Journal of Biogeography (J. Biogeogr.) (2015) 42, 630–640

ORIGINAL ARTICLE

# Decoupled post-glacial history in mutualistic plant–insect interactions: insights from the yellow loosestrife (*Lysimachia vulgaris*) and its associated oil-collecting bees (*Macropis europaea* and *M. fulvipes*)

Yann Triponez[1*†], Nils Arrigo[2†], Anahí Espíndola[3] and Nadir Alvarez[2]

[1]*Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, CH – 2000 Neuchatel, Switzerland,* [2]*Department of Ecology and Evolution, University of Lausanne, CH – 1015 Lausanne, Switzerland,* [3]*Department of Biological Sciences, University of Idaho, Moscow, ID 83843, USA*

*Correspondence: Yann Triponez, Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, CH – 2000 – Neuchatel, Switzerland.
E-mail: yann.triponez@bluewin.ch

†These authors contributed equally to the present work and are considered as joint first authors.

## ABSTRACT

**Aim** We take a comparative phylogeographical approach to assess whether three species involved in a specialized oil-rewarding pollination system (i.e. *Lysimachia vulgaris* and two oil-collecting bees within the genus *Macropis*) show congruent phylogeographical trajectories during post-glacial colonization processes. Our working hypothesis is that within specialized mutualistic interactions, where each species relies on the co-occurrence of the other for survival and/or reproduction, partners are expected to show congruent evolutionary trajectories, because they are likely to have followed parallel migration routes and to have shared glacial refugia.

**Location** Western Palaearctic.

**Methods** Our analysis relies on the extensive sampling of 104 Western Palaearctic populations (totalling 434, 159 and 74 specimens of *Lysimachia vulgaris*, *Macropis europaea* and *Macropis fulvipes*, respectively), genotyped with amplified fragment length polymorphism. Based on this, we evaluated the regional genetic diversity (Shannon diversity and allele rarity index) and genetic structure (assessed using STRUCTURE, population networks, isolation-by-distance and spatial autocorrelation metrics) of each species. Finally, we compared the general phylogeographical patterns obtained.

**Results** Contrary to our expectations, the analyses revealed phylogeographical signals suggesting that the investigated organisms demonstrate independent post-glacial trajectories as well as distinct contemporaneous demographic parameters, despite their mutualistic interaction.

**Main conclusions** The mutualistic partners investigated here are likely to be experiencing distinct and independent evolutionary dynamics because of their contrasting life-history traits (e.g. dispersal abilities), as well as distinct hubs and migration routes. Such conditions would prevent and/or erase any signature of co-structuring of lineages in space and time. As a result, the lack of phylogeographical congruence driven by differences in life-history traits might have arisen irrespective of the three species having shared similar Pleistocene glacial refugia.

**Keywords**
**Co-diversification, comparative phylogeography, genetic diversity, isolation-by-distance, mutualism, plant–insect interactions, population network, spatial autocorrelation, Western Palaearctic.**


http://wileyonlinelibrary.com/journal/jbi
doi:10.1111/jbi.12456


Journal of Biogeography

## INTRODUCTION

Plant–insect mutualisms offer unique opportunities to explore co-diversification processes (Bronstein, 1994). In particular, interactions between angiosperms and pollinating insects have led the evolution of floral morphology and physiology, as well as sensitive cues and mouthpart morphologies in insects (Krenn & Szucsich, 2005). The extent to which those coevolutionary processes are reflected in phylogenies is, however, highly variable among groups, depending not only on the tightness of the interaction but also on the evolutionary scale considered. For example, phylogenies of fig trees (genus *Ficus*) and their highly specialized pollinating wasps show evidence of co-cladogenesis at large phylogenetic scales. Here, the timing and position of lineage splits in the interacting plants and insects correlate more than expected by chance (Machado *et al.*, 2005; Rønsted *et al.*, 2005). In contrast, host shifts are more frequent within *Ficus* sections (e.g. *Galoglychia*; Renoult *et al.*, 2009), where co-cladogenesis becomes less frequent. Evolutionary processes occurring at shorter temporal scales, such as those happening at the intraspecific level (often referred to as the phylogeographical level), can also lead to co-cladogenesis. This is particularly true when plant–insect interactions evolve under climatic conditions that remain relatively stable through time and when biogeographical boundaries are strong enough to be shared by mutualistic partners, regardless of their respective dispersal abilities (Hoberg & Brooks, 2008). This is, for example, the case in the Joshua tree, *Yucca brevifolia*, and its associated yucca moth pollinators in the Mojave Desert (Smith *et al.*, 2011), as well as between the carnivorous plant *Roridula* and its hemipteran mutualist *Pameridea* (Anderson *et al.*, 2004). However, differences in life-history traits such as generation time, population sizes and dispersal abilities can cause mutualistic partners to evolve under different demographic regimes (Alvarez *et al.*, 2010). Such contrasts differentially affect the respective phylogenies or phylogeographies of the mutualistic partners and decrease the correlation of their spatial genetic structure (e.g. see Borer *et al.*, 2012 for a simulation study). This assumption is even stronger when considering organisms inhabiting high latitudes or elevations – characterized by high Pleistocene and Holocene climate-change velocity – that were selected for their aptitude to track available habitats and thus demonstrate high dispersal abilities. In plant–insect antagonisms distributed in the Western Palaearctic, and thus subject in the past to periods of rapid climate change, patterns of phylogeographical congruence have been found to be highly variable depending on the characteristics of the system and the dispersal abilities of the interacting species. For instance, the pollination antagonism involving lords-and-ladies plants (*Arum maculatum*) and psychodid flies were found to have disconnected phylogeographical histories, with the plant, but not the insect, displaying a north-west/south-east structuring of genetic variation (Espíndola & Alvarez, 2011). The absence of spatial genetic structure in the flies could be reasonably foreseen given that they harbour a combination of life-history traits driving high dispersal rates (i.e. several generations per year, population sizes reaching several thousands of individuals per hectare, ability to fly over dozens of kilometres). In contrast, the phytophagous alpine beetle *Oreina gloriosa* and its host plant *Peucedanum ostruthium* were found to have congruent phylogeographical histories when analysed at a coarse spatial scale (Borer *et al.*, 2012). Here, these flightless, poor-dispersing insects and their host plant are both spatially constrained by main biogeographical boundaries, in particular high mountain massifs, which shape congruent spatial structuring of genetic variation.

In contrast to those antagonistic interactions, one might argue that specialized mutualistic interactions should display a higher level of phylogeographical congruence, as each species largely relies on the co-occurrence of its partner for survival and/or reproduction. So far, however, comparative phylogeography of plants and insects interacting in a mutualistic manner has rarely been investigated in areas showing strong spatial gradients in climate (but see Espíndola *et al.*, 2014).

Here, we examine the extent to which species involved in specialized pollination mutualisms demonstrate congruent phylogeographies. We focus on oil-rewarding pollination, a strategy identified in 11 plant families worldwide (Renner & Schaefer, 2010; Schäffler *et al.*, 2012) and of putatively ancient origin (Michez *et al.*, 2008), involving highly specialized oil-collecting bees (Hymenoptera) from the Melittidae and Apidae families (Renner & Schaefer, 2010). We particularly focus on a mutualism involving the host plant yellow loosestrife, *Lysimachia vulgaris*, a Eurasian temperate Myrsinaceae, and its associated pollinators, *Macropis europaea* and *Macropis fulvipes*, two sister species within the Melittidae bee family. Whereas the specificity of this interaction is relaxed for the plant (i.e. they have other pollinators), *Macropis* species obligatorily depend on oil rewards to feed their larvae (Vogel, 1976). Here, we analyse the large-scale genetic variation in populations collected from the Western Palaearctic range of both the plant and the bees in order to: (1) document the respective phylogeographies of the plant and its associated insect species; (2) assess whether the mutualistic partners show similar phylogeographical signatures – and test the hypothesis that mutualisms are associated with parallel dispersal routes and overall congruent phylogeographies of interacting partners; and (3) discuss the results in the light of how the Western Palaearctic (post-)glacial history and evolutionary regimes have imprinted the genetic diversity of our focal organisms.

## MATERIALS AND METHODS

### Specimen collections

Plants and insects were sampled during the flowering periods of 2007 and 2008 (Table 1). The leaves of three to five specimens of *L. vulgaris* were collected in 95 European

**Table 1** Collection sites, with population code, geographical coordinates, country and number of specimens collected for each investigated species.

| Population | Latitude (N) | Longitude (E) | Country | *Lysimachia vulgaris* | *Macropis europaea* | *Macropis fulvipes* |
|---|---|---|---|---|---|---|
| JOH | 47.500 | 14.549 | Austria | 3 | — | — |
| RUC | 46.591 | 14.567 | Austria | 5 | 5 | — |
| BAU | 50.489 | 3.822 | Belgium | — | 3 | — |
| WAM | 50.110 | 5.435 | Belgium | 4 | — | — |
| RIL | 42.102 | 23.091 | Bulgaria | 4 | 4 | — |
| TUL | 42.574 | 25.576 | Bulgaria | 4 | 2 | — |
| LOK | 45.395 | 14.691 | Croatia | 5 | — | — |
| VRH | 45.334 | 13.920 | Croatia | 5 | 2 | — |
| HOS | 49.687 | 12.582 | Czech Republic | 4 | 5 | — |
| SUC | 49.486 | 16.762 | Czech Republic | 4 | — | 4 |
| LIS | 56.234 | 10.167 | Denmark | 5 | — | — |
| MAR | 54.769 | 11.506 | Denmark | 4 | 5 | — |
| UGG | 57.587 | 10.142 | Denmark | 5 | 1 | — |
| BAZ | 47.680 | −0.206 | France | 5 | 5 | — |
| BDL | 45.771 | 6.242 | France | 5 | 2 | — |
| BIG | 42.640 | 9.549 | France | 5 | — | — |
| BOR | 45.440 | 2.439 | France | 5 | — | — |
| BOV | 49.855 | 2.378 | France | 5 | 4 | — |
| CAP | 44.301 | −0.255 | France | 5 | 4 | — |
| COX | 47.973 | 2.316 | France | 5 | — | — |
| JUG | 48.405 | −2.325 | France | 5 | 1 | — |
| MAV | 49.421 | 0.533 | France | 5 | 1 | — |
| ROL | 47.951 | 5.257 | France | 5 | — | — |
| SCA | 43.606 | 4.336 | France | 4 | 3 | — |
| BAK | 41.765 | 43.483 | Georgia | — | — | 4 |
| KHA | 41.999 | 43.656 | Georgia | 5 | — | 4 |
| KOB | 41.858 | 41.786 | Georgia | 4 | — | — |
| TKI | 42.377 | 43.037 | Georgia | — | — | 1 |
| DIP | 50.919 | 13.681 | Germany | 5 | 5 | 1 |
| HOL | 53.368 | 9.642 | Germany | 5 | 2 | — |
| LEM | 51.723 | 6.995 | Germany | 4 | 3 | — |
| MUM | 48.601 | 8.192 | Germany | 5 | — | — |
| SCH | 53.711 | 10.838 | Germany | 5 | — | — |
| TRO | 51.683 | 11.041 | Germany | 5 | 2 | 3 |
| WOL | 48.421 | 10.599 | Germany | 5 | 4 | — |
| BEE | 55.001 | −3.721 | Great Britain | 5 | — | — |
| BIL | 51.022 | −0.478 | Great Britain | 4 | — | — |
| CLE | 51.429 | −2.832 | Great Britain | 4 | — | — |
| HEL | 53.457 | −2.683 | Great Britain | 2 | — | — |
| DIO | 47.932 | 19.062 | Hungary | 4 | 4 | — |
| HAJ | 47.695 | 21.665 | Hungary | 5 | 2 | — |
| HOD | 46.356 | 20.209 | Hungary | 4 | 4 | — |
| KBA | 46.660 | 17.126 | Hungary | 5 | 4 | — |
| AGH | 54.521 | −6.314 | Ireland | 5 | — | — |
| CLO | 53.332 | −7.980 | Ireland | 5 | — | — |
| AND | 46.504 | 11.238 | Italy | 5 | — | — |
| BAG | 44.312 | 8.048 | Italy | 5 | — | 1 |
| CAS | 43.777 | 10.628 | Italy | 5 | 5 | — |
| MAT | 41.406 | 14.406 | Italy | 5 | — | — |
| RIE | 42.510 | 12.753 | Italy | 3 | 4 | — |
| SOA | 45.209 | 10.734 | Italy | 4 | — | — |
| VIV | 45.441 | 8.006 | Italy | 5 | 5 | — |
| DZE | 56.655 | 24.933 | Latvia | 4 | 5 | 3 |
| LAU | 57.178 | 22.692 | Latvia | 3 | 3 | 5 |
| KOZ | 41.056 | 21.036 | Macedonia | 5 | 4 | — |
| LOO | 51.611 | 5.076 | Netherlands | 5 | — | — |
| TER | 53.394 | 5.313 | Netherlands | 5 | — | — |

**Table 1** Continued

| Population | Latitude (N) | Longitude (E) | Country | *Lysimachia vulgaris* | *Macropis europaea* | *Macropis fulvipes* |
|---|---|---|---|---|---|---|
| HAL | 59.130 | 11.489 | Norway | 5 | — | — |
| LAS | 59.170 | 10.178 | Norway | 4 | — | — |
| NOR | 61.395 | 7.284 | Norway | 4 | — | — |
| BOB | 53.947 | 16.599 | Poland | 4 | — | — |
| KET | 49.844 | 19.214 | Poland | 4 | 5 | 1 |
| FEL | 46.701 | 23.590 | Romania | 5 | — | 3 |
| FRA | 47.548 | 25.765 | Romania | 5 | — | — |
| OIT | 46.067 | 26.372 | Romania | 5 | — | — |
| PAS | 47.712 | 23.777 | Romania | 5 | — | 1 |
| DES | 44.042 | 21.537 | Serbia | 4 | 5 | 2 |
| KRU | 43.105 | 22.688 | Serbia | 5 | 3 | — |
| DOM | 49.016 | 21.673 | Slovakia | 5 | 1 | 5 |
| SLO | 48.746 | 19.241 | Slovakia | 5 | — | — |
| GJE | 45.727 | 14.408 | Slovenia | 4 | 4 | — |
| ARI | 42.991 | −3.975 | Spain | 4 | — | — |
| ASP | 42.715 | −1.158 | Spain | 5 | 4 | — |
| BEC | 40.403 | −5.627 | Spain | 5 | — | 4 |
| CAE | 42.221 | 3.105 | Spain | 5 | — | — |
| CAM | 42.226 | −2.627 | Spain | 5 | — | — |
| CER | 42.947 | −4.492 | Spain | 5 | — | — |
| CUE | 40.192 | −2.113 | Spain | 5 | 4 | — |
| HOR | 39.361 | −4.615 | Spain | 4 | — | — |
| LOZ | 40.902 | −3.863 | Spain | 3 | 3 | 2 |
| MES | 38.487 | −2.358 | Spain | 5 | — | — |
| MIN | 41.974 | −8.737 | Spain | 5 | 2 | — |
| PIE | 39.048 | −4.239 | Spain | 4 | — | 5 |
| REN | 42.504 | −4.729 | Spain | — | 3 | — |
| RIO | 40.234 | −6.646 | Spain | 5 | — | — |
| SAL | 40.975 | −5.671 | Spain | — | 4 | — |
| SAN | 42.115 | −6.734 | Spain | 4 | — | 5 |
| SON | 42.974 | −3.805 | Spain | — | — | 4 |
| ZAO | 40.978 | −2.155 | Spain | 5 | — | — |
| BOK | 56.403 | 13.600 | Sweden | 5 | 2 | — |
| LJU | 58.802 | 15.452 | Sweden | 4 | 5 | — |
| BAL | 47.402 | 9.619 | Switzerland | 4 | 5 | — |
| BEG | 46.427 | 6.242 | Switzerland | 4 | 4 | — |
| BOL | 46.161 | 8.863 | Switzerland | 4 | 3 | — |
| MON | 46.234 | 7.338 | Switzerland | 5 | 4 | — |
| PRE | 47.785 | 7.429 | Switzerland | 5 | — | — |
| VAU | 47.190 | 7.318 | Switzerland | — | — | 4 |
| ABA | 40.601 | 31.282 | Turkey | 5 | — | — |
| BEL | 38.264 | 34.291 | Turkey | 5 | — | — |
| DAD | 41.481 | 33.603 | Turkey | 5 | — | 4 |
| DAG | 39.574 | 39.864 | Turkey | 4 | — | — |
| ERF | 41.859 | 34.744 | Turkey | — | — | 4 |
| ILG | 41.140 | 34.064 | Turkey | — | — | 4 |
| YAY | 38.061 | 28.773 | Turkey | 5 | — | — |

populations (434 specimens in total) and dried in silica gel. Oil-collecting bees were sampled from 62 populations with the help of Christian Schmid-Egger, Denis Michez and Lucas Bassin, resulting in a total of 159 and 74 specimens for *M. europaea* and *M. fulvipes*, respectively. The bee specimens were caught whenever blossoming and weather conditions favoured pollinator activity and were preserved in 70% ethanol. *Macropis* species were identified based on morphology, according to Michez & Patiny (2005); see Appendix S1 in Supporting Information for the legal status of the bees in the study area.

### DNA extraction and AFLP fingerprinting

Plant DNA was extracted from 10 mg of silica-dried leaf fragments using the DNeasy Plant Kit (Qiagen, Hilden, Germany). Insect DNA was extracted from wing muscles (using the Qiagen DNeasy Blood & Tissue extraction Kit), to exclude DNA contamination from pollen grains stuck to the exoskeletons of the bees. All specimens were genotyped with amplified fragment length polymorphisms (AFLP), following Vos *et al.* (1995) with minor modifications. Both for

*L. vulgaris* and *Macropis* spp., a primer trial on a subset of samples from different origin was conducted using 12 (for plants) and six (for bees) different primer combinations to identify pairs of selective primers that were sufficiently repeatable and polymorphic. The final selective PCR products were analysed with an automated capillary-sequencer (ABI 3730, Applied Biosystems, Foster City, CA, USA; service provided by Macrogen Inc., Seoul, South Korea). The resulting electropherograms were analysed with the software PEAK SCANNER 1.0 (Applied Biosystems, Foster City, CA, USA) in order to detect and measure the size of AFLP bands, using default parameters. The presence/absence of AFLP bands was recorded for each specimen with RAWGENO 2.0-1 (Arrigo *et al.*, 2009), an automated R CRAN library (R Core Team, 2014), following recommendations (scoring range = 100–500 bp, minimum bin width = 1 bp, maximum bin width = 2 bp, minimum fluorescence intensity = 100 rfu, minimum reproducibility threshold = 80%) by Arrigo *et al.* (2012). The results of two primer pairs were combined to produce the respective AFLP matrices of *Lysimachia vulgaris* (*Eco*RI–ACA/*Mse*I–CTA and *Eco*RI–ATA/*Mse*I–CAC) and *Macropis* spp. (*Eco*RI–ACA/*Mse*I–CAA and *Eco*RI–ACA/*Mse*I–CGA; the two *Macropis* species were scored separately). All reactions were performed on 96-well PCR plates, in which specimens were randomly distributed. Ten to 15 specimens of each plate were replicated in order to evaluate reproducibility of results. The AFLP datasets were deposited in Dryad (doi:10.5061/dryad.p4n0j).

### Genetic diversity

We first estimated regional genetic diversity using a sliding window approach following Arrigo *et al.* (2010). Briefly, plant and insect specimens were assigned to the cells of a predefined 200-km resolution geographical grid, permitting estimation of cell-level diversity statistics (i.e. rarity index and Shannon information criterion; Ehrich, 2006). A jackknife resampling procedure was applied to account for heterogeneous sampling effort among cells (estimates were obtained by 1000 resamplings of five specimens per cell). Diversity statistics were estimated separately for *L. vulgaris*, *M. europaea* and *M. fulvipes*.

### Phylogeographical structure and population networks

The respective phylogeographical structures of the plant and insects were explored using the admixture model implemented in STRUCTURE 2.3.2.1 (Falush *et al.*, 2007). This algorithm uses a Bayesian framework to calculate the posterior probability of each specimen belonging to $K$ genetic groups ($K$ being defined *a priori* and ranging from 1 to 20, with 10 replicates being performed for each parameter setting) while optimizing Hardy–Weinberg equilibrium within groups. The program relies on a Markov chain Monte Carlo (MCMC) heuristic, set here to 1,000,000 steps to estimate model parameters. The final results were collected after removing the initial 500,000 steps as burn-in. We followed recommendations of Evanno *et al.* (2005) to optimize the number of groups used for reflecting the phylogeographical structure of each organism. Specimens were then assigned to each phylogeographical group using the majority-rule criterion, and summarized at the population level. Results were mapped (using the R CRAN package MAPS; Dyer & Nason, 2004) and phylogeographical congruence between species was quantified at the population level using the mean genetic structure similarity index (i.e. GSS; Alvarez *et al.*, 2008) and tested for significance using chi-square tests. To this end, we compared the frequencies of individuals assigned to each genetic cluster for the plant and the insects and tested whether these were drawn from the same distribution. $P$-values higher than 0.05 indicate a similar phylogeographical structure. The chi-square tests used STRUCTURE assignments for $K = 2$ when comparing the plant and *M. europaea* (best $K$ in both cases), and both $K = 2$ (best $K$ for *L. vulgaris*) and $K = 3$ (best $K$ for *M. fulvipes*) when comparing the plant and *M. fulvipes*.

We further investigated the respective phylogeographical structures of our focal organisms using population networks (Dyer & Nason, 2004), as implemented in the R CRAN package POPGRAPH 1.0 (Becker *et al.*, 2013). This approach displays populations and their pairwise genetic covariance as the nodes and edges of a network, respectively. Here, the number of displayed edges is minimized using dimensional reduction techniques, so that only edges that are the most representative of the overall covariance are retained. These were then displayed on maps, analysed using connectivity statistics (i.e. betweenness, defined as the number of minimal-length paths transiting through each population) and compared statistically among species (i.e. Pearson correlation among betweenness values and number of shared edges, computed from graphs including only locations shared by the compared organisms). Only populations with at least four specimens were included in the network analysis.

### Isolation-by-distance and spatial autocorrelation

The spatial genetic structure of species was further evaluated using isolation-by-distance and spatial autocorrelation. Both metrics were estimated separately for each investigated species. Isolation-by-distance was measured at the population level as a regression slope between pairwise $G_{ST}$ differentiation indexes and Euclidean geographical distances. Pairwise $G_{ST}$ were estimated with custom R scripts (available in Dryad, doi:10.5061/dryad.p4n0j), using a variance partitioning procedure based on the Shannon information index. Autocorrelation patterns were investigated with Moran's $I$, using SPAGEDI 1.4b (Hardy & Vekemans, 2002), in order to estimate the maximal geographical extent over which spatial autocorrelation was significant. Significance levels were assessed by permuting specimens among distance classes (999 permutations).

## RESULTS

### Regional genetic diversity

A total of 496, 119 and 83 polymorphic AFLP markers were retrieved for *L. vulgaris*, *M. europaea* and *M. fulvipes*, respectively. On average, each specimen yielded 173 (*L. vulgaris*), 85 (*M. europaea*) and 56 (*M. fulvipes*) distinct AFLP bands with an average error rate of 6%.

Regional diversity patterns differed markedly between species (Fig. 1). The rarity index outlined geographical areas enriched in rare alleles, with locations varying by species. For *L. vulgaris*, rare alleles were chiefly located in eastern Turkey/southern Caucasus (Fig. 1a), while for the two *Macropis* species (Fig. 1b, 1c) rare alleles were located mostly in Central Europe and around the Black Sea.

Complementary insights were retrieved from the Shannon information index. In *L. vulgaris*, several areas with increased diversity were detected in the Iberian Peninsula, the southern Balkans and southern Caucasus (Fig. 1d). The diversity of *M. europaea* (Fig. 1e) was geographically homogeneous, except for Spain and the Baltic region, which held the least and most diversified populations, respectively. The patterns detected for *M. fulvipes* were in better agreement with those described earlier, with areas of increased diversity being mainly located along a 2000-km-long belt extending from Germany to Turkey (Fig. 1f).

### Phylogeographical structure

According to the $\Delta K$ statistic (see Appendix S2), the optimal number of $K$ genetic groups was two for both *L. vulgaris* and *M. europaea* and three for *M. fulvipes* (Fig. 2).

The plant showed a low degree of genetic structure (Fig. 2a), with one of the two identified groups extending across Europe (blue group), whereas the other prevailed in the south-eastern distribution of the species (red). Similarly, the population network revealed that most *L. vulgaris* populations were closely related to each other. Accordingly, the obtained network extended over the complete range and connected populations belonging to distinct STRUCTURE groups across suture zones (e.g. Balkans).

The groups detected in *M. europaea* outlined a fuzzy phylogeographical structure (Fig. 2b) orientated along a west–east gradient. One group (blue) prevailed in the Iberian and Italian peninsulas, whereas the other (red) occurred mostly in the central and eastern range of the study area. These eastern and western genetic pools meet along a large suture zone spread from western France to Denmark in the north, to northern Balkans in the south. The population network revealed a poorly defined differentiation pattern, with many edges connecting the eastern and western genetic pools.

Finally, the genetic structure of *M. fulvipes* differed from that of *L. vulgaris* and *M. europaea*, with three well-defined

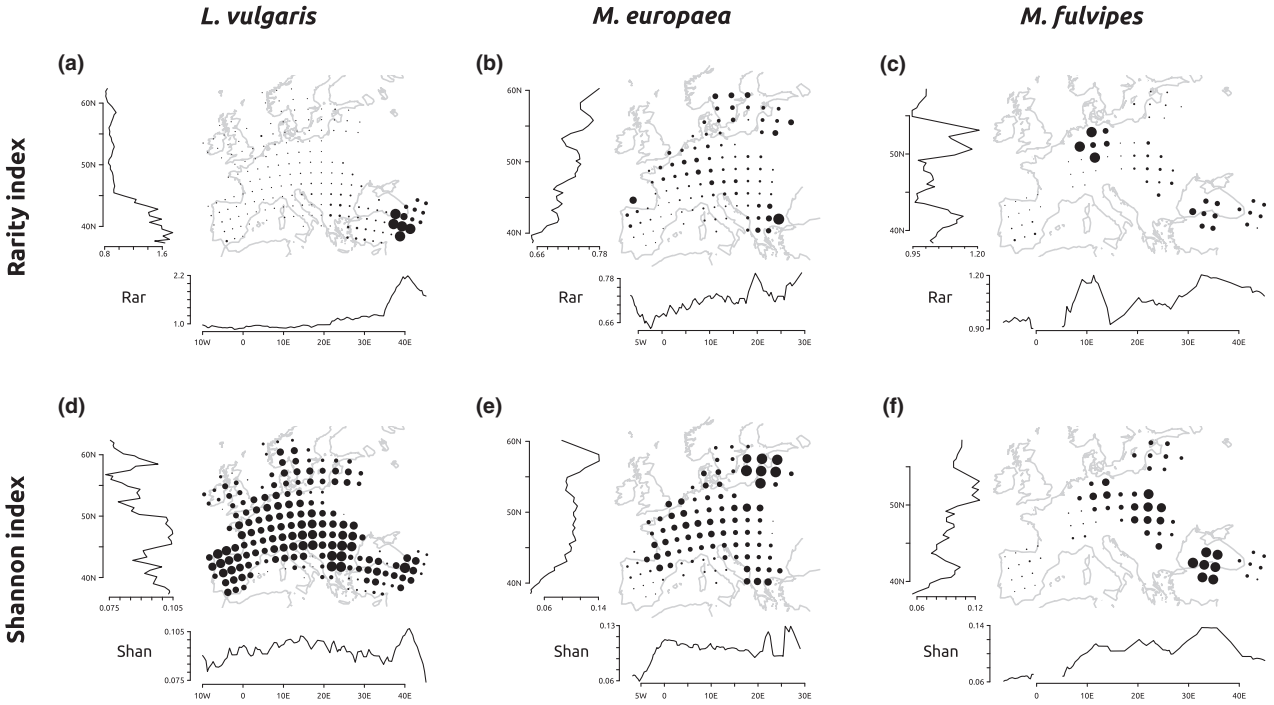


**Figure 1** Regional genetic diversity of *Lysimachia vulgaris*, *Macropis europaea* and *M. fulvipes*, computed from AFLP datasets. Distribution of rarity (a–c) and Shannon (d–f) diversity indexes, estimated on a 200-km resolution grid, using a rarefaction procedure correcting for sampling effort heterogeneity (diversity indexes are estimated by resampling five specimens per cell). Results are overlaid on geographical maps (with cell centroids shown as dots with diameter proportional to regional diversity) and averaged into latitudinal/longitudinal projections.

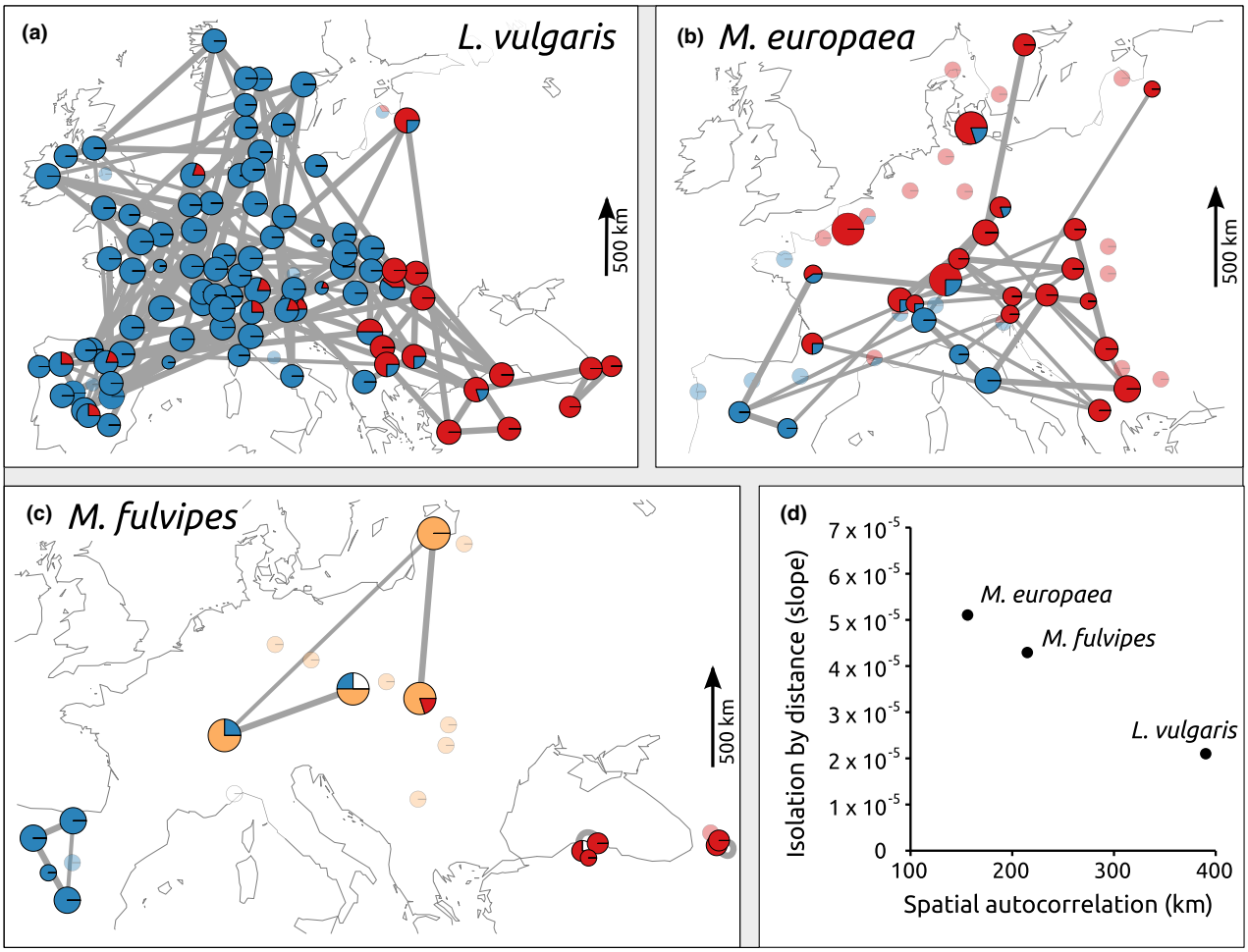


**Figure 2** Phylogeographical structure and population networks. Genetic clustering for (a) *Lysimachia vulgaris*, (b) *Macropis europaea* and (c) *M. fulvipes* estimated with STRUCTURE (optimal number of *K* groups identified following Evanno *et al.*, 2005). The proportion of specimens assigned to each cluster (using a majority-rule criterion) is shown with different colours and for each population as a pie chart. The white section of the pie charts indicates unassigned specimens. Results from population networks are shown as connections among populations. These represent the covariance relationships among populations that are representative of each dataset, at a 5% significance level. Note that only populations with at least four specimens were included in this analysis (the remaining populations are shown with transparent colours). Pie-chart sizes represent the network connectivity of each population, estimated using the 'betweenness index'. (d) Isolation-by-distance and spatial autocorrelation analyses. For each dataset, we estimated the regression slope of genetic (i.e. $G_{ST}$, based on the Shannon diversity index) over geographical distances among populations, as well as the maximal geographical extent over which significant spatial autocorrelation is detected (Moran's *I* among specimens, 5% significance level assessed from 1000 permutations).

groups showing consistent geographical clustering (Fig. 2c). Accordingly, one group (blue) prevailed in the Iberian Peninsula, another group (orange) clustered specimens from Central Europe and the last group (red) occurred mostly in the south-eastern distribution of the species. The population network confirmed these results and outlined connections similar to the clustering detected with STRUCTURE.

### Comparative phylogeography, insights from network connectivity, isolation-by-distance and spatial autocorrelation

The three investigated species differed markedly in the spatial distribution of their genetic structure. Thus, no significant association was detected using chi-square tests, between the respective phylogeographical signals of *L. vulgaris* and its associated pollinators (none between *L. vulgaris* and *M. fulvipes* – as well as weak genetic structure similarity indices equivalent to congruence levels obtained from random datasets – data not shown; Table 2). In addition, the respective population betweenness values and the network topologies of the three species were not congruent (i.e. non-significant Pearson correlation among betweenness values and only two network edges being shared by *L. vulgaris* and *M. europaea*; Table 2).

Isolation-by-distance and spatial autocorrelation metrics further outlined the distinctness of genetic structures among species (Fig. 2d, and see Appendix S3). The largest geographical extent of spatial autocorrelation and respectively the

**Table 2** Phylogeographical congruence among the investigated organisms, quantified according to population graphs and statistics based on STRUCTURE group assignments. $N_p$, number of shared populations among the compared organisms.

| Pairwise comparison | Edges ($N_p$)* | Corr. ($P$-value)* | GSS ($N_p$)† | $\chi^2$ (d.f.)‡ | $P$-value‡ |
|---|---|---|---|---|---|
| *Lysimachia vulgaris – Macropis europaea* | 2 (31) | −0.1 (0.62) | 0.46 (43) | 132.27 (42) | $2.80 \times 10^{-11}$ |
| *Lysimachia vulgaris – Macropis fulvipes* | 0 (11) | 0 (1) | 0.33 (17) | 51.98 (16) | $1.11 \times 10^{-5}$ |

*Population graph analyses assessing whether migration routes (Edges – number of shared network edges) or dispersal hubs (Corr. – Pearson correlation among the connectivity metrics of each population, i.e. betweenness) are shared among the compared organisms.

†Average genetic structure similarity (GSS) index, computed among STRUCTURE runs yielding $K = 2$ groups (considering the best run for the plant).

‡Congruence of STRUCTURE assignments among organisms. Computed from results obtained according to the most likely $K$ value of each species (i.e. $K = 2$, 2 and 3, for *L. vulgaris*, *M. europaea* and *M. fulvipes*, respectively). $H_0$ assumes congruent phylogeographical signals among the compared organisms.

lowest isolation-by-distance slope occurred in *L. vulgaris*. In contrast, *M. europaea* showed the lowest spatial autocorrelation extent and the strongest slope of isolation-by-distance. Finally, *M. fulvipes* showed intermediate values for these statistics.

## DISCUSSION

We compared the phylogeographical signals of a plant and two insect species involved in a mutualistic pollination interaction within the Palaearctic region. We expected the three species to display a trend towards overall phylogeographical congruence, as the presence of interacting partners increases the chances to survive (for the bees) and/or reproduce (for the plant). However, we found low levels of phylogeographical congruence between *L. vulgaris* and each of its mutualistic partners, despite the highly specialized oil-based diet of the investigated *Macropis* species and the specific pollination features displayed by *L. vulgaris* (Vogel, 1976). These results are in agreement with the relaxed nature of this mutualistic interaction, characterized by varying levels of interdependence between the pollinators and their host plant. For instance, although *M. europaea* feeds exclusively on *L. vulgaris*, *M. fulvipes* collects oil on other native *Lysimachia* species [e.g. *L. punctata*, which is, however, more frequent outside the investigated area (Michez & Patiny, 2005; Dötterl & Schäffler, 2007; Bassin *et al.*, 2011), and exceptionally on *L. nummularia* when the latter is nearby *L. vulgaris* populations (Bassin *et al.*, 2011)]. Further, *L. vulgaris* does not exclusively interact with *Macropis*, as it is also occasionally visited by bees within the genus *Lasioglossum* (Bassin *et al.*, 2011), as well as by hoverflies (Syrphidae; Bassin *et al.*, 2011). In addition, this plant species can reproduce vegetatively at its northern distribution margin (Vogel, 1976) and sustains some levels of autogamy (Simpson & Neff, 1983).

Although the *Lysimachia–Macropis* mutualism may not be fully obligate, phylogeographical decoupling could theoretically also arise in specialized and obligate mutualistic interactions, owing to differences in historical/demographic context and evolutionary processes that we discuss here. First, the historical and demographic background of each species might differ substantially from that of its interacting partner. For instance, the host plant *L. vulgaris* might have survived in glacial refugia, either together with its mutualist *Macropis* partners, or without them, relying on alternative pollinators. In such a scenario, these organisms would have achieved their respective post-glacial expansions through different routes. Although our approach has limited power to test this hypothesis, we note that all the investigated species show a longitudinal structuring of their genetic diversity. Accordingly, the Iberian Peninsula and the eastern margin of Europe appear as clear diversity and differentiation hotspots in *L. vulgaris* and *M. fulvipes*, while a corresponding west–east structure gradient is detected in *M. europaea*. These signatures are compatible with several well-documented European glacial refugia (Hewitt, 1999, 2011) and suggest that the investigated species could at least have shared two distinct refugia (i.e. the Iberian Peninsula and south-eastern Europe). An additional refugium located further east (in Anatolia, probably with *L. punctata* or *L. verticillaris* as host plants), might have also been involved in the history of *M. fulvipes*.

Second, we argue that the absence of clear congruent phylogeographical structures can reflect differences in life-history and life-cycle traits among the investigated species. Such differences would impact on the major evolutionary forces classically considered at the population scale (drift, migration, mutation and selection), and thus lead to distinct spatial genetic structures in each species. Here, we expect drift and migration regimes to be the major driver of patterns recovered in our AFLP datasets. In contrast, mutation and selection are assumed to have played a secondary role in the recovered differentiation patterns and are not discussed here, i.e. the shallow evolutionary time frame we consider provides limited opportunities for differential fixation of newly arisen alleles, and most AFLP loci are considered neutral (only 2–10% of AFLP loci are outlined as putative selection candidates using $F_{ST}$ outlier tests, according to Bierne *et al.*, 2011). As an example of the effect of life-history traits on demographic processes, we can consider the case of differing effective population sizes. All else being equal, differing effective population sizes among the plant and its associated oil-collecting bees would lead to distinct levels of genetic drift, and thereby different degrees of shared ancestral

polymorphisms, eventually reflected in incongruent phylogeographical signals. From the current literature, arthropods tend to have larger effective population sizes than land plants (Waples *et al.*, 2013). Yet, solitary bees show population diversities that are in the same order of magnitude than those of land plants (Graur, 1985), potentially because hymenopterans generally have smaller effective populations than other insects (Graur, 1985; Packer *et al.*, 2005). Field evidence indicates that *L. vulgaris* and *Macropis* species have comparable census population sizes (Y. Triponez, pers. obs.), suggesting that these organisms might have effective population sizes of similar magnitude.

In contrast, our results point towards contrasted migration regimes in *L. vulgaris* and both *Macropis* species. Indeed, the evaluation of the range occupied by the genetic clusters of each species (Fig. 2a–c), as well as measures of isolation-by-distance and spatial autocorrelation (Fig. 2d) suggest enhanced dispersal abilities in *L. vulgaris* compared with its associated oil-collecting bees. In addition, population networks suggest that the investigated species follow distinct dispersal hubs and migration routes. The dispersal abilities of *L. vulgaris* are well documented, with evidence of duck (Soons *et al.*, 2008) and cattle (Cosyns *et al.*, 2005) endozoochory. In addition, this species is associated with waterbodies and, as many other riparian species, exploits water-mediated seed dispersal to expand across the main rivers and waterways of Europe (DiTomaso & Healy, 2003). Accordingly, the yellow loosestrife is found invading in several regions of North America, and even listed as a noxious and quarantine weed in a few states where it has spread more intensively (Dillon & Reichard, 2014). Solitary bees are reputed to forage over long distances (Zurbuchen *et al.*, 2010) but the high level of patchiness of their narrowly distributed habitat (bank slopes of dry clay for *Macropis*) might select for features associated with an ecological island syndrome (e.g. see Pellissier *et al.*, 2012), with a high level of site fidelity and a spatially clustered genetic structure. As a result, it is likely that oil-collecting bees display lower migration abilities than their host plant (either because successful migration events are rarer, or occur over smaller spatial scales). Simulations have shown that phylogeographical coupling is quickly erased in such a context (Borer *et al.*, 2012), because the respective genetic diversity of interacting partners gets homogenized across space at different speeds. Finally, *M. fulvipes* appears to have a slightly broader ecological niche than *M. europaea* (Bassin *et al.*, 2011). This feature might explain the contrasted isolation-by-distance and autocorrelation patterns detected between the two bee species (see Appendix S3).

To conclude, the mutualistic partners investigated here are likely to be experiencing distinct and independent evolutionary dynamics as a result of their contrasting dispersal abilities, as well as distinct hubs and migration routes, a situation that would prevent and/or erase any co-diversification signatures. The absence of congruence in the phylogeographical signal driven by differences in life-history traits might arise irrespective of having shared similar glacial refugia. Given the overall incongruence in their phylogeographical patterns, these mutualistic species do not show any evidence of ongoing co-divergence. However, we cannot discard a scenario of geographical mosaic of coevolution, in which species experience varying levels of coevolution at different locations (Thompson, 2005), with adaptations at the local scale being spread or lost along dispersal events.

## ACKNOWLEDGEMENTS

The authors are grateful to R. Arnoux, A. Favre, I. Hiltpold, D. Kharazishvili and B. Prudat for their help during field collections, A. Sarr for support with AFLP analysis, D. Michez, C. Schmid-Egger and L. Bassin for providing additional *Macropis* specimens and Mila Pajkovic for providing helpful remarks on the manuscript. We are also very grateful to M. Aytekin, P. de la Rua, D. Michez, C. Schmid-Egger, M. Schwarz and B. Tomozei for providing information on biodiversity policies for Turkey, Spain, Belgium, Germany, Austria and Romania, respectively (see Appendix S1 for a summary of country-based protection policies for *Macropis* bees). This research was supported by the Swiss National Science Foundation (grant no. 3100A0-116778/1). Field collections were supported by the travel grants commission of the Swiss Academy of Sciences (SCNAT), and by the University of Neuchâtel (grant 'Marguerite Wütrich and A. Mathey-Dupraz'). N. Arrigo and N. Alvarez were funded by a Swiss National Science Foundation Professorship awarded to N. Alvarez (grant no. PP00P3_144870).

## REFERENCES

Alvarez, N., Arrigo, N. & Intrabiodiv Consortium (2008) SIMIL: an R (CRAN) scripts collection for computing genetic structure similarities based on STRUCTURE 2 outputs. *Molecular Ecology Resources*, **8**, 757–762.

Alvarez, N., Kjellberg, F., McKey, D. & Hossaert-McKey, M. (2010) Phylogeography and historical biogeography of obligate specific mutualisms. *The biogeography of host–parasite interactions* (ed. by S. Morand and B. Krasnov), pp. 31–39. Oxford University Press, Oxford, UK.

Anderson, B., Olivieri, I., Lourmas, M. & Stewart, B.A. (2004) Comparative population genetic structures and local adaptation of two mutualists. *Evolution*, **58**, 1730–1747.

Arrigo, N., Tuszynski, J.W., Ehrich, D., Gerdes, T. & Alvarez, N. (2009) Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics*, **10**, 33.

Arrigo, N., Felber, F., Parisod, C., Buerki, S., Alvarez, N., David, J. & Guadagnuolo, R. (2010) Origin and expansion of the allotetraploid *Aegilops geniculata*, a wild relative of wheat. *New Phytologist*, **187**, 1170–1180.

Arrigo, N., Holderegger, R. & Alvarez, N. (2012) Automated scoring of AFLPs using RawGeno v 2.0, a free R

CRAN library. *Methods in Molecular Biology*, **888**, 155–175.

Bassin, L., Alvarez, N., Pellissier, L. & Triponez, Y. (2011) Ecological niche overlap in sister species: how do oil-collecting bees *Macropis europaea* and *Macropis fulvipes* (Hymenoptera: Melittidae) avoid hybridization and competition? *Apidologie*, **42**, 579–595.

Becker, R.A., Wilks, A.R., Brownrigg, R. & Minka, T.P. (2013) *maps*: *Draw Geographical Maps*. R package version 2.3-6. Available at: http://CRAN.R-project.org/package=maps.

Bierne, N., Welch, J., Loire, E., Bonhomme, F. & David, P. (2011) The coupling hypothesis: why genome scans may fail to map local adaptation genes. *Molecular Ecology*, **20**, 2044–2072.

Borer, M., Arrigo, N., Buerki, S., Naisbit, R. & Alvarez, N. (2012) Climate oscillations and species interactions: large-scale congruence but regional differences in the phylogeographic structures of an alpine plant and its monophagous insect. *Journal of Biogeography*, **39**, 1487–1498.

Bronstein, J. (1994) Our current understanding of mutualism. *The Quarterly Review of Biology*, **69**, 31–51.

Cosyns, E., Claerbout, S., Lamoot, I. & Hoffmann, M. (2005) Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecology*, **178**, 149–162.

Dillon, K. & Reichard, S.H. (2014) Effect of temperature on the seed germination of garden loosestrife (*Lysimachia vulgaris* L.). *Natural Areas Journal*, **34**, 212–215.

DiTomaso, J.M. & Healy, E.A. (2003) *Aquatic and riparian weeds of the West*. Agriculture and Natural Resources, University of California, Oakland, CA.

Dötterl, S. & Schäffler, I. (2007) Flower scent of floral oil-producing *Lysimachia punctata* as attractant for the oil-bee *Macropis fulvipes*. *Journal of Chemical Ecology*, **33**, 441–445.

Dyer, R. & Nason, J. (2004) Population graphs: the graph theoretic shape of genetic structure. *Molecular Ecology*, **13**, 1713–1727.

Ehrich, D. (2006) AFLPdat: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes*, **6**, 603–604.

Espíndola, A. & Alvarez, N. (2011) Comparative phylogeography in a specific and obligate pollination antagonism. *PLoS ONE*, **6**, e28662.

Espíndola, A., Carstens, B.C. & Alvarez, N. (2014) Comparative phylogeography of mutualists and the effect of the host on the genetic structure of its partners. *Biological Journal of the Linnean Society*, **113**, 1021–1035.

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

Falush, D., Stephens, M. & Pritchard, J. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.

Graur, D. (1985) Gene diversity in Hymenoptera. *Evolution*, **39**, 190–199.

Hardy, O. & Vekemans, X. (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hewitt, G.M. (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica*, **139**, 617–638.

Hoberg, E.P. & Brooks, D.R. (2008) A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography*, **35**, 1533–1550.

Krenn, H. & Szucsich, N. (2005) Mouthparts of flower-visiting insects. *Arthropod Structure & Development*, **34**, 1–40.

Machado, C., Robbins, N., Gilbert, M. & Herre, E. (2005) Critical review of host specificity and its coevolutionary implications in the fig/fig-wasp mutualism. *Proceedings of the National Academy of Sciences USA*, **102**(Suppl. 1), 6558–6565.

Michez, D. & Patiny, S. (2005) World revision of the oil-collecting bee genus *Macropis* Panzer 1809 (Hymenoptera: Apoidea: Melittidae) with a description of a new species from Laos. *Annales de la Société entomologique de France (N.S.)*, **41**, 15–28.

Michez, D., Patiny, S., Rasmont, P., Timmermann, K. & Vereecken, J. (2008) Phylogeny and host-plant evolution in Melittidae s.l. (Hymenoptera: Apoidea). *Apidologie*, **39**, 146–162.

Packer, L., Zayed, A., Grixti, J.C., Ruz, L., Owen, R.E., Vivallo, F. & Toro, H. (2005) Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. *Conservation Biology*, **19**, 195–202.

Pellissier, L., Litsios, G., Guisan, A. & Alvarez, N. (2012) Molecular substitution rate increases in myrmecophilous lycaenid butterflies (Lepidoptera). *Zoologica Scripta*, **41**, 651–658.

R Core Team (2014) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Renner, S. & Schaefer, H. (2010) The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 423–435.

Renoult, J., Kjellberg, F., Grout, C., Santoni, S. & Khadari, B. (2009) Cyto-nuclear discordance in the phylogeny of *Ficus* section *Galoglychia* and host shifts in plant-pollinator associations. *BMC Evolutionary Biology*, **9**, 248.

Rønsted, N., Weiblen, G.D., Cook, J.M., Salamin, N., Machado, C.A. & Savolainen, V. (2005) 60 million years of co-divergence in the fig–wasp symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 2593–2599.

Schäffler, I., Balao, F. & Dötterl, S. (2012) Floral and vegetative cues in oil-secreting and non-oil-secreting *Lysimachia* species. *Annals of Botany*, **110**, 125–138.

Simpson, B.B. & Neff, J.L. (1983) Floral biology and floral rewards of *Lysimachia* (Primulaceae). *American Midland Naturalist*, **110**, 249–256.

Smith, C.I., Tank, S., Godsoe, W., Levenick, J., Strand, E., Esque, T. & Pellmyr, O. (2011) Comparative phylogeography of a coevolved community: concerted population expansions in Joshua trees and four yucca moths. *PLoS ONE*, **6**, e25628.

Soons, M., Van Der Vlugt, C., Van Lith, B., Heil, G. & Klaassen, M. (2008) Small seed size increases the potential for dispersal of wetland plants by ducks. *Journal of Ecology*, **96**, 619–627.

Thompson, J. (2005) *The geographic mosaic of coevolution*. University of Chicago Press, Chicago.

Vogel, S. (1976) *Lysimachia*: Ölblumen des Holartiks. *Naturwissenschaften*, **63**, 44–45.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Homes, M., Frijters, A., Pot, J., Peleman, J. & Kuiper, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Waples, R., Luikart, G., Faulkner, J. & Tallmon, D. (2013) Simple life-history traits explain key effective population size ratios across diverse taxa. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20131339.

Zurbuchen, A., Cheesman, S., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010) Long foraging distances impose high costs on offspring production in solitary bees. *Journal of Animal Ecology*, **79**, 674–681.

## SUPPORTING INFORMATION

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

**Appendix S1** Legal status of *Macropis* species in the study area.

**Appendix S2** Goodness of fit and $\Delta K$ statistics associated with the number of groups ($K$) tested throughout the STRUCTURE analyses.

**Appendix S3** Evaluation of the species' spatial genetic structure: isolation-by-distance and spatial autocorrelation.

## DATA ACCESSIBILITY

The dataset and scripts have been deposited in Dryad (doi:10.5061/dryad.p4n0j).

## BIOSKETCH

**Yann Triponez** is an evolutionary biologist specializing in plant–insect interactions. He is currently working as a scientific collaborator at a cantonal agency in charge of forests, nature and landscape conservation in Valais, Switzerland.

Author contributions: Y.T., N.Ar., A.E. and N.Al. conceived the ideas; Y.T. and A.E. collected the data; Y.T. and N.Ar. performed data analyses, Y.T., N.Ar., A.E. and N.Al. wrote the manuscript.

Editor: Liliana Katinas