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Honey bee interest in flowers with anemophilous characteristics: first notes on handling time and routine on *Fraxinus ornus* and *Castanea sativa*

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Abstract

Pollination syndrome theory should explain flower characteristics on the base of the pollen vector (wind, water, animal) involved in the plant reproductive strategy. Some flowers, although exhibiting morphological traits considered best suited for wind pollen dispersal, experience visits of insects. Data on such visits are often not detailed enough to establish if they are occasional or regular events. If visitors are foraging bees, morphological constraints of anemophilous flowers may affect handling ability and foraging effort. We investigated the foraging behaviour of honey bees on *Fraxinus ornus* L. and *Castanea sativa* Miller, two species showing a double pollination strategy (wind + insect), but whose flowers retain clear anemophilous characteristics. We recorded honey bee handling time and routines on the flowers of the two tree species providing a comparison when possible. Interestingly, our results confirm the visits to these plants to be regular events. Moreover, on each of them bees express an appropriate collection strategy, adapted to the different spatial organization of the flowers. Two are the implications discussed: on one side, bees can exploit abundant pollen resources, which could increase colony growth and strength, even if the unsuited flower morphology may limit resource collection. On the other, the plants may increase their reproductive success when climatic conditions are unfavourable to wind pollen dispersal.

Key words: Insect-flower interaction, honey bee handling ability, wind pollination, *Fraxinus ornus* L., *Castanea sativa* Miller.

Introduction

Hypothesis on the evolution of pollination include the well-known pollination syndromes (Faegri and Van der Pijl, 1971; Proctor *et al*., 1996), where functional groups of flowers show similar attractive strategies imposed by pollen vectors (wind, water, insect). For example, plants as the catkin-bearing trees (oaks, beeches, willow, elms and ashes) have an enormous number of staminate small sized flowers, often lacking or with a very reduced corolla, grouped into an inflorescence. These characteristics help pollen removal and dispersion by wind. Curiously, there are some records on insects paying visits to such trees. In the majority of cases, unfortunately, these records do not allow to better define the role of the visiting insect. Anemophily is a topic that elicits many questions in the scientific community, mainly on the reproductive success linked to this strategy, its evolution and its limitations (Whitehead, 1969; Ackermann, 2000; Friedman and Barrett, 2009). Less attention has been paid so far to critical species classified as anemophilous because of their floral and pollen characteristics, but cited in various papers dealing with insect visits. Some of these papers refer to the collection of resources exerted by honey bees (Pearson and Braiden, 1990; Cane *et al*., 1992; Suryanarayana *et al*., 1992; Coffey and Brean, 1997; Erickson and Atmowidjojo, 1997; Fortunato *et al*., 2006): due to its importance for the survival of the entire colony, bee behaviour on possibly unsuitable flowers is stimulating for its evolutionary implications.

Honey bees are often referred to as generalist pollinators. Data on honey bee resource collection come from direct observation of foraging individuals (Mamood *et al*., 1990; Chagnon *et al*., 1991; Giovanetti *et al*., 2006), samples as hive reserves or forager loads (de-Sa-Otero *et al*., 2007; Dimou and Thrasyvoulou, 2007; Bilisik *et al*., 2008), or pollen residuals into honey samples (Von der Hohe and Dustmann, 1990; Terrab *et al*., 2004; Staviarz, 2008; Aronne and De Micco, 2010). Foraging for pollen or nectar is mainly carried out by different individuals at different times and has already stimulated investigations on driving forces responsible for bee choices (Gonzalez *et al*., 1995; Camazine *et al*., 1998; Pernal and Currie, 2001; Pernal and Currie, 2002; Fernández and Farina, 2005). Traditionally, flower classifications depend on corolla characteristics as symmetry, shape, size, and colour. Honey bee performances have been recorded independently on numerous types of flowers (Duffield *et al*., 1993; de Sa Otero *et al*., 2007), but not directly compared. Foragers show some innate preferences for certain floral characteristics: for example, they prefer yellow and undamaged flowers, but may still make many mistakes (Goulson *et al*., 1997). They can use hydrocarbons for previous intra- and interspecific visitor's detection (Goulson and Stout, 2002) and are known to be spontaneously constant (Hill *et al*., 1997). Handling routines are usually poorly investigated in details, in spite of their importance for a successful resource collection and the overt link with theories on floral features.

Based on previous theories, as the pollination syndromes, and on the assumption that handling ability has to be relevant for high foraging efficiency, we would expect bees never/rarely to visit anemophilous flowers. Visits could turn out to be incidental and handling poorly successful. We tested these predictions, recording in some details handling time and handling routine of *Apis mellifera* L. on flowers of *Fraxinus ornus* L.

and *Castanea sativa* Miller. *Fraxinus* is a genus with a controversial reproductive strategy (possibly amphiphilic - wind and insects act in the process of pollen dispersion, according to Wallander, 2008), which pollen has been detected into honey samples and into hive provision (de Sá Otero *et al.*, 2004; Fortunato *et al.*, 2006; Aronne *et al.*, 2008; Aronne and De Micco, 2010). *C. sativa* is an important nectar and pollen source for bees, while holding floral characteristics due to anemophily and still dispersing pollen by wind (Sabugosa-Madeira *et al.*, 2008). We are aware of no direct observational record of honey bee dealing with flowers of anemophilous characteristics. Honey bee visits to such plants may raise interest from an adaptive point of view, for both the pollen collection (bee side) and the pollen dispersion (plant side). Through this preliminary work we aimed at displacing the idea that honey bees visit only occasionally flowers with characteristics of anemophilous species, defining handling routines and their potential to infer success in resource collection on such flowers.

Materials and methods

Area and standard observations

The site is located on a hilly area (Colli Berici, Vicenza, Italy).

F. ornus belongs to the family of Oleaceae. Its flowers are small and grouped into dense panicles; they have a corolla with four slender creamy white petals, two stamens and, those hermaphrodites, a pistil with a flat ovary (Fiori, 1929). Panicles are pending and anthesis is quite synchronous on a given plant. *C. sativa* belongs to the family Fagaceae. The male flowers have numerous stamens with a long filament, no corolla and are born on upright catkins. The female flowers show styles emerging from the enclosing scales (Fiori, 1929). Observations were conducted on flowering plants (in 2008, 7th, 8th, 9th of May on *F. ornus*, FO, and 20th, 21st, 22nd June on *C. sativa*, CS), located close to honey bee hives to limit variability due to flying efforts (distance: about 500 m). Before proceeding with data collection, we ascertained bee presence/activity (presence/absence; rough estimate of number of individuals per inflorescence at different times of the day; data not reported) and defined handling routines. On both tree species, observations took place from 8:00 to 12:00 (solar hour). Unmarked individual bees were followed at sight during their activity as long as possible. Data collection (handling time and handling routine) started with visual recording of a bee in action, while approaching a FO panicle or CS catkin.

Handling time

We recorded the number of FO panicles / CS catkins visited on the same plant by individual bees (FO n = 74 bees; CS n = 23 bees) and calculated average length of time spent a) during the entire visit on the plant and b) on each panicle/catkin. We also recorded interruptions due to pouncing insects, and compared activity of disturbed and undisturbed bees.

Handling routine

We recorded landing position and orientation, and resource collection (pollen: rolling the front legs; nectar: proboscis extension). Through preliminary observations we established unique behavioural units on each species of plant; these have been later grouped (approaching / landing; resource collection; approaching / leaving) to facilitate discussion. Details on the foraging behaviour on each plant species are given in details in table 1 (*F. ornus*) and table 2 (*C. sativa*). Grooming and pressing pollen into the corbiculae was a distinctive behaviour on FO, while it was not detected as an independent behavioural unit when observing bees foraging on CS: it was probably performed quickly while collecting pollen, or in flight. Each FO panicle / CS catkin was considered as a single unit during observations.

With the help of a stopwatch and a voice-recorder we listed behavioural units performed by 14 bees on FO and 23 bees on CS. We calculated frequencies of single *Fraxinus* Behavioural Units (FBU, n = 564 records) and of single *Castanea* Behavioural Units (CBU, n = 339) in two ways: as percentage of total records and as percentage of bees performing that behaviour. On FO we were also able to calculate average duration of each Behavioural Unit.

Activity (time spent foraging and number of panicles visited) of disturbed and undisturbed bees on FO was compared through the T-test, all conditions for applying a parametric analysis being supported. Differences on time and number of CS catkins visited by nectar and pollen foragers were tested using Mann-Whitney U-test. Statistical analysis was performed using the software Statistica (StatSoft Inc., Tulsa, Oklahoma, USA) and following Zar (1974) and Dytham (2003).

Table 1. Details on handling routine of honey bees on *F. ornus*. First Column: type of behaviour; second column: unique Behavioural Unit codes; third column: unique Behavioural Unit description.

Type of behaviour	Behavioural Unit codes	Description of activity
Approaching / landing	FBU-1	Entering a panicle, walking on or among the flowers, rolling the legs and vibrating the wings
Resource collection	FBU-2	Grooming the pollen and pressing it into the corbiculae, while hovering in front of the panicle
	FBU-3	Grooming the pollen and pressing it into the corbiculae, while hanging from a panicle
Approaching / leaving	FBU-4	Flying from one panicle to another, eventually grooming and pressing pollen into the corbiculae

Table 2. Details on handling routine of honey bees on *C. sativa.* First Column: type of behaviour; second column: unique Behavioural Unit codes; third column: unique Behavioural Unit description. In column four we splitted previous Behavioural Unit on the base of an extra feature (direction taken while performing the behaviour).

Type of Behaviour	Behavioural Unit codes	Description of activity	
Approaching / landing	CBU-1	Landing on the initial part of the catkin, directing first	towards the end: CBU-1a
			towards the base: CBU-1b
	CBU-2	Landing on the central part of the catkin, directing first	towards the end: CBU-2a
			towards the base: CBU-2b
	CBU-3	Landing on the terminal part of the catkin, directing first	towards the end: CBU-3a
			towards the base: CBU-3b
Resource collection	CBU-4	Collecting pollen, following a	straight line: CBU-4a
			sinuous line: CBU-4b
	CBU-5	Collecting nectar, following a	straight line: CBU-5a
			sinuous line: CBU-5b
	CBU-6	Collecting pollen + nectar, following a	straight line: CBU-6a
			sinuous line: CBU-6b

Results

On both species we collected data in order to: 1. outline complete visit to a single inflorescence, 2. define the approach to it, including landing and orientation, 3. describe individual flower manipulation. Bees proceeded with different tactics on the two species and the adopted strategies were not always comparable. Descriptive statistics are reported in table 3 and 4.

Complete visit

On FO, bees (n = 74) collected only pollen on both male and hermaphrodite plants, visiting a range of 1-14 panicles/plant and spending from a minimum of 13 seconds to a maximum of about 7 minutes to complete a visit. Disturbance was a frequent event interrupting resource collection on FO. Disturbance due to the pouncing of another insect occurred in 31% of the visits, mostly due to Diptera. Disturbed (D, n = 24) and undis-

Table 3. Handling time. Average ± SD of duration of visits to single plants and single panicle / catkin (in seconds) and average ± SD of number of panicles or catkins visited on the same plant.

		Number of bees	Handling time		Number of panicles or catkins visited / plant
			seconds / plant	seconds / panicle or catkin	
F. ornus	Pollen foragers	74	143 ± 100	27 ± 15	5 ± 3
C. sativa	Pollen foragers	14	143 ± 96	19 ± 8	7 ± 4
	Nectar foragers	7	319 ± 291	28 ± 11	10 ± 6
	All bees	23	203 ± 193	22 ± 10	8 ± 5

Table 4. Handling routine. Data on Behavioural Units on the two species, *F. ornus* (n = 564 records) and *C. sativa* (n = 339 records). Average duration of Behavioural Units is reported for FO. Frequencies of records and of number of bees performing each Behavioural Unit are reported for FO and CS.

			Handling routine		
			Average duration (seconds)	Frequency (% of total records)	Frequency (% of bees performing it)
F. ornus	approaching/landing	FBU-1	5.2 ± 4.0	41.1	100
	resource collection	FBU-2	2.9 ± 2.1	44.0	100
		FBU-3	4.7 ± 5.1	2.5	57.1
	approaching/leaving	FBU-4	3.2 ± 2.5	12.4	92.9
C. sativa	approaching/landing	CBU-1	-	12.9	52.2
		CBU-2	-	35.7	95.7
		CBU-3	-	51.5	91.3
	resource collection	CBU-4	-	49.4	73.9
		CBU-5	-	28.0	30.4
		CBU-6	-	22.6	60.9

turbed (UD, n = 50) individuals showed weak differences as time dedicated to resource collection (on average, D = 127 ± SD 95 sec; UD = 151 ± SD 103 sec) and number of panicles visited (on average, D = 5 ± SD 3 panicles; UD = 6 ± SD 3 panicles). However, bees activity was not significantly affected by the disturbance (time, T-test: $t = 0.962$, df = 72, p = 0.339; number of panicles, T-test: $t = 1.565$, df = 72, p = 0.122).

On CS, bees (n = 23) collected both pollen and/or nectar from the male flowers, visiting a range of 2-20 catkins/plant, spending from a minimum of 20 seconds to a maximum of about 15 minutes to complete a visit. Interruptions of bee activities due to other insects were not recorded. No visit were recorded to the female flowers.

Approach

On FO, bees landed/entered directly the massive group of flowers of a panicle or walked from one panicle to the other if close enough. Sometimes they grab any surface with any leg while still hovering in front of the panicle. No precise position or orientation on the panicle could be detected.

On CS, bees landed directly on the catkin most of the time, but they may also move from one to another simply by walking when catkins were very close. There was a spatial orientation: the initial part of the catkin showed the lowest values (table 4, CBU-1), both as percentage of records as well as bees visiting it. Once landed, the elected direction was the one offering the majority of flowers (CBU-1a: 63.6% and CBU-3b: 61.4%); if landing on the central part of the catkin, the elected direction was towards the apex (CBU-2a: 68.9%). In 17 cases out of 171, the bee did not clearly take any direction, visiting the flowers close to the place where it landed and then flying away.

Individual flower manipulation

On FO resource collection consisted in approaching the panicle and moving among the flowers, rolling the legs and vibrating the wings (FBU-1). This behaviour produced a visible release of pollen grains. The pollen covered completely the body of the bee, then prompting a cleaning behaviour (figure 1, FBU-2; or FBU-3). The alternation of two behaviours (table 4, FBU-1 and FBU-2) may occur up to 48 times resulting in the core strategy of pollen collection. No precise manipulation of single flowers was detected.

On CS, only two individuals performed CBU-6 (pollen and nectar collection at the same spot) as main/exclusive collection; the remaining 21 bees focused mainly on a single resource, performing CBU-6 no more than 4 times and could be cast as pollen or nectar foragers (table 4).

Figure 1. Honey bee forager in front of a *F. ornus* panicle, hovering while grooming the pollen and pressing it into the corbiculae (FBU-2).
(In colour at www.bulletinofinsectology.org)

Excluding CBU-6, pollen collection (CBU-4) accounts for the 63.8% of records while nectar collection (CBU-5) for the remaining 36.2%. Considering the higher number of foragers dedicating to pollen collection, in the context of our observations, pollen can be placed as top resource gathered on *C. sativa*. Nectar foragers spent more time on each plant and visited a higher number of catkins than pollen foragers, but the difference is not statistically significant (time spent: Mann-Whitney U = 26, n1 = 7, n2 = 14, p = 0.086; number of catkins: Mann-Whitney U = 36, n1 = 7, n2 = 14, p = 0.332). Pollen (CBU-4) or nectar (CBU-5) collection showed a significant correlation with the path (linear or sinuous) followed on the catkin ($\chi^2 = 51.6$, df = 1, $p < 0.0001$). When collecting pollen, bees followed a straight line (74.7% of CBU-4); when collecting nectar, they followed a sinuous path (91.5% of CBU-5). Resource collection was unclear in 11 visits.

Discussion

Notwithstanding the increased large-scale studies on pollination and bee floral preferences, detailed floral visits are the main evidence we can count on. Distinguishing between pollen and nectar visits may seem obvious, till this information is lacking in many studies and for many botanical species (Cane and Sipes, 2006). We expect a pollen-forager to look for abundant, available and easy to manipulate resources. Plants presenting flower with anemophilous characteristics produce high quantities of usually small pollen grains, stamens are usually large, well-exposed, mainly hanging freely from panicles/catkins and yield a high concentration of pollen/area (Proctor *et al.*, 1996). In fact, we found that honey bee activity on *F. ornus* and *C. sativa* is not incidental and that the bees approach the flowers with different strategies perfectly suited for the best pollen collection.

Honey bees actively collect pollen on *F. ornus* and *C. sativa*, even nectar on the latter. A panicle of *F. ornus* provides higher number of flowers than a catkin of *C. sativa*, which may explain the variations in number of units visited on each plant. Also the difference in handling routines may reflect flower spatial arrangement: *F. ornus*, lacking a landing surface, is approached apparently in a disorganized way. Honey bees in fact invest in hectic movements that result in a pollen cloud, covering their body with pollen grains that are groomed later. This strategy may also be necessary to maximize the pollen release from *F. ornus* anthers, considering that (as exhaustively described by Pacini and Hesse, 2004) some plant species evolved mechanisms to allow gradual removal of the grains.

F. ornus and *C. sativa* constitute a perfect pollen source, even more important from an apicultural point of view. In fact, early pollen collection during the blooming season improves colonies survival and allows achieving stronger beehives. Moreover, since honey is a product very appreciated on the market, hive locations could be scheduled according to the flowering phenology of the closest wind pollinated species to increase colony productivity, honey production and the survival of local communities (Castellini *et al.*, 2009).

On the other hand, plant fitness could be enlarged by insect visits. De Oliveira *et al.* (2001) tested experimentally the outcome of different vectors (wind, gravity, insects) over *Castanea* burr set, finding a higher chestnut production in presence of insects. Wallander (2008) reported *F. ornus* as pollinated by both wind and insects, as many other species of the same genus. Wind may be the main vector for pollen dispersal in both *F. ornus* and *C. sativa*. However, insects can still play a role when environmental conditions, as high humidity or absence of wind, are unfavourable to wind dispersal. Unfortunately, present literature is lacking more detailed studies on visits paid by insects to similar plants and we do not often know if the outcome is incidental or the result of a double (wind + insect) pollination strategy. Considering that anemophily in Angiosperms is of secondary derivation (Whitehead, 1969; Ackerman 2000, Friedman and Barret, 2009), the occurrence of insect attractive traits on wind pollinated species, such as nectar in *C. sativa* or petals in *F. ornus*, might be considered remnant features of a past entomophilous phase. Further observations in the field could help detecting ancestral or current adaptations and identifying the process we are examining.

In an evolutionary view of pollination strategies, the behavioural plasticity shown by honey bee foragers accounts for the possible exploitation of species presenting flowers with anemophilous characteristics. This may occur even in absence of changes in flower morphology, evinced in our assessment of flower handling routine. Insect visit to rather wind-pollinated species may uncover underestimated costs and benefits in the plant-animal interactions. Therefore researchers should drive their attention towards the stability and coexistence of different pollination strategies in the same plant species.

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