Ecology Letters, (2008) **11**: 564–575 doi: 10.1111/j.1461-0248.2008.01170.x

LETTER

Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization

Theodora Petanidou,[1]* Athanasios S. Kallimanis,[2] Joseph Tzanopoulos,[3] Stefanos P. Sgardelis[3] and John D. Pantis[3]

[1]Laboratory of Biogeography and Ecology, Department of Geography, University of the Aegean, University Hill, 81100 Mytilene, Greece

[2]Department of Environment and Natural Resource Management, University of Ioannina, 30100 Agrinio, Greece

[3]Department of Ecology, U.P.B. 119, School of Biology, Aristotle University, 54124 Thessaloniki, Greece

**Correspondence: E-mail: t.petanidou@aegean.gr*

Abstract

We analysed the dynamics of a plant–pollinator interaction network of a scrub community surveyed over four consecutive years. Species composition within the annual networks showed high temporal variation. Temporal dynamics were also evident in the topology of the network, as interactions among plants and pollinators did not remain constant through time. This change involved both the number and the identity of interacting partners. Strikingly, few species and interactions were consistently present in all four annual plant–pollinator networks (53% of the plant species, 21% of the pollinator species and 4.9% of the interactions). The high turnover in species-to-species interactions was mainly the effect of species turnover (*c.* 70% in pairwise comparisons among years), and less the effect of species flexibility to interact with new partners (*c.* 30%). We conclude that specialization in plant–pollinator interactions might be highly overestimated when measured over short periods of time. This is because many plant or pollinator species appear as specialists in 1 year, but tend to be generalists or to interact with different partner species when observed in other years. The high temporal plasticity in species composition and interaction identity coupled with the low variation in network structure properties (e.g. degree centralization, connectance, nestedness, average distance and network diameter) imply (i) that tight and specialized coevolution might not be as important as previously suggested and (ii) that plant–pollinator interaction networks might be less prone to detrimental effects of disturbance than previously thought. We suggest that this may be due to the opportunistic nature of plant and animal species regarding the available partner resources they depend upon at any particular time.

Keywords

Apparent vs. real specialization, coevolution, ecological networks, food web structure, Mediterranean scrub, nestedness analysis, network analysis, sampling effort.

Ecology Letters (2008) 11: 564–575

INTRODUCTION

The plant–pollinator relationship is among the most widely studied biotic interactions in terrestrial ecosystems. An extensive literature examines specialization vs. generalization of animals as pollinators of plants, on one hand, and of plants as hosts of flower visitors, on the other (e.g. Waser & Ollerton 2006 and references therein). At the community level, the pattern of specialization has been affirmed as highly asymmetrical with specialist plants usually being visited by generalist pollinators and specialist pollinators usually having generalist plant hosts (Petanidou 1991a; Petanidou & Ellis 1996; Vázquez & Simberloff 2002; Bascompte *et al.* 2003, 2006; Vázquez & Aizen 2004, 2006; Basilio *et al.* 2006; Petanidou & Potts 2006; Stang *et al.* 2007). At the species level, long-term studies on the same plant species have confirmed an opportunistic character of plant–pollinator relationships and thus demonstrated the plasticity these interactions may show through time (Herrera 1988; Horvitz & Schemske 1990; Cane & Payne 1993; Gómez & Zamora 1999; Fenster & Dudash 2001; Cane *et al.* 2005; Price *et al.* 2005). These observations appear to contrast with the belief that the reciprocally beneficial relationship between a plant and a pollinator typifies both a highly specialized mutualism and a tight coevolution between species. Indeed, Ollerton (1996) highlighted the apparent paradox that although floral morphology often appears to reflect evolutionary adaptation to specific pollinator morphology, implying specialized interactions, such highly specialized interactions are rare in nature.

To identify a plant or pollinator species as a specialist it is important to monitor its interactions for a substantial period of time and check whether it faithfully interacts with the same partner(s). However, most existing studies that deal with specialization in natural plant–pollinator interactions are based on a single year or even a single season. In such cases there is no certainty that recorded interactions do, indeed, remain constant over time; 'specialists' may not be so in reality, but may instead be opportunists that have been sampled too infrequently to judge their real dietary preferences or ability to attract flower visitors. This begs the question whether plant–pollinator interactions often qualify as tight coevolutionary relationships, i.e. relationships that lead to reciprocal adaptations in both interaction partners, as has commonly been assumed since the time of Darwin (1862). The alternative is that adaptations of flowers and pollinators are to multiple partners and that these partners could be functionally similar or even equivalent (e.g. Zamora 2000), perhaps leading to a view of communities similar to Hubbell's (2001), in which species are interchangeable on a per-capita basis (He 2005).

The main aim of this paper was to address the above questions by taking into account the temporal dynamics of a plant–pollinator interaction web. More specifically, using a highly resolved data set of plant–pollinator interactions over four successive years, we test whether species that appear as specialists in 1 year are real specialists or exhibit plasticity in their interactions when they are observed over longer time periods. To achieve this we assess the plasticity of the plant–pollinator network and investigate the effects of plasticity on the perception of specialization. The major objectives are to: (i) measure temporal variation in species composition in the network, (ii) estimate temporal variation in the identity of plant–pollinator interactions, (iii) examine how the measurement of specialization depends on the duration of sampling and (iv) test whether the temporal variation in species composition and interaction identities affect overall structural properties of the plant–pollinator network.

METHODS

Study site and sampling method

Our study uses an existing data set derived from an intensive investigation of plants and pollinators in a phryganic community within a nature reserve located at Daphni, *c.* 10 km west of the center of Athens, Greece (Petanidou 1991a). This data set is exceptional in that data were collected year-around across four consecutive years, making the data more extensive than those from any other study we know of. The data set has also been used in a number of previous publications (e.g. Petanidou & Ellis 1993, 1996; Buchmann & Nabhan 1996; Olesen & Jordano 2002; Medan *et al.* 2006; Petanidou & Potts 2006).

The study site is characterized by a xerothermo-mediterranean type climate with very hot and dry summers and mild to relatively cool and wet winters. The main vegetation type is Mediterranean low scrub, dominated by entomophilous plants, mainly annuals (Petanidou *et al.* 1995). Detailed description of the study site is provided by Petanidou & Ellis (1993, 1996), Petanidou *et al.* (1995) and Petanidou & Potts (2006).

Fieldwork took place between April 1983 and May 1987. Sampling focused on recording all interactions between all entomophilous plant species and their pollinators using a rigorous and systematic methodology (see Petanidou & Ellis 1993, 1996; Petanidou & Potts 2006 for details on the sampling methods). In brief, every angiosperm species whose flowers were visited by insects was followed throughout its flowering period for at least 2 days out of every 20. A number of individual plants of each species were visited three times per day (morning, noon and afternoon) and all pollinators were recorded or collected. We considered as 'pollinators' all flower visitors that visited the flowers repeatedly, irrespectively of their 'quality' (i.e. effectiveness in transferring pollen during each visit). This means that plant and pollinator species were recorded only when they participated in an interaction, and not if they were simply present (e.g. plants in flower) or patrolling around plants (e.g. male bees). In total, 5000 h of interaction-directed observations were conducted. Over 18 000 pollinator specimens were collected (except a few species including honeybees that were simply recorded) visiting 133 plant species. Not collecting honeybees might have introduced a very slight bias into the data by allowing all honeybee specimens to explore flowers freely vs. other insect species of which some specimens were collected and therefore deprived of the possibility to visit more flowers. All pollinators were insects, including bees, butterflies, flies, beetles, wasps, sawflies and true bugs, but excluding spiders and ants. Insect taxonomy engaged *c.* 50 European specialists over a total of 6 years. The pollinators (665 species for the total of 50 months of survey) are listed in Petanidou (1991a,b), and the plants in Petanidou *et al.* (1995). This sampling protocol yielded qualitative information on the identity of interactions between plants and pollinators throughout time, but did not provide estimates on the abundance of plant and pollinator species, or pollination efficiency. In this study, we did not use any quantitative measure of interaction strengths, i.e. the number of observed visits of a pollinator species to a particular plant species.

Data analysis

Although our survey covered a period of 50 months, this paper considers only four annual cycles (i.e. the first 48 months). As an annual cycle we considered the period from the 1 April (i.e. the date this study started in 1983) until the 31 March of the next calendar year. For presentation purposes, each annual cycle is named after the starting year, e.g. the period from April 1983 to March 1984 is termed 'year 1983' and so forth.

We calculated between-year similarity in (i) species present in the community (plants and pollinators separately) and (ii) observed plant–pollinator interactions, using the Jaccard index, i.e. the number of species (or interactions) present in both years divided by the total number of species (or interactions) recorded across the 2 years. The Jaccard index calculates how similar (or, conversely, how dissimilar) 2 years are. This dissimilarity could be due to either changes in species composition or changes in the number of species present. For instance, if 10 species are present in 1 year and only four of them continue to be present in the next with no new species, then the Jaccard index equals 0.4 (four common species divided by 10 species in total). To separate the richness change from the effect of the species turnover, we estimated a variation of the Simpson similarity index, proposed by Koleff *et al.* (2003). This index measures the similarity once differences from difference in richness (or number of interactions) have been removed. It is calculated as the number of species (or interactions) present in both years divided by the minimum species richness (or number of interactions) present in one of the two years, thus in the aforementioned example the modified Simpson index would be 1.0 (four common species divided by four species, i.e. the species richness of the poorest second year).

Regarding the plasticity in interaction identity, we tried to tease apart the effect of species turnover and of real species flexibility in making partnerships. For instance, comparing between years 1 and 2, the interactions of each year can be distinguished in: (i) interactions between species that are present in both years and (ii) interactions between species that are present in only one year or between a species that is present in both years and a species that is present in one year. Thus, we calculated the part of the dissimilarity due to the first set of interactions (real species flexibility) and the dissimilarity that is due to the second set of interactions (species turnover).

To investigate how sampling duration affects the estimation of the degree of specialization and how short periods of observation may lead to overestimation of specialization, we compared the level of specialization in data sets of increasing sampling duration. We split the entire data set into subsets of non-overlapping sampling periods as follows: (i) two subsets of 2 years each, (ii) four annual subsets, (iii) eight half-year subsets, (iv) 16 subsets each covering one season (spring, summer, autumn and winter) and (v) 12 randomly selected subsets of 1 month duration. The degree of specialization within each subset was calculated as the proportion of the species (plants or pollinators) that were recorded to interact with only one partner during that sub-period. We acknowledge that this procedure provided an estimate of 'extreme' specialization. However, because specialization and generalization are not a strict dichotomy, but lie along a continuum, we measured how variable the breadth of interaction partners were for species along the entire continuum. So, for each plant and pollinator species recorded in 3 or more years we recorded the minimum and maximum number of interaction partners per year and plotted them against each other. Further, we regressed the minimum number of interaction partners against the range of the number of interaction partners (i.e. the difference between maximum and minimum number of interaction partners). We expect that this approach can highlight whether species flexibility (i.e. the ability to change interaction partner number) is related to the species position along the specialization–generalization gradient.

To test whether temporal variation in species composition and interaction identity affects the overall structure of the plant–pollinator network, we compared a number of different network properties between the entire 4-year plant–pollinator network and the four annual networks. We applied Network Analysis using Pajek Software Version 1.15 (2007) to estimate the following properties: pollinator community size (A); plant community size (P); number of links (I); connectance (C), calculated as $I \times 100/(A \times P)$; degree centralization (DC), i.e. an index of the variation in the degree of generalization in a network; number of components (NC), i.e. clusters within the network; average distance (AvD), i.e. number of interactions along the shortest path between two species averaged over all pairs of species and network diameter (Dm), i.e. the longest of all shortest distances of any species pair in the network.

The effects of the high temporal turnover in species composition and interaction identity may also affect the nestedness of the plant–pollinator interaction network. To test this effect, we analysed the four annual networks using nestedness analysis (Bascompte *et al.* 2003) based on presence–absence data in the plants × animals matrix. In nested systems, smaller species assemblages are perfect subsets of larger species assemblages (Atmar & Patterson 1993; Guimaraes & Guimaraes 2006; Rodríguez-Gironés & Santamaría 2006). Pollination networks are perfectly nested if the most specialized plants are visited by perfect subsets of pollinator assemblages visiting more generalized plants. Similarly, the most specialized pollinators will use plants that are perfect subsets of those used by more generalized pollinators. We calculated nestedness using the metric N, an index of matrix nestedness, *sensu* Bascompte *et al.* (2003). N ranges from 1, indicating perfect nestedness to 0 indicating a system where interactions are distributed completely random. We calculated nestedness using the BINMATNEST software package and calculated associated significance values using their null model 3 (Rodríguez-Gironés & Santamaría 2006).

RESULTS

Temporal variation in species composition of plants and pollinators

Over the entire 48-month period included in this study we recorded 661 pollinator species and 133 plant species. However, comparison of the four annual plant–pollinator networks shows that there is a great variation in species composition between years (Table 1). Species richness of plant community ranged from 97 to 119 across the four annual plant–pollinator networks. The Jaccard similarity of plant communities between any pair of annual networks varied from 66% to 84% (Table 2). When taking into account the difference in species richness between years, the modified Simpson similarity index scored between 83% and 94%. Only a small number of plant species (71) were constantly represented in all four annual networks, which comprise only 53.4% of the total number of species recorded in the entire 4-year period. These plant species will be henceforth referred to as 'core' plant species. The pollinator community was even more variable, with species richness ranging from 305 to 430 species in the annual networks, while species richness in the entire 4-year pool was 661 (Table 1). The Jaccard similarity of pollinator species composition ranged between 39% and 45% for any pair of annual networks. When considering the difference in species richness between years, the modified Simpson similarity index scored between 60% and 73%. The number of 'core' pollinator species was 136, i.e. only 20.6% of the entire species pool.

Table 1 Values for properties of the plant–pollinator networks

	1983	1984	1985	1986	Total
P	97	113	119	104	133
P%	72.9	85.0	89.5	78.2	100
P specialists	22	16	12	19	4
P specialists %	22.7	14.2	10.1	18.3	3.0
P – core					71
A	348	341	430	305	661
A%	52.6	51.6	65.1	46.1	100
A specialists	166	165	183	133	238
A specialist %	47.7	48.4	42.6	43.6	36.0
A – core					136
I	868	989	1493	939	3006
I%	28.8	32.9	49.6	31.2	100.0
I – core spp.	446	522	655	535	1222
C	2.6	2.6	2.9	3.0	3.4
I – core spp.	446	522	655	535	1222
C – core spp.	4.6	5.4	6.8	5.5	12.7
DC	0.15	0.09	0.09	0.10	0.15
NC	6	3	2	1	2
AvD	4.04	4.00	3.79	3.94	3.49
Dm	8	8	7	9	7
N	0.982	0.977	0.971	0.981	0.970

Values are given for each annual observation as well as the entire 4-year study period.

P: number of plant species; A: number of pollinator species; I: number of interactions; P%: percentage of plant species over the total 133 species observed throughout the entire 4-year study period; A%: percentage of the pollinator species over the total 661 species observed throughout the entire 4-year study period.

Specialists are species interacting with only one species partner. Core species are those recorded in every single year of the entire study period.

C, connectance, is $I \times 100/(P \times A)$. DC, degree centralization; NC, number of components; AvD, average distance; Dm, network diameter; N, nestedness, calculated as $(100 - T)/100$, where T is the temperature. Values are explained in the text (*Methods*).

Temporal variation in plant–pollinator interactions at the community level

Over the entire 4-year study period we recorded 3006 different interactions between plant and pollinator species. However, only a fraction of these were observed within each annual pollination network, and no annual network was found to contain more than 50% of the total number of interactions (Table 1). It is important to mention that a large number of interactions (viz. 2170, i.e. 72.1% of the total pool) were observed only in one of the four annual networks. The number of interactions that were repeatedly observed in all four different annual networks was very small (148 interactions) accounting for only 4.9% of the total number observed in the entire study period. Similarity in the identity of interactions between any pair of annual networks was always

Table 2 Similarity between any pair of study years, given as number of common resources (plant species, insect species and interactions), and as Jaccard and modified Simpson indices

	1983 and 1984	1984 and 1985	1985 and 1986	1983 and 1985	1984 and 1986	1983 and 1986
Number of species/interactions observed in both years						
Plants	88	106	95	90	91	80
Insects	207	238	224	230	193	183
Interactions	282	407	355	331	288	246
Interactions of core species	223	290	276	254	248	221
Number of interactions 'lost' between years, i.e. observed only in one over two years						
Total number of interactions observed only in one year	1292	1665	1717	1697	1348	1314
Interactions lost among species present in both years	383 (29.6%)	603 (36.3%)	495 (28.8%)	510 (30.1%)	363 (26.9%)	289 (22.0%)
Interactions between a species present in both years and a partner species present in one year	687 (53.2%)	890 (53.4%)	919 (53.6%)	908 (53.5%)	694 (51.5%)	731 (55.6%)
Interactions between species that are present in only one year	222 (17.2%)	172 (10.3%)	303 (17.6%)	279 (16.4%)	291 (21.6%)	294 (22.4%)
Jaccard similarity index						
Plants	0.721	0.841	0.742	0.714	0.722	0.661
Insects	0.429	0.447	0.438	0.420	0.426	0.389
Interactions	0.179	0.196	0.171	0.163	0.176	0.158
Interactions of core species	0.299	0.327	0.302	0.300	0.307	0.291
Modified Simpson's similarity index						
Plants	0.907	0.938	0.913	0.928	0.875	0.825
Insects	0.607	0.698	0.734	0.661	0.633	0.600
Interactions	0.325	0.412	0.378	0.381	0.307	0.283
Interactions of core species	0.500	0.556	0.516	0.570	0.475	0.496

Core species are those recorded in every single year of the entire study period.

below 20% (i.e. Jaccard index), whereas the modified Simpson's index was always below 41% (Table 2).

Our results show that the among years dissimilarity in the identity of interactions was caused by species flexibility and turnover. Approximately 30% of the interactions 'lost' between any pair of years (i.e. observed in only 1 over 2 years) were interactions between species that were present in both years, but interacted only in 1 year (Table 2). The remaining 70% of the interactions were 'lost' either because one partner was missing (*c.* 50%) or both partners were missing (*c.* 20%) (Table 2).

Despite the temporal variation in species composition and in plant–pollinator interactions, the structural properties of the four annual networks were remarkably similar (Table 1). Network connectance ranged between 2.6% and 3.0%, and the degree centralization ranged between 0.09 and 0.15. Furthermore, all networks were highly nested, and the degree of nestedness varied only slightly among the annual networks (range: 0.971–0.982). Using BINMATNEST software we showed that the observed nestedness differs significantly from the nestedness of random matrices produced by any of the three types of null models, whose nestedness ranged from 0.846 to 0.958.

The entire 4-year network is characterized by a low connectance (3.4%), and connectance rises only if the analysis is limited to the core species alone (71 plant and 136 pollinator species, 1222 interactions and 12.7% connectance; Table 1). Even limiting ourselves to core species, 684 (56.0%) of the 1222 interactions were observed only in one of the four annual networks, while only 148 (12.1%) were recorded across all four annual networks. The similarity in identity of interactions among core species between any pair of annual networks was below 33%, using the Jaccard index, and below 57% using the modified Simpson index (Table 2).

Temporal variation in plant–pollinator interactions at the species level

We examined whether species were consistent in their interaction preferences throughout the four study years. We found that even for core species, the identities of interaction partners were highly variable from year to year. For example,

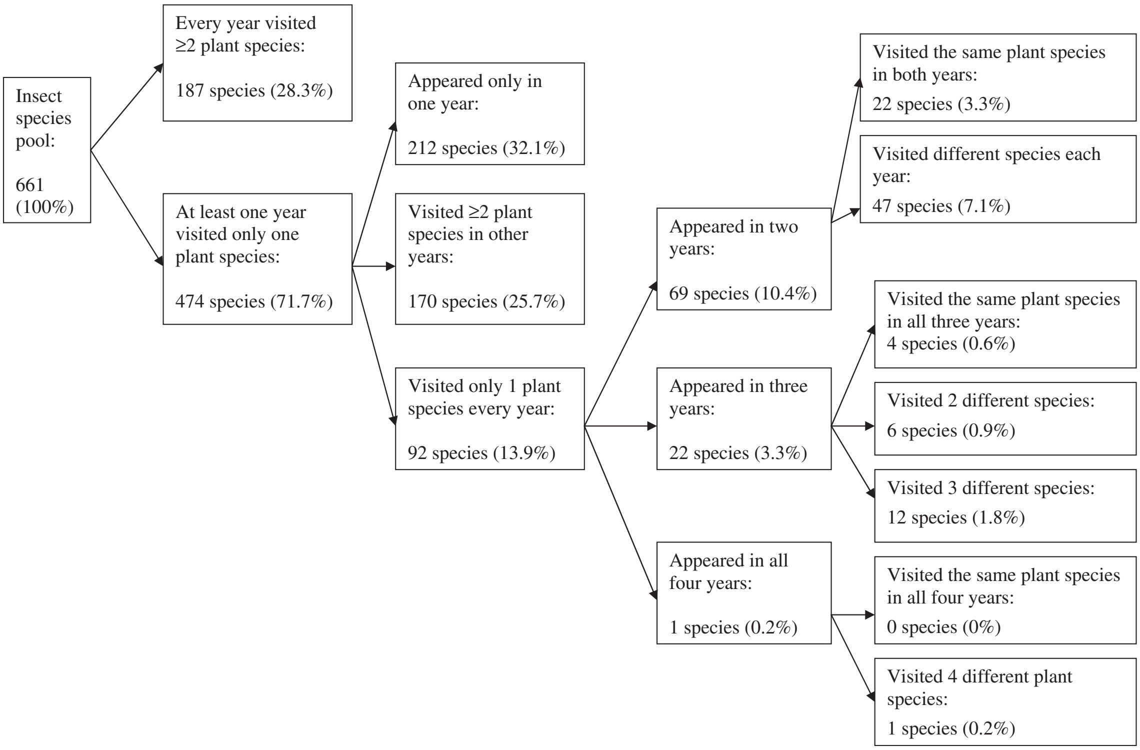

Figure 1 Flow chart showing all pollinator species, presented according to the number of plant species they visited. Beginning at the left-hand side, the total pool of insect species is subdivided into groups exhibiting different patterns of use of plants across years. Percentages are over the total species pool (661).

across all four years, the solitary bee *Lasioglossum malachurum* visited a total of 66 plant species (50% of the plant species pool), whereas within each single year it visited between 10 and 28 plant species (representing, 10.3% and 23.5% of the plant species encountered in the respective year). Thus, the identity of the plant species visited varied considerably among years, and the similarity between the visited species in any pair of years was < 47% (Jaccard index). In fact, of the 66 plant species encountered throughout the 4 years, *L. malachurum* interacted consistently with only five. Other bee species (e.g. *Apis mellifera, Amegilla albigena, Eucera bidentata, Anthidium florentinum, Osmia aurulenta* and *Rhodanthidium septemdentatum*) displayed similar unpredictable preferences. The same pattern could be seen in many plant species. For example, during the entire 4-year study period, thyme (*Thymus capitatus*) interacted with a total of 123 pollinator species, but the number of pollinators visiting the plant per year varied between 32 and 72 species. In addition, the identity of these pollinators varied markedly among years and only three of them were consistent visitors throughout the 4 years of sampling.

Temporal variation, sampling effort and specialization

We recorded 474 pollinator species as extreme specialists (i.e. interacting with a single mutualistic partner) across the four annual networks (i.e. the sum of the number of specialists within each annual network considered separately; Fig. 1). Out of them, 212 appeared only during 1 year, thus no conclusion can be drawn regarding their specialization. Among the remaining 262 pollinator species, only a limited number visited the same plant species in more than 1 year (viz. four species for 3 years and 22 species for 2 years; see right-hand side of Fig. 1), while the rest 236 species visited different plant species in other years ('apparent specialists'). In brief, none of the pollinators appeared to be completely consistent in its host plant choice across the 4 years.

Focusing on the annual networks, the fact that so many pollinator species (236) appeared as specialists in 1 year and as generalists in another (middle and right-hand side of Fig. 1) is alarming for studies discussing the specialization and generalization in plant–pollinator interactions. This can be illustrated by two examples: the hoverfly *Bombylius undatus* appeared to interact annually with only one, but a different plant species during the first 2 years, whereas it interacted with six plant species in the third year that were different from the previous years, and with 10 species in fourth year (modified Simpson's similarity index between years 3 and 4: 83.3%); and the oligolectic bee *Chalicodoma parietina nestorea*, which specialized on asphodel (*Asphodelus aestivus*) during

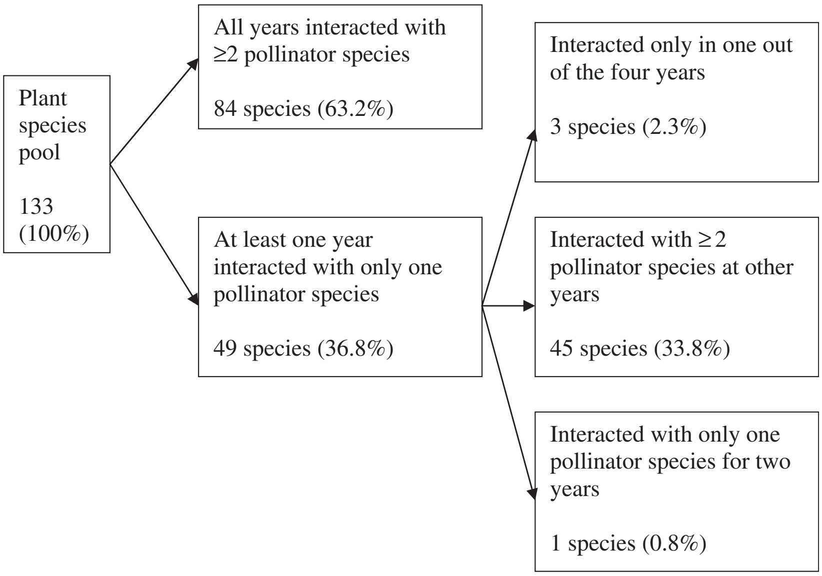

Figure 2 Flow chart showing all plant species presented according to the number of insect species they were visited by. Conventions follow Fig. 1. Percentages are over the total species pool (133).

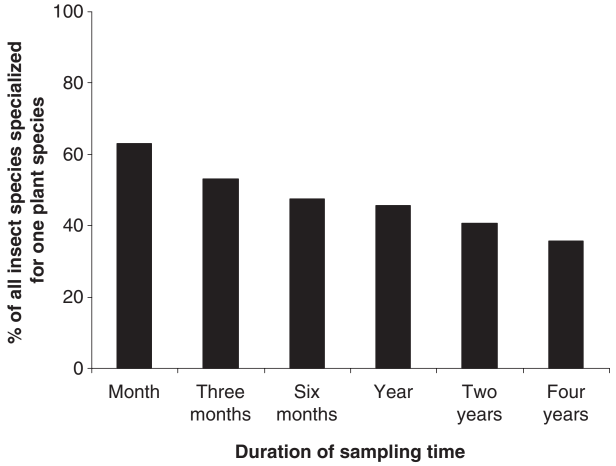

Figure 3 Time dependence of 'apparent specialization' of flower visitors. The values of 'apparent specialization' (i.e. per cent of insect species visiting one plant species over all insect species within a certain time span) were estimated over different non-overlapping sampling periods (see *Methods*).

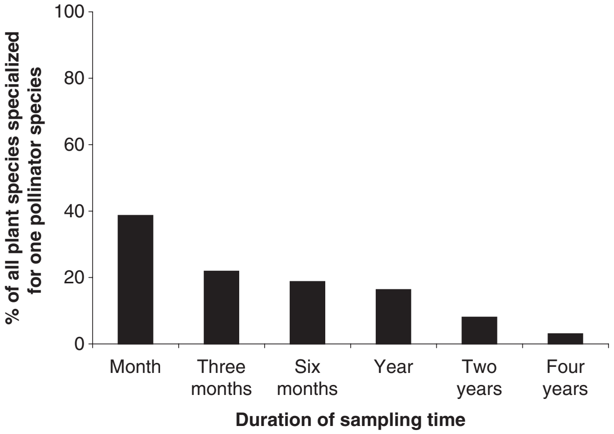

Figure 4 Time dependence of 'apparent specialization' of plants. The values of 'apparent specialization' (i.e. per cent of plant species visited by one insect species over all plant species in flower within a certain time span) were estimated over different non-overlapping sampling periods (see *Methods*).

2 years, but visited six totally different plant species in the other 2 years.

The picture is even clearer in the case of plants (Fig. 2). Out of the entire pool of 133 plant species, 49 interacted with only one pollinator species at least during 1 year, but only one plant species, Friar's cowl (*Arisarum vulgare*) interacted repeatedly – albeit not throughout the four study years – with the same pollinator species. As another example, the globe thistle (*Echinops microcephalus*) was observed to interact with only one pollinator species during the first year of observation, but with 36 species belonging to four different orders in another year.

These results suggest that the estimate of the degree of specialization of species depends strongly on the duration of sampling, and tends to be inflated in shorter time span. Indeed, our data (Figs 3 and 4) show that for both plant and pollinator 'apparent specialization' depends on the sampling time span, in other words the sampling effort. Figures 3 and 4 show that by reducing the sampling effort from 4 years to a single month the proportion of specialist species increased from 36% to 63% for pollinators and from 3% to 39% for

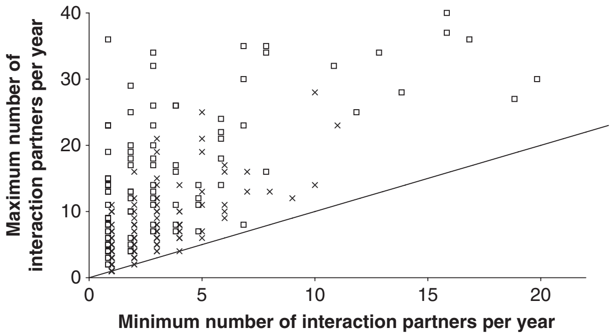

Figure 5 Minimum vs. maximum number of interaction partners per year for plants (☐) and pollinators (×). The line represents the slope of 1, under which values are impossible (maximum cannot be less than minimum). Species with high generalization (e.g. *Apis mellifera* and *Thymus capitatus*) were excluded as outliers.

plants. The fact that the percentage of specialists over the total number of species (plants or pollinators) decreases in an approximately linear trend as the sampling effort increases suggests that even after 4 years of sampling our data have not revealed the 'true proportion' of real specialists.

However, specialization vs. generalization is not a dichotomy, but refers to a continuum (e.g. Waser *et al.* 1996). To test whether there is any trend in this continuum, we estimated for each species present for more than 2 years the minimum and maximum number of interaction partners per year and plotted them against each other (Fig. 5). The figure shows that for all plant species and almost all pollinator species the maximum number of interaction partners is *much* greater than the minimum. This difference is recorded along the entire range of values from specialist to generalist. If species tended to interact with a similar number of partner species every year, the species would tend to accumulate close to the line of slope 1. This, however, is not the case, as seen in Fig. 5, where the whole range of possible combinations can be observed. This suggests that degree of 'apparent specialization' differs from degree of 'real specialization' not only for the extreme specialists, but for species along the entire continuum from extreme specialist to extreme generalist. The linear regression between the minimum number of interaction partners vs. the range of the number of interaction partners (maximum minus minimum) was significant for both partners (for pollinators: $R^2 = 0.355$, $P < 0.001$; for plants: $R^2 = 0.037$, $P = 0.027$).

DISCUSSION

Interannual plasticity in plant–pollinator networks

Both the plant and the pollinator communities displayed high temporal turnover in species composition. The plant community was the least variable, as the similarity in species composition across years was almost twice as high as in the pollinator community. Comparable high species turnover has also been observed in other studies of Mediterranean and other temperate zone ecosystems (e.g. Herrera 1988, 2005; Cane & Payne 1993; Marlin & LaBerge 2001; Williams *et al.* 2001; Quaranta *et al.* 2004; Cane *et al.* 2005; Price *et al.* 2005).

Even more striking was the turnover in the identities of interactions between specific plants and pollinators. Only a small fraction of the interactions recorded were observed repeatedly in all four years. Forup *et al.* (2008) have shown that when such fluctuations are taken into account and the data are weighted in terms of number of species constantly observed in all study years, the differences in species composition among years are less dramatic. However, our study shows that even if the analysis focuses only on the 'core species', the turnover in the interaction identities remains high. The mechanisms responsible for the fluctuation in identity of species-to-species interactions were species turnover and flexibility. Trying to tease apart their relative importance, we found that, to a large extent (*c.* 70% in pairwise comparisons among years) this reflected species, especially insects, appearing and disappearing from the local community across time. To a lesser extent (*c.* 30% in pairwise comparisons among years) it reflected species flexibility, i.e. species still present in the community and exhibiting changed behaviour or preferences. Therefore, we infer that observed annual changes in the interaction identities may also reflect opportunism in the reciprocal use of resources by either partner, as has also been suggested by Waser *et al.* (1996) and Waser & Price (1998). What remains unclear though is the behavioural base of such opportunism on the part of the pollinators, although the foraging theory (e.g. Stephens & Krebs 1986) is a very promising tool to re-activate in exploring such phenomena.

Despite the great differences in species composition and the identity of interactions within our annual networks, overall network properties such as connectance, nestedness and centralization remained quite constant through time. Furthermore, our estimates for these structural features are comparable to those found in other mutualistic networks of similar size (see e.g. Olesen *et al.* 2006). This implies that the network may remain equally functional in many aspects despite annual variation in its structural elements (i.e. species and links). The only other study we know of that has investigated the sensitivity of pollination network structural properties to sampling effort is that of Nielsen & Bascompte (2007) that showed nestedness to be less prone to sampling bias, when compared to the number of species and links in the network. Furthermore, they showed that nestedness increases as the sampling period increases, a trend that is not apparent in our study.

A limitation in our study is that the data are qualitative rather than quantitative. As we lack estimates of each interaction frequency, we cannot exclude the possibility that the high frequency links are fairly constant in time and account for the majority of the pollination interactions. Furthermore, it is uncertain whether the specialists (apparent or real) will be particularly important to the function of the network and thus to pollination as an ecosystem service.

During the review process of this manuscript we became aware of two more studies that have examined the temporal variation in plant–pollinator interaction networks. These studies by Alarcón (2004) and by Olesen *et al.* (2008) provide strikingly similar results. Alarcón (2004) studied a smaller plant–pollinator network over three summer seasons in the coastal mountains in California, USA, and also found high turnover in plant and pollinator species, and in the exact identities of the interactions realized. In his system drought seemed to be a possible driver of much of the variation. He also reported that basic properties of the network seemed to be quite stable, in spite of the high variation in species and interactions. Olesen and colleagues examined the day-to-day dynamics of an Arctic pollination interaction network over two consecutive seasons. They also report that despite high temporal turnover in species composition and identity of interactions in the pollination network, structure remained surprisingly stable.

Implications of network plasticity: apparent vs. real specialization

The high temporal turnover in species composition signifies a correspondingly high uncertainty as to whether a specific plant or pollinator species will be available to its interaction partners in the next year. This uncertainty may be sustained and amplified by different sets of factors shaping the flowering phenology of plants and affecting the life cycles and behaviour of different pollinator groups (O'Toole & Raw 1991; Petanidou *et al.* 1995; Michener 1979, 2000; Olesen *et al.* 2008). Given such conditions of variability or plasticity in the plant–pollinator network, specializing may be a very daring strategy (Waser *et al.* 1996). In our study community, a large number of plant and pollinator species appear as specialists in 1 year but tend to be generalists when observed across years. Such cases, where generalist species appear as specialists under certain conditions (e.g. undersampling or sampling during non-typical years), constitute cases of 'apparent specialization' rather than 'real specialization'. This appearance vs. essence is more evident in the case of plants. Within the total plant assemblage of 133 species, only one single 'specialist' species interacted with the same pollinator for more than a single year. Among the pollinators, most species appeared to interact with a single plant species in at least 1 year. However, few confirmed a truly specialized nature, visiting one and the same plant species for more than 1 year. In fact, no pollinator species interacted with only one particular species throughout all 4 years of the study. The difference between 'apparent specialization' and 'real specialization' does not apply only to extreme specialists but can be observed in the whole continuum from specialists to generalists. The differences between the maximum and minimum number of species interactions observed per year suggests that there is a high variation in the number of partners that a species may have from year to year.

One possible explanation for the incongruous estimates of specialization across years may be incomplete sampling that failed to record all species and interactions in the system. Several authors have raised questions regarding the validity of data on ecological networks, emphasizing problems of sampling effort also in systems other than plant–pollinator systems (e.g. Paine 1988; Polis 1991; Cohen *et al.* 1993; Martinez *et al.* 1999; Banasek-Richter *et al.* 2004; but see Nielsen & Bascompte 2007). Although sampling bias may play a role also in this study, we stress that this is one of the most intensively sampled plant–pollinator systems in the literature, and the only effort so far known to have focused on such a long consecutive time period (Buchmann & Nabhan 1996; Olesen & Jordano 2002). Indications that the observed turnover in species interactions is not a mere sampling error also comes from some key generalist species in the study. Such species, both plants and pollinators were found to be entangled within a different interaction network each year. For instance, *T. capitatus*, a key plant species of this phryganic community (Petanidou & Ellis 1996), was visited consistently by only three pollinator species through all four years, but by a total of 123 pollinator species over the same period. The trend for other generalist plants and pollinators was similar.

Reviewing the empirical evidence for specialization in plant–pollinator systems, we notice that almost all studies reported in the literature have been based on sampling that lasted less than a year, in most cases just a single season or even less. Our data showed that such short sampling periods will overestimate the proportion of species that are considered as specialists. As a matter of fact, the vast majority (> 90%) of our study species that appeared as specialists in one year, behaved as generalists in other years. Actually, no species recorded in all four years was truly specialist. This allows us to speculate that reported levels of specialization in the literature are overestimates of real specialization. This overestimation may have direct consequences for ecological and evolutionary theory as well as conservation recommendations and remedies.

If apparent specialization in plant–pollinator systems is often a sampling artefact, then the role of coevolution in shaping these mutualistic interactions should be reconsidered. Perhaps opportunism and availability of interaction partners may be more responsible for shaping interaction patterns than previously suspected. This might well explain the ability of alien plant and pollinator species to thrive in invaded areas (Memmott & Waser 2002; Bjerknes *et al.* 2007; Lopezaraiza-Mikel *et al.* 2007). We do not suggest, however, that coevolution is never a driver of plant–pollinator relationships, but rather that it might not be as frequent as previously thought. Another theoretical implication is that the community of plants and their pollinators is not so much assembled based on niche-restricted assembly rules, but, instead, species are more equivalent in their function. This is closer to the neutrality assumption of Hubbell's (2001) biodiversity theory.

The concept of specialization in plant–pollinator systems is inevitably connected to the notion of extinction cascades in natural ecosystems, i.e. the idea that if the pollinator of a specialist plant becomes extinct, then the plant is bound to follow and vice versa. However, we should point out two caveats. First, the nested structure and heterogenic nature of plant–pollinator networks can be important for the robustness and stability of the networks by retarding its collapse in the face of component species extinctions (Petanidou 1991a; Vázquez & Simberloff 2002; Bascompte *et al.* 2003; Jordano *et al.* 2003; Memmott *et al.* 2004; Fontaine *et al.* 2006; Fortuna & Bascompte 2006). Second, if estimates of specialization are compromised by a too short sampling period and swapping over to a new interaction partner is likely, then the probability of extinction will be considerably lower. This fits well with the conclusion of Vázquez & Simberloff (2002) that in such systems there may be no relationship between pollination specialization and response to disturbance. These considerations are important in the context of global climate change. For example, Memmott *et al.* (2007), assuming that the observed plant–pollinator specialization is real, estimated the proportion of plant–pollinator species and interactions that are expected to be affected by climate change. Based on our results we hypothesize that their assumption of invariant links between plants and pollinators is likely to be overly cautious (a point which these authors also raised). It is most likely that no mutualistic partner in pollination interactions depends on the presence of the other to survive and it is very likely that the great majority of both plants and pollinators will find new interaction partners if their present partners vanish. The high plasticity of the plant–pollinator interaction networks suggests that these networks may well be more robust to disturbance than we have usually assumed.

This study raises more questions than it can answer. What factors explain, for instance, the fluctuation in the identity of species (especially pollinators) and interactions? To what extent does this reflect changed foraging preferences? And if behaviour changes, why is this? Are there simple behavioural rules that explain the shifts? What, exactly, are the implications of relatively constant connectance, nestedness, etc. for the resilience of pollination networks, despite the immense temporal variation in species and interactions? We trust these questions will stimulate a lot of discussion, thought and further research.

ACKNOWLEDGEMENTS

We acknowledge the contribution of the insect taxonomists who identified to species level all the bugs from the Daphni site, and the cooperation of the directors of 'I. & A. Diomedes Botanical Garden of the University of Athens'. Also, acknowledge Nick Waser, for not remaining as an anonymous referee, and for re-writing the text to improve the English and the logical flow. Many thanks also to the three anonymous referees and the subject editor for constructive criticisms, and for infusion of new and original ideas and analyses that strengthened this work. The paper was partly supported by the European Commission Framework 6 Integrated project ALARM (Assessing LArge scale environmental Risks for biodiversity with tested Methods) (GOCE-CT-2003-506675).

REFERENCES

Alarcón, R. (2004). *The structure of plant–pollinator interactions in montane meadow environments*. PhD Thesis. University of California Riverside, Riverside, CA, USA.

Atmar, W. & Patterson, B.D. (1993). The measure of order and disorder in the distribution of species in fragmented habitats. *Oecologia*, 96, 373–382.

Banasek-Richter, C., Cattin, M.F. & Bersier, L.F. (2004). Sampling effects and the robustness of quantitative and qualitative food-web descriptors. *J. Theor. Biol.*, 226, 23–32.

Bascompte, J., Jordano, P., Melián, C.J. & Olesen, J.M. (2003). The nested assembly of plant-animal mutualistic networks. *Proc. Natl Acad. Sci. U.S.A.*, 99, 9383–9387.

Bascompte, J., Jordano, P. & Olesen, J.M. (2006). Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, 312, 431–433.

Basilio, A.M., Medan, D., Torretta, J.P. & Bartoloni, N.J. (2006). A year-long plant–pollinator network. *Austral Ecol.*, 31, 975–983.

Bjerknes, A.-L., Totland, Ø., Hegland, S.J. & Nielsen, A. (2007). Do alien plant invasions really affect pollination success in native plant species? *Biol. Conserv.*, 138, 1–12.

Buchmann, S.L. & Nabhan, G.P. (1996). *The Forgotten Pollinators*. Island Press, Washington, DC.

Cane, J.H. & Payne, J.A. (1993). Regional, annual, and seasonal variation in pollinator guilds – intrinsic traits of bees (Hymenoptera, Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). *Ann. Entomol. Soc. Am.*, 86, 577–588.

Cane, J.H., Minckley, R., Kervin, L. & Roulston, T.H. (2005). Temporally persistent patterns of incidence and abundance in a pollinator guild at annual and decadal scales: the bees of *Larrea tridentata*. *Biol. J. Linnean Soc.*, 85, 319–329.

Cohen, J.E., Beaver, R.A., Cousins, S.H., DeAngelis, D.L., Goldwasser, L., Heong, K.L. *et al.* (1993). Improving food webs. *Ecology*, 74, 252–258.

Darwin, C. (1862). *On the Various Contrivances by with British and Foreign Orchids are Fertilised by Insects and the Good Effects of Intercrossing*. John Murray, London.

Fenster, C.B. & Dudash, M.R. (2001). Spatiotemporal variation in the role of hummingbirds as pollinators of *Silene virginica*. *Ecology*, 82, 844–851.

Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. (2006). Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. *PLoS Biol.*, 4, 129–135.

Fortuna, M.A. & Bascompte, J. (2006). Habitat loss and the structure of plant–animal mutualistic networks. *Ecol. Lett.*, 9, 281–286.

Forup, M.L., Henson, K.S.E., Craze, P.G. & Memmott, J. (2008). The restoration of ecological interactions: plant–pollinator networks on ancient and restored heathlands. *J. Appl. Ecol.*, 45, doi :10.1111/j.1365-2664.2007.01390.x.

Gómez, J.M. & Zamora, R. (1999). Generalization vs. specialization in the pollination system of *Hormatophylla spinosa* (Cruciferae). *Ecology*, 80, 796–805.

Guimaraes, P.R. & Guimaraes, P. (2006). Improving the analyses of nestedness for large sets of matrices. *Environ. Model. Softw.*, 21, 1512–1513.

He, F. (2005). Deriving a neutral model of species abundance from fundamental mechanisms of population dynamics. *Funct. Ecol.*, 19, 187–193.

Herrera, C.M. (1988). Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biol. J. Linnean Soc.*, 35, 95–126.

Herrera, C.M. (2005). Plant generalization on pollinators: species property or local phenomenon? *Am. J. Bot.*, 92, 13–20.

Horvitz, C.C. & Schemske, D.W. (1990). Spatiotemporal variation in insect mutualists of a neotropical herb. *Ecology*, 71, 1085–1097.

Hubbell, S.P. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton.

Jordano, P., Bascompte, J. & Olesen, J.M. (2003). Invariant properties in coevolutionary networks of plant-animal interactions. *Ecol. Lett.*, 6, 69–81.

Koleff, P., Gaston, K.J. & Lennon, J.J. (2003). Measuring beta diversity for presence-absence data. *J. Anim. Ecol.*, 72, 367–382.

Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R. & Memmott, J. (2007). The impact of an alien plant on a native plant–pollinator or network: an experimental approach. *Ecol. Lett.*, 10, 539–550.

Marlin, J.C. & LaBerge, W.E. (2001). The native bee fauna of Carlinville, Illinois, revisited after 75 years: a case for persistence. *Conserv. Ecol.*, 5, U91–U116. Available at: http://www.consecol.org/vol5/iss1/art9/. Last accessed on 18 February 2008.

Martinez, N.D., Hawkins, B.A., Dawah, H.A. & Feifarek, B.P. (1999). Effects of sampling effort on characterization of food-web structure. *Ecology*, 80, 1044–1055.

Medan, D., Basilio, A.M., Devoto, M., Bartoloni, N.J., Torretta, J.P. & Petanidou, T. (2006). Measuring generalization and connectance in temperate, long-lasting systems. In: *Plant–pollinator Interactions: from Specialization to Generalization* (eds Waser, N.M. & Ollerton, J.). University of Chicago Press, Chicago, IL, pp. 245–259.

Memmott, J. & Waser, N.M. (2002). Integration of alien plants into a native flower-pollinator visitation web. *Proc. R. Soc. Lond. B*, 269, 2395–2399.

Memmott, J., Waser, N.M. & Price, M.V. (2004). Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B*, 271, 2605–2611.

Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecol. Lett.*, 10, 710–717.

Michener, C.D. (1979). Biogeography of the bees. *Ann. Mo. Bot. Gard.*, 66, 277–347.

Michener, C.D. (2000). *The Bees of the World*. Johns Hopkins University Press, Baltimore, MD, USA.

Nielsen, A. & Bascompte, J. (2007). Ecological networks, nestedness and sampling effort. *J. Ecol.*, 95, 1134–1141.

O'Toole, C. & Raw, A. (1991). *Bees of the World*. Blandford, London.

Olesen, J.M. & Jordano, P. (2002). Geographic patterns in plant–pollinator mutualistic networks. *Ecology*, 83, 2416–2424.

Olesen, J.M., Bascompte, J., Dupont, Y.L. & Jordano, P. (2006). The smallest of all worlds: pollination networks. *J. Theor. Biol.*, 240, 270–276.

Olesen, J.M., Bascompte, J., Elberling, H. & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, in press.

Ollerton, J. (1996). Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant–pollinator systems. *J. Ecol.*, 84, 767–769.

Paine, R.T. (1988). Food webs – road maps of interactions or grist for theoretical development. *Ecology*, 69, 1648–1654.

Pajek Software Version 1.15. (2007). *Program for Analysis and Visualization of Large Networks, Version 1.15*. Available at: http://vlado.fmf.uni-lj.si/pub/networks/pajek/. Last accessed on 9 September 2007.

Petanidou, T. (1991a). *Pollination ecology in a phryganic ecosystem*. PhD Thesis. Aristotelian University, Thessaloniki, Greece (in Greek, with English summary).

Petanidou, T. (1991b). Pollinating fauna of a phryganic ecosystem. *Versl. Tech. Geg., Amst.*, 59, 1–12.

Petanidou, T. & Ellis, W.N. (1993). Pollinating fauna of a phryganic ecosystem: composition and diversity. *Biodivers. Lett.*, 1, 9–22.

Petanidou, T. & Ellis, W.N. (1996). Interdependence of native bee faunas and floras in changing Mediterranean communities. In: *The Conservation of Bees* (eds Matheson, A., Buchmann, S.L., O'Toole, C., Westrich, P. & Williams, I.H.). Academic Press, London, pp. 201–226.

Petanidou, T. & Potts, S.G. (2006). Mutual use of resources in Mediterranean plant–pollinator communities: how specialized are pollination webs? In: *Plant–pollinator Interactions: from Specialization to Generalization* (eds Waser, N.M. & Ollerton, J.). University of Chicago Press, Chicago, IL, pp. 220–244.

Petanidou, T., Ellis, W.N., Margaris, N.S. & Vokou, D. (1995). Constraints on flowering phenology in a phryganic (East Mediterranean shrub) community. *Am. J. Bot.*, 82, 607–620.

Polis, G.A. (1991). Complex trophic interactions in deserts: an empirical critique of food-web theory. *Am. Nat.*, 138, 123–155.

Price, M.V., Waser, N.M., Irwin, R.E., Campbell, D.R. & Brody, A.K. (2005). Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology*, 86, 2106–2116.

Quaranta, M., Ambroselli, S., Barro, P., Bella, S., Carini, A., Celli, G. *et al.* (2004). Wild bees in agroecosystems and semi-natural landscapes: 1997–2000 collection period in Italy. *Bull. Insectology*, 57, 11–61.

Rodríguez-Gironés, M.A. & Santamaría, L. (2006). A new algorithm to calculate the nestedness temperature of presence-absence matrices. *J. Biogeogr.*, 33, 924–935.

Stang, M.P., Klinkhamer, G.L. & van der Meijden, E. (2007). Asymmetric specialization and extinction risk in plant-flower visitor webs: a matter of morphology or abundance? *Oecologia*, 151, 442–453.

Stephens, D.W. & Krebs, J.R. (1986). *Foraging Theory*. Princeton University Press, Princeton.

Vázquez, D.P. & Aizen, M.A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, 85, 1251–1257.

Vázquez, D.P. & Aizen, M.A. (2006). Community-wide patterns of specialization in plant–pollinator interactions revealed by null models. In: *Plant–pollinator Interactions: from Specialization to Generalization* (eds Waser, N.M. & Ollerton, J.). University of Chicago Press, Chicago, IL, pp. 200–219.

Vázquez, D.P. & Simberloff, D. (2002). Ecological specialization and susceptibility to disturbance: conjectures and refutations. *Am. Nat.*, 159, 606–623.

Waser, N.M. & Ollerton, J. (2006). *Plant–pollinator Interactions: from Specialization to Generalization*. University of Chicago Press, Chicago, IL.

Waser, N.M. & Price, M.V. (1998). What plant ecologists can learn from zoology. *Perspect. Plant Ecol. Evol. Syst.*, 1, 137–150.

Waser, N.M., Chittka, L., Price, M.V., Williams, N.M. & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043–1060.

Williams, N.M., Minckley, R.L. & Silveira, F.A. (2001). Variation in native bee faunas and its implications for detecting community change. *Conserv. Ecol.*, 5, 57–89.

Zamora, R. (2000). Functional equivalence in plant-animal interactions: ecological and evolutionary consequences. *Oikos*, 88, 442–447.

Editor, Jordi Bascompte
Manuscript received 18 December 2007
First decision made 15 January 2008
Manuscript accepted 29 January 2008