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Nina Joffard, François Massol, Matthias Grenié, Claudine Montgelard, Bertrand Schatz. Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids. *Journal of Ecology*, 2019, 107 (1), pp.478-490. 10.1111/1365-2745.13013 . hal-01806945

HAL Id: hal-01806945

<https://hal.science/hal-01806945v1>

Submitted on 18 Nov 2020

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Journal of Ecology

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Article type : Research Article

Handling Editor: Ignasi Bartomeus

Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2745.13013

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Abstract

1. Pollination niches are important components of ecological niches and have played a major role in the diversification of Angiosperms. In this study, we focused on Euro-Mediterranean orchids, which use diverse pollination strategies and interact with various functional groups of insects. In these orchids, we investigated the determinants of pollination niche breadth and overlap by analysing the orchid-pollinator network and the factors that may have shaped it.

2. We constructed a database reporting 1278 interactions between 243 orchid and 773 pollinator species based on a thorough literature review. We then focused on 153 orchid species for which phylogenetic data were available. We used Bayesian phylogenetic mixed models to study the relationship between specialisation (as estimated by the degree and degree in the projected network), pollination strategy and breadths of orchids' spatial and temporal distributions, while correcting for the effect of phylogenetic relationships among orchid species and sampling effort. We then used a singular value decomposition of the orchid-pollinator matrix combined to a redundancy and variation partitioning analyses to investigate the determinants of similarity in pollination niches between orchids.

3. Specialisation was higher in deceptive than in nectar-producing orchids and decreased with the breadth of orchids' spatial distribution. When interactions were considered at the insect family level, similarity in pollination niches between orchids was solely explained by their pollination strategy and phylogeny. By contrast, when they were considered at the insect species level, this similarity was primarily explained by their geographical range and flowering time, although other factors had significant effects as well, with orchids using the same pollination strategy, being closely related and growing in the same habitats sharing more insect species than expected.

4. *Synthesis.* Specialisation in orchid-pollinator interactions depends on orchids' pollination strategy and geographical range. The pool of insect families with which orchids interact depend on their pollination strategy and phylogeny, with consistent associations between some functional or phylogenetic groups of orchids and some families of pollinators. By contrast, the pool of insect species with which orchids interact depend on their spatio-temporal distribution, suggesting that at a finer scale, orchid-pollinator interactions are more opportunistic than previously thought.

Keywords: Plant-insect interactions, network, specialisation, phylogenetic conservatism, geographical range, flowering time, Orchidaceae

Translated abstract (French):

1. La niche de pollinisation est une composante importante de la niche écologique et elle a joué un rôle majeur dans la diversification des Angiospermes. Dans cette étude, nous nous sommes focalisés sur les orchidées Euro-Méditerranéennes, qui utilisent des stratégies de pollinisation diverses et interagissent avec des groupes fonctionnels d'insectes variés. Chez ces orchidées, nous avons exploré les déterminants de la largeur de la niche de pollinisation et du chevauchement de cette niche en analysant le réseau orchidées-pollinisateurs et les facteurs l'ayant potentiellement façonné.
2. Nous avons construit une base de données reportant 1278 interactions entre 243 espèces d'orchidées et 773 espèces de pollinisateurs à l'aide d'une revue de la littérature. Nous nous sommes ensuite focalisés sur 153 espèces d'orchidées pour lesquelles des données phylogénétiques étaient disponibles. Nous avons utilisé des modèles mixtes phylogénétiques bayésiens pour étudier la relation entre la spécialisation (estimée à travers le degré et le degré dans le réseau projeté) d'une part,

et la stratégie de pollinisation et la largeur de la distribution spatiale et temporelle des orchidées d'autre part, tout en corrigeant l'effet potentiel de la phylogénie et de l'effort d'échantillonnage. Nous avons ensuite décomposé la matrice orchidées-pollinisateurs en valeurs singulières et utilisé une analyse canonique de redondance afin d'explorer les déterminants de la similarité de niches de pollinisation entre orchidées.

3. Nos résultats montrent que les orchidées tricheuses sont plus spécialistes que les orchidées productrices de nectar, mais que le degré et le degré dans le réseau projeté augmentent aussi avec la largeur de la distribution spatiale des orchidées. Quand les interactions orchidées-pollinisateurs sont considérées à l'échelle de la famille d'insectes, la similarité de niches de pollinisation entre orchidées s'explique par leur stratégie de pollinisation et leur phylogénie. Au contraire, quand elles sont considérées à l'échelle de l'espèce d'insectes, cette similarité s'explique tout d'abord par l'aire géographique et la période de floraison des orchidées, bien que les autres facteurs (stratégie de pollinisation, phylogénie et habitat) entrent en jeu également.
4. *Synthèse.* A travers cette étude, nous montrons que s'il y a bel et bien des associations préférentielles entre certains groupes fonctionnels ou phylogénétiques d'orchidées et certaines familles d'insectes, à une échelle plus fine, les interactions orchidées-pollinisateurs sont plus opportunistes que décrites jusqu'à présent.

Introduction

Biotic interactions have played a major role in the generation of biodiversity in both the plant and animal kingdoms (Ehrlich & Raven, 1964; Schatz *et al.*, 2017). In particular, Angiosperms have provided ecological niches for pollinators to occupy, while pollinators have acted as selective agents promoting taxonomic and floral diversification in flowering

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plants (Grant, 1949; Stebbins, 1970; Van der Niet, Peakall & Johnson, 2014). Pollination systems can be seen as ecological niches, since pollinators are resources on which plants depend and sometimes compete for (Johnson, 2010; Pauw, 2013). The pollination niche of a plant is primarily defined by its pollinator assemblage (*i.e.* number, identity, relative abundance and efficiency of pollinators with which it interacts), although plants interacting with the same pool of pollinators may have different pollination niches through the divergent use of these pollinators (Pauw, 2013).

One of the major challenges in the study of plant-pollinator interactions is to determine what factors underlie similarity in pollination niches between plants, a question that is relevant both to ecology (*e.g.* to explain plant coexistence) and evolutionary biology (*e.g.* to explain convergent evolution). First, plants with the same spatial and temporal distribution (*i.e.* geographical range and flowering time) will have an increased probability to encounter the same regional pool of pollinators. At a local scale, however, sympatric plants often have distinct pollination niches (Armbruster & Herzig, 1984; Kephart & Theiss, 2004; Botes, Johnson & Cowling, 2008, but see Waser *et al.*, 1996), perhaps revealing a niche partitioning process due to competition (Mitchell *et al.*, 2009; Pauw, 2013). In addition, traits of plants and pollinators may promote (*i.e.* trait-matching hypothesis) or prevent (*i.e.* forbidden links hypothesis) some interactions (Vázquez, Chacoff & Cagnolo, 2009; Nuismer, Jordano & Bascompte, 2013; Dehling *et al.*, 2016). Pollinators often visit plants exhibiting particular suits of floral traits (Junker *et al.*, 2013; Schiestl & Johnson, 2013), and plants with similar phenotypes may thus attract the same pollinators. Conversely, plants relying on the same pollinators for sexual reproduction may undergo the same selective pressures and show evolutionary convergences (Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014). Because traits involved in plant-pollinator interactions are partly the legacy of their ancestors, one may expect a phylogenetic signal in these traits, and thus a phylogenetic conservatism in these

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interactions (Rezende *et al.*, 2007; Gómez, Verdú & Perfectti, 2010). By contrast, in plant groups in which speciation is mainly driven by pollinator shifts, such as sexually deceptive orchids (Peakall *et al.*, 2010; Breitenkopf *et al.*, 2014), closely related plants usually interact with different pollinators (Van der Niet & Johnson, 2012; Van der Niet, Peakall & Johnson, 2014).

In the past few years, ecological communities have increasingly been modelled as networks (Proulx, Promislow & Phillips, 2005; Bascompte & Jordano, 2007; Bascompte, 2010). These networks can be described using several metrics, such as modularity (*i.e.* extent to which they can be divided into weakly connected subsets of strongly connected species: Olesen *et al.*, 2007) or nestedness (*i.e.* extent to which specialists interact with subsets of species with which generalists interact: Bascompte *et al.*, 2003). Network approaches can also be used at larger spatial and temporal scales to address macro-ecological or macro-evolutionary questions (Gómez, Verdú & Perfectti, 2010). For example, Martos *et al.* (2012) and Taudière *et al.* (2015) have applied network tools to data gathered from large-scale bibliographical and molecular analyses to study mycorrhizal associations at the scale of the entire Reunion and Corsica islands, respectively, highlighting the major role of life-history traits and ecological successions in conditioning these associations. By contrast, network tools have seldom been used to characterise pollination niches at large spatial and temporal scales (but see Gómez *et al.*, 2013, 2015), despite the long-standing interest in the factors that may have shaped these niches, especially in taxonomically and functionally diverse plant groups such as orchids.

The Orchidaceae family is the most species-rich family of Angiosperms, with more than 25000 species (Dressler, 2005; The Plant List, 2013). Orchids show an extraordinary diversity of both pollination strategies and floral traits (Darwin, 1862; Jersáková, Johnson & Kindlmann, 2006). In particular, one third of these orchid species are deceptive (Cozzolino &

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Widmer, 2005), *i.e.* they do not offer any reward to pollinators but attract them using cues that pollinators typically associate with a food or sex promise (Jersáková, Johnson & Kindlmann, 2006). Pollinators have played a major role in the diversification of the Orchidaceae family (Inda, Pimentel & Chase, 2012; Breitkopf *et al.*, 2014; Givnish *et al.*, 2015) and are of primary importance for the conservation of most orchid species, which exclusively rely on insects for sexual reproduction (Swarts & Dixon, 2009). While orchid-pollinator interactions are described as extremely specialised in some groups, such as sexually deceptive or euglossine bee-pollinated orchids (Mant *et al.*, 2002; Ramírez *et al.*, 2011), they seem to be much more opportunistic in others, such as food deceptive orchids (Cozzolino *et al.*, 2005), suggesting that pollination niche breadth and overlap may vary among pollination strategies, phylogenetic groups and perhaps biogeographical zones. Orchid-pollinator interactions are well documented in some regions, especially in the Euro-Mediterranean region, where pollinator assemblages of several hundreds of orchids have been described in details (*e.g.* Van der Cingel, 1995; Paulus, 2006; Scopece *et al.*, 2009; Claessens & Kleynen, 2011). In addition, in the past few years, the phylogeny of several genera of Euro-Mediterranean orchids has been resolved (Bateman *et al.*, 2003; Schlüter *et al.*, 2007; Inda, Pimentel & Chase, 2012; Breitkopf *et al.*, 2014) and data on their spatial and temporal distribution are available in the literature (Delforge, 2005; Dusak & Prat, 2010). However, there is currently no study comparing pollinator assemblages of Euro-Mediterranean orchids and investigating the factors that may determine the composition of these assemblages at large spatial and temporal scales.

In this study, we thoroughly reviewed the literature on orchid-pollinator interactions in the Euro-Mediterranean region to construct a database linking 243 orchid and 773 pollinator species and to characterise pollination niches of 153 of these orchid species. Orchids' pollination niches were defined by the number and identity of their pollinators, regardless of

the relative abundance and efficiency of these pollinators. We then investigated the factors affecting pollination niche breadth (*i.e.* which orchids are specialists, which are generalists and why) and overlap (*i.e.* which orchids interact with the same pool of pollinators and why). More specifically, we addressed two main questions: (i) Does specialisation in orchid-pollinator interactions depend on orchids' pollination strategy and distribution? (ii) Does similarity in pollination niches between orchids depend on their pollination strategies, phylogenetic relationships and overlap in ecological and spatio-temporal distributions, and what is the relative importance of these factors?

Materials and methods

Orchid-pollinator interaction matrix

The review of Claessens and Kleynen (2011), reporting 1409 interactions between 298 orchid and 838 pollinator taxa, was used as a starting point to construct the orchid-pollinator interactions dataset, which was then improved in several steps. First, orchid and pollinator names were updated. Second, the following interactions were removed from this dataset: (i) duplicate interactions due to synonymy and interactions for which orchid or pollinator identification was doubtful (213 interactions), (ii) interactions reported in studies published before 1900, as we could not update species names in these cases (177 interactions), (iii) interactions involving pollinator genera or families (42 interactions), (iv) interactions involving orchid subspecies or varieties (16 interactions), (v) interactions observed outside the Euro-Mediterranean region (12 interactions) and (vi) interactions for which pollinia removal was not observed, as visitation may not be a good proxy for pollination (King, Ballantyne & Willmer, 2013), especially in orchids (Schatz, 2006; 11 interactions). Third, 340 orchid-pollinator interactions that were not reported in this dataset were added based on a thorough

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literature review using the Web of Science ® (Thomson Reuters) until December 2016 by searching for published studies containing the name of each orchid species and the terms “pollination” or “pollinators”. We obtained a dataset reporting 1278 interactions between 243 orchid and 773 pollinator taxa gathered from 173 studies published between 1933 and 2016 (Joffard *et al.*, 2018). We then extracted a subset of 1139 interactions involving 153 orchid species for which phylogenetic data were available and 726 pollinator species belonging to 87 families. The 90 orchids that were not taken into account in this dataset were mainly *Ophrys* (82 out of 90 species) and their removal was unlikely to affect our results. Indeed, most of these *Ophrys* do not share any of their pollinators with other orchids, meaning that they would not be taken into account in our analysis of similarity in pollination niches between orchids. A matrix was constructed from this dataset and a network from this matrix using the package *igraph* version 1.2.1 (Csardi & Nepusz, 2006) in R version 3.4.3 (R Core Team, 2017), and this network was graphically represented using GEPHI version 0.9.2 (Bastian, Heymann & Jacomy, 2009).

Explanatory matrices

Pollination strategy matrix. The pollination strategy of each orchid species was extracted from Claessens and Kleynen (2011). Orchid species were assigned to one of the following categories: (i) nectar production, (ii) food deception, (iii) shelter mimicry or (iv) sexual deception (see Table S1).

Phylogenetic correlation matrix. The phylogeny of the 153 orchid species was constructed using a supermatrix approach combining seven genes (de Queiroz & Gatesy, 2007). *Cypripedium calceolus* was chosen as the outgroup as it belongs to the Cypripedioideae subfamily, whereas the other species belong to the more closely related Epidendroideae and Orchidoideae families (Givnish *et al.*, 2015). Seven nuclear (BGP, ITS

and LEAFY), mitochondrial (COX1) and chloroplastic (psbA-trnH, rbcL and rpl16) genes were used to construct this supermatrix. One sequence per species was imported from GenBank (see Table S2 for accession numbers) and aligned using the Muscle algorithm (Edgar, 2004) as implemented in SeaView version 4.4.2 (Gouy, Guindon & Gascuel, 2010) prior to concatenation. This supermatrix was then analysed with both Maximum Likelihood (ML; RAxML version 7.0.3: Stamatakis, 2006) and Bayesian (MRBAYES version 3.1.2: Ronquist & Huelsenbeck, 2003) methods. The best partitioning scheme and model for each partition were chosen using the Bayesian Information Criterion (BIC) as estimated in PartitionFinder version 1.1.1 (Lanfear *et al.*, 2012). ML nodal support was calculated using 1000 bootstrap replicates. Bayesian analyses were conducted with two separate runs of four Markov Chain Monte Carlo (MCMC) chains for 25 million generations with tree sampling every 10000 generations. 25% of the sampled trees were discarded as burn-in and the 75% best scoring trees were used to calculate the consensus tree (see Fig. S1). A correlation matrix was then constructed from this tree, after having added an arbitrary length of 1.10^{-5} to null branch lengths, using the functions *vcv.phylo* and *cov2cor* from the R packages *ape* version 5.0 and *stats* version 3.6.0 (Paradis, Claude & Strimmer, 2004; R Core Team, 2017).

Geographical range matrices. Euro-Mediterranean countries in which each orchid species has been reported to occur were extracted from Delforge (2005) to characterise their spatial distribution. This matrix comprises all the European countries as well as all the countries from the Mediterranean basin, *i.e.* 48 geographical units. In addition, a finer-scale geographical range matrix was constructed by gathering georeferenced records extracted from GBIF for 134 orchid species (*i.e.* species for which more than five records were available). These records were projected onto the Common European Chronological Grid Reference System (CGRS, approximately 50 km × 50 km square grid) across the Euro-Mediterranean region. In order to generate orthogonal spatial predictors from the occupancy patterns of these

134 orchid species, Moran Eigenvector Maps (MEMs) were used (Dray *et al.*, 2006). Vectors obtained using this method aim at representing spatial relationships between sites in our redundancy analysis. MEMs were computed from the occupied grid cells using the function *mem* from the R package *adespatial* version 0.1-1 (Dray *et al.*, 2018). Significant MEMs (with $\alpha = 0.05$) were selected using a Moran permutation test (function *moran.randtest*). Because models explaining the cell-species matrix with significant positive MEMs had higher cumulative R^2 (over 70%) than those explaining this matrix with significant negative MEMs (around 20%), only the 48 first significant positive MEMs were selected (cumulative $R^2 = 45\%$). The cell-species matrix was then projected onto these MEMs to obtain the spatial predictors of each orchid species. In addition, for each orchid species, the area of the minimum convex polygon comprising the occupied grid cells was computed to estimate its extent of occurrence. In order to avoid overestimating this range by including water bodies, only land bodies were considered. Note that these two geographical range matrices were broadly congruent (*i.e.* the georeferenced records extracted from GBIF were located in the countries listed by Delforge). Subsequent analyses were performed using the fine-scale matrix, but the country-level matrix was also used for comparison.

Flowering time matrix. Months of the years in which each orchid species has been reported to flower from the northernmost to the southernmost tips of its geographical range were extracted from Delforge (2005) to characterise their temporal distribution.

Habitat matrix. Habitats in which each orchid species has been reported to occur were extracted from Delforge (2005) to characterise their ecological distribution. Orchid species were assigned to one or several of the following categories: (i) dry grasslands, (ii) mesic grasslands, (iii) wet grasslands, (iv) alpine grasslands, (v) garrigues and maquis, (vi) thermo-Mediterranean scrubs, (vii) woodlands, (viii) forests, (ix) forest edges and clearings, (x)

agricultural lands, (xi) fallows, (xii) urban lands, (xiii) marshes, (xiv) bogs, (xv) seeps, soaks and springs and (xvi) sand-dunes.

Correlation between specialisation and explanatory variables

For each orchid species, the degree (*i.e.* the number of pollinator species with which it interacts) and degree in the projected network (DPN; *i.e.* the number of orchid species with which it interacts through at least one pollinator species) were calculated based on the orchid-pollinator matrix. These two metrics were used as proxies of specialisation, the first describing whether orchids are specialist (low degree) or generalist (high degree), and the second whether they tend to share their pollinators with other orchids (high DPN) or not (low DPN).

Bayesian phylogenetic mixed models (BPMMs), as implemented in the R package *MCMCglmm* version 2.25 (Hadfield, 2010), were used to test the effect of one qualitative (pollination strategy) and two quantitative (breadths of spatial and temporal distributions) explanatory variables on the degree and DPN (*i.e.* Poisson-distributed response variables), while taking into account phylogenetic relationships among orchid species and sampling effort (as estimated by the number of field studies per orchid species) as potential confounding factors. The breadth of orchids' spatial distribution was estimated by their extent of occurrence in km² (see above), while the breadth of their temporal distribution was estimated by the number of months in which they flower. We built a full model including pollination strategy, breadths of orchids' spatial and temporal distributions, sampling effort and some of the interactions between these four variables (*i.e.* interactions between pollination strategy and spatial distribution; pollination strategy and temporal distribution; spatial and temporal distributions; spatial distribution and sampling effort; temporal distribution and sampling effort: see Tables S3 & S4) as fixed effects, and phylogenetic

relationships among orchid species as a random effect. We then built reduced models (always including phylogenetic relationships among orchid species and sampling effort as potential confounding factors) based on this full model, and we compared them based on their Deviance Information Criterion (DIC) values. All models were run for 500 000 iterations, with a thinning interval of 50 iterations and a burn-in of 5000 iterations, using the default priors for the fixed effects (normal distribution with a mean of 0 and a variance of $10E^{10}$) and an inverse-Gamma distribution with $V=1$ and $\nu=1$ for the random effect, using parameter expansion (with $\alpha.\mu=1$ and $\alpha.V=1000$) to speed up the rate of convergence. All models were run multiple times (>2) to check the repeatability of DIC values.

Correlation between similarity in pollination niches and explanatory variables

Using an adaptation of the method of Dalla Riva and Stouffer (2016) for bipartite networks, pollination niches of orchid species were estimated through a singular value decomposition (SVD) of the orchid-pollinator incidence matrix. Pollination niches were defined in the multidimensional space corresponding to the first 102 SVD vectors (*i.e.* the vectors corresponding to the largest 102 singular values of the incidence matrix), which accounted for more than 90% of the inertia (*i.e.* the sum of singular values) of the orchid-pollinator matrix. We thereby obtained a matrix containing scores on these 102 SVD vectors for each orchid species. This method was chosen over other methods, such as the use of module membership (*e.g.* Gómez *et al.*, 2013, 2015), to characterise orchids' pollination niches because it allows a much finer description of the orchid-pollinator matrix.

A redundancy analysis (RDA) was then performed with the pollination niche as the response variable, and the pollination strategy, spatio-temporal distribution, ecological distribution and six first eigenvectors of the phylogenetic correlation matrix (which accounted for more than 90% of its inertia) as the explanatory variables. Permutation tests for

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redundancy analyses were performed to assess the statistical significance of the effect of each explanatory variable (10000 permutations) and p-values were adjusted using the false discovery rate (*fdr*) method (Verhoeven, Simonsen & McIntyre, 2005). A variation partitioning analysis (function *varpart* from the R package *vegan* version 2.4-4; Oksanen *et al.*, 2017) was then performed to assess the respective explanatory power of each of these variables through the calculation of adjusted R squares (R^2_{adj}). The geographical range and flowering time matrices had to be merged into a single matrix as the function *varpart* does not take into account more than four explanatory matrices. To test for the robustness of our results to phylogenetic uncertainty, these analyses were also performed using a set of 100 randomly selected trees rather than the consensus tree.

Because the determinants of plant-pollinator interactions may depend on the taxonomic level at which these interactions are considered (*e.g.* insect species *versus* families), the same analyses (SVD decomposition of the orchid-pollinator matrix and subsequent RDA and variation partitioning analysis) were performed on another orchid-pollinator network, in which insect species were collapsed into families. This network comprised 153 orchid species, 87 insect families and 405 links between them, and was qualitative (*i.e.* the number of species per family was not taken into account). This time, pollination niches were defined in the multidimensional space corresponding to the first 30 SVD vectors, which accounted for more than 90% of the inertia of the orchid species-insect family matrix.

To test for the robustness of our results, we compared them to these obtained on randomised networks, using two randomisation models (*i.e.* with free or fixed degree distributions; see Comment S1 & Table S5). Finally, to check the proper functioning of these randomisations, we calculated and compared the degree assortativities of observed and randomised networks (see Comment S2).

Results

Network topology

The orchid-pollinator network contains 879 nodes (153 orchid and 726 pollinator species) and 1139 links (Fig. 1). The degree of orchid species ranges from 1 to 158 pollinator species, with a mean of 7.44 pollinators per orchid. Most orchids interact with one to a dozen pollinators: 129 (84.31%) of them interact with fewer than 10 pollinators and 70 (45.75%) of them are extreme specialists (*i.e.* they interact with one pollinator). By contrast, some are extreme generalists, such as *Neottia ovata* (158 pollinators) or *Epipactis palustris* (124 pollinators). The DPN of orchid species ranges from 0 to 56, with a mean of 11.20 and an heterogeneous distribution, with 39 orchids having a DPN of 0 (*i.e.* sharing none of their pollinators) and 49 orchids having a DPN of at least 10.

Specialisation

The best model to explain degree variations (DIC=576.57, see Table S3) took into account pollination strategy and breadth of orchids' spatial distribution, while the best model to explain DPN variations (DIC=657.26, see Table S4) took into account their interaction as well. Pollination strategy had a significant effect on both the degree and DPN (Table 1). Degrees ranged from an average of 1.56 (± 0.14 SE, N=90) pollinators for sexually deceptive orchids to an average of 23.22 (± 8.04 , N=23) pollinators for nectar-producing orchids, while orchids using food deception and shelter mimicry were characterised by intermediate degrees (mean of 11.60 ± 1.90 with N=35 and 10.25 ± 6.42 with N=4; Fig. 2). DPN averaged 2.30 ± 0.36 orchids for sexually deceptive species and 22.83 ± 3.54 orchids for nectar-producing species, with species using food deception or shelter mimicry being characterised by high (mean=26.09 ± 2.44) and low (mean=7.00 ± 2.68) DPN, respectively (Fig. 2). In addition, the

degree and DPN increased with the breadth of orchids' spatial distribution, while the breadth of their temporal distribution had no effect on their specialisation (Table 1). Using the country-level instead of the finer-scale geographical range matrix did not affect our results.

Similarity in pollination niches

We found a significant effect of the pollination strategy, phylogeny, ecological and spatio-temporal distributions on the pollination niche (p -values <0.05 ; Table 2 and Fig. 3). Altogether, these factors explained 42.37% of the variation in pollination niches among orchids. Spatio-temporal distribution was by far the most explanatory factor, accounting for 34.39% of the variation in pollination niches among orchids, while phylogeny, ecological distribution and pollination strategy respectively accounted for 5.77%, 4.72% and 2.58% of the variation in these niches. Using the country-level geographical instead of the finer-scale geographical range matrix did not affect our results, nor did using a set of 100 randomly selected trees rather than the consensus tree (*i.e.* 95% of the R^2_{adj} values associated with the “phylogeny” factor were comprised between 5.43% and 6.14%).

By contrast, when the same analysis was performed on the orchid species-pollinator family network, we found a significant effect of pollination strategy (p -value=0.01) and phylogeny (p -value=0.05), which accounted for 5.94% and 3.36% of the variation in pollination niches among orchids, but the effect of ecological and spatio-temporal distributions was no longer significant (p -values >0.05).

The results of our randomisation analyses (see Comment S1 & Table S5) suggest that correlations between pollination niches and explanatory variables may be underpinned by variations in the number of pollinators rather than by variations in the identity of these pollinators, but this last finding is difficult to test due to the strong disassortativity of our network (see Comment S2).

Discussion

In this study, we constructed the largest database on orchid-pollinator interactions in the Euro-Mediterranean region to date. We hope that this database, which is based on our current knowledge of orchid-pollinator associations and is meant to be completed over time, will stimulate studies about plant-pollinator interactions in the Orchidaceae family. By analysing this database, we provided new insights into the main ecological and evolutionary factors that have shaped the orchid-pollinator network in this region. More specifically, we demonstrated that specialisation depends not only on orchids' pollination strategy, but also on the breadth of their spatial distribution. We further showed that similarity in pollination niches between orchids studied here primarily depends on their spatio-temporal distribution, although pollination strategies, phylogenetic relationships and ecological distributions are correlated to pollination niche overlap as well.

Determinants of specialisation

Species from the sexually deceptive genus *Ophrys* are characterised by low degrees and DPN, as previously shown by other authors (Scopece *et al.*, 2007; Schiestl & Schlüter, 2009). In this genus, such a specialisation in plant-pollinator interactions is ensured by the emission of species-specific sexual pheromone-mimicking semiochemicals (Schiestl *et al.*, 1999; Joffard, Buatois & Schatz, 2016) that mediate reproductive isolation between *Ophrys* species (Xu *et al.*, 2011). However, it should be noted that *Ophrys* species' degrees often exceed one (1.56 on average), meaning that *Ophrys*-pollinator associations are not always strictly specific but sometimes involve one major pollinator species and one or several minor ones, which may promote local adaptation and speciation (Gaskett, 2011; Breitkopf *et al.*, 2013). At the other extremity of the specialisation spectrum, some rewarding orchids attract over a hundred of nectar-consuming insect species, while orchids using food deception or

shelter mimicry are typically characterised by intermediate degrees. In addition, food deceptive orchids are characterised by high DPN, meaning that they share at least some of their pollinators with other orchids, as previously demonstrated by other authors (Cozzolino *et al.*, 2005). This suggests that the role of pollinators in reproductive isolation may not be as important in food deceptive species as it is in sexually deceptive species (Scopece *et al.*, 2007; Schiestl & Schlüter, 2009). Interestingly, we also found an increase of the degree and DPN with the breadth of orchids' spatial distribution, suggesting that widely distributed species tend to be generalist, both at the regional and local (*i.e.* population) scales. It is worth noting that pollination strategy and breadth of orchids' spatial distribution are correlated (Kruskal-Wallis test: $\chi^2=74.097$, p-value<0.05). For example, the nectar-producing orchids *Neottia ovata* and *Epipactis palustris* are among the most widely distributed species in the Euro-Mediterranean region (32 and 29 geographical units, respectively), while sexually deceptive orchids from the genus *Ophrys* often have limited geographical ranges (see Table S1). However, in most cases, pollination strategy seems to be more important than breadth of orchids' spatial distribution in determining specialisation in orchid-pollinator interactions. For example, the rewarding orchid *Gymnadenia nigra* and *Spiranthes romanzoffiana*, both recorded in only two geographical units, respectively interact with 16 and 12 pollinator species, while the sexually deceptive orchid *Ophrys insectifera*, recorded in 24 geographical units, interact with only two pollinator species. Finally, it is worth noting that specialisation in orchid-pollinator associations is important in terms of conservation, as specialists may be more vulnerable to pollinator extinction than generalists (Pauw & Bond, 2011; Phillips *et al.*, 2015; Vogt-Schilb *et al.*, 2015; Reiter *et al.*, 2017). Our study thus suggests that the most vulnerable orchids are also the ones that typically have limited geographical ranges and whose conservation status is often difficult to assess due to major taxonomic confusions (Vereecken, Dafni & Cozzolino, 2010; Schatz *et al.*, 2014).

Determinants of similarity in pollination niches

Interactions between orchids and pollinators were found to be conditioned by several factors, namely orchids' pollination strategy, phylogeny, ecological and spatio-temporal distributions. Orchids using the same strategy to attract pollinators tended to interact with the same insect families, which is not surprising given that these strategies are often directed toward particular guilds of pollinators (Jersáková, Johnson & Kindlmann, 2006; Vereecken, Dafni & Cozzolino, 2010). For example, Euro-Mediterranean food deceptive orchids generally attract naive bees or bumblebees by exploiting their innate foraging behaviour (*i.e.* generalised food deception; Cozzolino *et al.*, 2005), while shelter mimics (*i.e.* *Serapias* species) usually attract solitary bees by mimicking a shelter in which they can sleep or hide during rainy days (Dafni, Ivri & Brantjes, 1981). By contrast, nectar-producing orchids can be pollinated by various functional groups of insects, as reflected by their morphological and chemical diversity. For example, some *Epipactis* species are pollinated by wasps, hornets or hoverflies attracted by green-leaf volatiles or alarm pheromones-mimicking semiochemicals (Brodmann *et al.*, 2008, 2009; Stökl *et al.*, 2011), while the long-spurred *Gymnadenia* and *Platanthera* species are mainly pollinated by butterflies and moths. We also found a phylogenetic signal in both orchid species-insect families and orchid species-insect species associations, suggesting that closely related orchids have more similar pollination niches than expected. For example, *Platanthera bifolia* and *P. chlorantha* share seven of their 12 and 39 pollinators, while *Spiranthes aestivalis* and *S. spiralis* share two of their three and five pollinators. Such a pollinator conservatism could be explained by the fact that some traits – floral size, shape, colour or odour for example – may constrain the evolution of orchid-pollinator interactions, and suggests that speciation is not always driven by pollinator shifts in Euro-Mediterranean orchids, as previously shown in other regions (Van der Niet, Johnson & Linder, 2006). It is worth noting that in the genus *Ophrys*, closely related orchids usually

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interact with different pollinators (N. Joffard, unpublished data). However, because *Ophrys* are often disconnected from the rest of the network (*i.e.* they do not share any of their pollinators with other orchids), many of them were not taken into account in our analysis, which may have caused an overestimation of the phylogenetic signal in orchid-pollinator associations. On the other hand, an even more important phylogenetic conservatism may have been detected if we had considered phylogenetic relationships among pollinator species too, as demonstrated in the Australian genus *Chiloglottis* for example (Mant *et al.*, 2002).

One of our main findings is that similarity in pollination niches between orchids is primarily due to the overlap of their spatio-temporal distribution, with geographical ranges and flowering times explaining almost 35% of this similarity. This suggests that orchid-pollinator interactions are more opportunistic than previously thought, pollination niches being primarily shaped by the pool of pollinator species that are available in a given region, at a given time of the year. However, other factors, either intrinsic (*e.g.* traits promoting some interactions and preventing others, for example morphological: Moré *et al.*, 2012) or extrinsic (*e.g.* variations in pollinators' local abundance: Sun *et al.*, 2014) may also be important in shaping these niches. Alternatively, the correlation between pollination niche and spatio-temporal distribution overlap could arise from orchids' distributions being limited by those of the same pollinators (Sargent & Ackerly, 2008; Pauw & Bond, 2011; Pellissier, Alvarez & Guisan, 2012).

The fact that non-orchid plants visited by pollinators were not included in our analyses may constrain the topology of our network and cause an artificial disconnection between its components, thereby underestimating similarity in pollination niches between orchids. The inclusion of these plants in future studies, together with a finer estimation of pollination niche breadth and overlap (*i.e.* taking into account the relative abundance and efficiency of each pollinator species, as well as their phylogenetic relationships and functional traits) could

provide new insights into the ecological and evolutionary factors that have shaped the orchid-pollinator network in the Euro-Mediterranean region.

Conclusion

The Orchidaceae family is extremely charismatic, due to its diversity in floral traits, reflecting its diversity in pollination strategies. Following the pioneer works of Van der Cingel (1995) and Claessens and Kleynen (2011, 2016), we provide here a database reporting over one thousand orchid-pollinator interactions in the Euro-Mediterranean region. By analysing the network constructed from this database, we show that the pool of insect families with which orchids interact solely depends on their pollination strategy. By contrast, the pool of insect species with which they interact primarily depends on their spatio-temporal distribution, although pollination strategy, phylogeny and ecological distribution are also correlated to similarity in pollination niches. These results suggest that associations between orchid and pollinator species are more opportunistic than previously thought, although it is not perfectly clear whether similarity in pollination niches between orchids reflects similarity in pollinators' number or identity, because of the particular topology of our network. Further studies are now needed to investigate spatio-temporal variations in pollinator assemblages among conspecific populations in Euro-Mediterranean orchids, as well as the factors responsible for these variations and the extent to which they may drive taxonomic and floral diversification in the Orchidaceae family (Moeller, 2005; Gómez *et al.*, 2007; Gómez *et al.*, 2013).

Acknowledgements

This study was funded by the HESAM Université and its Paris Nouveaux Mondes program, the CNRS, the OSU OREME and the ANR-funded ARSENIC project (grant no. 14-CE02-

0012). Many thanks to J. Claessens and J. Kleynen for sharing their data on orchid-pollinator interactions; to P. Geniez and D. Genoud for the taxonomic update of orchids' and pollinators' names, respectively; to I. Le Roncé, F. Munoz and P. de Villemereuil for statistical advices and to F. Kjellberg and A. Taudière for helpful discussions.

Authors' contributions

NJ, FM and BS conceived the study and designed the methodology; NJ and BS collected the data; NJ, FM, MG and CM analysed the data; NJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

The orchid-pollinator database constructed in this study is available online at the following address: https://data.oreme.org/orchid/orchid_map. This database, as well as the explanatory matrices used in this study are also deposited in the Dryad repository: <https://doi.org/10.5061/dryad.58n11j4> (Joffard et al. 2018).

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Tables

(a)

	Posterior mean	P-value
(Intercept)	-2.141E ⁻⁰¹	0.479
Nectar production	1.257	0.002
Food deception	1.011	0.006
Shelter mimicry	1.183	0.008
Spatial distribution	1.146E ⁻⁰⁷	0.013
Sampling effort	3.044E ⁻⁰¹	< 1.00E⁻⁰⁴
Spatial distribution: Sampling effort	-1.657E ⁻⁰⁸	0.020

(b)

	Posterior mean	P-value
(Intercept)	-2.800E ⁻⁰¹	0.632
Nectar production	1.624	0.038
Food deception	2.634	2.02E⁻⁰⁴
Shelter mimicry	8.691E ⁻⁰¹	0.326
Spatial distribution	2.479E ⁻⁰⁷	0.048
Sampling effort	2.594E ⁻⁰¹	0.010
Nectar production: Spatial distribution	-1.377E ⁻⁰⁷	0.388
Food deception: Spatial distribution	-2.078E ⁻⁰⁷	0.169
Shelter mimicry: Spatial distribution	-1.382E ⁻⁰⁸	0.962
Spatial distribution: Sampling effort	-1.622E ⁻⁰⁸	0.183

Table 1. Results of Bayesian phylogenetic mixed models on the effect of pollination strategy, breadths of orchids' spatial and temporal distributions and sampling effort on the degree (*a*) and DPN (*b*) of each orchid species (N=134). Significant effects in bold.

	Fraction	Df	R² (%)	R²_{adj} (%)	P-value
[aeghkln]	ps	3	4.43	2.02	1.12E ⁻⁰⁴
[befiklmo]	phy	6	7.77	3.38	3.17E ⁻⁰⁴
[cfgjlmno]	ed	15	14.76	3.83	1.12E ⁻⁰⁴
[dhijkmno]	std	65	65.02	31.08	1.12E ⁻⁰⁴
[abefghijklmno]	ps+phy	9	11.12	4.61	1.12E ⁻⁰⁴
[acefghijklmno]	ps+ed	18	17.97	5.02	1.12E ⁻⁰⁴
[adehijklmno]	ps+std	68	67.66	33.30	1.12E ⁻⁰⁴
[bcefgijklmno]	phy+ed	21	21.82	7.03	1.12E ⁻⁰⁴
[bdefhijklmno]	phy+std	71	70.09	35.27	1.12E ⁻⁰⁴
[cdfghijklmno]	ed+std	80	73.93	33.83	1.12E ⁻⁰⁴
[abcefgijklmno]	ps+phy+ed	24	24.71	7.98	1.12E ⁻⁰⁴
[abdefghijklmno]	ps+phy+std	74	72.60	37.64	1.12E ⁻⁰⁴
[acdefghijklmno]	ps+ed+std	83	76.46	36.60	1.12E ⁻⁰⁴
[bcdefghijklmno]	phy+ed+std	86	79.02	39.78	1.12E ⁻⁰⁴
[abcdefghijklmno]	ps+phy+ed+std	89	81.23	42.37	1.12E ⁻⁰⁴
<i>Individual fractions</i>					
[a]	ps phy+ed+std	3	-	2.58	3.00 ⁻⁰³
[b]	phy ps+ed+std	6	-	5.77	1.12E ⁻⁰⁴
[c]	ed ps+phy+std	15	-	4.72	1.12E ⁻⁰⁴

[d]	stdlps+phy+ed	65	-	34.39	1.12E ⁻⁰⁴
[e]	-	-	-	0.19	-
[f]	-	-	-	-1.43	-
[g]	-	-	-	-0.21	-
[h]	-	-	-	-1.63	-
[i]	-	-	-	-2.81	-
[j]	-	-	-	-1.36	-
[k]	-	-	-	0.05	-
[l]	-	-	-	-0.33	-
[m]	-	-	-	0.89	-
[n]	-	-	-	0.50	-
[o]	-	-	-	1.06	-
[p]	residuals	-	-	57.64	-

Table 2. Correlations between each combination of explanatory variables and pollination niches and their statistical significance. Each fraction is lettered as in Figure 2. Ps: pollination strategy; phy: phylogeny; ed: ecological distribution; std: spatio-temporal distribution; Df: degree of freedom; R²: coefficient of determination; R²_{adj}: adjusted coefficient of determination. P-value are indicated for testable fractions only.

Figures

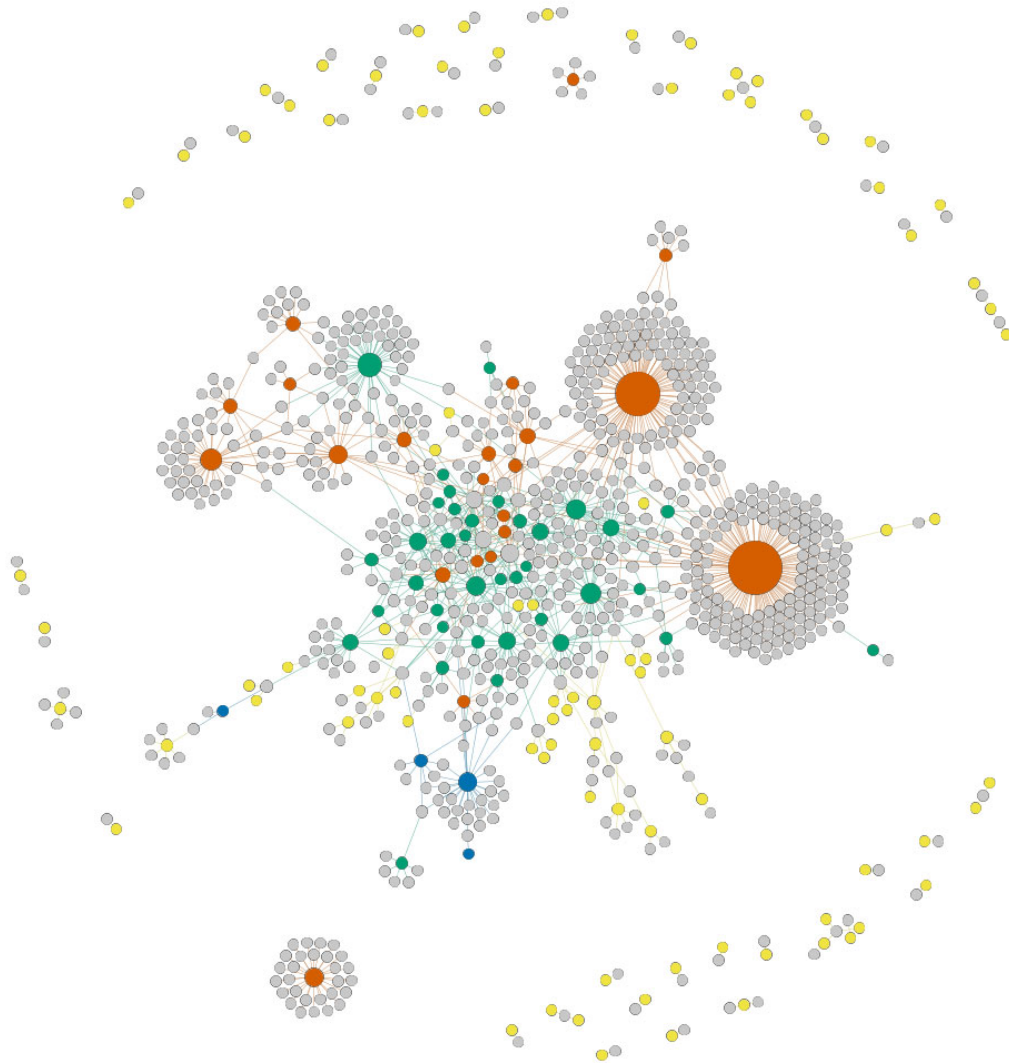


Figure 1. Graphical representation of the bipartite orchid-pollinator network based on the spatialisation algorithm *Force Atlas 2* (Jacomy *et al.*, 2014) as implemented in GEPHI (Bastian, Heymann & Jacomy, 2009). Circles correspond to orchid or pollinator species and their size is proportional to species' degrees. Vermillion circles represent nectar-producing orchids, bluish green circles food deceptive orchids, blue circles shelter mimics, yellow circles sexually deceptive orchids and grey circles pollinators.

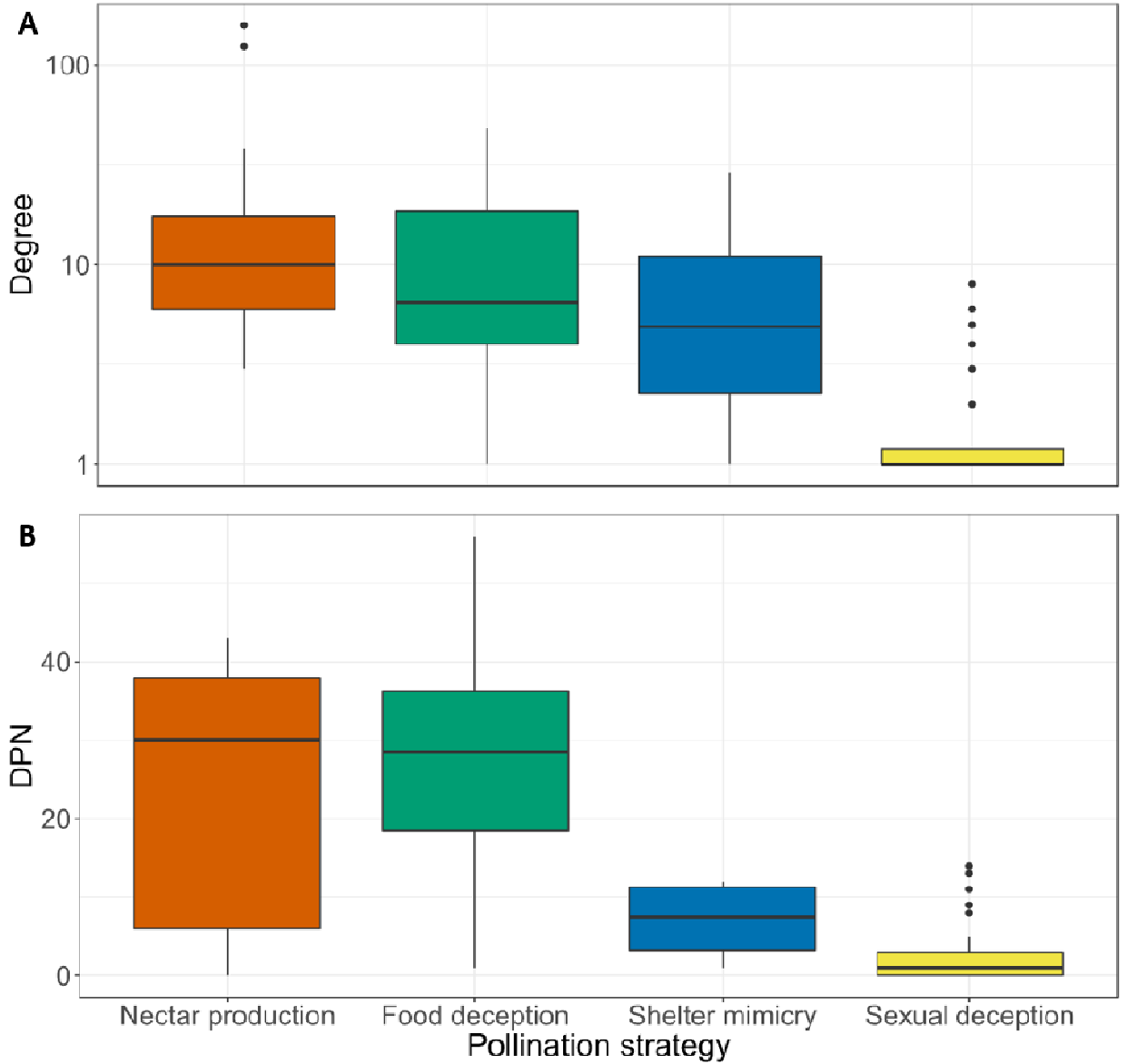


Figure 2. (A) Degree and (B) Degree in the Projected Network (DPN) variations among orchids' pollination strategies (nectar production, N=23; food deception, N=36; shelter mimicry, N=4; sexual deception, N=88).

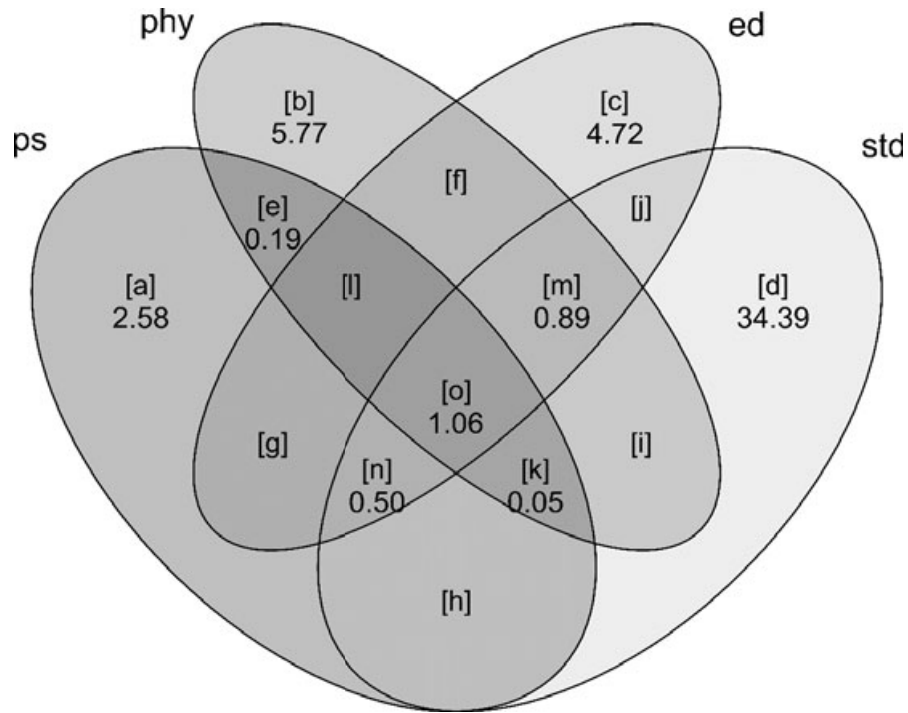


Figure 3. Venn diagram representing the results of the RDA and variation partition analysis testing the correlation between pollination strategy (ps), phylogeny (phy), ecological distribution (ed) and spatio-temporal distribution (std) on the one hand and pollination niches on the other hand. Each fraction is lettered as in Table 2. Adjusted coefficient of determination (R^2_{adj} , expressed in percentages) are indicated for each fraction. Negative values are not shown.