

# Evolution of pollination niches in a generalist plant clade

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## Summary

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- It is widely assumed that floral diversification occurs by adaptive shifts between pollination niches. In contrast to specialized flowers, identifying pollination niches of generalist flowers is a challenge. Consequently, how generalist pollination niches evolve is largely unknown.
- We apply tools from network theory and comparative methods to investigate the evolution of pollination niches among generalist species belonging to the genus *Erysimum*. These species have similar flowers.
- We found that the studied species may be grouped in several multidimensional niches separated not by a shift of pollinators, but instead by quantitative variation in the relative abundance of pollinator functional groups. These pollination niches did not vary in generalization degree; we did not find any evolutionary trend toward specialization within the studied clade. Furthermore, the evolution of pollination niche fitted to a Brownian motion model without phylogenetic signal, and was characterized by frequent events of niche convergences and divergences.
- We presume that the evolution of *Erysimum* pollination niches has occurred mostly by recurrent shifts between slightly different generalized pollinator assemblages varying spatially as a mosaic and without any change in specialization degree. Most changes in pollination niches do not prompt floral divergence, a reason why adaptation to pollinators is uncommon in generalist plants.

## Introduction

Divergent evolution is characterized by adaptive shifts between ecological niches that represent new ecological opportunities (Schluter, 2000; Nosil, 2012). Pollinators, like other resources, are important dimensions of the ecological niches of plants (Heithaus, 1974; Moldenke, 1975; Parrish & Bazzaz, 1978, 1979; Pleasants, 1980; Kephart, 1983; Armbruster & Herzig, 1984; Armbruster *et al.*, 1994; Ollerton *et al.*, 2007a; Johnson, 2010; Pauw, 2013). Consequently, it is widely assumed that adaptive floral diversification occurs in plants when there is a transition between pollination niches, that is, when a plant species pollinated by one group of pollinators shifts to a different group of pollinators (Wilson & Thomson, 1996; Whittall & Hodges, 2007; Smith *et al.*, 2008; Tripp & Manos, 2008; Harder & Johnson, 2009). Divergence in pollination niches between phylogenetically-related plant species is thereby a pre-condition for the occurrence of such pollinator-mediated floral divergence.

Variation in pollination niches among specialized plants is high, because the pollination niches are defined by a principal pollinator type and any change in this flower visitor is associated

with a change in the pollination system (Fenster *et al.*, 2004; Johnson, 2010). Specialized plant species are considered to be bat-pollinated, bird-pollinated, fly-pollinated, bee-pollinated, etc. (Grant & Grant, 1965; Anderson & Johnson, 2009; Ley & Claßen-Bockhoff, 2009; Martén-Rodríguez *et al.*, 2009; Alcántara & Lohmann, 2010; Wester & Claßen-Bockhoff, 2011; Forest *et al.*, 2014). Changes in specialized pollination niches, produced by a full replacement of the main pollinators, usually causes adaptive pollinator-mediated divergence in floral traits (Goldblatt & Manning, 2006; Whittall & Hodges, 2007; Smith *et al.*, 2008; Tripp & Manos, 2008; Fleming *et al.*, 2009; Knapp, 2010). Unrelated plants belonging to the same specialized pollination niche may even evolve a similar suite of traits adapted to their common pollinator type, the pollination syndromes (Faegri & van der Pijl, 1979; Willmer, 2011).

In contrast to specialized plants, differences between generalist plants in pollinator fauna is mainly quantitative rather than qualitative (Waser, 2001; Castellanos *et al.*, 2003; Wilson *et al.*, 2004; Aigner, 2005; Armbruster & Muchhala, 2009; Kay & Sargent, 2009). Generalized pollinator assemblages differ mostly in their relative abundances (Grant & Grant, 1965; Kephart, 1983;

Wilson *et al.*, 2004) rather than in the identity of floral visitors. There is not a single type of pollinator making most pollination events or acting as the main selective agent of generalist plants. Contrasting with the copious information on specialist systems, how generalist pollination niches evolve and drive floral divergence is largely unknown.

As a consequence of the boom in ecological network studies, there has been in the last decade a renewed interest in exploring the quantitative variation in pollinator fauna among sympatric plant species (Bascompte & Jordano, 2014). These studies have found that between-species quantitative variation in ecological interactions often causes the appearance of compartmentalized communities or modular networks (Olesen *et al.*, 2007; Bosch *et al.*, 2009; Martín González *et al.*, 2012). A modular network can be subdivided into modules, groups of species sharing most of their interactions with other species in the group and a few interactions with species from other groups (Guimerà *et al.*, 2007). In this scenario, two species can be considered to belong to the same interactive niche when they are in the same module (Gómez *et al.*, 2010a). Modularity has proven to be a good quantitative estimate of the interactive niches in many and disparate generalist systems (Gómez *et al.*, 2010a). In particular, this approach has been successfully applied to the study of the variation in the pollination niches in a generalist plant species, *Erysimum mediohispanicum* (Brassicaceae) (Gómez *et al.*, 2014a).

Here, by applying the same analytical procedure, we investigate the variation and evolution of pollination niches along a generalist clade belonging to the genus *Erysimum*. Our major question is to explore whether cladogenesis in this plant genus reflects different pollination niches. The specific objectives of this study are: to identify the pollination niches in this clade of *Erysimum* species; to explore how these niches have evolved along the evolutionary history of *Erysimum*; and to check whether there has been correlated evolution between the identity of the pollination niche and the generalization/specialization degree of the pollinator assemblages.

## Materials and Methods

### Study species

We have studied 40 *Erysimum* species from Western and Central Europe and Northwest Africa (Supporting Information Table S1). They represent >85% of the *Erysimum* species inhabiting this region (Tutin *et al.*, 1964; Polatschek, 1982, 2008; Nieto-Feliner, 1993; Giardina *et al.*, 2007; Abdelaziz *et al.*, 2011). These species inhabit diverse environments, from pure alpine habitats above the treeline in the Alps, Sierra Nevada, Pyrenees and Atlas mountains, to oak and pine forests in Mediterranean mountains, and lowland and coastal habitats in Central Europe and North Africa. The phylogenetic relationships of the studied species have been well established (Fig. S1; Abdelaziz *et al.*, 2011; Gómez *et al.*, 2014b).

*Erysimum* are annual, biennial, short-lived perennial herbs or sub-shrubs. They usually grow for 2–3 yr as vegetative rosettes,

and afterwards produce 1–15 reproductive stalks which can display between a few and several hundred hermaphroditic, hypogynous, ebracteate, slightly protandrous flowers (Nieto-Feliner, 1993). Flowers are very similar among different species, with a pedicel between 4 and 7 mm, four free saccate sepals, and a yellow, orange or purple corolla forming a nonfused tube. The breeding system of *Erysimum* has been studied in a few species, indicating that they are self-incompatible or, when self-compatible, require pollen vectors to produce full seed-set (Bateman, 1956; Gómez, 2005; West, 2008; Lay *et al.*, 2013; Abdelaziz, 2013).

*Erysimum* species studied so far are generalists in their interaction with pollinators (*E. cheiri* Knuth, 1908; *E. badghysi* Pesenko *et al.*, 1980; *E. scoparium* Hohmann *et al.*, 1993, Dupont *et al.*, 2003; *E. teretifolium* McGraw, 2004; *E. mediohispanicum* Gómez *et al.*, 2007, 2014a; *E. capitatum* Alarcón *et al.*, 2008, Lay *et al.*, 2013; *E. nevadense* Ortigosa & Gómez, 2010, Abdelaziz, 2013; *E. baeticum baeticum* Ortigosa & Gómez, 2010; *E. popovii* Fernández *et al.*, 2012). We have recently shown that the pool of species included in this study are also very generalized (Gómez *et al.*, 2014b).

### Pollinator survey

We conducted flower visitor counts in 1–3 georeferenced populations per species in each of 35 species (Table S1; we could not obtain data for pollinators of *E. incanum*, *E. linifolium*, *E. seipkae*, *E. sylvestre* and *E. virgatum*). We visited each population during the peak of the bloom, always at the same phenological stage and between 11:00 h and 17:00 h. Each survey was done by at least three researchers simultaneously, sampling each species for at least 18 person hours. Previous studies in some Iberian *Erysimum* indicate that a sample of 130–150 insects provides an accurate estimate of the diversity of the pollinator assemblages of a population of a site (Gómez *et al.*, 2009; Fernández *et al.*, 2012). Unfortunately, it was impossible to reach this amount of floral visitors in a few species where insects were scarce. However, we kept these species in our study because we observed that their removal did not change our main outcomes. Insects were identified in the field, and some specimens were captured for further identification in the laboratory. Insects included in this study were potential pollinators of *Erysimum* because:

- (1) We only recorded those insects contacting anthers or stigma and doing legitimate visits at least during part of their foraging at flowers (Rosas-Guerrero *et al.*, 2014). We did not record those insects only eating petals or thieving nectar without completing legitimate visits. This means that those insect species not observed contacting the sexual organs of a given *Erysimum* species during the surveys were not included as pollinators of that specific *Erysimum* species. In fact, many of the insects included here have been observed transporting pollen grains on their body (Gómez, 2003; Gómez *et al.*, 2009; Lay *et al.*, 2013).
- (2) They benefit the fitness of several *Erysimum* species by enhancing seed production (Pesenko *et al.*, 1980; Gómez, 2005; Gómez *et al.*, 2007; Ollerton *et al.*, 2007b; Fernández

& Gómez, 2012), decreasing pollen limitation intensity (Gómez *et al.*, 2010b; Fernández *et al.*, 2012; Abdelaziz, 2013), and modifying the plant mating network (Gómez *et al.*, 2011).

- (3) They can even act as selective agents of several *Erysimum* species (Abdelaziz, 2013; Gómez *et al.*, 2006, 2008a,b, 2009, 2014b; Ollerton *et al.*, 2007b; Lay *et al.*, 2011).

We grouped the insects visiting *Erysimum* flowers into functional groups, which are herein defined as comprising insects that interact with the flowers in a similar manner (Fenster *et al.*, 2004). We used criteria of similarity in body length, proboscis length, morphological match with the flower, foraging behaviour and feeding habits (Gómez *et al.*, 2014a). Table 1 describes the 19 functional groups used in this study. These functional groups differ in efficiency. Some functional groups, such as bees, beesflies, hoverflies and butterflies, are highly efficient pollinators of *Erysimum* (Ollerton *et al.*, 2007b; Gómez *et al.*, 2009, 2010b, 2011; Lay *et al.*, 2011; Fernández *et al.*, 2012). By contrast, beetles, flies, moths, bugs, small wasps and ants are low-efficiency pollinators (Knuth, 1908; Korotyaev & Gültekin, 2003; Gómez *et al.*, 2009, 2010b; Lay *et al.*, 2011, 2013; Metspalu *et al.*, 2011; Fernández *et al.*, 2012), although they can be very efficient in some particular *Erysimum* species (Abdelaziz, 2013).

### Discrimination of pollination niches

We determined the occurrence of different pollination niches in our studied populations using bipartite modularity, a complex-network metric. We constructed a weighted bipartite network including the *Erysimum* species with pollinator data. In this network, we pooled the data from the different populations in a species and did not consider the time difference involved in sampling across different species. We subsequently determined the modularity level in this weighted bipartite network by using the QuanBiMo algorithm (formerly QuaBiMo, Dormann & Strauss, 2014). This method uses a Simulated Annealing Monte-Carlo approach to find the best division of populations into modules. A maximum of  $10^{10}$  MCMC steps with a tolerance level =  $10^{-10}$  were used in 100 iterations, retaining the iterations with the highest likelihood value as the optimal modular configuration. We tested whether our network was significantly more modular than random networks by running the same algorithm in 100 random networks, with the same linkage density as the empirical one (Guimerà & Amaral, 2005). Modularity significance was tested for each individual iteration by comparing the empirical vs the random modularity indices using a *z*-score test (Dormann & Strauss, 2014). After testing the modularity of our network, we determined the number of modules using the approach proposed by Newman (2004). We subsequently identified the pollinator functional groups defining each module and the plant species that were ascribed to each module. Modularity analysis was performed using R package bipartite 2.0 (Dormann & Gruber, 2012).

We explored whether the module adscription of a given plant was a consequence of its geographic distribution by testing whether those plant species inhabiting the same geographic

region belonged to the same module. For this, we compared the geographic distances between and within modules by means of a permutational multivariate analysis of variance using distance matrices (permanova), performed with the 'adonis' function in R package vegan 2.0-10 (Oksanen *et al.*, 2013).

Once we had identified the pollination niches, we explored whether they occupied different positions in the pollination niche space. For this, we first tested their differences in the quantitative composition of the pollinator assemblages in terms of pollinator functional groups by means of a permanova, using Morisita–Horn index as distance matrix. Afterward, we calculated their overlap using the Pianka index (Pianka, 1973), which varies between 0 (no overlap) and 1 (complete overlap) (Krebs, 2014). Niche overlap significance was obtained by resampling the dataset 10 000 times and using the RA4 randomization algorithm. The RA4 algorithm is the most adequate when the species involved are super-generalist (Lawlor, 1980; Winemiller & Pianka, 1990). Niche overlap was calculated using the R packages EcoSimR 1.00 (Gotelli & Ellison, 2013) and spaa 0.2.0 (Zhang *et al.*, 2012).

### Relationship between pollination niche and pollinator diversity

We explored whether the pollination niches represent specialization toward subsets of pollinator functional groups or if they are different explorations of the pollination niche space without becoming specialists. For this, we tested the correlated evolution of pollination niche and pollinator diversity. We described the diversity of pollinators using the probability of interspecific encounter (PIE) (Hurlbert, 1971), the probability that two randomly sampled individuals from the pollinator assemblage pertain to different pollinator functional groups. This index has proven very useful to describe the diversity of *Erysimum* pollinator assemblages (Gómez *et al.*, 2014b). It was generated using the 'adpart' function in R package stratigraph (Green, 2012).

We first determined the relationship between the pollination niche of each *Erysimum* species and its generalization degree by means of phylogenetic generalized least square (PGLS) models (Nunn, 2011). In this analysis, lambda was optimized by maximum likelihood while fitting the model. PIE was included as a dependent variable and the module identity as an explanatory variable. To control for spatial dependence, we did a preliminary model including longitude and latitude of each species as additional explanatory variables. Because these two variables were nonsignificant, we re-ran a simpler model removing these variables. The PGLS was performed using the R package caper 0.2 (Orme *et al.*, 2013).

In addition, we performed a phylogenetic ANOVA (Garland *et al.*, 1993) to find the identity of the pollination niches varying in pollination diversity. This analysis was performed using the 'phylANOVA' function in R package phytools 0.3-72 (Revell, 2012). This function does a simulation-based phylogenetic ANOVA and allows for post-hoc tests about the group means.

**Table 1** Functional groups of the insects visiting the flowers of the studied *Erysimum* species

| FG | Functional Group                | Body length    | Resource              | Behavioural notes   | Type of visits            | Order       | Main families and genera   |
|----|---------------------------------|----------------|-----------------------|---|---------------------------|-------------|--|
| 1  | Long-tongued large bees         | ≥ 10 mm        | Nectar                | Partially introducing the head in the flower  | Legitimate                | Hymenoptera | Anthophoridae ( <i>Anthophora</i> ), Apidae ( <i>Apis mellifera</i> , <i>Bombus</i> )  |
| 2  | Short-tongued large bees        | > 10 mm        | Pollen + nectar       | Introducing the whole head in the flower  | Legitimate                | Hymenoptera | Halictidae ( <i>Lasioglossum</i> , <i>Halictus</i> ), Megachilidae ( <i>Osmia</i> ), Colletidae ( <i>Colletes</i> ), Andrenidae ( <i>Andrena</i> )                           |
| 3  | Short-tongued medium-sized bees | 5–10 mm        | Pollen + nectar       | Introducing the whole head in the flower  | Legitimate                | Hymenoptera | Halictidae ( <i>Lasioglossum</i> ), Colletidae ( <i>Hyleaus</i> ), Andrenidae ( <i>Andrena</i> ), Apidae Xylocopinae ( <i>Ceratina</i> ), Apidae Nomidinae ( <i>Nomada</i> ) |
| 4  | Short-tongued small bees        | 2–5 mm         | Pollen + nectar       | Mostly accessing the nectar from between the sepals but also introducing the whole body into the flower to reach the lower anthers and the nectar | Illegitimate + Legitimate | Hymenoptera | Halictidae ( <i>Lasioglossum</i> ), Colletidae ( <i>Hyleaus</i> ), Andrenidae ( <i>Andrena</i> ), Apidae Xylocopinae ( <i>Ceratina</i> ), Apidae Nomidinae ( <i>Nomada</i> ) |
| 5  | Short-tongued extra-small bees  | < 2 mm         | Nectar + pollen       | Introducing the whole body into the flower to reach the lower anthers and also accessing the nectar from between the sepals                       | Legitimate + Illegitimate | Hymenoptera | Halictidae ( <i>Lasioglossum</i> ), Colletidae ( <i>Hyleaus</i> )  |
| 6  | Large ants                      | > 2 mm         | Nectar                | Introducing the whole body into the flower to reach the nectar  | Legitimate + Illegitimate | Hymenoptera | Formicidae ( <i>Formica</i> , <i>Camponotus</i> , <i>Proformica</i> , <i>Cataphypis</i> )  |
| 6  | Small ants                      | < 2 mm         | Nectar                | Mostly nectaring from between sepals  | Illegitimate + Legitimate | Hymenoptera | Formicidae ( <i>Plagiolepis</i> , <i>Leptothorax</i> )   |
| 7  | Large pollen wasps              | Variable       | Pollen                | Partially introducing the head into the flower  | Legitimate                | Hymenoptera | Massarinae ( <i>Ceramius</i> )   |
| 7  | Large nectar-collecting wasps   | > 7 mm         | Nectar                | Partially introducing the head into the flower  | Legitimate                | Hymenoptera | Mainly <i>Polystes</i>   |
| 8  | Small nectar-collecting wasps   | Usually < 3 mm | Nectar                | Mostly nectaring from between sepals  | Illegitimate + Legitimate | Hymenoptera | Chalcidoidea and Ichneumonoidea  |
| 9  | Long-tongued beeflies           | Variable       | Nectar + pollen       | Hovering while nectaring and collecting some pollen   | Legitimate                | Diptera     | Bombyliidae ( <i>Bombylius</i> ), Nemestrinidae  |
| 10 | Short-tongued beeflies          | Variable       | Nectar                | Nectaring without hovering  | Legitimate                | Diptera     | Bombyliidae ( <i>Anthrax</i> )   |
| 10 | Large hoverflies                | > 5 mm         | Pollen                | Collecting pollen without entering the flower   | Legitimate                | Diptera     | Syrphidae (Eristalini)   |
| 10 | Small hoverflies                | < 5 mm         | Pollen + nectar       | Collecting pollen without entering the flower and sometimes nectaring from between the sepals   | Legitimate + Illegitimate | Diptera     | Syrphidae  |
| 11 | Large flies                     | > 5 mm         | Nectar + pollen       | Collecting pollen without entering the flower and nectar from between the sepals  | Legitimate + Illegitimate | Diptera     | Muscidae, Calliphoridae, Tabanidae, Scatophagidae, Anthomyiidae  |
| 12 | Small flies                     | < 5 mm         | Nectar + pollen       | Mostly nectaring from between sepals  | Illegitimate + Legitimate | Diptera     | Muscidae, Anthomyiidae, Micetophyllidae, Empididae, Bibionidae, Drosophilidae, Stratiomyidae   |
| 13 | Florivorous beetles             | > 7 mm         | Pollen + floral parts | Consuming not only pollen, but also anthers, petals and other floral parts  | Legitimate                | Coleoptera  | Mostly Cetonidae   |

Table 1 (Continued)

| FG | Functional Group     | Body length | Resource              | Behavioural notes   | Type of visits            | Order                       | Main families and genera  |
|----|----------------------|-------------|-----------------------|---|---------------------------|-----------------------------|---|
| 13 | Large beetles        | > 7 mm      | Mostly pollen         | Sometimes nectaring from the bottom part of the flower  | Legitimate + Illegitimate | Coleoptera                  | Lagridae, Mylabridae, Alleculinae   |
| 13 | Small beetles        | < 7 mm      | Pollen + nectar       | Consuming pollen during legitimate visits and also robbing nectar from the bottom part of the flowers | Legitimate + Illegitimate | Coleoptera                  | Melyridae (Malachidae, Dasytidae), Cleridae, Oedemeridae, Elateridae, Bruchidae, Buprestidae, Chrysomelidae |
| 13 | Small diving beetles | < 3 mm      | Nectar + Pollen       | Completely entering the flower, crawling down the corolla for nectar                                  | Legitimate                | Coleoptera                  | Nitidulidae, Dermestidae, Phalacridae   |
| 14 | Butterflies          | Variable    | Nectar                | Feeding on nectar both from inside the flower and between the sepals                                  | Legitimate                | Lepidoptera                 | Nymphalidae, Lycaenidae, Papilionidae, Pieridae   |
| 14 | Hawkmoths            | > 7 mm      | Nectar                | Hovering to sip nectar  | Legitimate                | Lepidoptera                 | Sphingidae  |
| 15 | Large moths          | > 3 mm      | Nectar                | Sipping nectar while landed onto the corolla  | Legitimate                | Lepidoptera                 | Crambidae, Noctuidae  |
| 15 | Small moths          | < 3 mm      | Nectar                | Nectaring without entering the flower   | Illegitimate + Legitimate | Lepidoptera                 | Adelidae, Plutellidae   |
| 16 | Bugs                 | Variable    | Nectar                | Nectaring without entering the flower. Also acting as sapsuckers in vegetative tissues                | Legitimate + Illegitimate | Hemiptera                   | Lygaeidae, Pentatomidae ( <i>Eurydema</i> )   |
| 17 | Thrips               | < 3 mm      | Pollen                | Feeding from inside the flowers   | Legitimate                | Thysanoptera                |   |
| 18 | Grasshoppers         | Variable    | Pollen + Floral parts | Mostly nymphs   | Legitimate                | Orthoptera                  |   |
| 19 | Others               | Variable    | Pollen + Floral parts |   | Legitimate + Illegitimate | Raphidioptera<br>Dermaptera |   |

We differentiated 28 functional groups based on behaviour at flowers and morphological fit. However, due to extremely low abundance of some groups, we subsequently pooled them in 19 functional groups identified in the first column ('FG'). All analyses have been performed considering these 19 groups.

To avoid type I error, we used the sequential Bonferroni post-hoc test.

### Exploring the evolution of pollination niche

In order to incorporate phylogenetic uncertainty, all comparative analyses were performed using not just the consensus phylogenetic tree (Fig. S1), but also a set of 6400 phylogenetic trees sampled from the stationary phase of the MCMC Bayesian analysis (see Gómez *et al.*, 2014b for details).

We explored the evolutionary models better describing the evolution of pollination niches using Pagel's lambda (Pagel, 1999; Münkemüller *et al.*, 2012). Pollination niche was considered a multi-state unordered discrete character with as many states as the number of modules that were obtained in the modularity analysis. We compared a model generating a ML estimate of Pagel's lambda for pollination niche with a model constraining lambda to 1 and another model constraining lambda to 0. A significant departure from the model with lambda 1 indicates that pollination niches are not evolving according to a BM model, whereas a significant departure from the model with lambda 0 indicates the occurrence of phylogenetic signal in the evolution of pollination niche (Nunn, 2011). These analyses were

performed using the 'fitDiscrete' command in the R package geiger 1.99-1 (Harmon *et al.*, 2008).

We also estimated the ancestral states of the pollination niches along the phylogenetic tree (Fig. S2). We used an updated version of the 'ancThresh' command implemented in the R package phytools 0.2-14 (Revell, 2012). This function uses Bayesian MCMC to estimate ancestral states and the threshold model to establish the thresholds for a discrete character to change between states (Felsenstein, 2012; Revell, 2014). Under the threshold model, the evolving discrete trait is considered to have a continuous underlying liability (Felsenstein, 2012; Revell, 2014). When the liability exceeds a threshold value, the discretely valued state of the observable character trait changes. We ran four chains of  $10^6$  generations each. The posterior probabilities for each character state at each internal node were obtained with the 'ace' command in R packages ape 3.0-6 (Paradis *et al.*, 2004) and 'anc.Bayes' in phytools 0.2-14 (Revell, 2012).

We explored the instantaneous transition rates between pollination niches along the phylogenetic tree by using the 'MuSSE' (multistate speciation and extinction) model in R package diversitree 0.9-3 (FitzJohn, 2012). In this model, as stated above, pollination niche was considered a multistate unordered discrete character. To explore the probability of the *Erysimum* species

evolving into different pollination niches, we tested the following hypotheses: (1) the transition to any of the modules from the other modules was equal for all modules and not different from zero ( $q_i = q_j = 0$ ); (2) the transition from any of the modules to the rest of the modules was equal for all modules and not different from zero ( $q_i = q_j = 0$ ). These hypotheses were tested by comparing the log-likelihood of the full MuSSE model with that of a set of submodels in which we constrained to zero the transition rates to (hypothesis 1) or from (hypothesis 2) each module (FitzJohn, 2012). We rejected the tested hypothesis when the log-likelihoods of the submodels differed from each other and were significantly larger than the log-likelihood of the full model (Nunn, 2011).

## Results

### Determining pollination niches

We recorded a total 13 724 flower visits in the studied populations from 746 insect species belonging to 99 families and 8 orders (Table S2). We observed visits by the 19 functional groups considered in this study, and most plant species were visited by more than 9 functional groups (Table S2). The most frequent functional groups were beetles (36.3% of the visits), long-tongued large bees (10.9%), ants (9.9%) and beeﬂies (9.1%) (Table S2).

The network between *Erysimum* species and the pollinator functional groups was significantly modular (Modularity =  $0.288 \pm 0.001$ ,  $N = 100$  iterations), because the empirical modularity values differed from random values in all replicates ( $z$ -scores =  $142.39 \pm 0.001$ , all  $P$ -values  $< 0.0001$ ,  $N = 100$  iterations; Table S3). Modularity analysis detected six modules in 96 iterations (Table S3), indicating that this was the most stable configuration. In fact, the modular configuration obtaining the highest likelihood ( $M = 0.293$ ) distributed the *Erysimum* species in six modules (Fig. 1), whereas the three iterations obtaining five modules were also those obtaining the lowest likelihood values (Table S3).

Modules were not associated with a single or a few pollinator functional groups. Rather, plants from every module were visited by most pollinator functional groups (Fig. 1; Table S4). Nevertheless, the frequency of interaction of each pollinator functional group varied among plant modules (Fig. 1; Table S4). Consequently, there was between-module quantitative difference in the composition of the floral visitor assemblages ( $F = 6.76$ ,  $R^2 = 0.54$ ,  $P < 0.001$ ; permanova). Similarly, the observed value of the between-module niche overlap (mean  $\pm 1$  SE =  $0.594 \pm 0.058$ ) was significantly lower than the expected value ( $0.755 \pm 0.001$ ;  $P < 0.0001$ ), despite some modules widely overlapping among them (Table 2). These findings suggest that the modules are associated with different regions of the niche space determined by the pollinator functional groups. The module identity of the *Erysimum* species was consistent across iterations. Only 8 species were assigned to different modules in more than 25% of the iterations: *E. merxmulleri*, *E. bonannianum*, *E. crassistylum*, *E. wilczekianum*, *E. mediobispanicum*, *E. baeticum bastetanum*, *E. popovii* and *E. etnense* (Table S5).

There was no spatial aggregation in the geographic distribution of the modules, because we found that the geographic distances

between and within modules were similar ( $F = 2.08$ ,  $R^2 = 0.26$ ,  $P = 0.155$ ; permanova; Fig. 2).

### Relationship between pollination niche and pollinator diversity

Despite the occurrence of high levels of generalization in the pollination systems of all studied plants, we found that some pollination niches differed in pollination diversity, estimated as the PIE index of the functional groups, both when estimated with PGLS ( $F_{6,27} = 5.32$ ,  $R^2 = 0.40$ ,  $P = 0.001$ ;  $\lambda = 0.0001$ , lambda  $P$  value  $> 0.5$ ) and phylogenetic ANOVA ( $F_{6,27} = 4.22$ ,  $P = 0.009$ ). In particular, according to the *post-hoc* sequential phylogenetically controlled Bonferroni test, pollinator diversity were significantly lower in plant species from module E (Fig. 3). Because the analyses were phylogenetically controlled, this difference was not due to an effect of the relatedness among plant species. All of these findings suggest that, without being extremely specialized, the pollination system of the plants from module E is more specialized than those of the rest of the plants.

### Evolution of pollination niche

Pollination niche evolution was consistent with Brownian motion (BM) evolution, because the likelihood of models where lambda was estimated (log<sub>e</sub>L:  $-54.11 \pm 0.05$ ; AIC:  $116.35 \pm 0.01$ ) did not differ from those where lambda was forced to equal one (log<sub>e</sub>L:  $-57.11 \pm 0.05$ ; AIC:  $116.35 \pm 0.01$ ; all  $P > 0.99$ ,  $N = 6400$  trees).

We did not detect phylogenetic signal in the evolution of pollination niches, as the ML estimate of lambda for module identity (log<sub>e</sub>L:  $-54.11 \pm 0.01$ ; AIC:  $116.35 \pm 0.01$ ) did not differ from trees where lambda was forced to be zero (log<sub>e</sub>L:  $-57.21 \pm 0.01$ ; AIC:  $116.57 \pm 0.01$ ; all  $P > 0.98$ ,  $N = 6400$  trees). As observed in Fig. 4, the pollination modules are scattered all over the *Erysimum* phylogenetic tree.

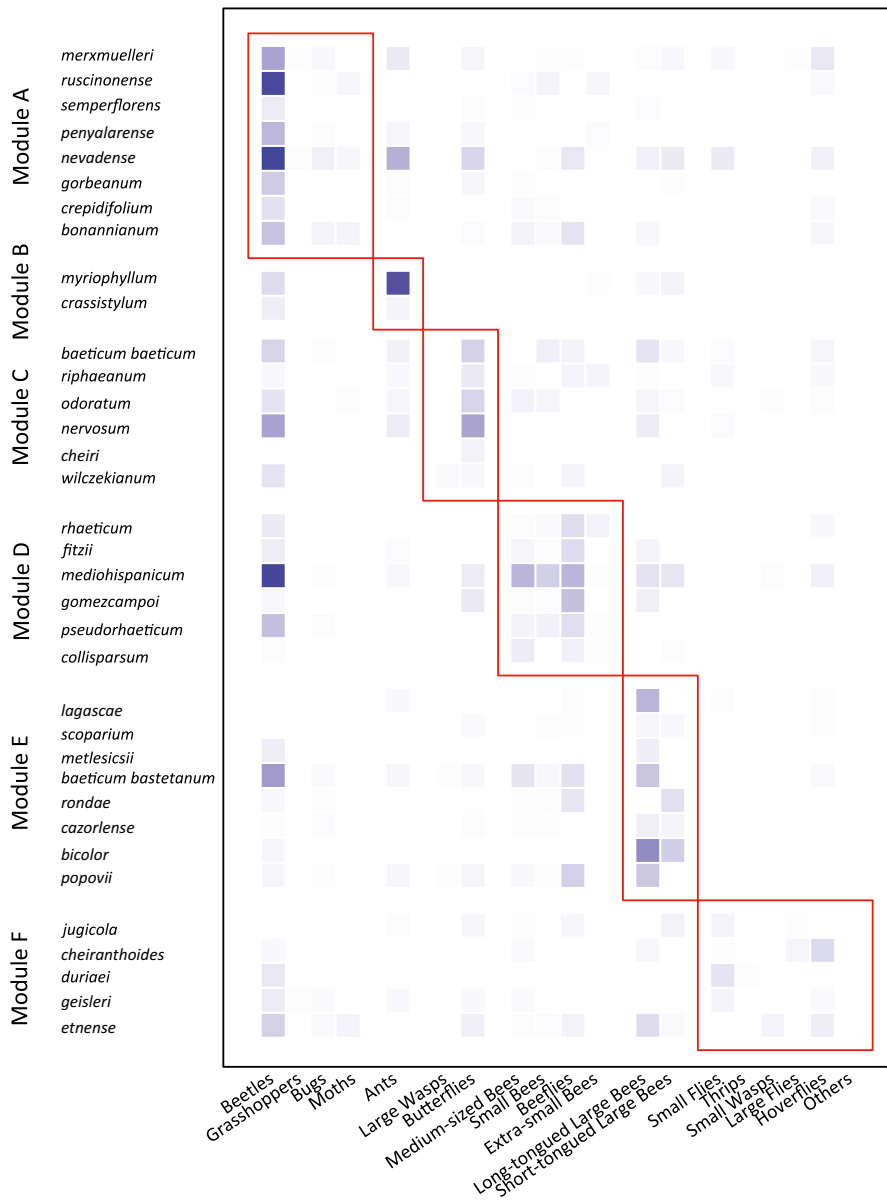
The reconstruction method suggests that the ancestral species could have belonged to any pollination niche (Fig. 4). Nevertheless, the highest posterior probability was obtained for modules E (posterior probability = 0.29) and A (posterior probability = 0.22; Fig. 4, Table S6), whereas the lowest probability was obtained for module B (posterior probability = 0.05). Moreover, it seems that the number of species belonging to module A has increased gradually along the evolutionary history of *Erysimum* (Fig. 4).

Although the instantaneous transition rates were very heterogeneous (Table S7), no transition rate to or from any module was different from zero (Table 3; the null hypothesis cannot be rejected because the AICs of the submodels were larger and the log-likelihoods were smaller than those of the full model).

## Discussion

### Generalist pollination niches in *Erysimum*

We envision the pollination niche space of *Erysimum* as a hypervolume described by  $n$  orthogonal axes, each one representing the



**Fig. 1** Plot showing the classification of the studied species into different pollinator modules, according to the analysis of bipartite modularity QunBiMo. The intensity of the colours indicates the relative abundance of each flower visitor's functional group per species. For more details, see Supporting Information Table S4.

abundance at flowers of a different pollinator functional group (Hutchinson, 1957; Pauw, 2013). Under this perspective, different pollination niches are associated with specific regions within that  $n$ -dimensional niche space. We have used a modularity algorithm derived from complex network theory to detect those pollination niches. Despite the high level of generalization of the *Erysimum* pollination systems (Gómez *et al.*, 2014b), the modularity algorithm has shown that the 35 *Erysimum* species can be grouped into six different pollination niches. That is, different *Erysimum* species may belong to similar pollination niches. The six pollination niches detected in this study occupied different regions of the overall niche space, as indicated by their low niche overlap and significant difference in pollinator composition. These findings suggest that different groups of plant species are exploring contrasting regions of the overall pollination niche space. However, niche segregation was not complete between

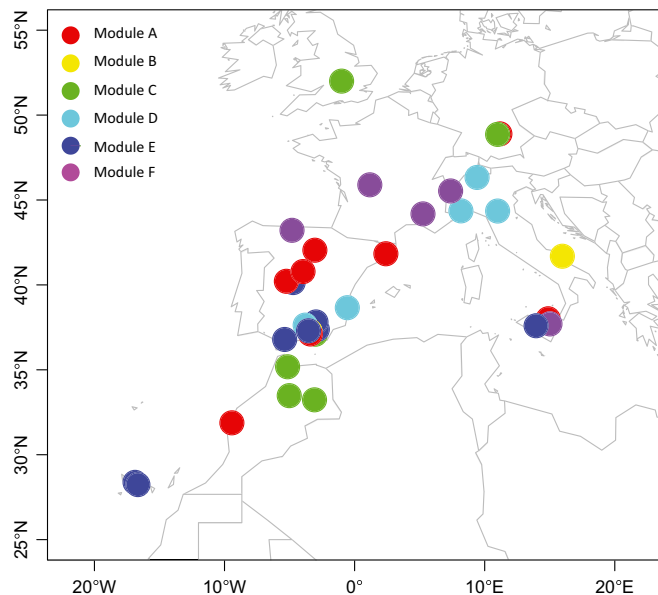
some pollination niches. This outcome is expected, taking into account the rampant generalization in the pollination system of the studied species. In fact, overlap in pollination niches has been found not only between generalist plants (Kephart, 1983; Wiens & Graham, 2005) but also between specialized ones (Muchhala, 2003).

It is widely assumed that specialization in the use of resources has recurrently evolved from generalist strategies and may represent evolutionary dead-ends (Futuyma & Moreno, 1988). In agreement with this idea, the evolution of pollination specialization from generalization has been demonstrated in several plant clades (Manning & Goldblatt, 2005; Pérez *et al.*, 2009). Phylogenetic studies have recently challenged this idea and shown the evolution toward generalization from specialized ancestors not only in plant–pollinator interactions (Armbruster & Baldwin, 1998; Tripp & Manos, 2008; Martén-Rodríguez *et al.*, 2010),

**Table 2** Pianka niche overlap between *Erysimum* pollination niches

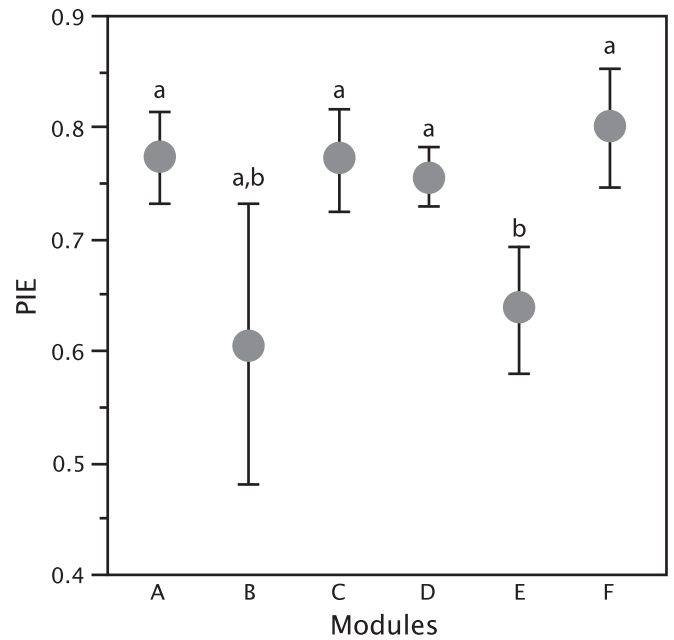
| Between-module pairwise comparisons | Observed niche overlap | Expected niche overlap |                           |                           | Significant overlap |
|-------------------------------------|------------------------|------------------------|---------------------------|---------------------------|---------------------|
|                                     |                        | Mean                   | Lower confidence interval | Upper confidence interval |                     |
| A–B                                 | 0.391                  | 0.206                  | 0.014                     | 0.704                     | No                  |
| A–C                                 | 0.776                  | 0.310                  | 0.064                     | 0.698                     | Yes                 |
| A–D                                 | 0.834                  | 0.291                  | 0.046                     | 0.696                     | Yes                 |
| A–E                                 | 0.630                  | 0.272                  | 0.043                     | 0.687                     | No                  |
| A–F                                 | 0.775                  | 0.356                  | 0.078                     | 0.719                     | Yes                 |
| B–C                                 | 0.331                  | 0.225                  | 0.021                     | 0.673                     | No                  |
| B–D                                 | 0.257                  | 0.222                  | 0.017                     | 0.686                     | No                  |
| B–E                                 | 0.239                  | 0.191                  | 0.013                     | 0.652                     | No                  |
| B–F                                 | 0.285                  | 0.267                  | 0.034                     | 0.659                     | No                  |
| C–D                                 | 0.793                  | 0.318                  | 0.074                     | 0.690                     | Yes                 |
| C–E                                 | 0.646                  | 0.301                  | 0.058                     | 0.704                     | No                  |
| C–F                                 | 0.786                  | 0.416                  | 0.164                     | 0.743                     | Yes                 |
| D–E                                 | 0.689                  | 0.280                  | 0.050                     | 0.703                     | No                  |
| D–F                                 | 0.760                  | 0.382                  | 0.118                     | 0.717                     | Yes                 |
| E–F                                 | 0.720                  | 0.352                  | 0.092                     | 0.689                     | Yes                 |

Significance in overlap is obtained by resampling.



**Fig. 2** Spatial location of the different pollination niches, estimated as the centroid of the populations sampled in a species.

but also in plant–herbivore (Nosil & Mooers, 2005) and parasitic interactions (Stireman, 2005). That is, generalization–specialization transitions may occur in both directions. One feature common to all of these study systems is the co-existence of specialist and generalist species in the same clade. However, all *Erysimum* species studied here are generalist in their interactions with pollinators, with no trend towards increased specialization or generalization (Gómez *et al.*, 2014b). We did not find strong association between pollination niche and generalization. *Erysimum* pollination niches were similarly generalized, with only module E composed of plant species interacting with pollinator assemblages



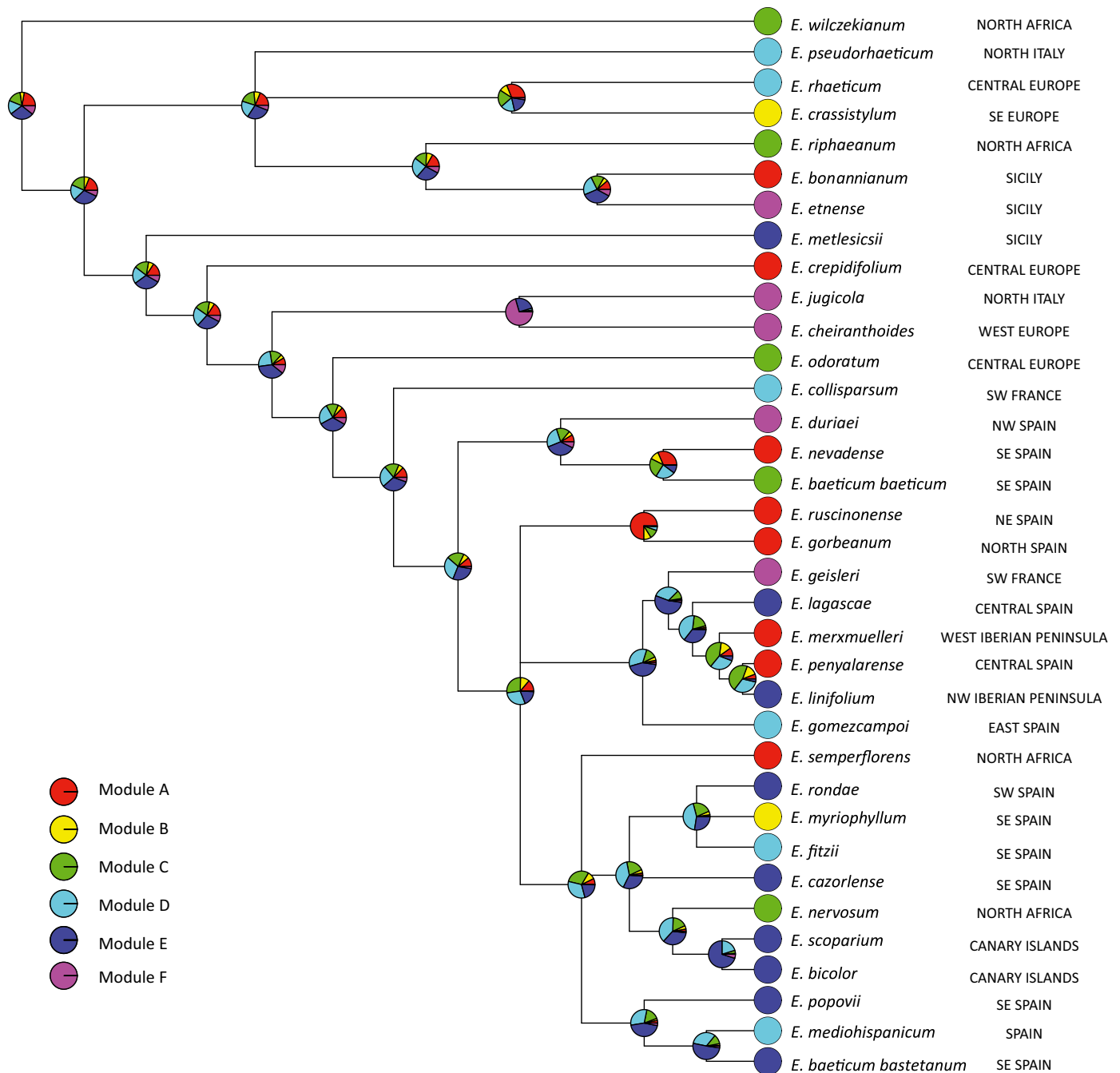
**Fig. 3** Between-pollination niche differences in pollinator diversity. Values of the probability of interspecific encounter (PIE) index with different lowercase letters were significantly different at  $\alpha = 0.05$  according to phylogenetic sequential Bonferroni tests. Error bars are  $\pm 1$  SE.

that had lower diversity. That is, the variation in the position within the niche space occupied by the six pollination niches was due to the variation in the relative abundance of pollinator functional types rather than the specialization to subsets of pollinators.

It is tempting to consider species from different pollination niches as belonging to different pollination syndromes (Danieli-Silva *et al.*, 2012; Martín González *et al.*, 2012). However, *Erysimum* pollination niches cannot be considered equivalent to syndromes. Thus, they were not caused by the replacement of major functional groups of pollinators. In addition, plants from different pollination syndromes differ in floral phenotype (Rosas-Guerrero *et al.*, 2014), but *Erysimum* species belonging to different pollination niches have similar floral traits (Polatschek, 1974, 1979, 2008; Blanca *et al.*, 1992). Nevertheless, further analyses are necessary to determine any relationship between floral traits and pollination niches in this generalist plant clade (J. M. Gómez *et al.*, unpublished).

It is necessary to remark that when describing pollination niches of *Erysimum*, we are assuming that the sampled flower visitor assemblages are the actual pollinator assemblages of the studied plants; that is, the sampling has been exhaustive and the pollinator assemblages does not fluctuate too much between years or populations. Spatio-temporal variation in pollinator assemblages has been recorded frequently (Herrera, 1988; Eckhart, 1992; Traveset & Sáez, 1997; Gómez & Zamora, 1999; Aigner, 2005; Price *et al.*, 2005; Ollerton *et al.*, 2006), and we have even observed between-population intraspecific changes in pollination niches for one *Erysimum* species (Gómez *et al.*, 2014a). We have tried to overcome this possibility by sampling pollinators in more than one population and several years per species. We are aware





**Fig. 4** Ancestral reconstruction of *Erysimum* pollination niches. The module identity of the terminal nodes (present-day species) was considered as a fixed state. Different colours in the pie graphs of the internal nodes represent their posterior probability of belonging to each of the six modules.

that intense sampling is necessary to accurately identify the pollination niches of generalist plants.

#### How do pollination niches evolve in *Erysimum*?

The methodological approach used in this study has been previously used to explore pollination niche variation among populations of *E. mediohispanicum* (Gómez *et al.*, 2014a). The current study indicates that the pattern observed within species extended across species. Johnson (2006, 2010) combined two kinds of

observations – that pollinators differ in effectiveness (Stebbins, 1970) and distribution range (Grant & Grant, 1965) – to suggest that geographic availability of pollinators may lead to mechanical and/or ethological isolation and pollinator-driven divergence. A corollary of this so-called Grant–Stebbins model is a congruence between the distribution of plant ecotypes or species and the distribution of pollinators (Anderson *et al.*, 2009; Van der Niet *et al.*, 2014). However, in *Erysimum*, as probably in many other generalist plants, the distribution of most pollinator functional groups exceeds the spatial location of the pollination niches. The

**Table 3** Outcome of the likelihood ratio tests analysing the difference from zero in the transition rate from any of the modules to a given module (hypothesis 1) or from a given module to the rest of the modules (hypothesis 2)

|   | df | Testing transition to a given module |        |          |         | Testing transition from a given module |        |          |         |
|---|----|--------------------------------------|--------|----------|---------|--|--------|----------|---------|
|   |    | AIC                                  | L      | $\chi^2$ | P value | AIC                                    | L      | $\chi^2$ | P value |
| Full model                                      | 42 | 180.66                               | -48.33 |          |         | 180.66                                 | -48.33 |          |         |
| Model constraining transition rates of module A | 37 | 185.87                               | -55.94 | 15.217   | 0.009   | 171.71                                 | -48.85 | 1.05     | 0.958   |
| Model constraining transition rates of module B | 37 | 181.96                               | -53.98 | 11.305   | 0.045   | 170.65                                 | -48.32 | 0.00     | 0.999   |
| Model constraining transition rates of module C | 37 | 184.84                               | -55.42 | 14.189   | 0.014   | 170.79                                 | -48.39 | 0.13     | 0.999   |
| Model constraining transition rates of module D | 37 | 172.01                               | -49.00 | 12.804   | 0.929   | 179.47                                 | -52.73 | 8.81     | 0.117   |
| Model constraining transition rates of module E | 37 | 190.22                               | -58.11 | 19.566   | 0.002   | 170.90                                 | -48.45 | 0.24     | 0.998   |
| Model constraining transition rates of module F | 37 | 186.10                               | -56.04 | 15.442   | 0.009   | 170.83                                 | -48.42 | 0.18     | 0.999   |

The chi-squares compare the full model with the models constraining to zero the transition rates of each module. Significance occurs only when the log-likelihood of a given model is lower than that of the full model. AIC, Akaike information criterion; L, log-likelihood.

absence of a geographic structure in pollination niches, the non-occurrence of phylogenetic signal, the lack of a clear pattern in the transitions between pollination niches, and the widespread overlap in the distribution of all *Erysimum* species together indicate that the evolution of pollination niches in *Erysimum* is not consistent with the Grant–Stebbins model.

Several features of the evolution of *Erysimum* pollination niches suggest that they have evolved in a way similar to that predicted by the adaptive wandering model (Wilson & Thomson, 1996; Dilley *et al.*, 2000; Wilson *et al.*, 2006; Thomson & Wilson, 2008). Although this model was intoned to explain floral divergence (Wilson & Thomson, 1996), it can also explain the change in pollination niches as a previous step to floral divergence. According to this model, different plant taxa will interact with pollinator assemblages differing quantitatively, in terms of relative abundance of pollinators. Under this scenario, any species spreading over a large region will face different pollination niches (Gómez *et al.*, 2014a). In fact, intra-specific variation in pollination niches is a common feature in many generalist plant species (Herrera, 1988; Inoue *et al.*, 1996; Dilley *et al.*, 2000; Füssel *et al.*, 2007). Some of these new pollination niches will be associated with speciation events, resulting in new species located in new niche positions. Our studies indicate that the variation in generalist pollination niches could occur both at intra- and interspecific levels, probably explaining why some *Erysimum* species were ascribed to more than one niche in our study. In addition, because the ecological requirements of each pollinator functional group are idiosyncratic, the spatial variation in pollinator assemblages will not have a clear geographic pattern. Rather, as observed in *Erysimum*, pollination niches will vary as a mosaic (Grant & Grant, 1965; Gómez *et al.*, 2014a), arising recurrently in different parts of the distribution range and evolutionary history of the genus, and with frequent divergence (sister species belonging to different pollination niches) and convergence (species from different lineages evolving similar niches). Although niche convergence is usually associated with adaptive evolution (Harmon *et al.*, 2005), we believe that in our current study neutral changes in pollinator assemblage compositions predominate. This neutral evolution is probably the reason

why the evolution of pollination niches in *Erysimum* fitted a BM model without phylogenetic signal and lacking any evolutionary trend towards a given trait state (Nunn, 2011). This evolution mode suggests that most generalist pollination niches are evolutionary labile and ephemeral (*sensu* Losos *et al.*, 2003; Losos, 2008).

We presume that the mosaic-like geographic variation in pollination niches will trigger pollinator-mediated fluctuating selection on floral traits (Herrera, 2006). Under these circumstances, only some niche shifts will result in pollinator-mediated phenotypic divergence (Gómez *et al.*, 2014a). Generalist pollination niches will evolve mostly by wandering, being adaptive only occasionally (recall that wandering is always adaptive when there is phenotypic divergence mediated by niche evolution, as suggested by Thomson & Wilson, 2008). We envision a geographic scenario composed of many plant species or populations belonging to pollinator niches that do not prompt floral evolution ('cold niches'), intermingled with a few species or populations belonging to specific pollination niches that cause divergence in plant phenotype ('hot niches'). This scenario would explain the lack of universal adaptation to pollinators in generalist systems.

In brief, our study suggests that the evolution of *Erysimum* pollination niches occurred mostly by recurrent shifts between slightly different generalized pollinator assemblages varying spatially as a mosaic and without much change in specialization degree. We believe that this mode of evolution is common to many other generalist plant clades. Unfortunately, the scarcity of studies on these type of systems precludes reaching any conclusion about how important or widespread this process is for the evolution of plant–pollinator interactions.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Phylogenetic relationships of the studied species.

**Fig. S2** Internal nodes of the phylogenetic tree.

**Table S1** Location and sample size of the studied *Erysimum* species

**Table S2** Frequency of flower visits by each insect functional group

**Table S3** Outcome of the QuanBiMo analysis

**Table S4** Among-modules differences in flower-visitor relative abundance

**Table S5** Consistency of the QuanBiMo analysis

**Table S6** Ancestral states of internal nodes

**Table S7** Instantaneous transition rates between modules

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