

Interaction frequency and per-interaction effects as predictors of total effects in plant–pollinator mutualisms: a case study with the self-incompatible herb *Linaria lilacina*

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Abstract It is widely recognized that pollinators vary in their effectiveness in pollination mutualisms, due both to differences in flower–pollinator morphological fit as well as pollinator behaviour. However, pollination webs typically treat all interactions as equal, and we contend that this method may provide misleading results. Using empirical and theoretical data, we present the case study of a self-incompatible herb in which the number of flowers visited by a pollinator cannot be used as a surrogate for the total effect of a pollinator on a plant due to differences in per-visit effectiveness at producing seeds. In self-incompatible species, the relationship between interaction frequency and per-interaction effect may become increasingly negative as more flowers per plant are visited due to geitonogamous pollen transfer. We found that pollinators making longer bouts (i.e. visiting more flowers per plant visit) had an overall higher pollination success per bout. However, per-interaction effects tended to decrease as the bout progressed, particularly for pollinators that cause higher pollen deposition. Since the same interaction frequency may result from different combinations of number of bouts (plant visits) and bout length (flowers visited/bout), pollinators making repeatedly shorter bouts may contribute more to

plant reproduction for the same number of flowers visited. Consequently, the magnitude of the differences in number of interactions of different insect types may be overridden by the magnitude of the differences in effectiveness as pollinators, even if the same pollinators consistently interact more frequently. We discuss two predictions regarding the validity of using interaction frequency as a surrogate for plant seed production (as a measure of total effect), depending on the degree of self-compatibility, plant size and floral display. We suggest that the role of interaction frequency must be tested for different species, environments, and across wider scales to validate its use as a surrogate for total effect in plant–pollinator networks.

Keywords Mutualistic networks · Plant–pollinator interactions · Visitation rate · Bout length · Pollen deposition

Introduction

In order to predict the effects of biotic interactions between species, it is important to take into account the qualitative and quantitative components of the interaction. The qualitative component accounts for differences in the traits of the interacting individuals (e.g. anatomical or behavioural; innate or learned). The quantitative component accounts for differences in the intensity of the interaction (e.g. frequency and/or strength; see Berlow et al. 2004; Vázquez et al. 2005). In plant–pollinator mutualistic interactions, both components may have a significant influence on the outcome of plant–pollinator interactions in terms of reproductive or demographic success of the interacting species (e.g. Herrera 1987, 1989; Colling et al. 2004; Aizen and Harder 2007). The need to take into account both the

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qualitative and quantitative components of plant–pollinator interactions was already acknowledged in the well-known “most effective pollinator principle” (Stebbins 1970), which emphasised the role of pollinators as determinants of the evolution of flower specialization, proposing that the most abundant and effective agents of pollen transfer between individual plants were also the most likely to determine their evolutionary pathways. Here we argue that qualitative and quantitative components of species interactions also need to be taken into account when analysing webs of interactions between plants and pollinators.

An increasing number of studies analyse whole plant–pollinator interactions networks (Olesen and Jordano 2002; Jordano et al. 2003; Vázquez and Aizen 2004). These interaction networks are generally characterised by binary matrices, where each row represents a plant species and each column a pollinator species, and the cell at the intersection between a row (plant) and column (pollinator) is set equal to zero (if the corresponding plant and pollinator species are not known to interact) or one (if the plant and pollinator are observed to interact). In this approach, no information is given about the qualitative and quantitative components that actually occur between interactors. This binary approach, however, may be insufficient to properly define the topology of an interaction network, because not all realised interactions are equally important, which has been pinpointed as one of the most recurrent problems in pollination network studies (e.g. Paine 1988, 1992; Memmott 1999; Blüthgen et al. 2006). A number of recent papers emphasise the need to include a suitable representation of the quantitative component of species interactions (Jordano 1987; Memmott 1999; Vázquez et al. 2005; see also Goldberg et al. 1999), to assess the properties of interaction networks beyond those addressed solely from binary matrices. Several approaches have been recently used to include a source of variation in quantitative components when characterising interaction networks, ranging from diversity indices to rarefaction methods (e.g. Vázquez and Simberloff 2002; Herrera 2005).

In plant–animal mutualistic networks, it has recently been suggested that one quantitative component, the interaction frequency (defined as the number of interaction events per time unit), may be used as a surrogate for the total interaction effect (defined as the per-capita reproductive or seed dispersal performance of a plant species), when the per-interaction effect is invariant (Vázquez et al. 2005). The use of interaction frequency as a quantitative estimate of total interaction effect has been proposed not only for plant–pollinator mutualistic networks but also for other interaction networks, including plant–seed dispersers (Jordano and Schupp 2000), plant–ant protection (Ness et al. 2006), and host–parasite (Poulin et al. 2008). However, a number of studies have shown that the most

abundant animal mutualists (i.e. those which presumably interact more frequently) are not necessarily the most effective ones on a per-visit basis (e.g. Herrera 1987, 1989; Schupp 1993; Mayfield et al. 2001). For plant–pollinator interactions, substantial variability in per-interaction effects may arise because of (1) varying abilities of different pollinator groups to pick up pollen from anthers and deliver it to stigma (related to their behaviour, size, mechanical fit, etc.; e.g. Wilson and Thomson 1991, Wilson 1995, Robertson et al. 2005); and (2) the composition of the pollen mixture delivered by those pollinator groups (most relevant in self-incompatible species, or in self-compatible ones with high levels of inbreeding depression; e.g. Ivey et al. 2003, Colling et al. 2004; Williams 2007). This variation in per-interaction effect is the result of pollinator foraging strategies and is strongly affected by the pollination environment (i.e. the biotic and abiotic conditions under which the plant–pollinator interaction takes place; see Rodríguez-Gironés and Santamaría 2010). In turn, the pollination environment may also affect the interaction frequency, for example through the number of flowers that individual pollinators visit per plant (hereafter referred to as bout length; e.g. Robertson and Macnair 1995; Grindeland et al. 2005; Williams 2007; see also Iwasa et al. 1995). Consequently, the per-interaction effect is not necessarily an invariant, inherent, parameter of a given plant–pollinator pair, and disregarding its influence may limit the validity of using interaction frequency as the main predictor of total effect.

In this paper, we present empirical data of a case study illustrating how the variation in per-interaction effect may limit the predictive value of interaction frequency as a surrogate for total effects (plant seed set) in plant–pollinator interactions. We used the plant *Linaria lilacina* (Plantaginaceae) and its pollinator assemblage. *L. lilacina* is a self-incompatible herb; thus, only outcrossed pollen is suitable for seed siring. *L. lilacina* is visited across its range by several bee species that differ in abundance, behaviour, body size and other morphological traits, which could affect their per-visit effectiveness. We also develop a general model for self-incompatible plant species aimed at calculating the relative contribution of different pollinators to seed production as a function of variable bout length and pollen deposition ability.

Methods

Study system and sites

Linaria lilacina Lange (Plantaginaceae) is a perennial herb endemic to mountains in southeastern Spain (Valdés 1970; Sáez and Crespo 2005). This study was carried out in 2005–2006 in the Jaén Mountains, in two *L. lilacina*

populations (Sillón del Rey [SR] and Otiñar [OT]) separated by ca. 3 km (further details can be found in Sánchez-Lafuente 2007). In these populations, plants sprout every season in midwinter and produce several to many simple or branched stems (range: 5–156; mean \pm SE stems/plant: 38.21 ± 26.66 ; $n = 195$) of ca. 22 cm. in length, some of which may produce a variable number of zygomorphic flowers clumped at their tops (range: 20–285; mean \pm SE flowers/plant: 102.49 ± 52.18 ; $n = 146$). Morphologically, the flowers consist of an occluded, tubular corolla, with upper and lower whitish lips, and coloured nectar guides (blue or yellow, depending on the population; Sánchez-Lafuente and Alcántara, unpubl. data). A spur is present to collect nectar produced by a nectary located under the ovary inside the corolla. Functionally, the flowers are hermaphroditic and self-incompatible. The fruit is a two-locule capsule, dehiscent by valves. After successful pollination, the capsule develops, producing numerous small, brown, flattened, winged seeds (range: 8–87; mean \pm SE seeds/fruit: 68.22 ± 23.24 ; $n = 2,541$). Fruit maturation takes between 18 and 24 days. In 2005, 15 plants were randomly selected in SR and 14 in OT. In 2006, the same plants were used in both populations, except for 2 plants lost in OT that were not substituted.

Composition, behaviour and effectiveness of the pollinator assemblage

In each season, we used the earliest 2–3 reproductive inflorescences of each tagged plant for a week of preliminary insect observations aimed at detecting the composition and abundance of the visitor assemblage, and analysing their behaviour while visiting plants. Overall, we detected seven insect species, of which four were analysed in further detail (see “Results” for a justification). From these preliminary observations, the experimental procedure proceeded as follows.

The remaining reproductive inflorescences of the tagged plants were covered with bags before flowering started to avoid uncontrolled flower visitation by insects while the plants were not observed and to deter floral herbivores that may damage corollas before abscission (Sánchez-Lafuente 2007). When the inflorescences started to flower, we removed the bags on each census day and observed each plant in random order twice a day in 20 min shifts during the time of maximum pollinator activity (12–18 h CET). Plants in both populations were observed for between 1 and 8 h each season, depending on their flowering phenology. Overall, pollinator censuses extended for 18 days in 2005 and 25 days in 2006, with 144 and 200 hours of observation, respectively (both populations pooled). During each shift, we recorded the activities of all insects visiting flowers. For each plant visit (i.e. each bout, hereafter defined as the

period in which an insect was visiting flowers on the focal plant), we identified the insect species and the flowers it visited (i.e. the bout length). In order to facilitate observations, only one insect was allowed at a time. Thus, when a plant was being visited by an insect, any other insect interested in that plant was gently waved away.

After each bout, we put a combination of coloured wires around the pedicel of each virgin flower visited; the combination depended on the visitor and the order in which they were visited. We considered the order in which flowers were visited to be a cue to assess the composition of the pollen mixture deposited on stigmas. We assumed that the first flower visited would receive the highest proportion of xenogamous pollen, while in subsequent flowers visited on the same plant (i.e. geitonogamous visitations), the pollen mixture deposited would be composed of an increasingly higher proportion of autogamous pollen (Karron et al. 2009). Using this method, we could identify the flowers visited in each bout, the order in which they were visited, the visitor species, and its relative abundance, based on visitation rates. As we only allowed one visit per virgin flower, each flower was immediately closed by inserting a small transparent glue drop to obstruct the opening to the corolla aperture after it had been visited once. At the end of each shift, the reproductive inflorescences were bagged again. When all flowers in an inflorescence had been visited, the pollinator bag was not removed again until corolla abscission.

Pollinator effectiveness was estimated from the number of seeds produced per fruit. All flowers were surveyed every third day to check for ovary enlargement. Fruits were collected before dehiscence, and the number of seeds counted. Plant size, measured as total flower production, was recorded at the end of the season. Developing fruits are used by *Gymnaetron* sp. (Curculionidae, Coleoptera) weevils for oviposition; thus, we sprayed all inflorescences with Syngenta Karate King® (a lambda-cyhalothrin-based insecticide) when the flowers were no longer functional for pollination. Spray was applied every third day. Previous observations demonstrated that this product is well tolerated by bees when diluted and applied according to the manufacturer’s instructions, and it is reasonably effective at preventing weevil attack (Sánchez-Lafuente 2007).

Data analyses

General linear mixed models were used to test differences among pollinator groups in (1) bout length, (2) seed production per plant in relation to the number of visits per plant, and (3) per-visit effectiveness (seeds produced per flower) in relation to flower visitation order in each bout. In the latter analysis, and since *Apis* made significantly longer bouts than the other pollinator groups (range: 1–9 flowers/

bout vs. 1–4 flowers/bout; see “Results”), bout length was considered a continuous variable, rather than a factor, to analyse the full range exhibited by *Apis*. All dependent variables were modelled as normal. Pollinator group, season and population were included as fixed factors, while plants were considered random blocks. Plant floral display (when testing for differences among flower or plant visits) or total flower production (when testing for differences among whole plants) were used as covariates.

All statistical analyses were conducted using R 2.11.1. (R Development Core Team 2010) and the *nlme* (Pinheiro et al. 2009) package.

Model of per-interaction effects

According to Vázquez et al. (2005), the total effect (T) of a pollinator type on a visited plant (in terms of reproductive performance; e.g. seed production) may be defined as the product of its interaction frequency (I , the number of flowers visited during a timed observation period) and its per-interaction effect (P , the per-interaction contribution to seed production): $T = IP$. In the data set that Vázquez et al. (2005) analysed, T was correlated with I , and there was no correlation between I and P ; thus, they concluded that the interaction frequency, I , can be used as a surrogate for the total interaction effect, T , regardless of differences in effectiveness among interactions, P . With the same premises, we develop a model addressing the effect of variation in per-interaction effects, and its eventual negative relationship with interaction frequency, with impacts on the total effect.

Using a deterministic expression, if a pollinator type i differs in effectiveness at seed production among the different flowers visited in each plant visit (i.e. in per-interaction effect), the total number of seeds (N_i) produced by pollinator type i after each bout may be calculated as:

$$N_i = \sum_{v=1}^b P_{i,v}, \quad (1)$$

where b is the bout length (i.e. number of flowers visited/bout) of pollinator type i , and $P_{i,v}$ is the number of seeds produced by the v th flower visited on the plant.

With a more general perspective, we can calculate N_i using a modified version of the model proposed by de Jong et al. (1992) to evaluate the likelihood of seed production by selfing. As our study species is self-incompatible, we have modelled the production of seeds by crossing as a measure of the per-interaction effect, assuming a linear relationship between xenogamous pollen deposition and seed set. While the relationship between pollen deposition and seed set must saturate when there is enough pollen to fertilise all ovules, the linear relationship constitutes a good

approximation when pollen is a limiting result, as was the case in our experiment (we only allowed a single visit per flower). Furthermore, unlike de Jong et al. (1992), we have also assumed that transfer of autogamous pollen from anthers to stigmas within the same flower is likely from the very first flower visited on a plant as a result of the flower handling by the visitor and given the close proximity of these reproductive structures in our study species.

We first considered a plant that produces one seed per flower. When a pollinator arrives at the plant, it carries a certain amount of xenogamous pollen from previously visited plants (E), and it may also remove a fraction (k_2) of the total autogamous pollen (A) produced by the currently visited flower. In these calculations, we ignore any pollen that is added to the bee’s “pollen basket” and hence removed from the pollination circuit. Because both E and k_2A are available for deposition, the proportion of xenogamous pollen deposited is $E/(E + k_2A)$. This is also the probability that a xenogamous pollen grain reaches and fertilises the ovule of the flower. If, in each flower, the pollinator deposits a fraction k_1 of the pollen it carries, and the pollinator goes on to visit a second flower on the same plant, upon arrival at that flower it will carry amounts $(1 - k_1)E$ and $(1 - k_1)k_2A$ of xenogamous and autogamous pollen, respectively. At this second flower, the pollinator will collect k_2A new (autogamous) pollen grains, and the proportion of xenogamous pollen available for deposition will be $(1 - k_1)E/[(1 - k_1)E + (1 - k_1)k_2A + k_2A]$. The same process is repeated at each new flower that the pollinator visits on the same plant. When it moves to a new plant, all the pollen can be considered xenogamous and the process is re-started. In general, upon arrival at the v th flower, the proportion of xenogamous pollen on the pollinator available for deposition will be

$$\frac{(1 - k_1)^{v-1}E}{(1 - k_1)^{v-1}E + k_2A \sum_{w=1}^v (1 - k_1)^{w-1}}.$$

Because this is also the probability that the v th visited flower is fertilised, a pollinator visiting b virgin flowers on a plant will lead to a cumulative seed set

$$M_i = \sum_{v=1}^b \frac{(1 - k_1)^{v-1}E}{(1 - k_1)^{v-1}E + k_2A \sum_{w=1}^v (1 - k_1)^{w-1}}. \quad (2)$$

Noting that

$$\sum_{w=1}^v (1 - k_1)^{w-1} = \frac{1 - (1 - k_1)^v}{k_1}, \quad (3)$$

Eq. 2 can be simplified to

$$M_i = \sum_{v=1}^b \frac{(1 - k_1)^{v-1}E}{(1 - k_1)^{v-1}E + k_2A \frac{1 - (1 - k_1)^v}{k_1}}. \quad (4)$$

In the steady state, the amount of pollen removed and deposited on each flower must be, on average, equal. In particular, this means that the total amount of pollen deposited on each flower must be independent of the number of flowers visited on a plant. Hence, from the first flower visited on a plant, we have that the amount of pollen removed (k_2A) must equal the amount of pollen deposited, $k_1(E + k_2A)$, and therefore $k_2A = Ek_1/(1 - k_1)$. Substituting into Eq. 4, and after some simplifications, we obtain

$$M_i = \sum_{v=1}^b (1 - k_1)^v = \frac{(1 - k_1) - (1 - k_1)^{b+1}}{k_1}. \quad (5)$$

For plants that can produce several seeds per flower, the seed set can be obtained by multiplying M_i by a certain constant. For this reason, we refer to M_i , the seed set divided by a constant, as the normalised cumulative seed set. If a pollinator deposits enough outcrossed pollen in a single visit to fertilize all ovules, the normalised cumulative seed set would equal the number of seeds produced divided by the mean number of ovules per flower. If this premise does not hold, normalization would be obtained by dividing the number of seeds produced by the first flower visited, but the model would yield only approximate results. Note that, in the steady state (in practice, except for the first handful of flowers visited in a foraging trip), the normalised cumulative seed set, M_i , depends only on the number of flowers per plant that the pollinator visits, b , and its pollen deposition ability (the proportion of carryover pollen that it deposits at each visited flower), k_1 .

Values of k_1 and b may be pollinator-specific for each target plant species, although b may also depend on the biotic or abiotic conditions in which the interactions take place (e.g. the spatial distribution of plants, population size, plant size, floral display, etc.). Variation in these parameters may, thus, influence variation in per interaction effects. As parameters for our model, we will use values of bout length (b) in the range 1–8 flowers per bout (those observed in our field study; see “Results”), and values of pollen deposition (k_1) obtained from published sources (de Jong et al. 1992, and references therein) for a number of hymenopteran (*Bombus* sp. and *Apis mellifera*) species. We will consider the cases of $k_1 = 0.02$, 0.19 and 0.47 (minimum, mean and maximum values, respectively).

Although the total amount of pollen deposited on each flower must be independent of the number of flowers visited on a plant, we assume that the proportion of autogamous pollen deposited increases with bout length, so that P_i decreases as the bout progresses. Since the same total number of flowers visited (i.e. the same interaction frequency) can be achieved by visiting a single flower in multiple plant visits per unit time, or many flowers in fewer

plant visits per unit time, the value of P_i must be corrected for the number of flowers visited per plant. Thus, for each bout length, the mean P_i may be estimated as

$$P_i = \frac{M_i}{b} = \frac{(1 - k_1) - (1 - k_1)^{b+1}}{bk_1}. \quad (6)$$

Results

Preliminary observations: pollinator composition, abundance and bout length

Seven insect groups were detected in 2005 and 2006. These were: *Apis mellifera*, *Bombus terrestris*, *Anthophora acervorum*, *A. dispar*, *Bombylius* sp., and two halictid bees. The most abundant visitor in 2005 was *Apis mellifera* (*Apis* hereafter; 50.67% of the visits, both populations pooled), followed by *Anthophora acervorum* (22.82%), *Anthophora dispar* (10.13%) and *Bombus terrestris* (*Bombus* hereafter; 10.38%). In 2006, the relative abundances were similar to the previous year (*Apis*, 45.32% of the visits; *Anthophora acervorum*, 21.23%; *Anthophora dispar*, 9.13%; *Bombus*, 12.32%). The rest of the insects were only rarely observed (*Bombylius* sp., 8.41% of the visits, both seasons pooled; halictid bees, 1.71% of the visits, only in 2006). *Bombylius* sp. never entered *L. lilacina* flowers, and only used its proboscis to attempt to collect nectar. For this reason, it was not considered an effective pollinator (Sánchez-Lafuente, unpubl. data; see also Vargas et al. 2010). The two halictid bees were disregarded given the small sample size available (only 9 flowers visited on 2 plants in 2006). We pooled both *Anthophora* species into a single functional group, given their similar sizes (mean \pm SE body and proboscis length, respectively; *A. dispar*: 23.70 \pm 0.85 mm, $n = 14$ bees; *A. acervorum*: 23.69 \pm 0.95 mm, $n = 17$ bees) and behaviours when visiting *L. lilacina* plants (in terms of flower handling time and effectiveness at seed production; see Sánchez-Lafuente 2007). Thus, we retained three functional groups for further analyses (*Apis*, *Anthophora* and *Bombus*).

Observations during the first week of each flowering season yielded significant differences among the three functional groups in bout length ($F_{2,488} = 323.38$, $p < 0.001$), with *Apis* making longer bouts (range 1–9 flowers/bout, mean \pm SE 7.90 \pm 1.31 flowers/bout) than *Bombus* (range 1–4 flowers/bout, mean \pm SE 3.50 \pm 0.16 flowers/bout) and *Anthophora* (range 1–4 flowers/bout, mean \pm SE 2.08 \pm 0.156 flowers/bout). Differences were consistent at both sites and seasons (no statistically significant interaction terms). Although overall mean bout length varied between sites ($F_{1,8} = 7.92$, $p < 0.03$), absolute differences were small (mean \pm SE; SR = 3.95 \pm 0.28 flowers/bout; OT = 3.30 \pm 0.19 flowers/bout). Bout length was positively

influenced by floral display ($F_{1,488} = 37.38, p < 0.001$), and its variation was almost exclusively found within plants (99.25% of the variance).

Differences among pollinator groups in effectiveness at seed production

We recorded 913 flower visits, in both populations and seasons, for the three pollinator groups considered. *Apis* visited 464 flowers producing 4,671 seeds; *Bombus* visited 143 flowers producing 2,168 seeds; and *Anthophora* visited 306 flowers producing 7,015 seeds. The visited flowers produced 13,854 seeds overall. Table 1 presents a detailed summary of the number of flowers visited, and the seeds produced by each pollinator group. These results indicate that the three groups differed significantly (post hoc Tukey's tests) in both the number of flowers visited ($F_{2,147} = 6.13, p < 0.003$) and the number of seeds produced by those flowers after a single visit ($F_{2,145} = 8.60, p < 0.001$).

We found that the influence of the number of flowers visited on seed production varied among pollinator groups (pollinator \times flowers visited interaction; $F_{3,145} = 39.09, p < 0.001$). Thus, while *Apis* was the pollinator that visited the most flowers/plant (mean \pm SE; *Apis*: 16.57 ± 2.05 flowers/plant, *Anthophora*: 10.98 ± 0.94 flowers/plant, *Bombus*: 5.10 ± 1.62 flowers/plant), *Anthophora* was the pollinator that produced more seeds/plant (mean \pm SE; *Apis*: 158.91 ± 8.18 seeds/plant, *Anthophora*: 257.92 ± 9.92 seeds/plant, *Bombus*: 81.48 ± 6.11 seeds/plant). However, *Apis* made significantly longer bouts than the other two pollinator groups, and we analysed how differences in bout length influenced seed production (Table 2). In this case, we found that *Apis*, with the longest bouts, produces (mean \pm SE) 71.97 ± 2.34 seeds/bout, while *Bombus* and *Anthophora*, with shorter bouts, produced 56.37 ± 1.64 and 51.37 ± 0.86 seeds/bout, respectively (all seasons and populations pooled). As found in the case of the relationship between flowers visited/plant and seed production/plant, a significant interaction (pollinator \times no. of bouts; Table 2) suggested that the strength of the relationship between number of bouts and seed production/bout differed among pollinator groups.

To further understand the combined effect of number of flowers visited/plant and bout length on seed production, we analysed the per-visit effectiveness (i.e., seeds produced/flower visited) of each pollinator group. We found increasing differences in per-visit effectiveness at seed production among pollinator groups as bouts progressed (pollinator \times flower visitation order interaction; Table 3). Thus, the effectivenesses of the three pollinator groups seemed to be comparable for the first flower visited in each bout, but *Bombus* and *Anthophora* did increasingly better at producing seed than *Apis* in subsequent flowers (Fig. 1). Overall, because *Apis* made significantly longer bouts than *Bombus* and *Anthophora*, the mean number of seeds/flower (i.e. the per-interaction effect) was higher for the latter groups (mean \pm SE: 16.42 ± 0.41 and 24.47 ± 0.38 seeds/flower for *Bombus* and *Anthophora*, respectively) than for *Apis* (mean \pm SE: 9.21 ± 0.36 seeds/flower; all seasons and populations pooled).

Using the total number of flowers visited per plant by each pollinator group as an estimate of interaction frequency, we examined the relationship between interaction frequency and plant seed production for each group as a measure of pollinator total effect using linear regression. We found that the slope for *Apis* was more gradual (6.75 seeds produced per plant per flower visited) than that obtained for *Bombus* (10.72 seeds produced per plant per flower visited), while that for *Anthophora* was the steepest (12.39 seeds produced per plant per flower visited) (test for slope comparison; *Apis* vs. *Bombus*: $t_{56} = 2.15, p < 0.01$; *Apis* vs. *Anthophora*: $t_{56} = 6.33, p < 0.001$; *Bombus* vs. *Anthophora*: $t_{58} = 2.02, p < 0.02$). These results suggest an increasingly strong influence of these groups on seed production as the total number of flowers visited increases (Fig. 2).

Theoretical variation in per-interaction effect, and estimation of total effect

Figure 3 plots the normalised cumulative seed set of our hypothetical insect types. Cumulative seed set increases with the number of flowers visited per plant. However, there are considerable differences in effectiveness among

Table 1 Summary of the number of *Linaria lilacina* flowers visited, and their seed production, by three pollinator groups (*Apis*, *Bombus* and *Anthophora*), in two populations (SR and OT) and two seasons (2005 and 2006)

Pollinator group	2005				2006			
	SR		OT		SR		OT	
	Flowers (n)	Seeds (n)	Flowers (n)	Seeds (n)	Flowers (n)	Seeds (n)	Flowers (n)	Seeds (n)
<i>Apis</i>	125	1,377	112	975	130	1,512	97	807
<i>Bombus</i>	31	438	32	489	43	667	37	592
<i>Anthophora</i>	81	1,914	70	1,524	73	1,725	82	1,852

Table 2 Results of a mixed linear model testing the influences of the number of bouts, pollinator group, population and season on the number of seeds produced per plant

Fixed effects	<i>df</i>	<i>F</i>	<i>P</i>
Pollinator group (PO)	2, 147	4.46	0.02
No. of bouts (NB)	1, 147	281.95	0.001
Population	1, 147	0.66	0.44
Season	1, 147	0.36	0.55
Flower production	1, 147	16.15	0.001
PO × NB	1, 147	92.85	0.001
Block effect		% variance	
Among plants		22.89	
Within plants		77.11	

Individual plants were considered blocks. Total flower production was included as a covariate

P < 0.05 in bold

Table 3 Results of a mixed linear model testing the influences of the flower visitation order along a given bout, pollinator group and population on seed production per flower

Fixed effects	<i>df</i>	<i>F</i>	<i>P</i>
Pollinator (PO)	2, 837	52.17	0.001
Visitation order (VO)	1, 837	189.17	0.001
Population	1, 837	3.23	0.08
Season	1, 837	1.21	0.27
Floral display	1, 837	0.33	0.56
PO × VO	2, 837	51.26	0.001
Block effect		% variance	
Among plants		21.97	
Within plants		88.13	

Individual plants were considered blocks. Floral display was included as a covariate

P < 0.05 in bold

insects with the same bout length, depending on their pollen deposition ability, k_1 . The normalised cumulative seed set asymptotes to $(1 - k_1)/k_1$, a decreasing function of k_1 , so that variation in pollen deposition ability yields higher or lower asymptotic values, thus approaching the asymptotes for shorter or longer bout lengths, respectively.

We used Eq. 6 to calculate the variation in per-interaction effects related to bout length and pollen deposition ability. Because per-interaction effects (P_i) are proportional to M_i/b , its average value decreases with bout length and with the pollen deposition ability of the pollinator (Fig. 4). Overall, the results show that variations in mean per-interaction effect (P_i) may occur in relation to the number of flowers visited/plant, and the behaviours and abilities of different pollinator types.

From Vázquez et al. (2005), we know that the total effect of a pollinator type i (T_i) results from the product of

its interaction frequency (I_i) and per-interaction effect (P_i). However, the latter parameter (Eq. 6) depends, at least in self-incompatible plant species, upon variation in bout length (b) and pollen deposition ability (k_1). With this information, the total effect of a pollinator type i may be calculated with the expression

$$T_i = I_i \frac{(1 - k_1) - (1 - k_1)^{b+1}}{b k_1}. \quad (7)$$

Using this equation, and a range of interaction frequency (I_i) of 1–120 flowers visited per observation period, we have calculated the expected absolute total effects of our hypothetical insect types. Values of bout length (b) and deposition ability (k_1) were the same as used in previous analyses (Figs. 3, 4). The results representing the expected total effect are plotted in Fig. 5.

Discussion

Using empirical data, we have performed a case study in which the most frequent pollinator of a plant species in two different populations and seasons, and hence the one visiting more flowers overall, does not contribute the most to plant reproduction in terms of seed production. Our results show that the foraging strategy of the pollinators, in particular the number of flowers visited before leaving a plant, has a strong effect on their pollination effectiveness. At least with self-incompatible plants, this effect may be sufficiently strong that the most common pollinators do not always contribute the most to the reproductive success of the plants, even though they may visit more flowers per plant than less common pollinators. By visiting more flowers per plant, pollinators are increasing their flower visitation rate (within-plant travel time is shorter than between-plant travel time) and simultaneously decreasing their expected effectiveness per visit, presumably due to geitonogamous pollen transfer. This foraging behaviour of the pollinators introduces a negative correlation between their quantitative (interaction frequency, I_i) and qualitative (per-interaction effect, P_i) components of pollinator effectiveness. Thus, if the mean per-interaction effect is higher when pollinators visit fewer flowers per plant, a higher number of short plant visits may yield more effective results, in terms of seed production, than a lower number of longer plant visits, even when the total number of flowers visited by each pollinator is the same. When this is the case, the use of interaction frequency as a surrogate for total effect may be suspect.

The empirical evidence of within-plant variation in per-interaction effect was obtained after we analysed the relative contributions of different pollinator groups (namely,

Fig. 1 Relationship between mean seed production (seeds flower⁻¹) and bout length (flowers visited per bout) for three pollinator groups in two populations (SR and OT) of *Linaria lilacina* in (a–b) 2005 and (c–d) 2006. Data are averaged at each flower position in the bout. Standard errors were not plotted to enhance the readability of the figure. See “Results” for a summary of sample sizes

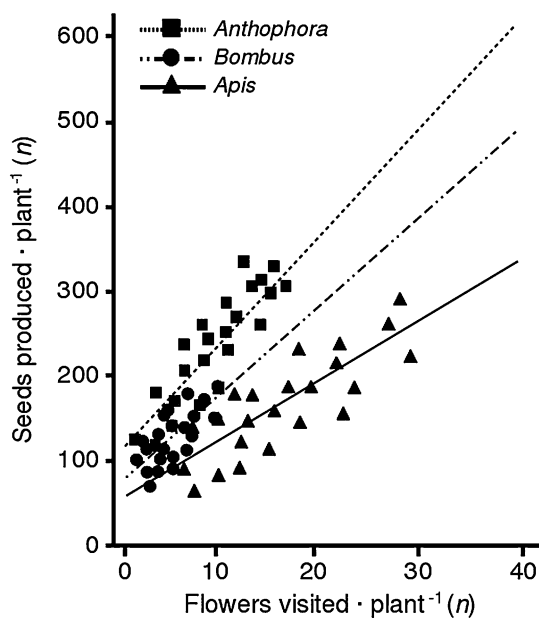
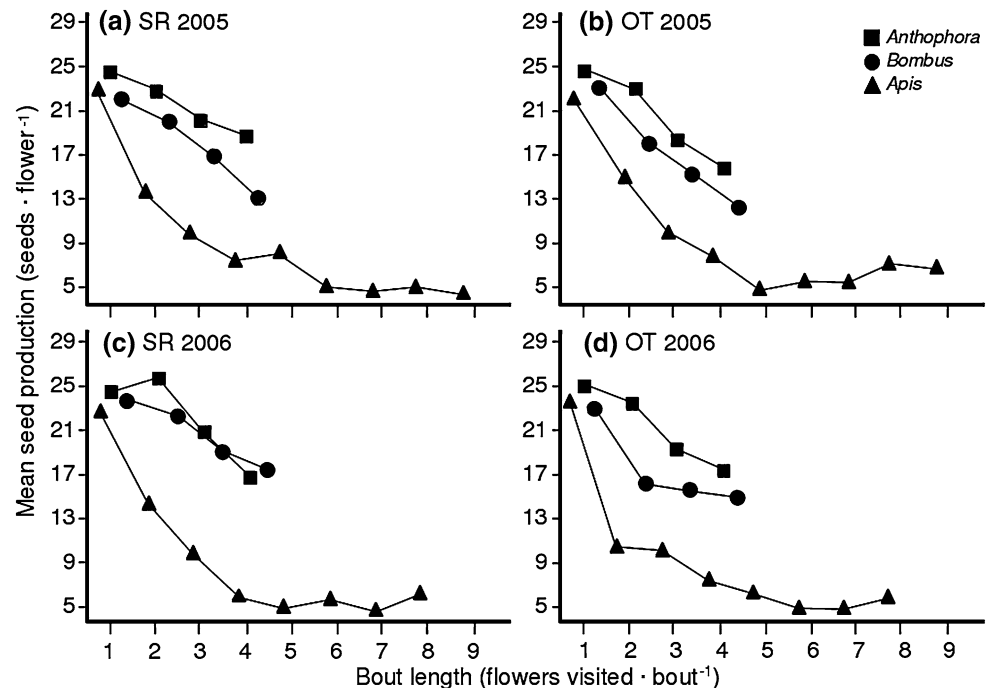


Fig. 2 Relationship between number of flowers visited per plant and seed production per plant in *Linaria lilacina* for each pollinator group (*Anthophora*: solid squares, dashed line; *Bombus*: solid circles, dashed-dotted line; *Apis*: solid triangles, solid line). Lines represent the predicted seed production from linear regression. Data are averaged across seasons pooled for both study populations

Bombus, *Anthophora* and *Apis*) to seed production, using *L. lilacina*, a self-incompatible herb, as a study system. Because the proportion of xenogamous pollen on the pollinator decreases (and that of autogamous pollen increases) as more flowers are visited on a plant (e.g. Strickler and

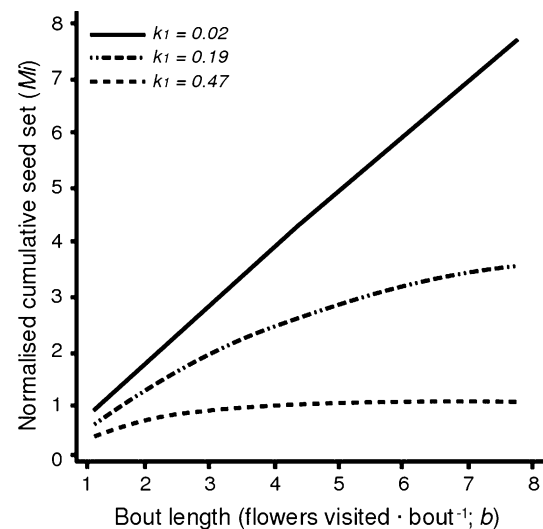


Fig. 3 Normalised cumulative seed set (M_i) related to bout length (b) for a number of hypothetical pollinator groups with different pollen deposition capabilities (k_1). Values were calculated from Eq. 5

Vinson 2000; Karron et al. 2009; see also Rodet et al. 1998), the seed set of flower visit $n + 1$ is lower than the seed set of flower visit n . Therefore, average pollinator effectiveness is a decreasing function of the number of flowers visited per plant. Thus, the three pollinator groups seemed to be functionally equivalent in the first flower visited in each bout (Kendall and Smith 1975, 1976; Dieringer 1992), while effectiveness progressively decreased in subsequent flowers visited. The rate at which effectiveness decreases depends on the proportion of carryover pollen deposited at each flower: the higher the proportion

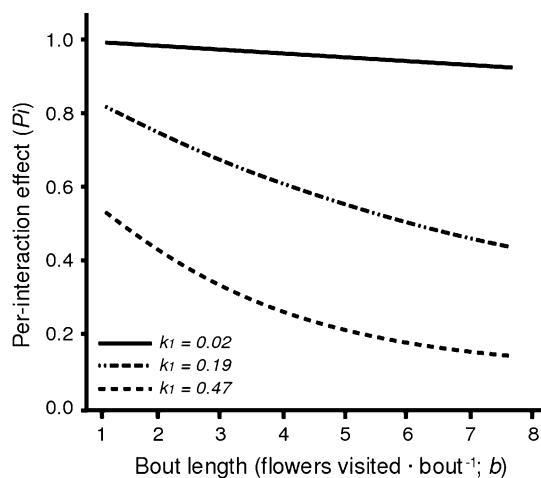


Fig. 4 Variation in per-interaction effect (P_i) related to bout length (b) for a number of hypothetical pollinator groups with different pollen deposition capabilities. Values were calculated from Eq. 6

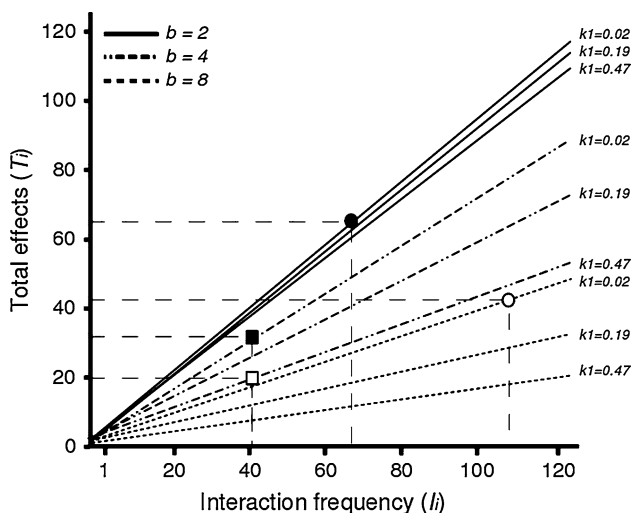


Fig. 5 Estimation of total effect (T_i) from interaction frequency (I_i) related to bout length (b). Interaction frequency was estimated in terms of number of flowers visited per observation period. We represented a number of hypothetical pollinators with different capabilities for pollen deposition (k_1) for each bout length (b). Values were calculated from Eq. 7. We show two examples of how the total effect may depend on variations in bout length for pollinators with similar pollen deposition abilities (solid and empty circles) and on variations in pollen deposition ability for pollinators with similar bout lengths (solid and empty squares)

of pollen deposited at each visit, the faster the proportion of xenogamous pollen approaches zero and the faster pollinator effectiveness decreases. In our system, pollinator effectiveness decreased faster for *Apis* than for *Anthophora* and *Bombus*. As we only allowed one visit per pollinator group per virgin flower, and there are no differences in the amount of total pollen deposited among flowers for each pollinator group (Sánchez-Lafuente and Parra, unpubl. data). The most probable explanation for this result is that

Apis deposits a higher fraction of autogamous pollen on each flower (e.g. Rodet et al. 1998; Ivey et al. 2003; but see Chamberlain and Schlising 2008) than *Bombus* and *Anthophora*, most likely as a result of the ways in which it handles flowers, spending more time per visit and penetrating the flower deeper than *Bombus* and *Anthophora* (Sánchez-Lafuente 2007; Sánchez-Lafuente and Parra, unpubl. data; see also Harder 1990; Thostesen and Olesen 1996).

A theoretical model allows us to predict how per-flower seed set changes with the number of flowers already visited on a plant and with the pollen deposition ability of the pollinators. Controlling for bout length, pollinators with higher pollen deposition capabilities (larger k_1 ; including both outcrossed and autogamous pollen) have lower normalised cumulative seed sets per bout, since the cumulative seed set approached its asymptotic value after the first 3–4 flowers visited/bout (e.g. Karron et al. 2009). In contrast, lower deposition abilities lead to higher asymptotic cumulative seed set, which is approached after a greater number of visits. For example, the relationship between bout length and normalised cumulative seed set is almost linear for bout lengths of up to 9 visits (Fig. 3), and the average pollinator effectiveness is little affected by bout length.

Comparing model predictions with data on seed set per flower, we can also estimate the proportion of available pollen that bees deposit at each flower. When pollen deposition ability (k_1) is large, the effect of bout length on pollinator effectiveness can be sufficiently high to break the typical positive correlation between interaction frequency and total effect. For example, fitting our model to the seed production data in Fig. 1 leads to pollen deposition values of 0.12, 0.16 and 0.31 for *Anthophora*, *Bombus* and *Apis*, respectively. With these values of pollen deposition, we found that, while the interaction frequency of *Apis* was the highest among all the visitor groups, a less abundant pollinator (*Anthophora*), which made shorter bouts, contributed more to seed production. Although *Bombus* also made shorter bouts than *Apis*, and therefore had higher average per-interaction pollination effectiveness, the effect was not sufficiently strong to compensate for the much lower interaction frequency of *Bombus*, which consequently had the lowest total effect of the three groups. Similar results have been found for another self-incompatible plant species in which pollinator effectiveness has been studied. Mayfield et al. (2001) compared the effectiveness at seed production and pollen export in the self-incompatible *Ipomopsis aggregata* (Polemoniaceae) of *Bombus appositus* and several hummingbird species for which this plant species was supposedly adapted. They found that *B. appositus* visited fewer flowers per plant than hummingbirds. However, they were 4.04 times more

effective at seed production, and 2.75 times more effective at pollen export per flower visit, than the birds. Consequently, although their overall visitation frequency could be between a third and a quarter that of hummingbirds in some seasons, *B. appositus* had a higher total effect on plant reproductive success.

Differences among pollinator groups in bout length and pollen deposition ability may influence per interaction effect independently of each other. In turn, variation in pollinator abundance may be related to the total number of interactions of each pollinator group. The outcome in terms of the measured total effect may result from a combination of these three parameters. For instance, in Fig. 5, the total effect of a pollinator with a bout length (b) of 8 flowers per plant visit and a pollen deposition ability (k_1) of 0.02 (empty circle) is actually comparable to that of a pollinator with $b = 4$ and $k_1 = 0.47$, despite the large differences in both parameters. However, differences in pollen deposition ability may eventually be more important than differences in bout length in generating variation in per-interaction effects. Thus, while we would not expect floral display and/or plant size to set a limit on the pollen deposition ability of a given pollinator group when visiting plants of the same species, those parameters may set a limit on the variation in bout length (e.g. Waser and Price 1991; Di Pasquale and Jacobi 1998; Strickler and Vinson 2000; Grindeland et al. 2005; Karron et al. 2009). For example, Pellmyr and Thompson (1996) found differences in the number of interactions and effectiveness among several insect groups visiting the self-incompatible herb *Lithophragma parviflorum* (Saxifragaceae). However, because no differences occurred among insect types in their bout lengths, given the small floral display exhibited by the study species at any given time, no variation in per-interaction effect associated with variation in bout length would be expected. Thus, only the number of bouts per plant (not the bout length) was responsible for the variation in interaction frequency among pollinator groups, and the total effect could be accurately predicted from the interaction frequency alone.

Many ecological processes related to reproductive success, demography or natural selection (among others) in wild populations are context dependent. This implies the occurrence of a number of variable outcomes depending on the particular conditions in which these processes take place (e.g. Rey et al. 2006; Alonso et al. 2007; see also Thompson 1994, 2005). Understanding the relationship between pollinator effectiveness and bout length is also important if we are to explain variability in reproductive success between plants, populations and years. The theoretical model can be applied not only to assess the seed production of self-incompatible plant species, but also to estimate the proportion of selfed and outcrossed seeds produced in self-compatible species. In the presence of

later-acting inbreeding depression, these estimates are essential to quantify pollinator effectiveness, as outcrossed seeds are more valuable than selfed ones. By measuring the seed set of a random sample of flowers visited by a single pollinator of a given plant species, we can obtain an unbiased estimate of this species' pollination effectiveness. However, plants and environmental traits, such as floral display and plant density, may affect bout length (e.g. Ohara and Higashi 1994; Ivey et al. 2003). The estimate per interaction effect obtained in one population will obscure the fact that the same pollinator species has different pollinator effectivenesses for plants of different sizes, and it will be impossible to extrapolate from one population measured one year to other populations or years. For example, we found that bout length was directly related to floral display (see also Robertson and Macnair 1995; Grindeland et al. 2005; Williams 2007), while plant visitation was not. Consequently, differences in flowering phenology or synchrony among plant populations of the same plant species, originating by environmental or biotic factors, or by differences among plants sizes, may directly be responsible for the variation in bout length and number of bouts of the same pollinator species (e.g. Strickler and Vinson 2000), with presumed influences on the among-flower variation in seed number and/or quality (e.g. Karron et al. 2009).

At least two complementary and testable predictions for plant–pollinator interactions may arise from our results. First, we predict that interaction frequency will be a worse predictor of total effect in self-incompatible than in self-compatible species; while, in self-compatible species, the predictive value of the interaction frequency as a surrogate for total effects will decrease as the level of inbreeding depression increases. In both cases, a lower mean per-interaction effect may be expected as more flowers are visited per bout, because the amount of autogamous pollen will increase with bout length. Second, because bout length is of interest to define the variation in per-interaction effects, and hence its influence on the total effect, the validity of the interaction frequency as a predictor of total effect in such plant species may be reduced as the plant size and/or floral display increase, as a larger number of flowers open at any one time may allow pollinators to increase their bout lengths. These predictions could also arise from the dataset used by Vázquez et al. (2005), even though they combined self-compatible and self-incompatible species. Thus, although most of the 22 species included in the dataset used were self-compatible (ca. 70%), the correlation between interaction frequency and per-interaction effects was negative for most of the self-incompatible ones. In cases with positive correlations, this could be due to the absence of differences among pollinators in bout length, as suggested above. However, the correlation

between interaction frequency and total effect was positive in all cases, and Vázquez et al. (2005) concluded that per-interaction effects could be disregarded when explaining the total effect. However, as shown in Fig. 2 from our empirical data, a positive correlation between interaction frequency and total effect does not guarantee that the pollinator with the highest frequency would also contribute the most to the total effect. A critical parameter to evaluate whether a pollinator group contributes more than another to seed production may be the ratio of mean per-visit effectiveness (i.e. the mean per-interaction effect). The ratio of mean per-interaction effects of *Anthophora* and *Apis* is 2.66. For any given interaction frequency, *Apis* would be more valuable than *Anthophora* for seed production if it visits more than 2.66 times as many flowers as *Anthophora*. However, below this critical value, *Anthophora* would be of greater value, despite the higher interaction frequency of *Apis*. As the ratio of the difference in the number of flowers visited by these pollinator groups was below the critical value in both seasons (in 2005: 237 flowers visited by *Apis*/151 flowers visited by *Anthophora* = 1.57; in 2006: 227 flowers visited by *Apis*/155 flowers visited by *Anthophora* = 1.46), *Anthophora* consistently contributed more to seed production than *Apis*, despite the latter visiting more flowers. However, if we compare *Bombus* to *Apis*, we find a critical value of 1.78. As the ratio of the difference in number of flowers visited by these pollinator groups is always above this critical value (in 2005: 237 flowers visited by *Apis*/63 flowers visited by *Bombus* = 3.76; in 2006: 227 flowers visited by *Apis*/80 flowers visited by *Bombus* = 2.84), the pollinator making more visits (*Apis*) is, in this case, more valuable for seed production. Thus, as in Vázquez et al. (2005), we found positive correlations between interaction frequency and total effect for all of the pollinator groups tested, but it was the ratio between per-interaction effects that finally determined which of the pollinators contributed the most to the total effect.

Overall, because differences in interaction frequency may be overridden by differences originating from variations in bout length and/or pollen deposition ability, it is clear that behaviour (Herrera 1987; Rodríguez-Gironés and Santamaría 2010) may explain pollinator contribution to plant reproduction better than the number of interactions. Our results suggest that, at least for self-incompatible plant species, the predictive value of the interaction frequency in plant–pollinator interactions may be a “moving target” because it is context dependent. In other words, we do not expect the same distribution for all its components under different environmental conditions, even when the same participants are involved. For simplicity, our model assumes a linear relationship between pollen deposition and seed production. That is, it fits species with post-zygotic mechanisms of self-incompatibility, while the results may be

approximate in cases with pre-zygotic mechanisms. However, even in these cases, the relationship between pollen deposition and seed set may still be linear (depending on each particular system), and the model may accurately predict the total effects, as observed for our example species.

Our study assessed the importance of different insect types as pollinators based upon their effectiveness at seed production at the plant level, as measured through behavioural and morphological features. However, our study does not address the synergistic effect of each insect type in the presence/absence of the rest of the insect types. In other words, we have not tested how interactions among different insect types may influence their value as pollinators, as suggested by Aigner (2001). Because plants are pollinated by a number of insect types constituting an assemblage, these interactions may be important to define functional groups, rather than individual pollinator types, as valuable contributors to plant fitness (see Fenster et al. 2004). A precondition for the definition of a functional group of pollinators is that all pollinators in such group should have additive effects on plant fitness, and this can only be tested by varying the presence/absence or abundances of different insect types and then analysing the fitness consequences of such variations. Consequently, we suggest that the role of the interaction frequency as a suitable predictor of total effect must be tested for different pollination systems, in different pollination environments, for different pollinator assemblages, and across wider scales, to validate its generalised use as a quantitative measure in plant–pollinator interaction networks.

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Conflict of interest None.

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