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Comparative phylogeography of mutualists and the effect of the host on the genetic structure of its partners

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Whether or not species participating in specialized and obligate interactions display similar and simultaneous demographic variations at the intraspecific level remains an open question in phylogeography. In the present study, we used the mutualistic nursery pollination occurring between the European globeflower *Trollius europaeus* and its specialized pollinators in the genus *Chiastocheta* as a case study. Explicitly, we investigated if the phylogeographies of the pollinating flies are significantly different from the expectation under a scenario of plant–insect congruence. Based on a large-scale sampling, we first used mitochondrial data to infer the phylogeographical histories of each fly species. Then, we defined phylogeographical scenarios of congruence with the plant history, and used maximum likelihood and Bayesian approaches to test for plant–insect phylogeographical congruence for the three *Chiastocheta* species. We show that the phylogeographical histories of the three fly species differ. Only *Chiastocheta lophota* and *Chiastocheta dentifera* display strong spatial genetic structures, which do not appear to be statistically different from those expected under scenarios of phylogeographical congruence with the plant. The results of the present study indicate that the fly species responded in independent and different ways to shared evolutionary forces, displaying varying levels of congruence with the plant genetic structure. *Biological Journal of the Linnean Society*, 2014, **113**, 1021–1035.

ADDITIONAL KEYWORDS: Approximate Bayesian computation – *Chiastocheta* – climatic oscillations – coalescent modelling – coevolution – Last Glacial Maximum – nursery pollination mutualism – spatial genetic structure – *Trollius europaeus*.

INTRODUCTION

The high number of species and enhanced rate of diversification in insects and angiosperms is often explained by reciprocal adaptive radiation of these two groups (Simpson, 1953; Schluter, 2000; Lunau, 2004; Futuyma & Agrawal, 2009). In the coevolutionary model of Ehrlich & Raven (1964), the 'escape and radiate' process can promote codiversification of plants and associated herbivore insects. From the plant side, protection against such an exploitation can arise by two different mechanisms: (1) by the development of chemical or physical defences and (2) by an evolution towards a cooperative interaction in which the cost of insect exploitation is balanced by an ecological service imposed by the plant (Dufaÿ & Anstett, 2003). The latter case is notably encountered in mutualistic pollination systems, in which costs and benefits for plants and insects tend to be equilibrated (Thompson, 2009).

Specialized and obligate interactions, although much rarer than generalist relationships (Ollerton *et al.*, 2007), represent simple cases in which coevolutionary hypotheses can be tested and have

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therefore been frequently exploited as models by evolutionary biologists. Most studies interested in understanding the history of organisms in a context of strict coevolution have focused on large spatiotemporal scales, showing long-term processes putatively leading to cocladogenesis and codiversification (Agosta, 2006), such as, for example, in the case of the fig and fig wasps (Jousselin *et al*., 2008) or *Yucca* and *Yucca* moths (Pellmyr & Leebens-Mack, 1999). However, phylogenetic investigations at smaller evolutionary scales (e.g. at the intraspecific level) hold the potential to inform us on the origin and maintenance of specific mutualistic interactions because they can provide an insight into the first steps of codivergence and the intraspecific population dynamics leading to it.

Indeed, similar to higher taxonomic levels, codivergence could also be expected to occur at the intraspecific level because speciation ultimately results from population-level processes. Practically, this can be observed in species displaying similar genetic structures, and experiencing similar and simultaneous demographic variations (e.g. population contractions and expansions, migration). Furthermore, because of the tight nature of the ecological relationship occurring between species in specific and obligate interactions, one might anticipate the phylogeographical history of interacting organisms to be more similar than expected by chance. So far, this idea has been tested in several studies that report relatively different findings. Although Tsai and Manos (2010) demonstrated that the phylogeography of the host *Fagus* and its parasites *Epifagus* were not similar but mostly depended of the host abundance through time, Smith *et al*. (2011) showed that the mutualistic *Yucca* and *Yucca* moths experienced similar and simultaneous demographic expansions. Finally, a recent investigation of a guild of oak gall wasps and associated parasitoids (Stone *et al*., 2012) revealed the presence of a lag-time in the recolonization dynamics of parasitoid species, postdating the population expansion experienced by their host herbivore by approximately 1000 years.

Whether or not a pattern of shared common history is expected in ecologically interdependent organisms remains an open question. In the present study, we investigated the fate of cold-adapted species involved in a tight mutualistic relationship in the context of post-glacial range contraction by studying the European globeflower and its associated pollinating flies.

The nursery pollination interaction featuring the European globeflower *Trollius europaeus* L. (Ranunculaceae) and flies of the genus *Chiastocheta* Pokorny (Diptera: Anthomyiidae) represents a unique widespread example of a specialized mutualism between cold-adapted plants and insects (Pellmyr, 1989). The European globeflower is a West-Palearctic hemicryptophyte displaying a closed flower morphology. This floral shape has been demonstrated to be adapted to the specialized and obligate nursery pollination interaction that it maintains with *Chiastocheta* (Pellmyr, 1992; Louca *et al*., 2012). Indeed, the plant is visited and specifically pollinated by the small Anthomyiids, whose larvae feed exclusively on the plant seeds. Because the flies are the only globeflower pollinators, plant reproductive success depends on the insect visits. Furthermore, because the globeflower is the only host-plant for these insects, their reproductive success also depends on the interaction they maintain with the plant. Based on morphology, eight *Chiastocheta* species have been described as interacting with the European globeflower (Michelsen, 1985; Pellmyr, 1992; Jaeger & Després, 1998). However, a recent phylogenetic study (Espíndola, Buerki & Alvarez, 2012a) has shown that the nominal species are not consistent with the pattern of variation in the genetic data, with several taxa likely exhibiting hybridization. From the eight initially described species, only three appear to be consistently delimitated by both genetic and morphological grounds: *Chiastocheta rotundiventris*, *Chiastocheta lophota*, and *Chiastocheta dentifera* (Espíndola, 2010; Espíndola *et al*., 2012a). From the plant side, its European spatial genetic structure has been investigated recently (Espíndola *et al*., 2012b), demonstrating the presence of four genetic clusters that have likely diverged during one of the last glacial terminations (Raymo, 1997).

In the present study, we exploit this previous knowledge and explore phylogeographical congruence between cold-adapted mutualists by focusing on the three later species and using the phylogeography of *T. europaeus* to test scenarios of post-glacial phylogeographical history. Using highly variable mitochondrial markers in combination with coalescent modelling approaches, we statistically test whether the phylogeographical patterns of the insects are more similar to a model fitting the plant's phylogeographical pattern than would be expected by chance. To do so, we apply the recently developed statistical phylogeographic analytical approach, which allows the achievement of a deeper evolutionary insight than simply describing and comparing the distribution of lineages in space (Hickerson *et al*., 2010). Simultaneously, we apply approximate Bayesian computation (ABC; Beaumont, Zhang & Balding, 2002) to evaluate a set of phylogeographical models and identify the one that offers the best fit to the phylogeographical data of the fly species. We hypothesize that *T. europaeus* and *Chiastocheta* spp. experienced concerted and contemporaneous demographic

responses to the last Quaternary glacial terminations, as reflected by congruent phylogeographical patterns and similar demographic variation.

MATERIAL AND METHODS

Sampling and DNA amplification

Chiastocheta flies were collected in 38 locations (see Supporting information, Table S1) across Europe during springs 2006–2008 and preserved in 70% ethanol. Samples were identified at the species level (see Supporting information, Table S1) *sensu* Hennig (1976) and further confirmed by the European specialist of Anthomyiids, V. Michelsen (Natural History Museum of Denmark). Only the three taxa presenting a consistent phylogenetic clustering (i.e. *C. rotundiventris*, *C. dentifera*, and *C. lophota*; Espíndola *et al*., 2012a) were further selected to infer phylogeographical patterns and demographic parameters (e.g. theta, migration; see below).

DNA from 87 *C. rotundiventris*, 38 *C. dentifera*, and 47 *C. lophota* samples was extracted using the DNeasy Animal Tissue extraction kit (Qiagen) in accordance with the manufacturer's protocol. Three mitochondrial regions (*COI*, *COII*, and *D-loop*) were amplified using the primers shown in Table 1. Polymerase chain reactions were conducted in a 20-μL mix consisting of 0.5 × buffer, between 1 and 2.5 mM $MgCl_2$, 10 mM dNTPs, 1 unit of GoTaq DNA polymerase (Promega), 0.5 μM primers and 3 μL of DNA, and run in a TGradient thermocycler (Biometra). Two types of thermocycling programmes were used, depending on the region amplified: for *COI* and *COII*, we applied a programme consisting of 90 s at 95 °C, followed by 40 cycles of 35 s at 95 °C, 1 min at 52 °C, 45 s at 72 °C, and a final elongation of 8 min at 72 °C; for the A-T rich *D-loop*, we used a programme consisting of 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 60 °C, and a final elongation of 5 min at 60 °C. Amplified fragments were purified and sequenced at Macrogen Inc. (South Korea) and Fasteris SA (Switzerland). Chromatograms were corrected using CHROMASPRO, version 1.41 (Technelysium Pty Ltd), aligned either using a Clustal–Wallis algorithm for *COI* and *COII* (Thompson, Higgins & Gibson, 1994) as implemented in BIOEDIT, version 7.0.4.1 (Hall, 1999) or applying the moderately accurate option proposed by the MAFFT, version 6, online alignment service for the *D-loop* (Katoh & Toh, 2008). All alignments were further visually checked and corrected if necessary. Gaps were coded applying the simple gap-coding method of Simmons & Ochoterena (2000), as implemented in FASTGAP, version 1.2 (Borchsenius, 2009). Total, variable, constant, and parsimony informative sites were calculated per species and mitochondrial region (mt)DNA region using MEGA, version 4.0 (Tamura *et al*., 2007).

Phylogenetic inference and haplotype networks

We used MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003) to separately infer Bayesian phylogenies for each of the three fly species. For each single species alignment, we parameterized two Markov chain Monte Carlo (MCMC) runs with two chains, consisting of 50 000 000 generations, a temperature of 0.5 and one sampled tree every 1000 generations. Data was partitioned for each mtDNA region using specific models of evolution following the results obtained with MrAIC (Nylander, 2004). A restriction model of evolution was assigned to the partition corresponding to gaps (coded as binary data). Convergence was assumed when standard variation between chains fell below 0.01, when the potential scale reduction factor index (Gelman & Rubin, 1992) reached at most 1.002, when a unimodal distribution of sampled parameters was retrieved, and when sampling for all parameters presented an effective sampling size greater than 200 (checked with TRACER, version 1.4; Rambaut & Drummond, 2004). Half-compatible consensus trees were calculated applying burn-ins of 10 000 000 (*C. dentifera*) and 20 000 000 (*C. lophota*) generations.

Maximum likelihood (ML) searches were conducted using RAXML, version 7.2.6 (Stamatakis, 2006) with

Table 1. Names, sequences, annealing temperatures, and references of the primers used for sequencing mitochondrial regions in *Chiastocheta* spp.

Region	Primer	Sequence	Annealing	Reference
COI	COI-2171	TTG ATT TTT TGG TCA YCC NGA AGT	52	Després and Jaeger (1999)
	tRNAleu-3048	TGG AGC TTA AAT CCA TTG CAC	52	Després and Jaeger (1999)
COII	tRNAleu-3023	GAT TAG TGC AAT GGA TTT AGC TC	52	Després and Jaeger (1999)
	COII-3683	CCR CAA ATT TCT GAA CAT TGA CC	52	Després and Jaeger (1999)
D-loop	TM-N-193	TGG GGT ATG AAC CCA GTA GC	55	Simon *et al*. (1994)
	SR-J-14612	AGG GTA TCT AAT CCT AGT TT	55	Simon *et al*. (1994)

a 10 000 rapid bootstrap analysis followed by the search of the best-scoring ML tree in one single run. Here, we considered the three mtDNA regions as one single partition and did not account for gap information. A majority-rule consensus tree was further inferred from the node information. This analysis was conducted using the facilities offered by the CIPRES portal (San Diego, CA, USA).

Parsimony-based haplotype-networks were constructed using TCS, version 1.21 (Clement, Posada & Crandall, 2000), applying a connectivity threshold of 95% (i.e. networks were unlinked if they differed by more than 5% of their sequences); gaps were considered as missing data.

All topologies were rooted based on the general relationships previously inferred by Espíndola *et al.* (2012a) and all supported clusters were plotted on maps using ArcMap, version 9.3 (ESRI).

Phylogeographic hypothesis testing

We used a statistical phylogeographic approach to hypothesis testing, as proposed by Knowles & Maddison (2002), to test whether or not the spatial genetic structure of the insects was congruent with that of the plant. This parametric simulation technique is derived from the theory of allele coalescence (Kingman, 1982) and explicitly models the stochasticity inherent to the coalescent process. The approach requires the definition of explicit phylogeographical hypotheses under which genealogies and sequence data are simulated. In formulating our hypotheses, we considered the spatial genetic structure detected by previous investigations on the host plant (Espíndola *et al.*, 2012b). Specifically, population trees were defined by assigning insect samples based on the genetic cluster to which the plant populations had been previously assigned (Fig. 1). This assignment was straightforward since samples from insects and plants had been collected simultaneously in all visited localities (see Supporting information, Table S1). Because species involved in this mutualism are cold-adapted species (*sensu* Stewart *et al.*, 2010) and because the current distribution of the plant has been driven by the last range contraction following the Last Glacial Maximum (LGM; in our case related

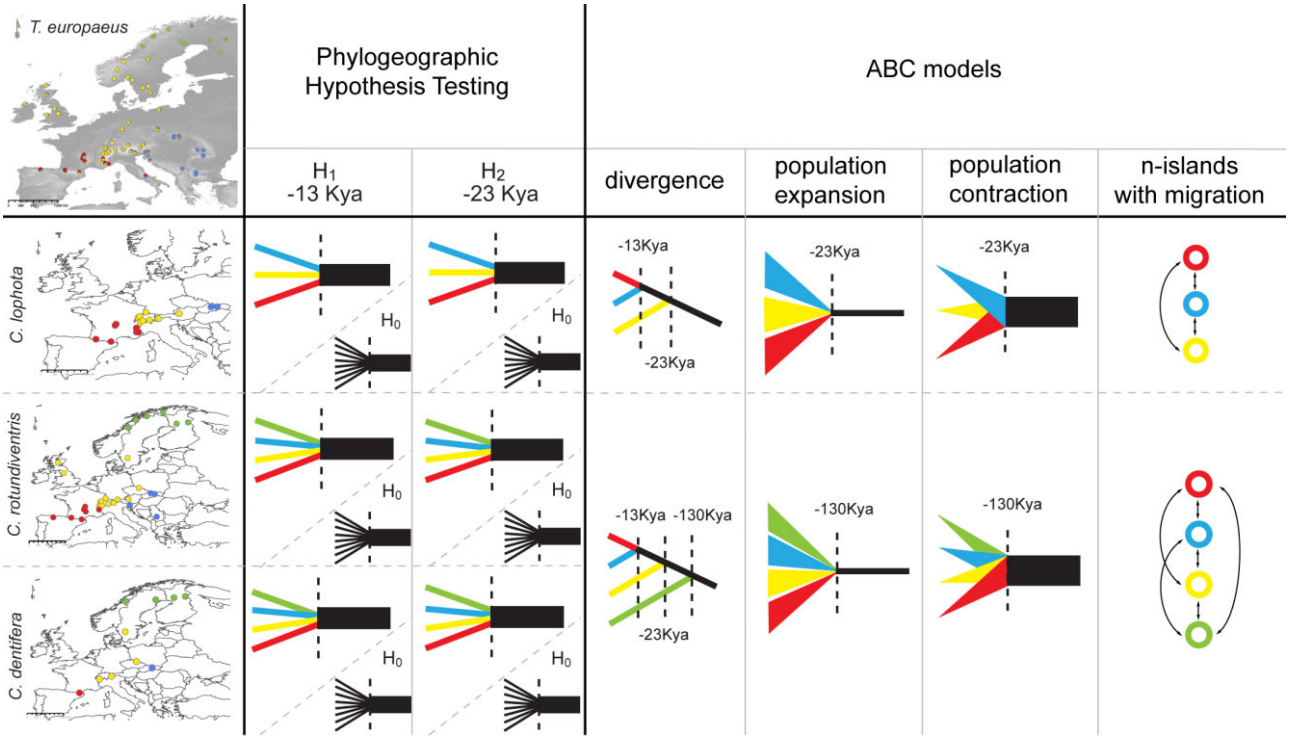

Figure 1. Tested phylogeographical hypotheses and compared approximate Bayesian computation (ABC) models for *Chiastocheta rotundiventris*, *Chiastocheta lophota*, and *Chiastocheta dentifera*, considering the inferred genetic identity of populations for *Trollius europaeus* based on Espíndola *et al.* 2012a. Maps for *C. rotundiventris*, *C. lophota*, and *C. dentifera* show the position of sampled populations and the expected genetic identity they should have if the genetic structure of the fly was congruent with that of the plant (top map). Fly populations included in each population tree branch are identified with colours reported on maps of expected spatial genetic structure (left panels).

to the termination of the last glacial period; Raymo, 1997), we used splitting times of lineages (Fig. 1): in the working hypothesis H_1, the genetic clustering of the insect was hypothesized to have followed contraction of plant lineages after complete deglaciation of the main European lowlands (13 kya, Raymo, 1997), whereas, in the alternative hypothesis, H_2, the genetic clustering was hypothesized to have followed contraction of plant lineages immediately after the previous Glacial Maximum (23 kya, Boulton *et al*., 2004). The null hypotheses H_0 for each of these scenarios were that the splitting of insects' genetic clusters was not defined by the distribution of plant lineages after the last glacial period.

To simulate the null distributions, we estimated the demographic parameter $\theta = 4Nem$ from the genetic data using MIGRATE-n (Beerli & Felsenstein, 1999, 2001) assuming that gene flow was not present. Two runs of ten short and three long chains were used for 50 000 000 generations, sampling one value every 1000 generations and applying a burnin period of 10 000 000 generations. Convergence between runs was verified using the Gelman criterion (Gelman & Rubin, 1992) (option 'gelman-convergence= YES:PAIRS'). Once this parameter was inferred, MS (Hudson, 2002) was used to simulate 1000 tree topologies fitting the coalescent models represented by our hypotheses. Based on these topologies, sequences carrying the same characteristics as the empirical data (i.e. sequence length, model of evolution, number of variable sites) were simulated for each simulated tree using SEQ-GEN, version 1.3.2 (Rambaut & Grassly, 1997). We followed the approach proposed by Carstens *et al*. (2005) and considered two rates of mutation (μ): either 1×10^{-7} or 1×10^{-8}. These rates were chosen considering that they include the rate inferred for *Drosophila* (6.2×10^{-8}) but still allow for some variation because no direct estimations are available for *Chiastocheta* (Haag-Liautard *et al*., 2008). Using these sequences, heuristic searches were performed with PAUP*4b10 (Swofford, 2003), with tree bisection–reconnection branch swapping, maxtrees = 1000 and 10 random-addition sequence replications. A strict consensus topology of the most parsimonious trees was computed for each dataset and the resulting trees (1000) were used to create a null distribution of the test statistic.

To estimate the fit or discordance between gene (i.e. simulated and empirical data) and species (i.e. phylogeographical scenarios) trees (Knowles & Maddison, 2002), we used the *S*-statistic of Slatkin & Maddison (1989), which indicates the minimum number of migration events (parsimony steps) required to fit the phylogeny into the population tree. Because our hypotheses consider that dispersal events should have happened in parallel among the different partners, using a measure of migration appears appropriate. Calculations of *S* for simulated trees and empirical data were performed using MESQUITE, version 2.72 (Maddison & Maddison, 2009). We considered rejection of hypotheses H_1 or H_2 if the empirical value fell outside the 2.5%–97.5% range of the simulated data (i.e. $\alpha = 5\%$).

Approximate Bayesian computation

In addition to the phylogeographic hypothesis testing approach described above, we also evaluated the relative posterior probability of the data given different phylogeographical models. After preliminary explorations suggested limited power to differentiate models that were heavily parameterized (i.e. > 8 parameters used in model description), we designed and compared four models (Fig. 1) that were constructed based on the phylogeography known for the host plant (Espíndola *et al*., 2012b). The first was a model of population divergence, using the divergence times (13 kya, 23 kya, common ancestry: 130 kya for *C. dentifera* and *C. rotundiventris*; 13 kya, common ancestry: 23 kya for *C. lophota*) and structure inferred from both the genetic and range variation identified in the plant. In this case, prior intervals were defined based on palaeoclimatic data (Raymo, 1997) and on evaluations of changes in the host range across the last millennia (Espíndola *et al*., 2012b). The second was an expansion model from an ancestral population, starting at 13 kya and leading to four populations, which corresponded to the plant genetic groups. The third model was similar to the second but considered contraction, instead of expansion. The fourth model was an island model with migration. In this case, the tested populations were defined based on the plant genetic groups, and migration among populations was parameterized to have equal rates.

A perl script was written to generate the prior distribution for the four models in each species: (1) parameter values (Table 2) were drawn from a uniform distribution; (2) MS (Hudson, 2002) was used to simulate a data set given these values; and (iii) DNASP (Librado & Rozas, 2009) was used to calculate nucleotide diversity (π), the number of segregating sites (s) and Tajima's D. Prior distributions contained 1×10^{-6} datasets for each species. Once the prior distributions were generated, MSREJECT (Hickerson, Stahl & Takebayashi, 2007) was used to filter the prior such that the posterior distribution represented the small proportion (0.0001) of the prior distribution that was most similar to the empirical data. The relative contribution of each model to the posterior distribution represents its posterior probability (Pritchard *et al*., 1999).

Table 2. Prior distribution of parameters used to construct the models compared in the ABC approach, for each fly species

	Prior distribution (uniform)		
Parameter	*Chiastocheta dentifera*	*Chiastocheta lophota*	*Chiastocheta rotundiventris*
$T_{1\ (27–140\ kya)}$	0.9–1.5	NA	0.9–1.5
$T_{2\ (21–27\ kya)}$	0.15–0.9	0.35–0.45	0.15–0.9
$T_{3\ (11–15\ kya)}$	0.03–0.15	0.02–0.35	0.05–0.15
*T*e	0.003–1.5	0.006–0.5	0.1–0.25
θ	0.1–10.0	0.1–5.0	0.1–25.0
γ	0.1–0.9	0.5–3.0	0.1–0.6
m	0.08–0.12	0.075–0.125	0.05–0.15

T_1, T_2, T_3, population splitting times (in units of 4*N*e generations); *T*e, time of expansion or contraction (in units of 4*N*e generations); θ, demographic parameter defined as 4*N*eμ per locus; γ, rate of expansion and contraction, *m*, migration rate, NA, not applicable.

RESULTS

The geographical distribution of the sampled species covered their entire known European ranges (Figs 2, 3, 4) (Pellmyr, 1992). Although all DNA regions were polymorphic, the *D-loop* was by far the most variable and its corresponding alignment was the only one containing gaps (Table 3; GenBank accession numbers KM255207-KM255664 and available at http://purl.org/phylo/treebase/phylows/study/TB2:S16187). Moreover, although *C. rotundiventris* and *C. lophota* presented relatively moderate levels of genetic diversity, *C. dentifera* displayed the lowest (Tables 3, 4).

Phylogeographical inferences

Chiastocheta rotundiventris

No convergent analyses could be retrieved using the Bayesian inference approach: independent runs yielded different topologies and likelihoods, even though trials with different MCMC parameters, models of evolution, and linkage among-partitions were performed. Consequently, Bayesian topologies were not taken into consideration for *C. rotundiventris*. The ML estimate of the genealogy indicates that resolution is very low in this taxon (Fig. 2A), with extremely short and polytomous branches, as well as low node support, although approximately 5% of the nucleotides among the three regions were variable. When analyzing patterns of the haplotype network inferred for *C. rotundiventris*, some spatial structure could be recovered among the 44 haplotypes (of which eight were shared by several samples and 36 were private to single samples) (Fig. 2B): the most abundant haplotype (red) was absent from Scandinavia, whereas two other haplotypes (purple and yellow) were restricted to this region. Other haplotypes were private to some populations, as those found in south Scandinavia (grey), northern Poland (blue), and the Balkans (black).

Chiastocheta lophota

Unlike *C. rotundiventris*, phylogenies (both Bayesian and ML) supported three main clades (Fig. 3A). The same three groups were also retrieved in the haplotype network analysis (Fig. 3B) and relationships between clades were congruent with topological analyses [i.e. haplotypes from the blue and red groups presented closer relationships (a maximum of three steps) than those relating them with the yellow clade]. The distribution of clades indicated a clear, geographically structured genetic distribution (Fig. 3C). Although one clade (blue) was restricted to eastern populations, another was exclusive to the southwestern Alps (yellow) and the last (red) was present mainly in the western species range and on the northern edge of the Alps.

Chiastocheta dentifera

Both phylogenetic approaches yielded similar topologies and node supports (Fig. 4A). Analyses retrieved four main clades (orange, red, yellow, and purple) supported by at least one method, and two additional groups placed in polytomies (green and blue). In the haplotype network, the three shared and 15 unique haplotypes were tightly interconnected, with a maximum of five steps between the two more distant haplotypes (Fig. 4B). Some of the haplotypes matched the clades found in the phylogenetic approach (i.e. red clade), whereas others only partially did (i.e. green and yellow). The geographical distribution of clades indicated a trend towards a spatial genetic structure, with samples from several clades restricted to particular European regions (Fig. 4C): one clade (yellow) was present over southern Scandinavia, as well as at several locations in the Alps; two other (orange and red) were restricted to Scandinavia, whereas the Alpine and Sudete locations harboured individuals clustering in spatially-restricted areas (blue and purple). The remaining group (green) was widespread but was the only one present in the Pyrenees and in north-eastern populations.

Phylogeographic hypothesis testing

Maximum likelihood estimates of theta for *C. rotundiventris*, *C. dentifera*, and *C. lophota* were relatively low, at 0.02004, 0.01049, and 0.00594, respectively, providing *N*e values equal to: 50 010 ($\mu = 1 \times 10^{-7}$) and 500 100 ($\mu = 1 \times 10^{-8}$) for *C. rotundiventris*, 26 220 ($\mu = 1 \times 10^{-7}$) and 262 200 ($\mu = 1 \times 10^{-8}$) for

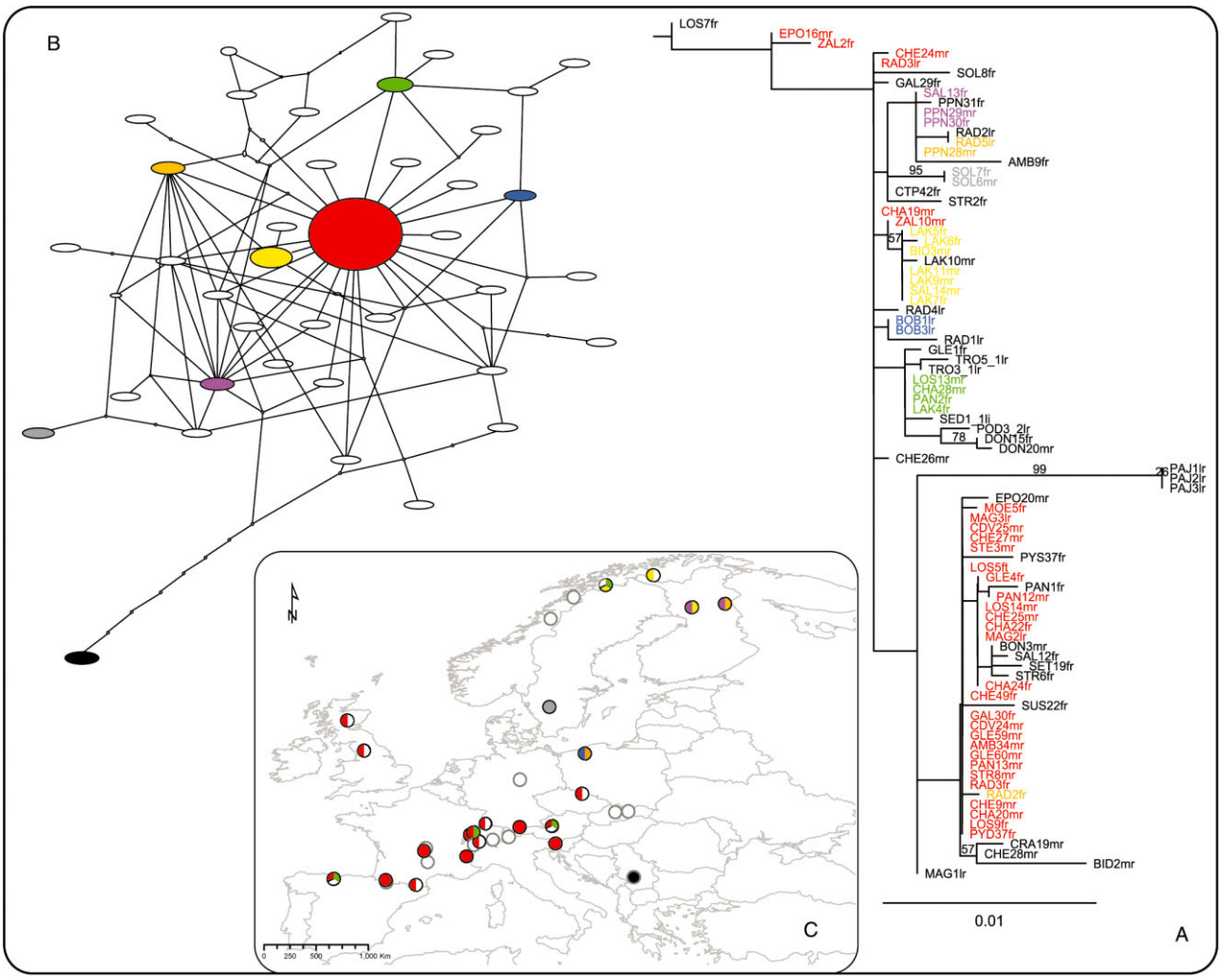

Figure 2. Topological inferences (A, B) and geographical distribution of haplotypes (C) in *Chiastocheta rotundiventris*. A, maximum likelihood (ML) phylogeny. Values on branches indicate ML supports higher than 50, based on 10 000 bootstraps. Colours indicate haplotypes. B, haplotype network. Colours show shared haplotypes; empty circles indicate unique haplotypes. C, geographical distribution of haplotypes. Colours correspond to (B). Sites with several colours indicate locations comprising different haplotypes.

C. dentifera; 14 860 ($\mu = 1 \times 10^{-7}$), and 148 600 ($\mu = 1 \times 10^{-8}$) for *C. lophota* (Table 4). Calculated *S*-values for the empirical data were 71 for *C. rotundiventris*, 24 for *C. dentifera*, and 27 for *C. lophota* (Table 4).

The hypothesis testing approach resulted in different results for each species. In *C. rotundiventris*, only $H_{0(H2)}$ was rejected using the high mutation rate (Table 5), whereas, in *C. lophota*, all models were rejected except for $H_{0(H1)}$ and $H_{0(H2)}$ using that same rate (Table 5). In *C. dentifera*, $H_{0(H1)}$ and $H_{0(H2)}$ were rejected using both mutation rates (Table 5). At most, it could be argued that the results were similar in *C. lophota* and *C. dentifera* if a lower rate of mutation is assumed (i.e. 1×10^{-8}).

Approximate Bayesian computation

The results from the ABC analysis suggest that the genetic variation within the three fly species (Table 6) results from differing population processes. Although the analysis could not distinguish between three of the four tested models in *C. rotundiventris*, the results are compelling in the other species. The divergence model has the highest posterior probability given the data in *C. lophota* ($P = 1.0$), whereas an *n*-island model with migration has the highest posterior probability given the data in *C. dentifera* ($P = 1.0$). As in the phylogeographic hypothesis testing, we do not see concordance in patterns across species.

Table 3. Number of total, constant, variable and parsimony informative (PI) sites, as well as the number of identified gaps per amplified region and species in *Chiastocheta*

Species	Region	Sites (base pairs)				Gaps
		Constant	Variable	PI	Total	
Chiastocheta rotundiventris						
	COI	645	12	4	657	0
	COII	457	22	5	479	0
	D-loop	865	56	27	1168	87
	Total	1967	90	36	2304	87
Chiastocheta dentifera						
	COI	653	4	1	657	0
	COII	475	4	0	479	0
	D-loop	883	32	15	1168	75
	Total	2011	40	16	2304	75
Chiastocheta lophota						
	COI	644	13	6	657	0
	COII	470	9	6	479	0
	D-loop	768	60	29	855	62
	Total	1882	82	41	1991	62

Table 4. Estimated demographic parameters ($\theta = 4Ne\mu$ per site; and *Ne* calculated considering two different mutation rates), calculated *S* of Slatkin statistics, and diversity indexes for *Chiastocheta rotundiventris*, *Chiastocheta dentifera*, and *Chiastocheta lophota*

	θ (MLE)	*Ne* ($\mu = 10^{-7}$)	*Ne* ($\mu = 10^{-8}$)	*S* (obs)	π	*S*	*D*
Chiastocheta rotundiventris	0.02004	50010	500100	71	0.00427	39	0.328
Chiastocheta dentifera	0.01049	26220	262200	24	0.00045	4	0.202
Chiastocheta lophota	0.00594	14860	148600	27	0.00507	39	0.945

MLE, maximum likelihood estimate.

DISCUSSION

Although the phylogeography of European organisms has been revisited several times in the last two decades (Taberlet *et al*., 1998; Hewitt, 1999; Schmitt, 2009), only a few studies (e.g. Espíndola & Alvarez, 2011; Borer *et al*., 2012) have examined organisms involved in specialized and obligate–antagonistic interactions in a framework of large-scale comparative phylogeography. In the present study, we evaluated and compared the phylogeographical patterns of three Anthomyiid species (i.e. *C. rotundiventris*, *C. lophota*, *C. dentifera*) involved in the specialized nursery pollination of the European globeflower. In contrast to our initial expectation, the spatial genetic structures of the three species were not congruent (Figs 2, 3, 4) and thus each species appeared to have experienced independent and distinct demographic processes (Tables 5, 6).

Chiastocheta rotundiventris is a species that has long been considered the 'most mutualistic' of all those interacting with *T. europaeus* (Pellmyr, 1992). Indeed, in addition to visiting flowers at the beginning of the flowering period (Pellmyr, 1989), this species lays only one egg per flower head, meaning that a lower price is to be paid by the flower in terms of number of larvae to host (Pompanon, Pettex & Després, 2006). Among the three studied pollinators, *C. rotundiventris* appears as the species with the weakest spatial genetic structure, not agreeing with any of the tested phylogeographical hypotheses (Table 5) or models (Table 6), and indicating high levels of admixture between populations (Fig. 2). This could be explained by either: (1) different long-distance dispersal abilities or (2) a scenario of incomplete lineage sorting in which colonization of the current range of this fly originated from one or a few neighbouring regions harbouring large levels of diversity. In Europe, an absence of spatial genetic structure has been also unravelled in other cold-adapted organisms such as *Ligusticum mutellinoides* (Alvarez *et al*., 2009) or *Ranunculus pygmaeus* (Schönswetter, Popp & Brochmann, 2006).

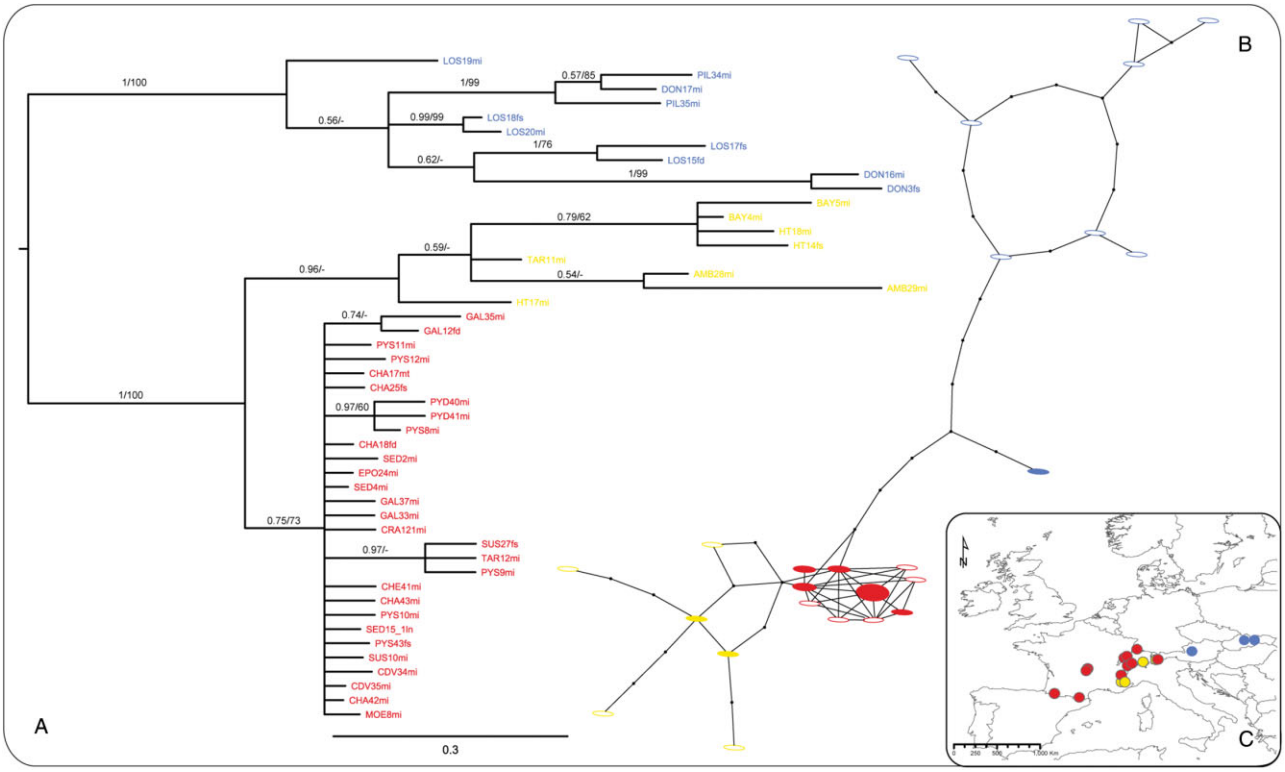

Figure 3. Topological inferences (A, B) and geographical distribution of haplotypes (C) in *Chiastocheta lophota*. A, half-compatible Bayesian phylogeny. Values on branches indicate Bayesian posterior probabilities and maximum likelihood supports. Colours indicate main clades. B, haplotype network. Colours indicate clades identified in (A). Empty circles indicate unique haplotypes; filled circles indicate shared haplotypes. C, geographical distribution of clades. Colours correspond to (A).

Unlike *C. rotundiventris*, *C. lophota* is restricted to the Southern range of the sampled area. Its absence from Northern areas might be explained by the presence of other *Chiastocheta* species (e.g. *Chiastocheta inermella*; Espíndola, 2010) who could be outcompeting *C. lophota* through similar floral choice and exploitation patterns (Pompanon *et al*., 2006). From a phylogeographical perspective, *C. lophota* exhibits indications of demographic contraction (i.e. highest *D* estimates; Table 4) and a strong spatial genetic structure (Fig. 3C), albeit one that did not reject the phylogeographical null hypotheses (Table 5). Additionally, in *C. lophota*, the ABC analysis indicates the divergence model as the best fit to the data (Table 6). Taken together, these results suggest that the clades in this species have been isolated for a long time, with their historical ranges restricted to the Southern European mountains before recently coming into secondary contact. This scenario is supported by the divergence between the Eastern (blue) and Western (yellow and red) clusters (Fig. 3A). Similar spatial genetic structure has also been observed in other arctic–alpine European organisms, such as the butterfly *Erebia epiphron* (Schmitt, Hewitt & Muller, 2006) or the gentian *Comastoma tenellum* (Schönswetter, Tribsch & Niklfeld, 2004).

Finally, diversification in *C. dentifera* appears to be more recent (i.e. short branch lengths and star-like topology) than that observed in *C. lophota*, and the spatial pattern of genetic variation is indicative of geographical structure (Fig. 4C), especially in Scandinavia (red and orange groups), and the Tatra range (purple). However, similar to *C. rotundiventris*, some lineages are widespread, such as the group shown in green. Also, haplotypes appear highly interconnected (Fig. 4B), probably indicating current or recent genetic exchange between populations. This scenario is supported by the results of the ABC analysis, which favours the *n*-island with migration model (Table 6). Finally, unlike the other species, we could not reject any of the alternative hypotheses (Table 5).

The fact that the three species present dissimilar spatial genetic structures indicates that they have distinct demographic histories, and likely have distinct dispersal capabilities, even though they share a common habitat (and thus have similar ecological requirements). Three non-exclusive explanations for this are: (1) that they have responded in very

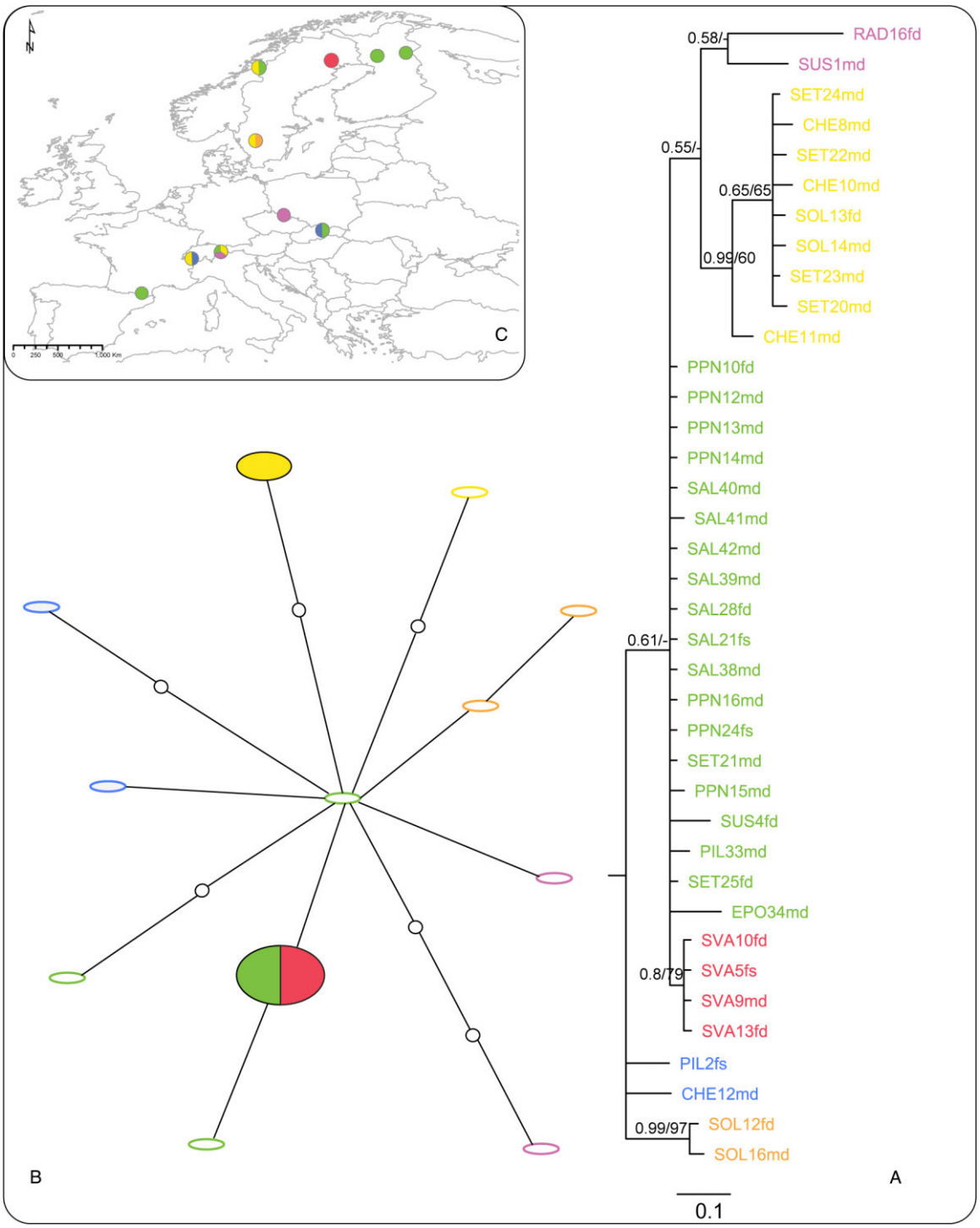

Figure 4. Topological inferences (A, B) and geographical distribution of haplotypes (C) in *Chiastocheta dentifera*. A, half-compatible Bayesian phylogeny. Values on branches indicate Bayesian probabilities and maximum likelihood supports. Colours indicate main groups. B, haplotype network. Colours indicate clades identified in (A). Empty circles indicate unique haplotypes; filled circles indicate shared haplotypes. C, geographical distribution of clades. Colours correspond to (A).

Table 5. Hypothesis testing results for *Chiastocheta dentifera*, *Chiastocheta lophota*, and *Chiastocheta rotundiventris*

	Phylogeographic hypothesis testing							
	H_1		$H_{0(H1)}$		H_2		$H_{0(H2)}$	
	1×10^{-7}	1×10^{-8}	1×10^{-7}	1×10^{-8}	1×10^{-7}	1×10^{-8}	1×10^{-7}	1×10^{-8}
Chiastocheta rotundiventris	$P = 0.84$	$P = 0.40$	$P = 0.99$	$P = 0.26$	$P = 0.96$	$P = 0.28$	**< 0.001**	$P = 0.38$
Chiastocheta lophota	**< 0.001**	**< 0.001**	$P = 0.39$	**< 0.001**	**< 0.001**	**< 0.001**	$P = 0.55$	**< 0.001**
Chiastocheta dentifera	$P = 0.76$	$P = 0.39$	**< 0.001**	$P =$ **0.002**	$P = 0.77$	$P = 0.52$	**< 0.001**	**< 0.001**

P-values and levels of significance are indicated for each species, scenario, and mutation rate (μ) considered. Values in bold indicate models significantly different from real data.

Table 6. Model comparison results for *Chiastocheta dentifera*, *Chiastocheta lophota* and *Chiastocheta rotundiventris*

	Posterior probability			
	Divergence	Expansion	Contraction	*n*-islands + m
Chiastocheta rotundiventris	0.3	0.34	0.34	0.02
Chiastocheta lophota	**1.0**	0	0	0
Chiastocheta dentifera	0	0	0	**1.0**

Posterior probabilities are shown for the four compared models. Values in bold indicate the highest unequivocal posterior probabilities obtained for each species.

dissimilar manners to the environmental variations associated to glacial cycles; (2) that interspecific evolutionary processes as resource competition are affecting the capabilities of successful migration and survival, in particular areas already occupied by other *Chiastocheta* (Després & Cherif, 2004; Pompanon *et al*., 2006), or (3) that life-history traits (such as generation time, dispersal capacities, population sizes) intrinsic to each species might be driving the distinct genetic structure of these species. Future investigations should aim to identify species-specific life-history traits and explore how strongly these characteristics affect their phylogeographical history (Alvarez *et al*., 2010).

The effect of the plant on the genetic structure of mutualistic flies

In contrast to our working hypothesis (i.e. congruence in the spatial genetic structures of the mutualistic partners), two of the three pollinator species (*C. rotundiventris*, *C. dentifera*) exhibit phylogeographical patterns that are incongruent with the demographic history inferred from the spatial genetic structure of the host plant. In *C. rotundiventris*, the high admixed diversity makes it unlikely that any phylogeographical hypothesis based on the pattern of diversification could be rejected, suggesting that these results are equivocal at best. For *C. dentifera*, it was not possible to differentiate between the phylogeographical hypotheses constructed on a scenario of congruence with the host plant (Table 5) and the ABC approach identified a genetic structure mainly explained by a set of isolated genetic groups experiencing migration (Table 6). Finally, *C. lophota* shows timing and patterns of diversification fitting the post-LGM one observed in the plant based on the ABC analysis (Table 6), but lacks congruent demographic history when applying statistical phylogeographic analysis (Table 5).

Statistical phylogeography enables us to quantitatively test phylogeographical hypotheses (Knowles & Maddison, 2002). It is thus an ideal tool for identifying drivers of phylogeographical patterns and for explicitly testing phylogeographical models. ABC (Csillery *et al*., 2010) is complementary to phylogeographic hypothesis testing, and allows comparison of phylogeographical models in a Bayesian framework. In our case, this was particularly useful because the data from each species appeared to contain differing amounts of variation, and cases where multiple models are equivocal (e.g. *C. rotundiventris*) indicate that the data lack resolution to discriminate among hypotheses.

Compared to other recent studies on interactions, the present study is the first to explicitly contrast the genetic structures of cold-adapted mutualisms.

Indeed, previous studies included interacting organisms from temperate (*Fagus* and *Epifagus* parasites: Tsai & Manos, 2010; *Arum* and Psychodids: Espíndola & Alvarez, 2011) or desertic regions (*Yucca* and *Yucca* moths: Smith *et al*., 2011; *Euphorbia* and *Araptus*: Garrick *et al*., 2013). By contrast to the findings of Espíndola & Alvarez (2011), we identify some congruent phylogeographical signals in at least some species pairs using an ABC approach (*C. lophota* and the host-plant) and, unlike Smith *et al*. (2011), we could not observe similar phylogeographical patterns in all pollinators. Our results also partially agree with the observations of Tsai and Manos (2010) and Garrick *et al*. (2013), although those previous studies investigated parasitic interactions, and thus their phylogeographical expectations differed. In this framework, we demonstrate that there is much variation across interacting systems and species. From a more general perspective, we show the potential of applying explicit phylogeographical testing when studying the phylogeographical patterns of interacting organisms, and indicate the need to move forward from the traditional visual comparison of spatial genetic structure when assessing interspecific phylogeographical congruence.

Conclusions

The results of the present study indicate that three insect species tightly linked to their host plant in an ecological setting (Pellmyr, 1992): (1) have experienced distinct demographic processes, leading to different phylogeographical patterns (Figs 2, 3, 4) and (2) are not equally congruent with the plant's spatial genetic structure and history (Tables 5, 6). Although it was not possible to identify likely phylogeographical scenarios for *C. rotundiventris* (Fig. 2, Tables 5, 6), *C. lophota* exhibited a genetic structure compatible with a divergence scenario similar to that of the plant (Table 6) and *C. dentifera* had a genetic structure likely explained by a set of isolated genetic groups experiencing migration (Table 6).

In the case of *T. europaeus* and its *Chiastocheta* flies, one explanation for those contrasting results may be the biological features of the interaction. Because all fly species exploit the plant carpels for larvae development, there is high interspecific competition (Després & Jaeger, 1999) and a strong evolutionary pressure towards niche differentiation (Pompanon *et al*., 2006). Such behavioural and developmental differences can potentially lead to the establishment of contrasted population dynamics, dispersal capabilities, generation times, and population sizes, which are key factors in the definition of the genetic signature left by range changes in species. In the *Trollius–Chiastocheta* interaction, it is possible that the different ecological and biological features displayed by each species are producing the very different genetic signals we identified in the present study. Unfortunately, only little information is currently available on such developmental and population parameters in both *Chiastocheta* spp. and *T. europaeus*, such that the effect of such variation on the phylogeographical histories of the partners cannot be explicitly examined. From a coevolutionary perspective, the complete phylogeographical congruence between the interacting species could be expected only under a situation of strict mutualistic coevolution across the studied range. However, such a situation might not be realistic, and we might rather expect a case of a coevolutionary mosaic, in which species experience different levels of coevolution at different locations (Thompson, 2005). Under such a scenario, we could expect species interacting at each population to respond differently to environmental variation, which could also lead to the pattern identified in the present study. To our knowledge, no empirical or theoretical study has investigated the (co-)phylogeographical expectations under the presence of geographic mosaics of coevolution. In the future, such questions need to be more thoroughly explored using explicit simulations, analytical approaches, and tests on empirical data.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Locations, geographical coordinates, number of sampled flies in each visited location, and host plant genetic group.