

## GENERALIZATION VS. SPECIALIZATION IN THE POLLINATION SYSTEM OF *HORMATHOPHYLLA SPINOSA* (CRUCIFERAE)

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**Abstract.** This study describes the interaction between *Hormathophylla spinosa*, a crucifer shrub, and its pollinators, analyzing the spatiotemporal variability of the pollinator assemblage as well as the foraging behavior and effectiveness of the common pollinators. The study was carried out in the high mountains of the Sierra Nevada, Spain, over four years (1988–1991). We selected three populations of *H. spinosa* located along a wide altitudinal gradient. This plant species was visited during the four years of the study by at least 70 species of insects belonging to five orders and 19 families. The assemblage of floral visitors of *H. spinosa* was composed mainly of ants and different species of solitary bees and flies, although most pollinators were comparatively rare. There were striking similarities between most pollinator species in the distributions of flight distance between consecutive flowers, which were leptokurtic and highly skewed. Moreover, almost all visitors acted as pollinators, depositing pollen grains enough for seed production. Insects visiting flowers of *H. spinosa* can be divided into two guilds: nectarivores (small flies and ants) and pollinivores (bees and hoverflies), which differ in pollination attributes. Flower-visitation rates were quite different between the faster pollinivores and the slower nectarivores. All nectarivorous species moved almost exclusively between flowers of the same plant, whereas pollinivores sometimes moved between plants; and pollinivores deposited more pollen grains per visit than did nectarivores. *H. spinosa* interacted most frequently with the nectarivorous *Proformica longiseta*, *Fannia scalaris*, and *Exechia dorsalis* in the two lowest elevation populations of Sierra Nevada, and with the pollinivorous *Colletes* sp. and *Eristalis tenax* in the highest elevation population. The mutualistic interaction between *H. spinosa* and its pollinators is a generalized system, where the traits of the flower allow almost every floral visitor to act as a true, effective, pollinator. The lack of differences in per-visit pollination effectiveness and the functional equivalence of very different pollinators may help to maintain this generalized system.

**Key words:** Cruciferae, generalization in pollination system; guild; high-mountain ecology; *Hormathophylla spinosa*; mutualism; pollination ecology; pollinator effectiveness and foraging behavior.

### INTRODUCTION

Most theoretical and empirical studies on plant–pollinator interactions use specialization as a central idea. Nevertheless, recent studies point out that generalization is at least as frequent as specialization in pollination systems, with increasing evidence indicating that most flowering plants are pollinated by a moderate to high number of insect species, and that most floral visitors usually visit many different hosts (Schemske 1984, Herrera 1996, Waser et al. 1996).

Several factors favor the maintenance of these types of generalized pollination systems. A high spatial variability in the distribution and abundance of pollinator species might restrict the potential evolutionary response to particular pollinators in those plant species with gene flow among subpopulations (Brantjes 1981, Thomson 1981, Aker 1982, Howe 1984, Herrera 1988, Horvitz and Schemske 1990, Eckhart 1991). Never-

theless, a geographic structure of specialization can appear when plants without or with limited gene flow among populations are pollinated by different species in different populations, with the result of each population being locally specialized to one or a few pollinators (Thompson 1994). Generalization is also favored within each plant population by temporal unpredictability in pollinator assemblage (Herrera 1988, 1996, Horvitz and Schemske 1990, Eckhart 1992, Fishbein and Venable 1996, Waser et al. 1996, and references therein). Indeed, when local floral visitor assemblages change between years, pollinator-mediated selective pressures become inconsistent, and the appearance of morphological and physiological traits of plants shaped to the demand of particular visitors is unlikely (Herrera 1988, Petterson 1991).

In addition to the importance of the spatiotemporal heterogeneity in promoting generalization, we should also consider that there is an opportunity for specialization only when different pollinators, due to interspecific differences in individual effectiveness, produce different outcomes from the plant's viewpoint

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TABLE 1. Differences between plant populations in the Sierra Nevada with respect to some morphological and reproductive traits of the labeled *H. spinosa* plants.

| Trait                         | Population                 |                            |                          |
|-------------------------------|----------------------------|----------------------------|--------------------------|
|                               | A                          | B                          | C                        |
| Plant size (cm <sup>2</sup> ) | 1603 ± 145 <sup>a</sup>    | 1225 ± 351 <sup>a</sup>    | 1739 ± 188 <sup>a</sup>  |
| Number of flowers/plant       | 6739 ± 585 <sup>a</sup>    | 11 621 ± 2693 <sup>b</sup> | 7744 ± 635 <sup>a</sup>  |
| Number of stamens/flower      | 5.78 ± 0.16 <sup>a,b</sup> | 4.69 ± 0.39 <sup>a</sup>   | 5.84 ± 0.20 <sup>b</sup> |
| Flower diameter (mm)          | 6.02 ± 0.15 <sup>a,b</sup> | 5.64 ± 0.21 <sup>a</sup>   | 6.72 ± 0.15 <sup>b</sup> |
| Pollination failure           | 25.6 ± 2.6 <sup>a</sup>    | 43.4 ± 2.6 <sup>b</sup>    | 10.2 ± 1.8 <sup>c</sup>  |
| Seed : ovule ratio            | 29.6 ± 0.1 <sup>a</sup>    | 30.5 ± 0.1 <sup>a</sup>    | 40.3 ± 0.1 <sup>b</sup>  |

Notes: Plant size is the major diameter. Pollination failure is expressed as a 3-yr average of the percentage of abscission of unpollinated flowers. Seed : ovule ratio is the proportion of ovules setting seeds per ripe fruit. Data are mean ± 1 SE. For the Sierra Nevada populations, means in each row followed by different superscript letters are different at  $P < 0.05$  according to Scheffé's test after one-way ANOVA analyses.

(Schemske and Horvitz 1984, 1989, Herrera 1987, 1989, Eckhart 1991, 1992, Stanton et al. 1991, Fishbein and Venable 1996). A different scenario can develop when very different pollinator species have the same effect on plant fitness (Feinsinger 1983). Indeed, there is evidence suggesting that taxonomically unrelated pollinators can forage at flowers in similar ways, and thereby have similar individual effectiveness (Motten et al. 1981, Waser 1982, Feinsinger 1983, Vaughton 1992, Conner et al. 1995, Fishbein and Venable 1996). In this case, the evolutionary process might be expected to result in a more generalized response to whole groups of species interacting in a similar way.

In the present paper, we study the interaction between *Hormathophylla spinosa* (Cruciferae) and its pollinators in the high mountains of the Sierra Nevada (Granada province, SE Spain). We quantified the number of species visiting the flowers of this plant by surveying the entire assemblage without any prior selection of species (Waser et al. 1996). Moreover, to test whether *H. spinosa* is locally specialized to only a few taxa, despite being visited by many different species throughout its distribution area, we analyzed the spatial variability of the main visitors by studying the pollination assemblage in three different populations. Additionally, we assessed year-to-year fluctuations in the local visitor assemblage during 4 yr for all three plant populations. Finally, we quantified the per-visit pollination effectiveness for the main visitors to determine whether they interacted similarly with the plant. By studying simultaneously multiple sources of potential variation in pollination success, we seek to ascertain whether this plant species has a generalized pollination system and, if so, the possible factors by which this generalization is maintained.

## MATERIALS AND METHODS

### Plant natural history

*Hormathophylla spinosa* (L.) Küpfer (Cruciferae) is an abundant stunted shrub of the high mountains of southern Spain and the western Mediterranean (Küpfer

1993). In the Sierra Nevada, this plant grows at an altitude of between 1600 and 3340 m.

During flowering, from June to August (Gómez 1993), a typical individual of this mass-flowering species produces ~8000 flowers grouped in inflorescences, each bearing 4 to 22 flowers (Gómez and Zamora 1992), with  $6.1 \pm 1.2$  flowers (mean ± 1 SD) open at the same time in each inflorescence. Each flower lasts  $3.6 \pm 1.9$  d. The white to pink flowers are actinomorphic, hermaphroditic, and slightly protandrous. The flower has four nectaries that produce traces of nectar ( $0.039 \mu\text{L}$  per flower per day) and  $769 \pm 460$  pollen grains per anther (Gómez and Zamora 1992). Each flower invariably has four ovules. *H. spinosa* can produce seeds by geitonogamy (Gómez and Zamora 1996).

### Study area

The study was carried out in the Sierra Nevada for 4 yr (1988–1991). We selected three populations of *H. spinosa* above the timberline, situated at 2160 m (Population A), at 2550 m (Population B), and at 3130 m (Population C) (see Gómez 1993 for a full description).

At the beginning of the study (1988), we tagged 80 randomly chosen reproductive plants (40 in Population A and 20 each in Populations B and C). The size distribution of the plant sample reflects the natural frequency distribution of plant size in populations. All the observations during the four study years involved these plants. The key plant traits related to the pollination interaction are shown in Table 1 for each plant population.

### Analysis of the diversity and abundance of the pollinator assemblage

The sampling procedure used to determine the composition and abundance of the pollinator species in each plot was to count all flower visitors seen in 1-min periods on each tagged plant. Each census thus corresponded to 20 min of total observation (in population A, we divided the 40 tagged plants in two groups of 20 each). Every 1–2 d during flowering peak (>60% of plants at flower) in each plot, we carried out several

censuses from sunrise to sunset. We made a total of 320 censuses (80 every year) evenly distributed per population corresponding to 106.7 h of net observation. During the censuses, we stayed ~1 m from the flowering plant, to monitor all the floral visitors but not disturb their foraging behavior. At the beginning of the study in 1988, we collected all possible pollinators of *H. spinosa* for identification. Each new species appearing in the following years of the study was collected and identified. Any insect seen on the flowers that could make contact with the anthers and/or stigma was considered to be a flower visitor and was recorded in the censuses. In addition, a subsample of each species was also measured in length, using a magnifying glass and micrometer. In this paper, we express pollinator abundance as the number of insects per *H. spinosa* individual and per 10 minute, instead of per 1 minute as they were censused, due to scarcity of most floral visitors. Voucher specimens of floral visitors have been deposited in the Department of Animal Biology and Ecology, University of Granada.

#### Pollinator foraging behavior

The foraging behavior of the pollinators was studied during 1988 and 1989, by observing flower visitors until they left the patch. The behavioral characteristics of flower visitors analyzed were flower-visitation rate, flight distance between flowers of the same plant, and percentage of flights and distance between flowers of different plants. We took special care in following all observed insects when they left a focal plant, in order to obtain accurate estimates of interplant flight proportion. "Flower-visitation rate," number of flowers visited per minute (Herrera 1989), was calculated by counting the number of flowers visited by an arbitrarily chosen insect during a foraging bout divided by the observation time.

In addition, using magnifying glasses, we recorded the type of food collected by the floral visitors (pollen vs. nectar) for most of the species censused. In total, 903 foraging bouts (30–300 s) were recorded, with 12 324 between-flower movements for a total of 1133 min 33 s of observation.

#### Pollinator effectiveness

We analyzed two main components of effectiveness, the frequency of interaction and per-visit pollen deposition (Herrera 1987, 1989, Waser and Price 1990, Fishbein and Venable 1996), for the most abundant visitors of *H. spinosa* flowers. Frequency of interaction has been estimated as the number of flowers of one *H. spinosa* individual visited by the population of a specific pollinator taxon in one minute ("number of flowers visited" [NFV] sensu Herrera 1989, see also Waser and Price 1990, Fishbein and Venable 1996). Since we quantified how many insects of each species visited flowers of a given plant per minute, and estimated how many flowers were visited per minute by each insect

species, we could estimate the number of flowers per plant visited each minute by the entire population of a focal insect species. We calculated NFV for 1 min by multiplying insect abundance (insects/plant) and flower-visitation rate (flowers/insect). The analysis of the NFV was made in 1988 and 1989, the only years for which we had estimates of the flower-visitation rate.

Per-visit pollen deposition was studied for all the common flower visitors of *H. spinosa*. We bagged several floral buds, and, after flowering, permitted insects to collect pollen or nectar from these virgin flowers. After each visit, the flowers visited were immediately removed and placed in small glass vials. At the end of each observation session (usually 1–2 h) we counted the number of pollen grains deposited on the stigmas by each pollinator species, using a dissecting microscope (pollination intensity sensu Waser and Price 1990, Dafni 1992). These quantities were compared with control flowers (same treatment, but with no insect visits). We also noted the sexual phase (whether female or male) of the flower visited.

To estimate deposition efficiency, we calculated the stigmatic pollen:ovule ratio (SP:O ratio), the ratio of the net number of pollen grains deposited on the stigma to the total number of ovules in the flower (Kearns and Inouye 1993). *Hormathophylla spinosa* has hermaphroditic flowers, and pollen auto-deposition occurs (Gómez and Zamora 1996). To calculate SP:O ratio accurately, we subtracted the SP:O ratio of control flowers (no visits) from the value of each species. We estimated the efficiency of eight pollinator species of *H. spinosa*, which accounted for >99% of the floral visits.

#### Analysis

We compared the abundance of insects between sites, years, and taxa using a three-way mixed-model ANOVA with interaction terms. Population and year were random effects in the models and censuses were the sampling units. In this model, we introduced only data from the three main orders of floral visitors (Hymenoptera, Diptera, and Coleoptera), since the other, rare, orders were not present in all populations  $\times$  years combinations, to avoid missing cells (Mead 1988, Underwood 1997). We used Type III of sums of square because we had unbalanced data, with twice the number of censuses in population A than in the other populations. Although this type of ss does not correct for unbalanced design, it provides the most readily interpretable test of the null hypotheses of no main effect of factors when all treatment combinations are observed but the number of observations varies among cells and is preferred over the other types with unbalanced data (Potvin 1993, Shaws and Mitchell-Olds 1993). Additionally, where interactions were significant, we compared the means of every factor separately at each level of the other factors by multiple comparisons (Underwood 1997:331). For these comparisons, we first tested the equality of variances using the rel-



ative Levene's test (SAS Institute 1994). Then, we used Tukey-Kramer HSD tests if variances were equal (Day and Quinn 1989) and Welch's robust ANOVA when variances were unequal (Day and Quinn 1989, SAS Institute 1994). To control for the experiment-wise Type I error produced by multiple comparisons, we adjusted the probabilities of error to  $\alpha = 0.05$ , by using the sequential Bonferroni technique (Day and Quinn 1989, Rice 1989). Prior to all these parametric analyses, we arcsine-transformed the ratio variables and log-transformed the remaining variables (Zar 1996).

We have used nonparametric statistics to analyze the foraging behavior of the pollinators because: (1) data departed sharply from normality, (2) distributions were highly skewed, (3) sample size was quite low for some pollinator species, and (4) there were pronounced differences in sample sizes across insect species. All these caveats, common to most behavioral data, suggest the use of distribution-free statistics (Martin and Bateson 1986). We used Mann-Whitney *U* test when comparing medians between two unpaired groups, and Kruskal-Wallis when comparing more than two unpaired groups. For comparing flight-distance distribution, we used the Kolmogorov-Smirnov test.

## RESULTS

### *Diversity and spatiotemporal variation in the floral-visitor assemblage*

The flowers of *H. spinosa* were visited by at least 70 insect species belonging to five orders, with Diptera (44% of the total of species) and Hymenoptera (32%) being the most diverse orders represented (Appendix). The most abundant species were *Proformica longiseta*, which represented 81.5% of overall floral visits, *Fannia scalaris* (5.7%), *Colletes* aff. *nigricans* (*Colletes* sp. hereafter; 2.3%), and *Eristalis tenax* (1.1%). Most species (97.3% of pollinator taxa) had an abundance of  $<1$  visit-plant<sup>-1</sup>·10 min<sup>-1</sup> (Appendix).

The number of insect species visiting the flowers of *H. spinosa* ranged from 35 in 1988 to 43 in 1990. The order represented by the most species was Diptera in all years. The number of visitor species gradually declined with rising altitude, from 57 species in population A, to 39 species in population B, and 36 in population C. Only 18 species were present in all study years, and 13 species were present in all populations (see Appendix).

In the three-way ANOVA on insect abundance, the main factors and interactions were significant (Table 2). The multiple comparisons show that insect abundance varied among populations and among taxa ( $P < 0.05$ , most pairwise comparisons with Tukey-Kramer HSD and Welch's test with Bonferroni correction), but not between years. Floral visitors were much more abundant in populations A ( $18.26 \pm 18.73$  insects-plant<sup>-1</sup>·10 min<sup>-1</sup>) and B ( $36.05 \pm 8.94$ ) than in C ( $5.87 \pm 1.63$ , see Appendix). Moreover, Hymenop-

TABLE 2. Summary of the three-way ANOVA ( $n = 250$  censuses) on log (1 + pollinator abundance) for the three main insect orders for four years and three populations.

| Source     | df  | ss     | F      | P       |
|------------|-----|--------|--------|---------|
| Taxon      | 2   | 61.67  | 226.30 | 0.00001 |
| Population | 2   | 7.67   | 28.13  | 0.00001 |
| Year       | 3   | 0.57   | 1.40   | 0.241   |
| T × P      | 4   | 32.50  | 59.63  | 0.0001  |
| T × Y      | 6   | 4.12   | 5.04   | 0.0001  |
| P × Y      | 6   | 4.71   | 5.76   | 0.0001  |
| T × P × Y  | 12  | 4.83   | 2.96   | 0.0001  |
| Residuals  | 923 | 125.49 |        |         |

tera (ants) were much more abundant than any other order. The significant population × taxon interaction resulted from the variability of the abundance of insect orders among populations. The most abundant flower visitors in populations A and B were Hymenoptera (primarily *P. longiseta*). In contrast, plants of population C were visited most frequently by Diptera (primarily *E. tenax* and *F. scalaris*), and solitary bees (mainly *Colletes* sp.).

### *Pollinator foraging behavior*

All floral visitor species made contact with anthers and stigma during their visits, acting as potential pollinators. Most of the insect species visited *H. spinosa* flowers to collect pollen (63%; Appendix). Bees and large hoverflies usually gleaned pollen while crawling over the flowering surface. There were also several species of nectarivores, such as *P. longiseta*, *F. scalaris*, *Rhamphomyia tenuirostris*, and *Exechia dorsalis*. We observed only one species, *Sphaerophoria scripta*, collecting both pollen and nectar in the flowers.

All pollinators moved mainly among flowers on the same plant, with only *Eupeodes corollae* and *Apis mellifera* making >5% of flights between flowers of different plants (Table 3). Pooled Hymenoptera made as few between-plant flights as Diptera ( $P > 0.017$ , Mann-Whitney *U* test). Similarly, the proportion of between-plant flights did not differ between nectarivorous and pollinivorous ( $P > 0.017$ , Mann-Whitney *U* test), although pollen-collecting insects made an average ( $\pm 1$  SE) of  $2.23 \pm 0.48\%$  (up to 5%) flights between plants, whereas nectar-collecting insects made only  $0.71 \pm 0.48\%$  flights between plants.

The within-plant flight-distance distributions were similar (i.e., highly leptokurtic and skewed to the right) among pollinator species ( $P > 0.15$ , all pairwise comparisons, Kolmogorov-Smirnov test). Most species moved between flowers of the same inflorescence or between flowers from nearby inflorescences (Table 3).

Flight-distance distributions were similar between pooled pollen-collecting and pooled nectar-collecting visitors ( $D = 0.21$ ,  $Z = 0.61$ ,  $P = 0.54$ , Kolmogorov-Smirnov test), although pollen-collecting pollinators tended to make long trips between different points within the same plant, whereas nectar-collecting pol-

TABLE 3. Behavioral characteristics of the pollinators of *H. spinosa* (pooled across all populations and years).

| Species                         | Size | Visitation rate | Between plant flights (%) | Within-plant flight distance |          |          |      | Between plant flight distance (maximum) |
|---------------------------------|------|-----------------|---------------------------|------------------------------|----------|----------|------|---|
|                                 |      |                 |                           | Mean $\pm$ 1 SE              | <i>n</i> | Kurtosis | Skew |   |
| <i>Proformica longiseta</i>     | 2.9  | 3.19            | 0.00                      | 1.98 $\pm$ 0.09              | 470      | 15.5     | 2.9  |   |
| <i>Colletes</i> sp.             | 10.5 | 27.62           | 1.76                      | 3.76 $\pm$ 0.12              | 2513     | 22.0     | 3.8  | 57.1                                    |
| <i>Lasioglossum</i> sp.         | 5.7  | 16.11           | 0.00                      | 2.83 $\pm$ 0.26              | 160      | 4.9      | 2.1  |   |
| <i>Andrena ocreata</i>          | 12.2 | 29.61           | 0.51                      | 2.13 $\pm$ 0.21              | 194      | 19.9     | 4.0  | 30.0                                    |
| <i>A. afrensis</i>              | 14.5 | 23.37           | 1.91                      | 2.01 $\pm$ 0.23              | 586      | 178.8    | 11.4 | 101.4                                   |
| <i>A. niveata</i> female        | 7.0  | 21.26           | 0.95                      | 2.76 $\pm$ 0.31              | 166      | 11.9     | 3.3  | 15.0                                    |
| <i>A. niveata</i> male          | 7.0  | 23.34           | 0.00                      | 3.32 $\pm$ 0.22              | 228      | 7.5      | 2.5  |   |
| <i>A. ovatula</i>               | 9.7  | 23.82           | 3.20                      | 2.94 $\pm$ 0.28              | 226      | 19.4     | 3.8  | 30.2                                    |
| <i>Dufourea paradoxa</i>        | 10.9 | 27.00           | 0.00                      | 2.58 $\pm$ 0.58              | 44       | 10.4     | 3.1  |   |
| <i>Apis mellifera</i>           | 13.0 | 24.74           | 5.54                      | 2.34 $\pm$ 0.14              | 586      | 15.5     | 3.5  | 70.0                                    |
| <i>Eristalis tenax</i>          | 13.4 | 20.64           | 4.06                      | 2.35 $\pm$ 0.10              | 2008     | 35.0     | 4.9  | 104.8                                   |
| <i>E. arbustorum</i> pale       | 10.7 | 19.50           | 4.03                      | 1.92 $\pm$ 0.23              | 438      | 158.7    | 10.8 | 65.4                                    |
| <i>E. arbustorum</i> dark       | 9.9  | 14.82           | 1.61                      | 2.09 $\pm$ 0.14              | 901      | 24.6     | 4.5  | 85.0                                    |
| <i>Scaeva selenitica</i>        | 12.3 | 15.45           | 1.29                      | 3.59 $\pm$ 0.17              | 777      | 9.2      | 2.6  | 80.8                                    |
| <i>S. albomaculata</i>          | 11.7 | 18.89           | 4.69                      | 3.97 $\pm$ 0.86              | 60       | 15.6     | 3.7  | 83.3                                    |
| <i>Chrysotoxum intermedium</i>  | 11.9 | 22.56           | 2.64                      | 2.27 $\pm$ 0.21              | 490      | 56.4     | 6.3  | 65.8                                    |
| <i>Platycheirus manicatus</i>   | 9.8  | 20.33           | 1.47                      | 2.47 $\pm$ 0.38              | 67       | 5.7      | 2.2  | 75.0                                    |
| <i>Eupeodes corollae</i>        | 9.7  | 18.79           | 6.61                      | 4.08 $\pm$ 0.47              | 113      | 5.6      | 2.0  | 42.5                                    |
| <i>Sphaerophoria scripta</i>    | 7.6  | 11.44           | 1.62                      | 4.16 $\pm$ 0.31              | 390      | 15.4     | 3.3  | 38.6                                    |
| <i>Anthrax</i> sp.              | 12.1 | 26.13           | 4.27                      | 2.88 $\pm$ 0.15              | 724      | 14.0     | 3.1  | 52.1                                    |
| Bombyliidae 1                   | 13.1 | 9.43            | 0.00                      | 3.04 $\pm$ 0.60              | 33       | 3.5      | 2.0  |   |
| Bombyliidae 2                   | 11.7 | 19.73           | 0.00                      | 3.21 $\pm$ 0.64              | 48       | 5.3      | 2.4  |   |
| <i>Fannia scalaris</i>          | 3.4  | 8.99            | 0.48                      | 1.47 $\pm$ 0.14              | 377      | 47.6     | 6.2  | 15.0                                    |
| <i>Muscina assimilis</i>        | 5.0  | 16.74           | 0.00                      | 1.38 $\pm$ 0.29              | 66       | 26.5     | 4.7  |   |
| <i>Rhamphomyia tenuirostris</i> | 3.1  | 5.48            | 0.00                      | 1.27 $\pm$ 0.20              | 130      | 64.4     | 7.4  |   |
| <i>Exechia dorsalis</i>         | 2.2  | 4.86            | 0.00                      | 1.61 $\pm$ 0.21              | 66       | 7.7      | 2.6  |   |
| <i>Protocalliphora azurea</i>   | 6.5  | 12.86           | 0.00                      | 1.92 $\pm$ 0.04              | 445      | 9.0      | 2.6  |   |

Note: Size is the body length in mm. Visitation rate is the number of flowers visited per minute. Within-plant and between-plant flight distances are in centimeters.

TABLE 4. Spatial variation in the frequency of interaction (number of flowers visited, NFV) between *H. spinosa* and its main pollinator species.

| Species                         | Population  |             |             |
|---------------------------------|-------------|-------------|-------------|
|                                 | A           | B           | C           |
| <i>Proformica longiseta</i>     | <u>5.18</u> | <u>8.60</u> |             |
| <i>Andrena afrensis</i>         | 0.22        |             |             |
| <i>Andrena ocreata</i>          | 0.02        | 0.06        | 0.13        |
| <i>Andrena niveata</i>          | 0.05        | 0.02        | 0.04        |
| <i>Lasioglossum</i> spp.†       | 0.29        | 0.18        | 0.05        |
| <i>Apis mellifera</i>           | 0.10        |             | 0.05        |
| <i>Colletes</i> sp.             |             |             | <u>5.36</u> |
| <i>Eristalis tenax</i>          | 0.19        | 0.04        | <u>2.67</u> |
| <i>Eristalis arbustorum</i>     | 0.46        |             | 0.03        |
| <i>Chrysotoxum intermedium</i>  | 0.15        |             | 0.15        |
| <i>Scaeva selenitica</i>        | 0.09        | 0.04        | 0.07        |
| <i>Eupeodes corollae</i>        | 0.02        |             | 0.16        |
| <i>Sphaerophoria scripta</i>    | 0.06        | 0.04        |             |
| <i>Platycheirus manicatus</i>   |             |             | 0.16        |
| <i>Anthrax</i> sp.              | 0.27        |             |             |
| Bombyliidae sp. 1               |             | 0.01        |             |
| <i>Fannia scalaris</i>          | <u>1.11</u> | <u>1.84</u> | 0.92        |
| <i>Protocalliphora azurea</i>   | 0.40        | 0.01        | 0.10        |
| <i>Rhamphomyia tenuirostris</i> | 0.04        | 0.07        | 0.01        |
| <i>Exechia dorsalis</i>         | 0.40        | <u>1.00</u> | 0.42        |

Note: NFVs > 1 are underlined.

† Includes *Lasioglossum aeratum* and *L. smeathmanellum*.

linators moved invariably within nearby inflorescences. Between-plant flight distances were always ~100 cm or less (Table 3).

The highest flower-visitation rates were recorded for *Andrena ocreata* and *Anthrax* sp., and the lowest for *P. longiseta*, *E. dorsalis*, and *R. tenuirostris*. Flower-visitation rate differed significantly between nectarivores ( $11.82 \pm 2.89$  flowers/min) and pollinivores ( $20.96 \pm 1.13$ ;  $U = 26$ ,  $P = 0.003$ ; Mann-Whitney  $U$  test).

#### Pollinator effectiveness

Only five species, *P. longiseta*, *Colletes* sp., *F. scalaris*, *E. dorsalis*, and *E. tenax*, had high frequencies of interaction (NFV > 1) in any population of the Sierra Nevada (Table 4). There were among-population differences in the species showing the greatest NFVs: *P. longiseta*, *F. scalaris*, and *E. dorsalis* exhibited the highest NFVs in populations A and B whereas NFVs of *Colletes* sp. and *E. tenax* were greatest in population C (Table 4). Visitors in populations A and B registered high NFVs because of their abundance (particularly *P. longiseta*), despite having the lowest flower-visitation rates. It is noteworthy that no species registered both high abundance and high flower-visitation rates in populations A and B. However, in population C, *Colletes* sp. and *E. tenax* had both high flower-visitation rate and high abundance.

TABLE 5. Pollen deposition of the most common insect species visiting *H. spinosa* flowers in the Sierra Nevada.

| Species                         | Food   | N  | PI                   | SP : O ratio |
|---------------------------------|--------|----|----------------------|--------------|
| Control                         |        | 22 | 82.64 $\pm$ 10.1     |              |
| <i>Rhamphomyia tenuirostris</i> | Nectar | 5  | 97.00 $\pm$ 19.5 NS  | 3.6:1        |
| <i>Muscinia assimilis</i>       | Nectar | 8  | 99.12 $\pm$ 17.4 NS  | 4.1:1        |
| <i>Fannia scalaris</i>          | Nectar | 10 | 95.00 $\pm$ 06.1 NS  | 3.1:1        |
| <i>Proformica longiseta</i>     | Nectar | 4  | 102.25 $\pm$ 18.1 NS | 4.9:1        |
| <i>Eristalis tenax</i>          | Pollen | 17 | 136.23 $\pm$ 13.4*** | 13.4:1       |
| <i>Scaeva selenitica</i>        | Pollen | 11 | 148.73 $\pm$ 18.5**  | 16.5:1       |
| <i>Colletes</i> sp.             | Pollen | 4  | 146.25 $\pm$ 22.2 NS | 15.9:1       |
| <i>Dufourea paradoxa</i>        | Pollen | 8  | 174.00 $\pm$ 18.7*** | 22.8:1       |

Notes: PI is pollination intensity, estimated as the number of pollen grains ( $\pm 1$  SE) deposited onto *H. spinosa* stigmas after one insect visit (Dafni 1992). The PI of every pollinator species is tested against self-deposition (control) PI by a Bonferroni-Dunn test; NS = not significant, \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  ( $\alpha = 0.05$  adjusted by Bonferroni technique). SP : O ratio is (sensu Kearns and Inouye 1993) the ratio of pollen load size to number of ovules. N is the sample size.

All of the observed insects delivered a measurable quantity of pollen grains during a single visit. However, only *E. tenax*, *D. paradoxa*, and *S. selenitica* (all pollen harvesters) deposited significantly higher amounts of pollen grains than autodeposition in control flowers after a single visit. There was also a significant difference in the number of pollen grains deposited by pollinators from different trophic guilds ( $U = 0.001$ ,  $n = 8$ ,  $P = 0.02$ , Mann-Whitney  $U$  test). Nectar-collecting pollinators deposited fewer pollen grains per visit ( $98.34 \pm 1.55$ , mean  $\pm 1$  SE) than did pollen-collecting ones ( $150.92 \pm 6.23$ ). However, within each trophic guild the quantity of pollen grains delivered on the stigma per visit was similar among species ( $H = 0.26$ ,  $P = 0.97$  for nectar-collecting pollinators,  $H = 3.22$ ,  $P = 0.36$  for pollen-collecting pollinators; Kruskal-Wallis test, Table 5). SP:O ratios were also higher for pollen-collecting than for nectar-collecting insects (Table 5).

The amount of pollen deposited per visit was significantly greater for flowers in the female phase ( $139.8 \pm 51.8$ ) than in the male phase ( $102.6 \pm 54.6$ ;  $U = 241$ ,  $n = 62$ ,  $P = 0.016$ , Mann-Whitney  $U$  test).

#### DISCUSSION

The flowers of *H. spinosa* were visited by a phylogenetically diverse insect assemblage (70 species, five orders, and 22 families) composed mainly of ants, flies (Syrphidae, Empididae, Mycetophylidae, and Muscidae), and solitary bees (Andrenidae and Halictidae). Most of these abundant visitors concurrently visit the flowers of other high-mountain plants in the Sierra Nevada (Gómez et al. 1996). The pollinator assemblage of *H. spinosa* was composed of a few highly abundant species, with most other pollinators comparatively rare (see also Herrera 1989 and references therein). Thus, as in other mutualistic assemblages (Schemske 1976, Motten 1983, Campbell 1985, Jordano 1987, Herrera 1988, Fishbein and Venable 1996), the resulting web of interactions between *H. spinosa* and pollinators in

all populations has a core of strong interactions, and a large network of weak relationships.

There is spatial variability in the interaction between *H. spinosa* and its pollinators. In populations A and B of the Sierra Nevada, a high number of pollinations were actually carried out by nectarivorous ants (as well as by small flies), for which poor per-visit effectiveness was offset by high abundance (Gómez and Zamora 1992). In these two populations, abundance and pollinator quality were decoupled (see also Herrera 1987, 1988, Pettersen 1991). In contrast, in population C the most abundant pollinators, pollinivorous *Colletes* sp. and *E. tenax*, were also the most speedy foragers, delivering the greatest pollen loads per visit and making the most distant trips between different plants. In this population, the most frequent pollinators were also the most efficient ones. Thus, different pollinators predominated in different plant populations. The resulting spatial variation in pollinator assemblages and in the identity of the best pollinators fosters generalization at the plant species level (Waser et al. 1996). At the population level, however, this spatial variability may produce a mosaic of selective regimes, leading to local specialization on particular pollinator species (Thompson 1994). This possibility is reinforced, given that the main pollinator species were present every year of the study with similar abundances in each plant population. According to this scenario (spatial variability but temporal predictability), we would expect to find specialization within each plant population towards the most effective pollinators (Thompson 1994, Wilson and Thomson 1996).

For local specialization of *H. spinosa* on one specific pollinator species, each taxonomic species must, from the plant's perspective, correspond to a distinguishable unit of interaction (Waser et al. 1996). When several species interact in the same way with the plant, however, the possibility of species-to-species coadaptation is strongly limited (Feinsinger 1983). Despite the high taxonomic diversity, all pollinators of *H. spinosa* pro-



duced similar pollen-dispersal patterns by moving between flowers in a similar way. Insects often spent considerable time on the same plant, visiting from 10 to 50 flowers (up to 200 flowers) before leaving. Thus, most of the movements for all pollinators were between flowers of the same plant and even of the same inflorescence, with very few trips between flowers on different plants. A likely consequence of this foraging behavior is a high level of self-pollination (whether autogamous or geitonogamous, Gómez and Zamora 1996), a common feature noted for other mass-flowering species (Augspurger 1980, Stephenson 1982, Frankie and Haber 1983, Harder and Barrett 1995, Snow et al. 1996). Furthermore, the unspecialized actinomorphic flower of *H. spinosa* favors effective pollen transfer in all pollinator visits. Since the minimum number of pollen grains required for fruit set to occur is generally equal to or less than the total number of ovules in the ovary (Silander and Primack 1978, Mulcahy et al. 1983, McDade and Davidar 1984, Herrera 1987), the pollen load delivered by all pollinator species could promote fruit set after a single visit to a flower (one *H. spinosa* flower has four ovules). Moreover, although pollen deposition by nectarivorous insects did not differ from self-pollinated flowers, those figures represent the deposition after only single visits. We have shown that a flower of *H. spinosa* has a high probability of being visited more than once by insects (Gómez and Zamora 1992), increasing transfer of pollen from different flowers. In fact, *P. longiseta* pollen deposition was statistically similar to that of the control, although previously we experimentally demonstrated the ability of this ant to pollinate this plant species (Gómez and Zamora 1992).

The above features promote generalization in the *H. spinosa*–pollinator interaction, and make specialization and fine morphological adjustment between the plant and one particular pollinator species very unlikely. Nevertheless, a possibility of matching might exist when pollinator species with similar effectiveness are taxonomically related (i.e., “pollination syndromes,” Faegri and van der Pijl 1979). In our system, however, no evidence of fitting was found at any taxonomic level (from species to order) because of the heterogeneous taxonomic composition of the entire pollinator assemblage (five orders, 22 families), and the most frequent floral visitors (two orders, several families). However, unrelated species can share critical features that select for floral morphology and behavior. In this respect, a functional pattern of plant–pollinator interactions stands out in our system when considering pollinator trophic habit (nectarivorous vs. pollinivorous), regardless of taxonomic identity. For example, (1) flower-visitation rates were quite different between the larger, faster pollinivores and the smaller, slower nectarivores; (2) all nectarivorous species moved almost exclusively between flowers of the same plant, whereas the low percentage of interplant flights made by several pol-

linivorous insects could be important to ensure some xenogamous pollination; (3) most importantly, interspecific differences in per-visit pollination effectiveness were evident only in relation to the trophic habit: pollen-collecting pollinators deposited significantly more pollen grains per visit than did nectar-collecting pollinators. Because there were between-population differences in guild abundance, some interpopulation differences in plant reproductive traits may be related to the guild dominant in each population. For example, plants from C had significantly lower pollination failure and a higher seed:ovule ratio (per ripe fruit) than did those in populations A and B (Table 1). In population C, pollinivorous insects, aided by wind pollination (Gómez and Zamora 1996), registered high pollination rates and an increase in the quantity of seeds produced by xenogamy. Furthermore, *H. spinosa* had larger flowers with more anthers in population C, than in populations A and, especially, B (Table 1), where nectarivores outnumbered pollinivores. These between-guild differences contrast with an evident within-guild interspecific similarity in foraging behavior and pollen-deposition rate. Pollinator species are functionally equivalent within guilds, and thus, they could be redundant, replaceable resources (sensu Tilman 1982) from the plant's viewpoint. As a result, all species belonging to the same guild could exert comparable selective pressures, constituting more powerful and coherent units of selection than could individual species. Our functional-group approach stresses similarities between taxonomically unrelated species (e.g., ants and small flies as nectarivores or bees and hoverflies as pollinivores), which interact similarly with the plant.

In conclusion, the mutualistic interaction between *H. spinosa* and its pollinators can be considered a generalized system, since the traits of the flower allow almost every floral visitor to act as an effective pollinator. The lack of differences in per-visit pollination effectiveness and the functional equivalence between very different pollinators may help to maintain this generalized system. The best pollinators were the most frequent at flowers, both at the species as well as at the guild level. These generalist features of the plant enable the spatiotemporal replacement of pollinators, and maintain an ecological interaction without any apparent species-specific coevolutionary consequences.

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## APPENDIX

Absolute abundance of the different species of insects visiting *H. spinosa* flowers (average number of insects per 10 min and per plant  $\pm$  1 SD) in the Sierra Nevada (Spain) during the four years of the study, all years pooled.

| Insect species                                     | Population    |               |             | Food   |
|--|---------------|---------------|-------------|--------|
|  | A             | B             | C           |        |
| Hymenoptera  |               |               |             |        |
| <i>Proformica longiseta</i> (Formicidae)           | 14.23 ± 18.92 | 33.33 ± 10.09 |             | Nectar |
| <i>Leptothorax tristis</i> (Formicidae)            | 0.05 ± 0.00   |               |             | Nectar |
| <i>Tapinoma nigerrimum</i> (Formicidae)            |               | 0.051 ± 0.00  |             |        |
| <i>Andrena carbonaria</i> (Andrenidae)             | 0.06 ± 0.00   |               |             | Pollen |
| <i>Andrena afrensis</i> (Andrenidae)               | 0.04 ± 8.75   |               |             | Pollen |
| <i>Andrena ocreata</i> (Andrenidae)                | 0.02 ± 0.03   | 0.02 ± 2.36   | 0.02 ± 1.41 | Pollen |
| <i>Andrena niveata</i> (Andrenidae)                |               |               |             | Pollen |
| male   |               |               | 0.01 ± 0.01 |        |
| female   | 0.03 ± 0.00   | 0.05 ± 6.74   | 0.03 ± 0.05 |        |
| <i>Andrena ovatula</i> (Andrenidae)                | 0.01 ± 0.02   | 0.04 ± 0.00   |             | Pollen |
| <i>Andrena nigroaenea</i> (Andrenidae)             | 0.06 ± 0.16   |               |             | Pollen |
| <i>Andrena</i> sp. (Andrenidae)                    |               |               | 0.01 ± 0.01 | Pollen |
| <i>Lasioglossum</i> spp.† (Halictidae)             | 0.09 ± 0.01   | 0.15 ± 0.01   | 0.05 ± 0.00 | Pollen |
| <i>Lasioglossum mediterraneum</i> (Halictidae)     | 0.01 ± 0.00   | 0.01 ± 0.01   | 0.05 ± 0.09 | Pollen |
| <i>Lasioglossum malachorum</i> (Halictidae)        |               | 0.04 ± 0.08   |             | Pollen |
| <i>Lasioglossum soror</i> (Halictidae)             | 0.01 ± 0.01   |               |             | Pollen |
| <i>Halictus</i> sp. (Halictidae)                   | 0.01 ± 0.01   |               |             | Pollen |
| <i>Dufourea paradoxa</i> (Halictidae)              |               |               | 0.02 ± 0.38 | Pollen |
| <i>Apis mellifera</i> (Apidae)                     | 0.02 ± 0.03   |               | 0.01 ± 0.03 | Pollen |
| <i>Colletes</i> aff. <i>nigricans</i> (Colletidae) |               | 0.02 ± 0.04   | 1.79 ± 0.56 | Pollen |
| <i>Hylaeus euryscapus</i> (Colletidae)             |               | 0.07 ± 0.14   |             | Pollen |
| <i>Nomada</i> sp. (Anthophoridae)                  | 0.01 ± 0.00   | 0.01 ± 0.05   |             | Pollen |
| <i>Polistes biglumis</i> (Vespidae)                | 0.03 ± 0.06   |               |             | Pollen |
| <i>Ancistrocerus longispinus</i> (Eumenidae)       | 0.01 ± 0.01   | 0.01 ± 0.05   | 0.02 ± 0.04 | Pollen |
| Eumenidae gen. sp.                                 |               |               | 0.03 ± 0.03 | Pollen |
| Diptera  |               |               |             |        |
| <i>Eristalis tenax</i> (Syrphidae)                 | 0.14 ± 0.14   | 0.05 ± 0.03   | 1.21 ± 0.27 | Pollen |
| <i>Eristalis arbustorum</i> (Syrphidae)            |               |               |             | Pollen |
| pale   | 0.13 ± 0.11   | 0.01 ± 0.01   | 0.01 ± 0.01 |        |
| dark   | 0.04 ± 0.05   |               |             |        |
| <i>Chrysotoxum intermedium</i> (Syrphidae)         | 0.02 ± 0.05   |               | 0.03 ± 0.04 | Pollen |
| <i>Chrysotoxum vernale</i> (Syrphidae)             | 0.01 ± 0.01   |               |             | Pollen |
| <i>Scaeva selenitica</i> (Syrphidae)               | 0.04 ± 0.05   |               |             | Pollen |
| <i>Scaeva albomaculata</i> (Syrphidae)             | 0.01 ± 0.01   | 0.03 ± 0.01   | 0.33 ± 0.25 | Pollen |
| <i>Eupeodes corollae</i> (Syrphidae)               | 0.02 ± 0.02   | 0.01 ± 0.01   | 0.05 ± 0.11 | Pollen |
| <i>Sphaerophoria scripta</i> (Syrphidae)           | 0.03 ± 0.03   | 0.02 ± 0.03   |             | Both   |
| <i>Platycyrtus manicatus</i> (Syrphidae)           |               |               | 0.06 ± 0.07 | Pollen |
| <i>Paragus strigatus</i> (Syrphidae)               | 0.01 ± 0.01   | 0.02 ± 0.03   |             | Pollen |
| <i>Merodon aeneus</i> (Syrphidae)                  | 0.01 ± 0.01   | 0.01 ± 0.01   |             | Pollen |
| <i>Volucella elegans</i> (Syrphidae)               | 0.01 ± 0.01   |               |             | Pollen |

## APPENDIX Continued.

| Insect species                                     | Population    |              |             | Food   |
|--|---------------|--------------|-------------|--------|
|  | A             | B            | C           |        |
| Syrphidae gen. sp.                                 | 0.01 ± 0.01   |              |             | Pollen |
| <b>Anthrax sp.</b> (Bombyliidae)                   | 0.11 ± 0.19   |              |             | Nectar |
| <i>Bombylius major</i> (Bombyliidae)               | 0.01 ± 0.03   | 0.01 ± 0.01  |             | Nectar |
| <i>Systoechus</i> sp. (Bombyliidae)                | 0.01 ± 0.01   |              |             | Nectar |
| Bombyliidae gen. sp. 1                             | 0.02 ± 0.03   | 0.01 ± 0.01  |             | Nectar |
| Bombyliidae gen. sp. 2                             | 0.01 ± 0.02   | 0.03 ± 0.05  |             | Nectar |
| Bombyliidae gen. sp. 3                             | 0.01 ± 0.01   |              | 0.01 ± 0.01 | Nectar |
| Bombyliidae gen. sp. 4                             | 0.01 ± 0.01   |              | 0.01 ± 0.01 | Nectar |
| <b><i>Rhamphomyia tenuirostris</i></b> (Empididae) | 0.26 ± 0.35   | 0.30 ± 0.21  | 0.06 ± 0.04 | Nectar |
| <b><i>Exechia dorsalis</i></b> (Mycetophilidae)    | 0.39 ± 0.38   | 0.11 ± 0.11  |             | Nectar |
| <b><i>Fannia scalaris</i></b> (Muscidae)           | 1.50 ± 1.94   | 1.15 ± 1.23  | 0.39 ± 0.50 | Nectar |
| <b><i>Muscinia assimilis</i></b> (Muscidae)        |               | 0.02 ± 0.04  | 0.21 ± 0.18 | Nectar |
| <i>Musca domestica</i> (Muscidae)                  | 0.10 ± 0.12   | 0.02 ± 0.03  | 0.08 ± 0.16 | Nectar |
| <i>Protocalliphora azurea</i> (Calliphoridae)      | 0.22 ± 0.28   | 0.01 ± 0.01  | 0.03 ± 0.06 | Nectar |
| <i>Chrysomia albiceps</i> (Calliphoridae)          | 0.01 ± 0.01   | 0.01 ± 0.01  |             | Nectar |
| <b><i>Calliphora vomitoria</i></b> (Calliphoridae) | 0.01 ± 0.01   |              | 0.18 ± 0.32 | Nectar |
| <b>Calliphoridae gen. sp.</b>                      | 0.05 ± 0.01   | 0.01 ± 0.01  | 0.03 ± 0.02 | Nectar |
| Sarcophagidae gen. sp. 1                           | 0.01 ± 0.01   |              |             | Nectar |
| Sarcophagidae gen. sp. 2                           | 0.01 ± 0.01   |              |             | Nectar |
| Diptera gen. sp. 1                                 | 0.01 ± 0.01   |              |             | ...    |
| Diptera gen. sp. 2                                 | 0.01 ± 0.01   |              |             | Nectar |
| Diptera gen. sp. 3                                 | 0.01 ± 0.01   |              |             | ...    |
| Diptera gen. sp. 4                                 |               | 0.01 ± 0.01  |             | ...    |
| Lepidoptera  |               |              |             |        |
| <i>Aglais urticae</i> (Nymphalidae)                | 0.01 ± 0.01   |              | 0.02 ± 0.01 | Nectar |
| <i>Macroglossum stellata</i> (Sphingidae)          |               |              | 0.01 ± 0.01 | Nectar |
| Pyrilidae gen. sp. 1                               |               | 0.05 ± 0.01  |             | Nectar |
| Pyrilidae gen. sp. 2                               |               | 0.01 ± 0.01  |             | Nectar |
| Coleoptera   |               |              |             |        |
| <b><i>Axinotarsus varius</i></b> (Malachidae)      | 0.22 ± 0.27   | 0.14 ± 0.10  | 0.02 ± 0.01 | Pollen |
| <i>Attalus tristis</i> (Malachidae)                |               |              | 0.25 ± 0.30 | Pollen |
| <i>Malachius laticollis</i> (Malachidae)           | 0.10 ± 0.01   |              | 0.01 ± 0.01 | Pollen |
| <i>Allotarsus baduii</i> (Dasytidae)               | 0.03 ± 0.01   | 0.03 ± 0.01  | 0.01 ± 0.01 | Pollen |
| <i>Aplocnemus andalusiacus</i> (Dasytidae)         | 0.01 ± 0.01   | 0.01 ± 0.01  |             | Pollen |
| <i>Labidostomis nevadensis</i> (Chrysomelidae)     |               |              | 0.05 ± 0.09 | Pollen |
| <i>Omophlus</i> sp. (Alleculidae)                  | 0.01 ± 0.01   |              |             | Pollen |
| <i>Heliotaurus ruficollis</i> (Alleculidae)        | 0.03 ± 0.01   | 0.01 ± 0.01  |             | Pollen |
| <i>Proctenius granatensis</i> (Alleculidae)        | 0.01 ± 0.01   |              |             | Pollen |
| <i>Mylabris scutellata</i> (Meloidae)              | 0.01 ± 0.01   |              |             | Pollen |
| Heteroptera  |               |              |             |        |
| <i>Lygaeus militaris</i>                           | 0.01 ± 0.01   | 0.05 ± 0.01  |             | Nectar |
| Total  | 18.26 ± 18.73 | 36.05 ± 8.94 | 5.87 ± 1.63 |        |

† Includes *Lasioglossum aeratum* and *L. smeathmanellum*. Names in bold correspond to insect species appearing in all of the four years of the study.