

# A new pollination probability index (PPI) for pollen load analysis as a measure for pollination effectiveness of bees

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## SUMMARY

Conventional pollen load analyses have calculated the proportion of pure to mixed pollen loads, or the average percentage of conspecific pollen on bees captured at their nests or hive. These indices can not distinguish 'flower' constancy from 'pollen' constancy, and are poor estimates for the probability of pollination. This study proposes a new index for pollen load analysis which reflects floral constancy at the pollinator population level and therefore may be an estimation for pollination probability (PPI). This index is the product of the mean proportion of conspecific pollen on bees captured on a certain forage flower species multiplied by the proportion of bees carrying that conspecific pollen. The PPI can be used to estimate the probability of pollination in natural plant populations as well as in crop plants. This study calculated the PPI for bumble bees foraging on five keynote species of a mediterranean phrygana: two polleniferous (*Hypericum triquetrifolium* and *Myrtus communis*) and three nectariferous (*Cephalaria joppensis*, *Echinops adeno-caulos* and *Salvia fruticosa*) plant species which are the main food sources of *Bombus terrestris* under the local conditions.

**Keywords:** pollination by bumble bees, pollen load analysis, floral constancy, *Bombus terrestris*, pollination probability index

## INTRODUCTION

The patterns of floral choice by bees have been the focus of numerous studies (reviews by Waser, 1986; Free, 1993). Floral constancy, or floral fidelity, refers to the tendency of a pollinator to restrict its visits to flowers of a single species or morph (Waser, 1986). However, Faegri & Pijl (1979), refer to constancy as a convenient general term describing all the relationships in which visitors (species as well as individuals) are visiting a limited number of plant species.

Constancy has been studied by a variety of means: observation of foraging behaviour in the field (review by Proctor *et al.*, 1996); choice experiments involving artificial flower arrays (e.g. Thomson, 1981); theoretical modeling (e.g. Oster & Heinrich, 1976; Waddington & Holden, 1979) and the examination of pollen purity (e.g. Betts, 1920, 1935; Percival, 1947; Free, 1963; Spencer-Booth, 1965).

Darwin (1876) pointed out that constancy to flower species is advantageous to the plant by facilitating cross-pollination. It is also advantageous to the bee (Free, 1993), enabling it to forage more quickly than if it had to learn the sites of the nectaries of each flower it visited, and enabling it to keep to a species yielding abundant nectar and pollen. Zahavi *et al.* (1984) explained that honey bees avoid collecting mixed pollen loads and hypothesized that honey bees face difficulties in packing together pollen of different species due to their different size and shape.

The foraging behaviour of bumble bees has been studied intensively (reviews by Heinrich, 1979a; Plowright & Laverty, 1984) and floral constancy well documented for this group (Heinrich, 1977; Teras, 1981). Betts (1920) found that 66% of bumble bee pollen loads were pure, while Spencer-Booth (1965) found that only 17% of bumble bees that collected from gardens and allotments had pure loads compared to 49% of those collected in homogeneous crops. Heinrich (1979b) observed that bumble bees tended to specialize on one flower species (their 'major') but also occasionally forage on other species (their 'minor'). Combining data from three large studies Heinrich (1976) estimated that about 57% of bumble bee pollen loads were pure, 32% comprised two pollen species and the rest had more than two species. The flowers visited by bumble bees for pollen may be very different from those used for nectar (Lui *et al.*, 1975; Free 1993). Bees require energy supplies during pollen foraging and so pollen and nectar gathering are usually intimately related (Plowright & Laverty, 1984).

Traditionally, pollen load analyses are calculated as the proportion of pure to mixed pollen loads (Free, 1970; Zahavi *et al.*, 1984; Dafni, 1992), or the average percentage of conspecific pollen present on the bees (e.g. O'Rourke & Buchmann, 1991; Degrandi-Hoffman *et al.*, 1992). However, the first type of analysis ignores the proportion of conspecific pollen in each load, while the

second ignores the proportion of bees that carried conspecific pollen.

The aim of the present paper is to re-examine the methodology of pollen load analyses as an index for floral constancy with a special reference to pollen vs. nectar foraging activity. We propose a new index for pollen load analysis which may reflect floral constancy at the pollinator species level. This index may also serve as an estimation for pollination probability and efficiency and could be used to examine bees' foraging behaviour in natural as well as agricultural environments.

## MATERIALS AND METHODS

### Study area

The study was carried out on Mount Carmel (Israel) 3–7 km east of Haifa, 9–12 km from the Mediterranean sea, at an elevation of 300 m. The study area was in open phrygana vegetation, dominated by the dwarf shrubs *Sarcopoterium spinosum* and *Cistus* spp., with scattered trees of *Quercus calliprinos* and *Pinus halepensis*.

### Pollen analysis

Bumble bees (*Bombus terrestris*) were captured in spring 1995 on flowers of five keystone plant species. Two were polleniferous species (*Hypericum triquetrifolium* and *Myrtus communis*) and three were nectariferous species (*Cephalaria joppensis*, *Echinops adenocaulos*, and *Salvia fruticosa*) which are the main food sources of the bees under the local conditions.

Bees were captured early in the morning, during their main activity period, and placed immediately in separate vials, to prevent possible pollen contamination. Pollen was first washed from the bees by rinsing them three times with absolute ethanol and then it was acetylated (Erdtman, 1969). Each bee from the same plant species was treated as a replicate. Pollen was identified to the closest possible taxon and the number of grains of each type were counted. This procedure was carried out to get the qualitative and quantitative pollen spectrum of each individual bee separately. Nomenclature is after Feinbrun-Dotan & Danin (1991).

### Data analysis

The mean number of pollen sources for each individual load (species richness) was calculated for each forage flowering plant species on which the bees were captured as a component of Simpson's diversity index. It was calculated for each individual pollen load and the mean was determined for each flowering species on which *B. terrestris* foraged. The value of Simpson's diversity index reflects less rare species than common ones (Begon *et al.*, 1996). One-way ANOVA was used to examine the effect of the forage flowering species on species richness and the Simpson index of pollen

**TABLE 1.** The number of *Bombus terrestris* captured on flowers of each nectar or pollen forage plant species, the mean total number of pollen grains on *B. terrestris* captured on these species and the mean diversity (Simpson's diversity index) of pollen grain species in pollen loads of these bees. Means with different letters (within each variable) are different (Tukey post-hoc test,  $P < 0.05$ ).

Forage plant species	Reward	n	Total number of pollen grains			Simpson diversity index		
			mean	± s.e.	Tukey	mean	± s.e.	Tukey
<i>Cephalaria joppensis</i>	nectar	34	86	5	B	3.19	0.28	A
<i>Echinops adenocaulos</i>	nectar	19	54	13	B	3.45	0.35	A
<i>Hypericum triquetrifolium</i>	pollen	16	225	56	A	1.31	0.02	B
<i>Myrtus communis</i>	pollen	32	254	42	A	1.59	0.14	B
<i>Salvia fruticosa</i>	nectar	76	25	6	B	3.14	0.15	A

**TABLE 2.** The mean proportion of conspecific pollen (PCP) (out of the total pollen grains in pollen loads of each bee), the proportion of bees (out of total number of observed bees) carrying that conspecific pollen (PBP) captured on the flowers of each forage flowering plant species, and the calculated pollination probability index (PPI) for each forage plant species.

Forage plant species	Reward	PCP	PBP	PPI
<i>Cephalaria joppensis</i>	nectar	0.04	0.21	0.01
<i>Echinops adenocaulos</i>	nectar	0.10	0.63	0.06
<i>Hypericum triquetrifolium</i>	pollen	0.98	1.00	0.98
<i>Myrtus communis</i>	pollen	0.82	1.00	0.82
<i>Salvia fruticosa</i>	nectar	0.42	1.00	0.42

diversity in pollen loads. A post-hoc Tukey test was used for the comparison of both variables among the flowering species (table 1). All statistical analyses were performed by SYSTAT v. 5 (Wilkinson *et al.*, 1992).

The mean proportion of conspecific pollen in each load for each forage flowering plant species was calculated (PCP in table 2). The proportion of bees carrying conspecific pollen for each forage flowering species (PBP in table 2) was also calculated. The pollination probability index ( $PPI = PCP \times PBP$  in table 2) was calculated for conspecific pollen for each forage flowering plant species. The PPI varies from 0 in cases where bees did not collect any pollen, to 1 in cases where all bees collected only conspecific pollen, thus reflecting the probability of pollination of a particular flowering plant species by the examined bee species.

## RESULTS

The total number of pollen grains on bumble bees captured on polleniferous vs. nectariferous flowering plant species is presented in table 1. The total size of the

pollen loads varied among the flowering plant species and it was significantly ( $F_{4,172} = 8.37$ ,  $P < 0.01$ ) larger in the polleniferous species than in the nectariferous ones (table 1). The average Simpson's diversity index for pollen species diversity was significantly ( $F_{4,172} = 17.75$ ,  $P < 0.01$ ) different among the forage flowering plant species. The diversity of pollen species was significantly higher (Tukey  $P < 0.05$ ) in bees captured on nectariferous than polleniferous ones (table 1).

Table 2 presents the mean PCP, PBP and PPI for the bumble bees on each species of forage flowering plant. The most obvious result is the high average PCP, PBP and the very high PPI for the two polleniferous plant species (*M. communis* and *H. triquetrifolium*). In contrast, PCP, PBP and mainly PPI were much lower in the nectariferous plant species (*S. fruticosa*, *E. adenocaulos* and *C. joppensis*).

The PCP and PBP differed for the same pollen species in bees captured on nectariferous species. Moreover, no significant correlation ( $r = -0.063$ , Barleti chi square = 0.105, d.f. = 1,  $P = 0.746$ ), was found between PCP and PBP for all pollen species on all tested forage

flowering species, showing the independence of these variables.

## DISCUSSION

Eickwort & Ginsberg (1980) noted that bees generally display greater host specificity in their pollen collecting behaviour than in nectar foraging. Our results (table 2) corroborate this view. Females of oligolectic species maintain host specificity throughout the range of the species and in the presence of other pollen sources (Linsley & MacSwain, 1957), while polylectic bees are generalized in their pollen collection.

Pollen load analysis as an indication of floral constancy has been addressed in several groups of bees: honey bees (Free, 1963; Suryanaryana *et al.*, 1991), bumble bees (Betts, 1920; Spencer-Booth, 1965; Free, 1970) and solitary bees (Gross, 1992; Schlindwein & Wittmann, 1995). This indirect measure, however, has several drawbacks, especially the inability to distinguish between 'flower' constancy and 'pollen' constancy. Bees often switch between floral rewards (nectar only, pollen only and nectar plus pollen) collected during foraging trips (Zahavi *et al.*, 1984; Free 1993). Consequently the species richness of pollen loads may underestimate the number of plant species actually visited within a single foraging bout. Using the proportion of different pollen types within a particular load is a valid estimator of pollination probability only if one assumes that each flower visited will deposit an equal amount of pollen on a bee irrespective of plant species. This is clearly unrealistic. Our data show explicitly that the amount of pollen on bees captured on polleniferous flowers was much larger than that on bees from nectariferous flowers. Thus, there is a low correlation between the proportion of pollen on a bee's body and the proportion of visits to a mixture of polleniferous and nectariferous flowers. Moreover, honey bees may acquire pollen from their nestmates that have visited different flowers (Free, 1963) and a similar situation may occur in a nest of bumble bees. Therefore, it is inaccurate to draw conclusions about behaviour, or to measure floral constancy simply from analyses of pollen on bodies of bees (Dafni, 1992).

Successful pollination is a result of a multifactorial process. However, if one concentrates on a population of a single flowering species and a population of a single pollinator species that forages on it (and on other co-flowering species), it can be assumed that the probability of pollination is directly related to the proportion of conspecific pollen on the body of each bee, and to the proportion of bees in the population that carry that conspecific pollen. However, each of these independent proportions by itself will fail as a measure for pollination probability. This is the theoretical justification of our proposed PPI which functions as a simple tool for the estimation of the effectiveness of a population of a certain bee species as pollinators of a flowering species.

The size of the pollen loads of bees foraging on polleniferous flowers was larger than that of bees from nectariferous flowers. A large part of the pollen was located in the corbiculae for transport to the hive and was therefore less useful for pollination. However, this pollen was initially distributed over the body of the bees and only later combed into the corbiculae; it can be assumed therefore that a large number of pollen grains were still scattered on the bees' bodies. The PPI for conspecific pollen was larger for bees collected on polleniferous species than for bees from nectariferous species. The species diversity of pollen loads was lower for bees from polleniferous species than for bees from nectariferous species. Together these results suggest that bumble bees were more efficient as pollinators of polleniferous plants.

The differences in PPI of *B. terrestris* within the nectariferous species indicates that it has a higher probability of pollinating *S. fruticosa* than *C. joppensis* or *H. triquetrifolium*. The very low PPI for conspecific pollen on bumble bees captured on *C. joppensis* and *E. adenocaulos*, may be an indirect indication of nectar thieving rather than legitimate foraging behaviour and pollination. This suggestion is supported by observation of bumble bees inserting their proboscis from the side of the flowers, thus avoiding contact with either the anthers or the stigmas (Dafni, personal observation).

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