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# Influence of Bee Species (Hymenoptera: Apiformes) with Contrasting Behaviors on Pollen Movement in a Mustard, Brassica rapa (Brassicaceae) and the Muskmelon Cucumis melo (Cucurbitaceae)

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Pollen removal and deposition to two crop species are measured as a preliminary screening tool to compare pollination by two commercially available bee species. The ratio of pollen deposited to pollen removed offers a rough estimate of pollinator effectiveness per visit. Differences among pollinators in these measures can help direct future study. Compared was the pollen deposition and removal by *Apis mellifera* (Linnaeus 1758) and *Bombus impatiens* (Cresson 1863) for a mustard (*Brassica rapa* Linnaeus 1753 [Brassicaceae]), and muskmelons (*Cucumis melo* Linnaeus 1753 [Cucurbitaceae]). As in some other systems, bumble bees and honey bees provided similar pollen transfer when they adopted the same behaviors; differences in pollen-transfer efficiency arose primarily when the bees adopted different behaviors. In *B rapa*, *B impatiens* and *A mellifera* deposited similar amounts of pollen on stigmas, with pollen-collecting visits resulting in more pollen deposited than nectar-collecting visits for both species. *A mellifera* removed significantly more pollen from *B rapa* flowers than *B impatiens*, largely because *B impatiens* removed little while nectar collecting. The greater deposition:removal ratio of *B impatiens* suggests it is a better pollinator, at least when visits are frequent, because it removes less pollen from circulation per visit. In *C melo*, the reverse was found: the two bee species differed little in pollen deposition, but *B impatiens* removed significantly more pollen. The ratio of deposition:removal was higher for *A mellifera*, suggesting it is a better pollinator than *B impatiens*. When visits are infrequent, however, *B impatiens* is likely to mobilize more pollen and be a more effective pollinator.

**Key words:** *Apis mellifera* (Linnaeus 1758) – *Bombus impatiens* (Cresson 1863) – bumble bee – crop pollination – foraging behaviour – honey bee – pollen deposition – pollen flow – pollen removal – pollen transfer

GOODELL K [Dept Evol Ecol Org Biol, Ohio State Univ, Newark, OH 43023, USA] & THOMSON J D [Ecol Evol Biol, Univ Toronto, ON MS53G5, Canada]: **La Movilización de polen de una mostaza, Brassica rapa (Brassicaceae), y melón, Cucumis melo (Cucurbitaceae), es afectado por especies de abeja (Hymenoptera: Apiformes) con comportamientos diferentes.** – Entomol Gener **29** (2/4): 237–252; Stuttgart 2007-01. – – – [Artículo]

Se midió la recolección y depósito de polen de dos cultivos como una forma de comparar preliminarmente la polinización de dos especies de abejas comerciales. La relación de polen depositado/recolectado muestra una idea aproximada de la efectividad del polinizador por visita.

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Diferencias de esta medida entre los polinizadores puede ser aplicada directamente en investigaciones futuras. Comparamos el deposito y recolección de polen de abejas, *Apis mellifera* (Linnaeus 1758) [Hymenoptera: Apidae], y abejorros, *Bombus impatiens* (Cresson 1863) [Hymenoptera: Apidae] en una mostaza, *Brassica rapa* (Linnaeus 1753 [Brassicaceae]), y melón (*Cucumis melo* Linnaeus 1753 [Cucurbitaceae]). Como en otras especies de plantas, *Bombus* y *Apis* proveyeron similar transferencia de polen cuando adoptaron el mismo comportamiento. Las diferencias en la eficiencia en la transferencia de polen resultaron cuando las abejas adoptaron comportamientos diferentes. En *B rapa*, *B impatiens* y *A mellifera* depositaron cantidades similares de polen en los estigmas. Para las dos especies de abejas, las visitas para recolectar polen dieron como resultado mayor deposito de polen que recolección de néctar. *A mellifera* recolectó significantemente más polen de flores de *B rapa* que *B impatiens*, debido a que *B impatiens* recogió menos polen mientras estaba recolectando néctar. La mayor relación depósito/recolección de *B impatiens* sugiere que es más efectiva como polinizador que *A mellifera*, por lo menos cuando las visitas son frecuentes, debido a que recolecta menos polen del sistema por visita. En *C melo*, encontramos lo contrario: aunque *B impatiens* recolectó más polen, las dos especies de abeja difirieron poco en el depósito de polen. La relación depósito/recolección de polen fue más alta para *A mellifera*, sugiriendo que es un polinizador más efectivo que *B impatiens*. No obstante, cuando las visitas son menos frecuentes, *B impatiens* probablemente movilice más polen y sea más eficiente como polinizador.

**Palabras clave:** *Apis mellifera* (Linnaeus 1758) – *Bombus impatiens* (Cresson 1863) – abeja – abejorro – comportamiento de forrajeo – depósito de polen – movimiento de polen – polinización de cultivos – recolección de polen – transferencia de polen

## 1 Introduction

Certain pollinating species confer greater fitness than others on host plants. This idea has been implicitly clear since DARWIN [1877] and has provided a satisfactory explanation for SPRENGEL's [1793] numerous observations of harmonious matches between flowers and insects.

Analogously, agriculture has a long tradition of investigating alternative insect pollinators for crops, where a 'good' pollinator confers not higher fitness, but higher yield or superior market quality of the produce. Such investigations tend to be completely empirical studies (as opposed to theoretical), and domesticated honey bees (*Apis mellifera* Linnaeus 1758) are the standard to which other insects are compared [STANGHELLINI et al 1997, FREITAS & PAXTON 1998]. A few 'alternative' pollinators have been developed for crops that are poorly served by honey bees, such as the leafcutter bee *Megachile rotundata* (Fabricius 1793) as a pollinator of alfalfa [STEPHEN 1962, BOHART 1972] or domesticated bumble bees for pollination of tomatoes in greenhouses [RUIJTER 1997]. Further research can produce more such successes [BATRA 1982, PARKER et al 1987, TORCHIO 1991, CANE 1997].

Candidate pollinators must be painstakingly tested to determine whether they can be managed in agricultural environment and how their visitation affects yield of particular crops. The great expense of developing and testing a new pollinator will limit the number of such inquires. Variation among pollinators in their pollination effectiveness must ultimately relate to differences in their patterns of pollen removal and delivery [WILSON & THOMSON 1991]. Mathematical models of pollen delivery show that differences among pollinators in per-visit removal and deposition of pollen grains are important predictors of their influence on plant fitness [THOMSON & THOMSON 1992]. They can also predict conditions under which particular pollinators may provide inadequate pollination. For example, under some conditions the models show that a less efficient pollinator can act to the plant's detriment by taking away pollen that would otherwise be deposited by a more efficient pollinator. Possible negative interactions between pollinator species, such as pollen-depletion [WILSON & THOMSON 1991], or even positive interactions between pollinator species, such as those reported by GREANLEAF & KREMEN [2006] are rarely considered in agricultural contexts.

Pollen removal and deposition can also depend on the foraging strategies adopted by the bees, which can be influenced by factors such as condition of the colony [HARDER 1990, PLOWRIGHT et al 1993], the surrounding plant community, and the foraging of other floral visitors in the community. Knowledge of how pollination services may respond to shifts in foraging strategies can be important for crop producers.

At the level of single visits, the ratio of deposition to removal is a rough indicator of relative pollination efficiency. Therefore, measuring removal and deposition rates is useful early step in deciding whether an alternative pollinator is worth investigating further. Pollen deposition can be a poor surrogate for fruit or seed set, however, both because the method cannot distinguish self- from outcross pollen for self-incompatible species (as for the *B rapa* used here), and also because pollen loads in excess of any fruiting response (fruit set, seed set) gives a false impression of pollinator superiority [CANE & SCHIFFHAUER 2003]. Nevertheless, it has the great advantage of immediate convenience and avoids post-pollination factors that can confound results (e.g maternal resource limitation, herbivory, weather, etc). Although pollen removal and deposition measures have been employed extensively in ecological and evolutionary research to compare pollinator efficiencies, their use in agriculture remains rare [although see GOODELL & THOMSON 1997, FREITAS & PAXTON 1998, THOMSON & GOODELL 2001].

The recent widespread commercial availability of bumble bees makes them an obvious target for such assessment. Here, such measurements are presented for honey bees (*Apis mellifera* Linnaeus 1758) and bumble bees (*Bombus impatiens* Cresson 1863) on two plant species with contrasting reproductive strategies and floral biology: a mustard (*Brassica rapa* Linnaeus 1753) and muskmelon (*Cucumis melo* Linnaeus 1753). The implications of the results for the relative values of these two insects as pollinators will be discussed.

## 2 Materials and methods

### 2.1 Study systems

The *B rapa* that was used here (Wisconsin Fast Plants) is not a crop variety, but the flowers and mating system are similar those of some commercial canola varieties, and also similar to those of wild radish (*Raphanus raphanistrum* Linnaeus 1753 [Brassicaceae]), for which honey bee and bumble bee pollination have previously been shown to differ [YOUNG & STANTON 1990]. *Brassica rapa* produces perfect flowers that are self-incompatible. Commercial pollination of related varieties is typically achieved with honey bees, although they are also visited by a variety of wild bee species [MCGREGOR 1976, MORANDIN & WINSTON 2005]. MORANDIN & WINSTON [2005] reported pollen limitation of cultivated *B rapa* and showed that wild, unmanaged bee density was positively correlated with seed yield. Their results suggest exploration of alternative pollinators may be fruitful. Comparative pollinator research aimed at improving pollinator services to *Brassica* crops is especially important for varieties with special pollination requirements and pollen containment issues, such as transgenic varieties and male sterile varieties [DANIELL 2002].

The muskmelon (*C melo*) was chosen because growers are concerned about achieving adequate pollination [MCGREGOR 1976] and we had access to suitable study fields. Muskmelons are andromonoecious and, although self-fertile, perfect flowers will not set fruits without being pollinated [BOHN & DAVIS 1964]. Furthermore, cross-pollinated flowers yield heavier fruits than self-pollinated flowers [MCGREGOR 1976]. Honey bees are typically used for muskmelon pollination, although wild bumble bees are cited as frequent, if not dominant pollinators in some muskmelon plots [HANDEL 1982 and references therein]. Cultivation in greenhouses has stimulated experimentation with pollination by alternative bee species, such as commercial bumble bees and mason bees [FISHER & POMEROY 1989, INCALCATERRA et al 2003].

Wild, unmanaged bees can be important pollinators of related watermelon (*Citrullus lanatus* Thunberg 1959) [KREMEN et al 2002]. It seems reasonable that other bee species could adequately service muskmelons under some growing conditions.

## 2.2 *Brassica* cultivation and visitation

*Brassica rapa* seed was obtained from the Wisconsin Fast Plants Program, (University of Wisconsin, Madison, WI, USA) and was sown in trays and grown under 24h light according to developer's protocols (Wisconsin Fast Plants Program 2006). Bred for rapid growth, this strain of *B rapa* produces diminutive plants about 30 cm tall, but the flowers are large, normally formed, and attractive to flower-visiting insects. On fair weather days, trays of flowering *B rapa* plants were placed into a meadow outside of the greenhouse on the State University of New York at Stony Brook campus, Long Island, NY, USA. A hive of honey bees was placed in the meadow to augment honey bee availability; the *B impatiens* were resident unmanaged bees. The experimental flowers were removed from plants held within the greenhouse to prevent bee visitation. They were carefully transported outside in covered Petri dishes to prevent loss of pollen grains. Experimental flowers were held with forceps and offered to foraging bees. One visit by either a *B impatiens* or an *A mellifera* worker was allowed. For each experimental flower we noted the species of forager, the behavior (pollen or nectar collection), and timed the visit using a hand-held stopwatch. Following the visit the anthers were removed from the experimental flower over a vial and stored in 70% alcohol for pollen analysis. On other flowers the stigmas were mounted on microscope slides in a drop of glycerine gel infused with basic fuchsin to stain the pollen grains. Stigmas and anthers were collected from different experimental flowers to prevent deposition of pollen by researchers while removing anthers and loss of pollen from anthers as the stigmas was manipulated. Data on *A mellifera* and *B impatiens* were collected on separate days. It was attempted to eliminate self-pollen deposition from the same flower by presenting emasculated flowers, but the bees appeared to reject emasculated experimental flowers. A small data set for *A mellifera* visits to emasculated flowers was included, but it was not possible to entice *B impatiens* to visit emasculated flowers. Emasculation of the flowers may have affected foraging behavior of *A mellifera* in ways that could influence pollen deposition.

## 2.3 *Cucumis* cultivation and visitation

Muskmelon plants were grown at the Cornell University Agricultural Research Station, Long Island, NY, USA. Fine mesh bags were placed over unopened flower buds, both perfect and staminate, to prevent insect visitation. A plastic ring around the flower prevented the bag from brushing the flower. Flowers were removed from the plants on the morning that they opened and placed in a small vial of water attached to the end of a 1 m long stick. The stick was used to position the flower in the path of a foraging bee and allowed a bee to visit the each flower. The *A mellifera* workers were from managed colonies and the *B impatiens* from wild, unmanaged populations. For each experimental flower, the species of forager and the behavior (pollen or nectar collection) were noted, and the visit was timed using a hand-held stopwatch. Following the visit, the anthers of the experimental flower were collected into separate vials of 70% ethanol. The stigmas were removed and mounted onto a microscope slide as described above. As for *B rapa*, separate flowers were used to collect stigmas and anthers.

## 2.4 Pollen deposition and removal data

The numbers of pollen grains deposited on *B rapa* and *C melo* stigmas were determined using a compound light microscope at the lowest magnification at which the pollen grains could be identified. All grains visible over the entire stigmatic surface were counted.

Control flowers that had not been visited by bees, but that were otherwise treated identically to the experimental flowers, were used to determine how many pollen grains were deposited as a result of handling the flowers by the experimenters. It was not possible to distinguish between self and outcross pollen, so the estimates of pollen deposition include both.

The number of pollen grains removed by *B impatiens* and *A mellifera* was estimated by subtracting the number of pollen grains remaining in the anthers after the visit from the average number of pollen grains in unvisited control flowers collected using the same methods as for the experimental flowers. The number of pollen grains per experimental and control flowers were estimated using an Elzone 280-PC electronic particle counter (Micromeritics, Norcross, GA, USA, http:// www.micromeritics.com/default.aspx ) For both control and experimental flowers, the vials containing the anthers were filled with 5 ml of 1% saline solution and sonicated at 9 watts (RMS) using a Virsonic 60 sonicating wand (VirTis Co., Inc. Gardiner, NY, USA, http://www.virtis.com) for 20 s just before counting to ensure that all pollen grains were separated and suspended in the liquid. All pollen grains in three sub samples of approximately 1 ml were counted for each flower, the vial gently shaken between sub samples to prevent settling of the grains. The exact volume counted was determined by subtracting final from initial weight. The total number of grains per sample was determined by multiplying by the total volume of liquid (initial weight – vial weight) by the average concentration of pollen grains in the sub samples.

### 2.5 Analyses

Differences among bee species in their pollen deposition and removal on the two crop species were tested separately using model I analysis of variance. The analyses were conducted using Proc GLM in SAS 9.1 [SAS 1999–2001]. Bee species and the type of visit and the two way interaction were considered fixed independent variables; the number of grains deposited or removed was the dependent variable. Most bees visiting melons probed for nectar, but did not collect pollen, so we did not examine visit type for melons. Melon removal data were square root transformed to meet the assumptions of the analyses. T-tests were used to test for differences between pollen removal following specific visit types and unvisited control flowers of *B rapa*.

Differences in the durations of visits to flowers among bee species and visit types were examined using a model I analysis of variance in which bee identity, visit type, and their interaction were fixed independent variables and duration of the visit was the dependent variable. Any visit type class that lacked replicates from both bee species was omitted from the analyses. Separate analyses were conducted for flowers on which deposition and removal were measured because both sets of data were not collected from each experimental flower. These analyses were conducted using Proc GLM in SAS 9.1 [SAS 1999–2001]. Finally, the relationship between pollen removal or deposition and the duration of the visit was examined using least squares linear regressions performed in Origin 7 [OriginLab 1991–2002]. The conventional criterion of $p < 0.05$ is used for reporting significance, but attention is also drawn to some non-significant results for which $0.05 < p < 0.10$. Because sample sizes are small, these tests have little power to support retaining the null hypothesis, and further investigation would be valuable.

## 3 Results

### 3.1 *Brassica* pollen deposition and removal

The number of pollen grains deposited on the stigmas of *B rapa* flowers depended on the type of resource sought by the bee visitor, but not on the species identity of the visitor nor their interaction (**Tab 1A**, **Fig 1**).

**Tab 1:** Pollen deposition on *Brassica rapa* Linnaeus 1753 [Brassicaceae]. Analysis of variance table showing (**A**) effects of bee type (*Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] or *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae]) and visit type (pollen or nectar) and (**B**) effect of visitor type (*A mellifera*, *B impatiens*, or unvisited control) on the number of pollen grains deposited on the stigmas of *Brassica rapa* plants. A planned mean contrast tested the difference between pollen deposition on the unvisited control compared to the visited flowers.

| Source | DF | MS | F | P |
|---|---|---|---|---|
| (A) | | | | |
| Bee type | 1 | 504.77 | 0.00 | 0.97 |
| Visit type | 1 | 3601150.47 | 13.79 | 0.001 |
| Bee X Visit type | 1 | 46759.10 | 0.18 | 0.68 |
| Error | 26 | 6788195.70 | | |
| (B) | | | | |
| Visitor type | 2 | 1221890.25 | 4.02 | 0.026 |
| Error | 37 | 11237199.09 | | |
| Contrast: Control vs visited | 1 | 2423639.77 | 7.98 | 0.008 |

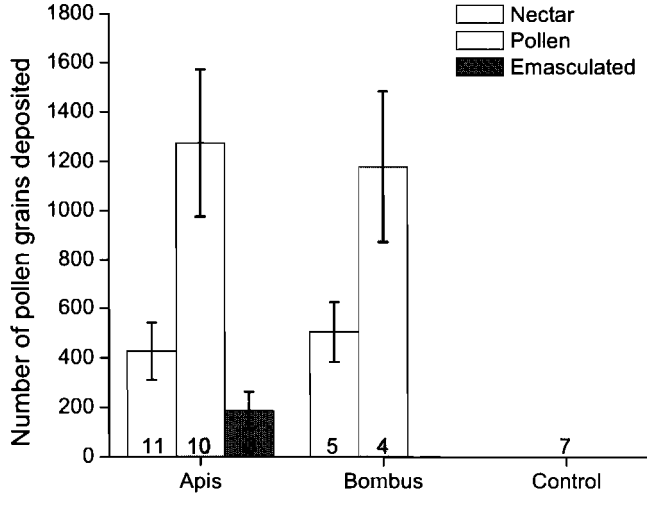


**Fig 1:** Pollen deposition on *Brassica rapa* Linnaeus 1753 [Brassicaceae] stigmas by *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae] visitors compared to unvisited controls. Mean number of pollen grains are given for nectar-collecting and pollen-collecting bees, and nectar-collecting *A mellifera* to emasculated flowers. Error bars represent ± 1 SE; numbers at the base of the bars show sample size.

Nectar visits deposited 62% fewer pollen grains than pollen visits (Nectar: mean = 464.57 grains, n=21, SE = 84.01; Pollen: mean = 1231.33, n = 9, SE = 214.67). Both *A mellifera* and *B impatiens* visits resulted in significantly greater pollen deposition than the unvisited controls (**Tab 1B**). Only *A mellifera* could be induced to visit emasculated flowers and the sample size is small, so these data were omitted from the analysis of variance. Emasculated flowers received on average 40% of the pollen grains deposited during a nectar visit by *A mellifera* (mean = 188.33, n = 3, SE = 76.21) (**Fig 1**). Although nectar visits made by *A mellifera* to emasculated flowers were slightly shorter on average (mean = 6.87 s, SE = 1.59, n = 3) than those to unmanipulated flowers (mean = 7.80, SE = 2.08, n = 11), this difference was not statistically significant (T-test,: T = 0.22, df = 12, p = 0.83). The shorter visits, therefore, are unlikely to cause the difference in pollen deposition, but rather suggest that 60% of the pollen deposited during a regular nectar visit is self-pollen from the same flower. It was not possible to distinguish between outcross pollen deposition and geitonogamous pollen deposition originating from other flowers on the plant, however.

Pollen removal from the anthers of *B rapa* varied with both the visitor identity and the type of visit (**Fig 2**). Pollen-collecting visits removed almost twice as much pollen as nectar-collecting visits (ANOVA $F_{1,81} = 22.55$, $p < 0.0001$). *A mellifera* in general removed more pollen than *B impatiens* (ANOVA $F_{1,81} = 7.02$, $P < 0.01$), largely because *A mellifera* nectar visits resulted in 99% more pollen removed than *B impatiens* nectar visits. This large difference in pollen removal by the two bee species for nectar visits, combined with the nearly identical pollen removal for pollen visits, resulted in a significant interaction term (ANOVA $F_{1,81} = 7.66$, $p < 0.01$). The number of pollen grains removed following a *B impatiens* nectar visit did not differ from those removed from unvisited control flowers (T-test: t = -0.11, df = 24, p (2-tailed) = 0.92). In addition to the typical nectar-collecting visit in which the bee enters the front of the flower, some *A mellifera* approached the flowers from below, inserting their proboscises between the petals.

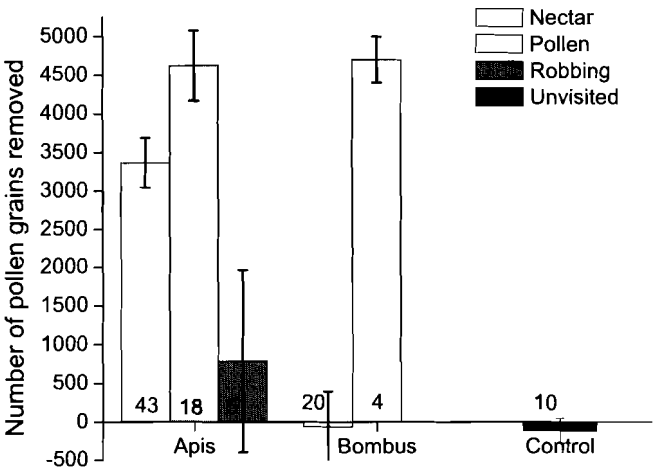


**Fig 2:** Pollen removal from *Brassica rapa* Linnaeus 1753 [Brassicaceae] anthers by *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae] compared to unvisited control flowers. Mean number of pollen grains removed per anther following a single visit shown for nectar-collecting, pollen-collecting and "robbing" bees. Error bars represent ± 1 SE; numbers at the base of the bars show sample size.

**Tab 2:** Visit duration to *Brassica rapa* Linnaeus 1753 [Brassicaceae]. Analysis of variance table testing for effects of bee visitor identity (*Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] or *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae]) and visit type (pollen or nectar) on the duration of visits to *Brassica rapa* flowers shown for deposition (**A**) and removal (**B**) experimental flowers separately.

| Source | DF | MS | F | P |
|---|---|---|---|---|
| A. Deposition | | | | |
| Bee type | 1 | 351.63 | 13.92 | 0.0009 |
| Visit type | 2 | 9.03 | 0.36 | 0.70 |
| Bee X Visit type | 1 | 13.57 | 0.54 | 0.47 |
| Error | 28 | 25.26 | | |
| B. Removal | | | | |
| Bee type | 1 | 1223.15 | 31.29 | <0.0001 |
| Visit type | 2 | 57.82 | 1.48 | 0.23 |
| Bee X Visit type | 1 | 25.41 | 0.65 | 0.42 |
| Error | 85 | 3322.31 | | |

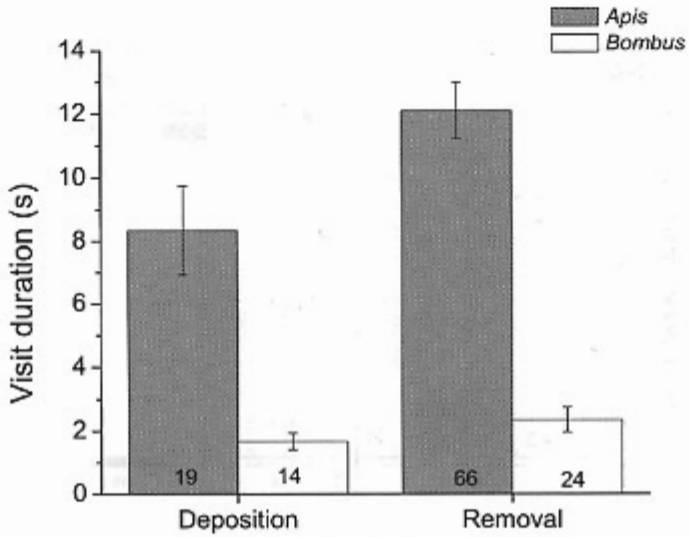


**Fig 3:** Duration of visits to *Brassica rapa* Linnaeus 1753 [Brassicaceae] anthers by *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae] visitors for experimental flowers on which pollen deposition and removal were measured. Columns represent means and error bars represent ± 1 SE. Numbers at the base of the bars show sample sizes.

This type of visit is referred to here as 'robbing' because the bee extracted nectar without contacting the sexual parts of the flowers. Robbing visits by *A mellifera* resulted in fewer pollen grains removed than legitimate pollen and nectar visits and did not differ from unvisited controls (T-test: t = 0.68, df = 4, p = 0.54).

The duration of visits to *B rapa* by *A mellifera* were 80% longer than those made by *B impatiens* on flowers measured both for pollen deposition and pollen removal (**Fig 3**, **Tab 2**). The duration of visits did not vary with the type of visit or the interaction between bee identity and visit type for neither removal nor deposition flowers, however (**Tab 2**). The duration of visits was positively associated with pollen deposition in *A mellifera* if all visits were considered ($R^2$-adj = 0.27, n = 19, p < 0.02), but not when nectar or pollen visits were analyzed separately (Nectar: $R^2$-adj = 0.22, n = 11, p = 0.08; Pollen: $R^2$-adj = 0.08, n = 5, p = 0.33), although sample sizes are small (**Fig 4A**). Pollen removal also increased with visit duration for *A mellifera* (**Fig 4C**). This trend was strongest for pollen visits ($R^2$-adj = 0.60, n = 18, p = 0.0001), but was also significant for nectar visits ($R^2$-adj = 0.13, n = 43, p = 0.009) and all visits combined ($R^2$-adj = 0.24, n = 66, p = 0.0001). Visit duration was not significantly associated with pollen deposition or removal for *B impatiens* (Deposition: $R^2$-adj = -0.08, n = 14, p = 0.78, Removal: $R^2$-adj = -0.04, n = 24, p = 0.74) (**Fig 4B, D**).

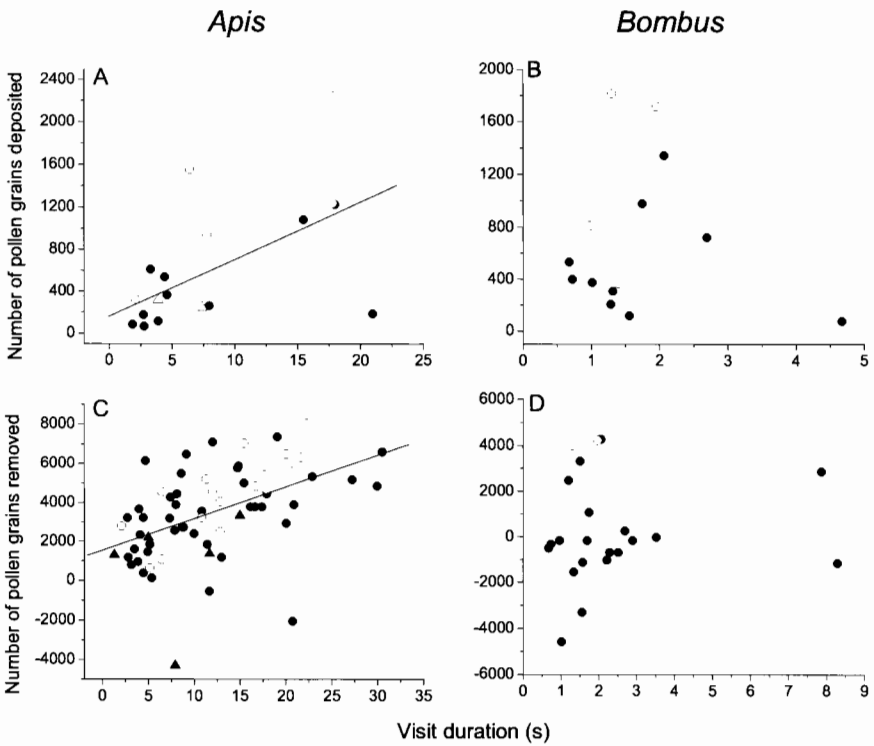


**Fig 4:** The number of pollen grains deposited on stigmas (**A** and **B**) or removed per anther (**C** and **D**) of *Brassica rapa* Linnaeus 1753 [Brassicaceae] flowers per visit as a function of the duration of the visit shown separately for *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] (**A** and **C**) and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae] (**B** and **D**). Symbols represent nectar-collecting visits (●), pollen-collecting visits (○), nectar-collecting visits to emasculated flowers (△), and "robbing" visits (▲). The trend line represents a least squares linear regression with all visit types combined.

### 3.2 *Cucumis* pollen deposition and removal

*Cucumis melo* visitors did not actively collect pollen from the flowers, but received grains passively while probing for nectar. On average, *B impatiens* tended to deposit about 50% more grains than *A mellifera* (**Fig 5**). However, large variances meant that deposition did not differ statistically between *A mellifera, B impatiens* and unvisited controls (ANOVA $F_{2, 23} = 1.64$, $p = 0.22$).

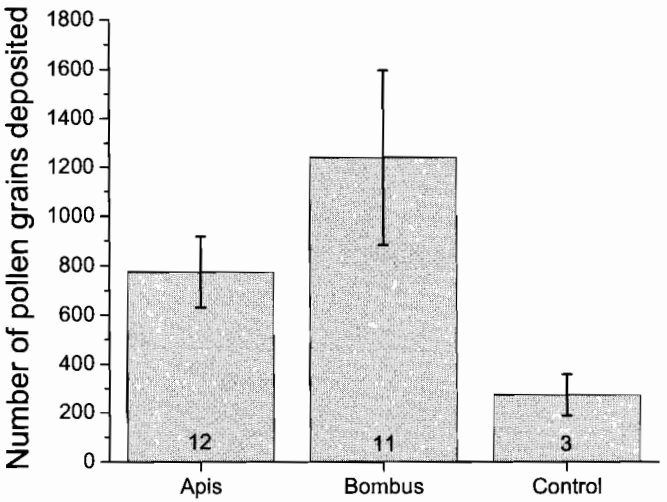


**Fig 5:** Pollen deposition on *Cucumis melo* Linnaeus 1753 [Cucurbitaceae] stigmas following single visits by *Apis* or *Bombus*. The Control flowers were unvisited but otherwise treated as experimental flowers. Columns represent mean number of pollen grains, error bars represent ± 1 SE, and numbers at the base of the bars show sample size.

*B impatiens* removed more pollen per visit from perfect *C melo* flowers than did *A mellifera* (ANOVA $F_{1, 32} = 6.49$, $p = 0.02$) (**Fig 6**). *A mellifera* did not actually remove negative amounts of pollen, as **Fig 6** seems to suggest; this is an artifact of the methods used for estimating removal by subtracting from a total number of pollen grains estimated from other flowers. Honey bees definitely carried pollen because of their deposition rates (**Fig 5**), so their removal rates were neither zero nor negative, although they were certainly small on average. Pollen removal by *B impatiens* was less from staminate flowers than that from perfect flowers (ANOVA $F_{1, 39} = 5.80$, $p = 0.02$) (**Fig 6**). Staminate flowers also produced less pollen than perfect flowers (unpublished dataset). It was not possible to obtain visits by *A mellifera* to staminate flowers.

*B impatiens* visits to melon flowers were 90% longer than those made by *A mellifera* for flowers on which removal was measured (ANOVA $F_{1, 32} = 4.28$, $p < 0.05$) (**Fig 7**). *B impatiens* visits lasted 88% longer than *A mellifera* visits to flowers on which pollen deposition was measured, but this difference was only marginally significant (ANOVA $F_{1, 21} = 3.64$, $df = 1$, $p = 0.07$). *B impatiens* visits to staminate flowers were significantly shorter than visits to perfect flowers (ANOVA $F_{1, 39} = 5.46$, $df = 1$, $p = 0.03$). Visit duration was weakly positively related to the number of pollen grains deposited by *A mellifera* ($R^2$-adj = 0.19, $n = 12$, $p = 0.09$), but not by *B impatiens* ($R^2$-adj = 0.24, $n = 8$, $p = 0.12$).

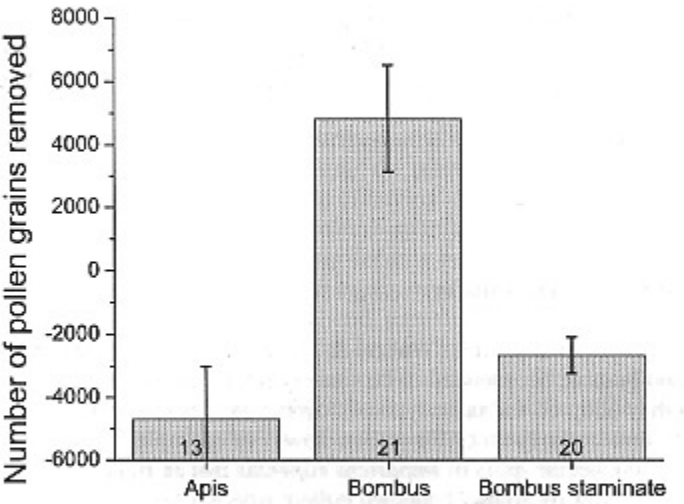


**Fig 6:** Pollen removal from *Cucumis melo* Linnaeus 1753 [Cucurbitaceae] flowers by *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae]. Data are mean numbers of pollen grains removed per flower shown for perfect flowers, and staminate flowers (*B impatiens* only). Error bars represent ± 1 SE; numbers at the base of the bars show sample size.

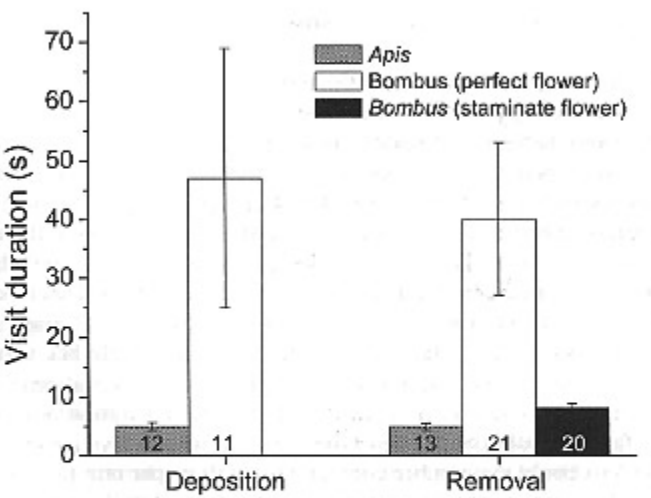


**Fig 7:** Duration of visits to *Cucumis melo* Linnaeus 1753 [Cucurbitaceae] flowers by *Apis mellifera* Linnaeus 1758 [Hymenoptera: Apidae] and *Bombus impatiens* Cresson 1863 [Hymenoptera: Apidae] visitors for experimental flowers on which pollen deposition and removal were measured. Columns indicate means and error bars represent ± 1 SE. Numbers at the base of the bars show sample sizes.

Visit duration did not explain variation in the number of pollen grains removed by *A mellifera* ($R^2$-adj = -0.05, n = 13, p = 0.52), by *B impatiens* from perfect flowers ($R^2$-adj = -0.05, n = 21, p = 0.85), or by *B impatiens* from staminate flowers ($R^2$-adj = -0.03, n = 20, p = 0.49).

## 4 Discussion

### 4.1 Comparative pollen transfer

In other comparative studies [WILSON & THOMSON 1991, THOMSON & GOODELL 2001], honey bees and bumble bees tended to be similar in their propensity to remove and deliver pollen when they adopted the same type of flower visit. Different types of visits, however, produced different consequences for pollen flow. For example, honey bees seemed to be unable to reach the nectar spurs of *Impatiens capensis* Boj ex Baker 1883 [Balsaminaceae] flowers, so they almost invariably collected pollen, whereas the longer-tongued bumble bees almost invariably probed for nectar [WILSON & THOMSON 1991]. In consequence, a honey bee visit removed far more pollen than a bumble bee visit and delivered less. In certain varieties of apples, honey bees may opt to extract nectar laterally through gaps in the ring of filaments, a behavior called 'sideworking'. Sideworking bees seldom touch stigmas and therefore deliver significantly less pollen than 'frontworking' bumble bees [THOMSON & GOODELL 2001]. When honey bees work from the front, however, their deposition does not differ from that of bumble bees. In cashews, native *Centris tarsata* Smith 1874 [Hymenoptera: Apidae] and introduced *A mellifera* remove similar amounts of pollen while nectar collecting at hermaphroditic flowers, but pollen collecting, which was only performed by *C tarsata*, removed on average more than both nectar-collectors [FREITAS & PAXTON 1998].

### 4.2 Pollen removal and deposition in *Brassica*

In *Brassica rapa*, however, a different pattern emerges. These two bees differ sharply in pollen removal when they perform the same behavior of nectar feeding, but not when they perform the same behavior of pollen collecting (**Fig 2**). The longer visits made by *A mellifera* (**Fig 3**) combined with the positive relationship between visit duration and pollen removal for *A mellifera* but not *B impatiens* (**Fig 4**), helps explain the contrasting contributions to pollen removal between these bee species. It is uncertain what the mechanism for this interaction is, but it seems likely that the longer tongue of the bumble bee allows it to extract nectar without bringing its head into contact with the anthers. This mechanism would likely decouple the visit duration from pollen removal for nectar-foraging *B impatiens*. CRESSWELL [1999] reported a similar relationship between bumble bee visit duration and pollen transfer in *Brassica napus*. Despite large differences in the duration of pollen-collecting visits between *A mellifera* and *B impatiens*, they removed similar amounts on average (**Fig 2**). Several factors could contribute to this result. Larger body size and faster working speed of *B impatiens* could mean more contact with anthers per unit time than *A mellifera*. Gradual anther dehiscence could mean that only a fraction of pollen was available, setting a cap on removal.

Whether this difference in pollen removal makes *B impatiens* a 'better' pollinator than *A mellifera* of *B rapa* depends on other variables that have not been measured here, such as pollen carryover patterns.

Results from a comparison of *A mellifera* and *Bombus* spp pollinators of oilseed rape (*Brassica napus* Linnaeus 1753 [Brassicaceae]) indicate similar pollen carryover patterns in which the majority of the pollen removed from a flower is deposited on the next flower visited [Cresswell et al 1995]. Subsequently visited flowers received diminishing proportions of that pollen in a classic leptokurtic distribution. If similar patterns of pollen carryover exist for *A mellifera* and *B impatiens* for *B rapa*, the results here suggest that on average, *B impatiens* will be more efficient in the sense of having a higher deposition: removal ratio. However, the nectar-feeding behavior that gives them this efficiency advantage puts so little pollen into circulation that consistently nectar-feeding bumble bees may not move enough pollen to provide full seed set. If individual *B impatiens* alternate between pollen and nectar feeding within foraging bouts, on the other hand, they may be considerably more advantageous to the plants than the more wasteful honey bees.

The results from emasculated flowers suggest that, at least for nectar-collecting *A mellifera*, approximately 60% of the pollen deposited on a stigma is from the same flower, and will not contribute to seed set (**Fig 1**). Because it was not possible to induce *B impatiens* to visit emasculated flowers, it is not known if they show similar patterns of self-deposition. Furthermore, without the use of pollen dimorphisms [eg Thomson & Thomson 1989], genetic markers [e.g Handel 1982, Cresswell 1994] or pollen analogs [e.g Campbell & Waser 1989], it is not possible to compare geitonogamous pollen transfer of these two species for either visit type. Other components of foraging behavior, however, such as the number of flowers visited per plant influence geitonogamous pollen transfer [Di Pasquale & Jacobi 1998]. Investigation of such easily measured aspects of foraging behavior can offer further insights into the relative quality of bee species to pollination in a self-incompatible plant like *B rapa*. More investigation into the feeding repertoires of individual bees on *B rapa* would be worthwhile.

### 4.3 Pollen removal and deposition in melons

*B impatiens* moved more pollen than *A mellifera* in muskmelons: they removed significantly more and appeared to deposit more, although the deposition difference was not significant for the sample sizes used here. The longer visits (almost an order or magnitude longer) made by *B impatiens* compared to *A mellifera* could contribute toward greater deposition by *B impatiens*. Although visit duration did not significantly explain variation in pollen deposition or removal measures within species, pollen delivery tended to be positively associated with duration. Greater explanatory power would likely result from larger sample sizes. Longer visits by *B impatiens* seem unlikely to explain the large differences in pollen removal, however, because duration of visits was not significantly associated with variation in removal for either species. Pollen removal tended to be greater for *B impatiens* than *A mellifera* even for visits of similar duration. Again, there are no direct observations to explain the mechanism at work here, but it seems likely that the larger body sizes of bumble bees means more contact with the anthers. Melon anthers are small relative to the flower size so that the position the visitor adopts within the corolla may have a large influence on how much contact the bee has with anthers.

In muskmelons, *A mellifera* appear to have a slight advantage in efficiency (measured as the deposition to removal ratio), but over a range of visitation rates, the pollination services provided by the two bee species are likely to be similar. If visits are few, *Bombus* will put more grains in circulation [Thomson & Thomson 1992]. If visits are many, *A mellifera* will ultimately deliver more grains because they will waste fewer. The relative value of these two species in pollinating melons will also depend on the ratio of perfect to staminate flowers produced.

Management techniques such as delaying pollination can boost the relative numbers of perfect flowers [EISCHEN et al 1994]; in this situation, a pollinator that wastes less pollen could provide a bigger advantage. Low pollinator density on melons grown under cover, often in winter, presents just the circumstance in which *Bombus* is predicted to outperform *Apis* as a pollinator. In an empirical study that compared three species of managed bees pollinating muskmelons INCALCATERRA et al [2003] found that *Bombus terrestris* (Linnaeus 1758), and *Osmia cornuta* (Latreille 1805) resulted in higher yields than *A mellifera*. This result is consistent with predictions for pollen transfer under low pollinator densities derived from the results obtained here.

## 4.4 General conclusions, insights, and limitations

Comparisons of pollen removal and deposition between pollinator species offer a powerful screening tool to determine not only estimates of efficiency of particular pollinators at transferring pollen, but also insights into conditions suitable for use of particular species or combinations of species. These comparisons provide only rough predictions, and can sometimes exaggerate differences between pollinators as measured by yield [FREITAS & PAXTON 1998, CANE & SCHIFFHAUER 2003], but their speed and versatility make them an attractive first approach. They do not require waiting until experimental plants have produced fruits, which takes longer and can be influenced by myriad other factors unrelated to pollination. Nor do they do not require the availability of special markers to track actual gene flow, which limits which plant species can be investigated without committing substantial time and resources to developing markers. Pollen deposition and removal data are also well-developed parameters in theoretical pollination biology. Their further use in applied studies will make those data available for use with mathematical models of the evolution of floral morphology and plant-pollinator interactions [e.g HARDER & THOMSON 1989, THOMSON & THOMSON 1992]

Practical applications of the results obtained from the pollen removal and deposition studies can help direct subsequent research for that system. For example, the slight advantage of *B impatiens* over *A mellifera* in *B rapa* is contingent on *B impatiens* are making both pollen-collecting visits in addition to nectar-collecting visits. Management of bumble bee colonies for particular foraging behaviors might be one way to enhance pollination [PLOWRIGHT et al 1999]. The advantage of *B impatiens* over *A mellifera* in *B rapa* would be likely to increase in situations in which pollen might be limiting, such as in the propagation of male sterile varieties.

In *C melo*, the dynamics of pollen removal by *A mellifera* and *B impatiens* are reversed with *A mellifera* having a slightly higher deposition:removal ratio, driven mainly by its lower removal rates. The consequences of this difference for pollen receipt and ultimately yield are potentially milder than that for *B rapa* because of differences in the floral biology between the two plants. Andromonoecy probably prevents extensive pollen-limitation. For *C melo*, then, comparative pollinator research is most needed for special circumstances such as very low pollinator densities or low ratios of male:hermaphroditic flowers.

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