all regions (the three natural distributional regions and the recently colonized region), haplotype diversity was high (0.74–0.91) while nucleotide diversity was low (0.004–0.012). This pattern is characteristic of a rapid demographic expansion from a small effective population size or may also be the result of transient bottlenecks in large ancestral populations (Avise, 2000). In other dragonflies that have experienced a rapid population expansion during their dispersal, such as the migratory *Anax junius*, similar contrasting levels of nucleotide and haplotype diversity were detected (see Freeland et al. 2003). In this study, the Tajima's D test was negative for all except southern Europe, although significantly negative values were only

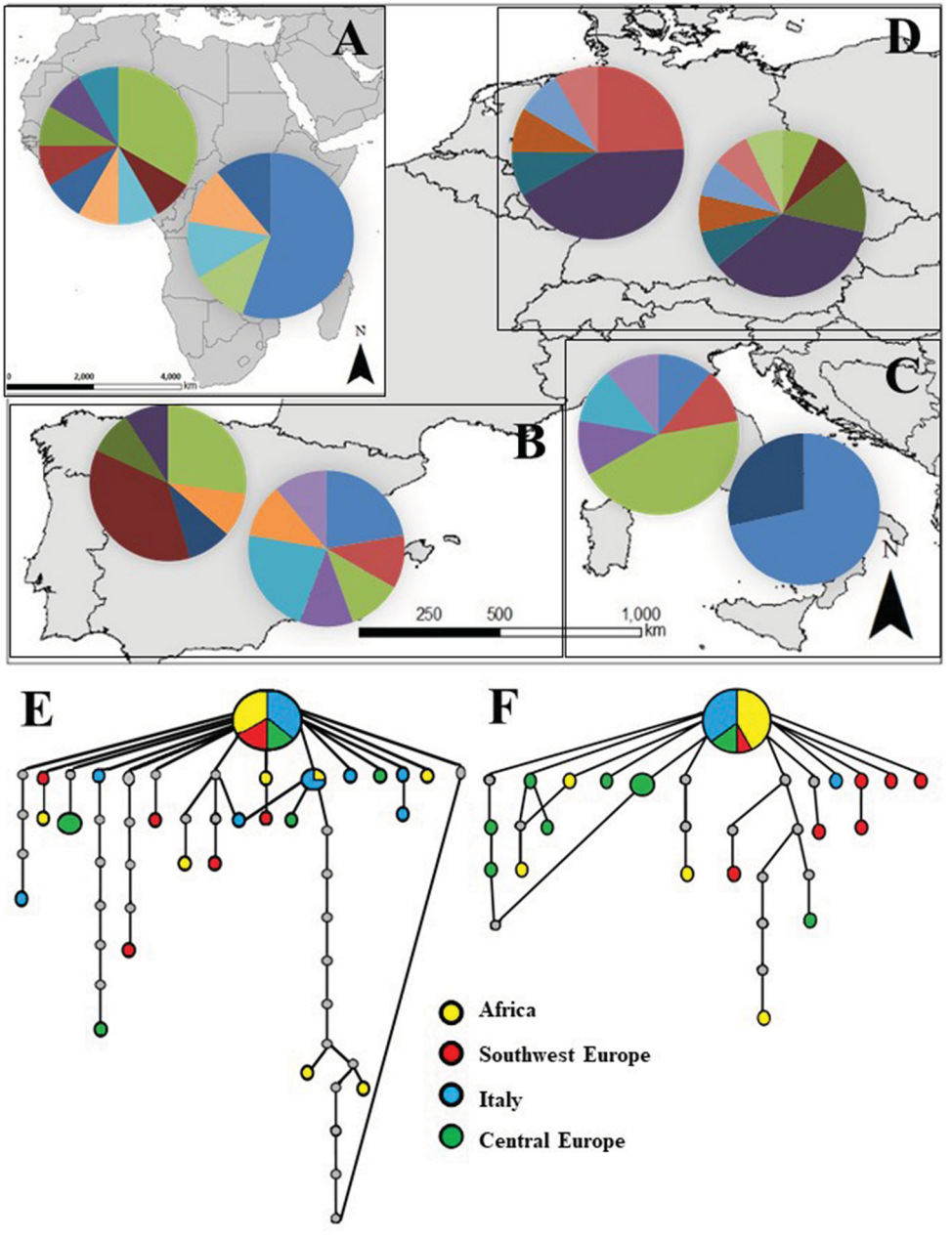

Figure 2. Haplotype map and parsimonious phylogenetic network. Upper-left diagram of haplotypes represent CYTB sequences, and lower-right diagrams of haplotypes represent ND1 sequences in Africa (A), south-west Europe (B), Italy (C) and central Europe (D). Phylogenetic networks are based on 95% connection limit for (E) CYTB and (F) ND1 sequences. Different colours represent different geographical populations (see legends). Circle sizes are proportional to the number of individuals sharing each haplotype. Grey nodes represent inferred mutational events occurring between haplotypes.

detected in Africa (for CYTB and ND1) and south-west Europe (CYTB). Negative values indicate an excess of rare mutations in populations, which can imply a recent population expansion in these regions.

The patterns of spread within the species' novel range can also be inferred by the phylogeographic analysis of the intraspecific genetic variation (Hewitt, 2001). In *C. erythraea* the phylogeographic analysis of the intraspecific genetic variation (of the central European populations) revealed threefold evidences of a rapid and recent range expansion from several fronts: (i) increased number of derived sequences due to "allele surfing", where some genetic variants increased in frequency due to founder effects (see Klopfstein, Currat, & Excoffier, 2006); (ii) lack of a phylogeographic structure (see Hewitt, 2004); and (iii) a haplotype network showing mainly single nucleotide differences between haplotypes (see Hewitt, 2004). The damselfly *Erythromma viridulum* (Watts et al., 2010) and the bark beetle *Ips typographus* (Mayer, Björklund, Wallén, Längström, & Cassel-Lundhagen, 2014) are current examples of the lack of a phylogeographic structure after recent range expansions.

The evolutionary potential of an invasive species is improved with an increase of genetic diversity by both elevating standing adaptive genetic variation (which reduces the negative effects of genetic bottlenecks and drift (Hahn & Rieseberg, 2017; Rieseberg et al., 2007)) and increasing phenotypic variation for ecologically important traits (see Harper & Pfennig, 2007; Hellberg, Balch, & Roy, 2001). Evidence of local adaptations such as changes in phenology and in voltinism (from univoltinism to bivoltinism), earlier emergence, faster larval development and higher trends to migration have been identified in the central European populations of the dragonfly *C. erythraea* (see Ott, 2007, 2009, 2010). Recently they also invaded formerly unpopulated water types, such as moorlands, and so widened their spectrum of potential habitats, possibly leading to competition with typical moorland species, such as *Leucorhinia dubia* (Suhling & Suhling, 2013). Other odonates are also experiencing similar changes in phenology (see Hassall, Thompson, & French, 2007). In the damselfly *I. elegans* an increased adaptive thermal tolerance towards the northern, expanding range edge has been found (Lancaster, Dudaniec, Hansson, & Svensson, 2015).

In conclusion, our study, although limited because of the small sample size, provides a much-needed framework for the ongoing investigation of the range expansion of *C. erythraea*. More studies – including more populations and increased sample sizes – will help to develop suitable management strategies for expanding species, such as the prioritization of sites to protect them (those sites including the highest levels of genetic diversity), that would allow the reduction of costs and conflicts with competing land uses (see Neel & Ellstrand, 2003).

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