The pollination potential of free-foraging bumblebee (*Bombus* spp.) males (Hymenoptera: Apidae)

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Abstract – Bumblebee workers are efficient pollinators. However, despite their flower visits and less intense grooming the role of males as pollen vectors is largely unexplored. We compared the quantity and diversity of pollen on the bodies (pollination-active pollen) of free-foraging workers and males of two bumblebee species (*Bombus lapidarius* and *Bombus terrestris*) to assess their pollination potential. In both species, males exhibit worker-like flower constancy, but differ significantly from workers in the predominantly collected pollen types. Mean pollen loads of approximately 10,000 grains/individual suggest that males can contribute to the colony pollination service. Bumblebee males add to the diversity of pollinators, associated to increased crop pollination and facilitate pollen flow in specific ways, worthwhile further investigation.

bumblebee males / pollen loads / colony pollination service / flower constancy

1. INTRODUCTION

Pollination of flowering plants by animal pollinators is an essential ecosystem function (Midgley and Bond 1991; Sargent and Ackerly 2008). Among the diverse suite of flower-visiting insects, bumblebees (*Bombus* spp.) are among the most efficient pollinators in the temperate and cold climate zones (Bingham and Orthner 1998; Goulson, 2003). Numerous studies on pollination efficiency and pollen dispersal illustrate the outstanding pollination potential of bumblebee workers (Campbell 1991; Kwak, et al. 1998), which well exceeds

that of honeybees (*Apis mellifera*) for many wild and managed plants (Westerkamp 1991). In contrast to the vast number of studies conducted on foraging behaviour of bumblebee workers (Heinrich 1976; Pyke 1978; Cartar and Dill 1990; Chittka, et al. 1997; Goulson, et al. 1997; Osborne, et al. 2008; Wolf and Moritz 2008), male bumblebees have been addressed only rarely in this context. Although the patrolling behaviour of bumblebee males was already recognized by Darwin (1886), studies on males focus almost exclusively on reproductive physiology (Alcock, et al. 1978; Eickwort and Ginsberg 1980; Duvoisin, et al. 1999) with few exceptions addressing foraging and flower visitation (Ranta and Lundberg 1981; Bertsch 1984; Ostervik, et al. 2010). Indeed, apart from recent work on two species of orchid (*Ophrys normanii* and *Ophrys chestermanii*), which are pollinated by males of the cuckoo-bumblebee *Bombus* (*Psithyrus*) *vestalis* (Gögler, et al.

2009) and insightful lab-experiments on male *Bombus impatiens* (Ostervik, et al. 2010), we are not aware of any report on the potential impact of free-flying bumblebee males on pollination.

In contrast to workers, males do not return to the nest. While establishing and monitoring their patrol routes to attract receptive queens (Darwin 1886; Haas 1949; Svensson 1979; Alcock, et al. 1978; Eickwort and Ginsberg 1980; Bergman and Bergström 1996; Ayasse, et al. 2001), the males also need to visit flowers to fuel this energetically expensive behaviour (Svensson 1979). Pollen deposited on the males' body while foraging on a flower may be transferred to the next flower visited on the foraging bout. Ostervik, et al. (2010) showed that the prolonged flower handling times of *B. impatiens* males under laboratory conditions increased pollen transfer. The same study also found more frequent between-patch flights of males potentially increased out-crossing (Ostervik, et al. 2010).

Additionally, the population level male flight range has been estimated to exceed the flight range of foraging workers by a factor of at least 1.6 (*B. terrestris* and *Bombus lapidarius*; Wolf, et al. 2012) and up to as much as 17-fold (*B. terrestris*, Kraus, et al. 2009). Although this increase may not directly reflect flower-to-flower movement, the much higher lifetime dispersal rates are still likely to increase the spatial scale of male-mediated pollen flow compared to that of the colony-based workers.

Foraging workers frequently groom their body to collect pollen in their pollen baskets on the tibia (i.e. corbiculae; Thorp 1979). This pollen is lost for pollination (Free and Williams 1972; Thomson 1986) and causes a considerable discrepancy between workers foraging efficiency and their pollination service (Westerkamp 1991). Males, in contrast, lack corbiculae and grooming is less intense (e.g. Thomson and Plowright 1980), therefore, they are often "pollen powdered" after flower visits potentially promoting pollen flow.

Pollen dispersal, however, does not per se imply efficient pollination. Whenever pollen is not transferred to the flower of a conspecific plant, pollination has failed. Foraging workers are preferably attracted to one plant species ("majoring") and display a high level of flower constancy (Heinrich 1979a; Waser 1986; Gegear and Laverty 2005; Raine and Chittka 2005). This short-term fidelity to particular plant species forms the basis of the pollination efficiency of bumblebee workers (e.g. Laloi, et al. 1999, Goulson 2003) and increases the efficiency of foraging and colony provisioning. Bumblebee males, however, do not forage for the colony but exclusively for themselves to obtain energy for mating flights and mating (Svensson 1979; Goulson 2003). The motivation of flower visits may therefore be very different between males and workers.

Here we assess the quantity and quality of pollen loads of bumblebee males in comparison to workers in order to estimate the pollen flow and flower constancy as measures for pollination potential of males of two common bumblebee species (*B. lapidarius* and *B. terrestris*) in relation to their workers. Given the importance of (long-distance) pollen flow for insect-pollinated plants in especially fragmented ecosystems (Sork, et al. 1999; Couvet 2002; Ghazoul 2005; Aguilar, et al. 2008), it is important to investigate the role of bumblebee males as potential pollinators (Thomson and Plowright 1980; Ackerman, et al. 1982).

2. MATERIALS AND METHODS

2.1. Bee sampling

Workers and males of *B. terrestris* and *B. lapidarius* were sampled from June 18th to July 27th, 2007 in the flower-rich urban park "Heide-Süd" in Halle/Saale, Germany (51°29′30 N; 11°56′10 W). Bees were randomly collected from flowers using an insect net. Flower visitation was the only criterion for sampling disregarding potential foraging specializations of individual bees (i.e. pollen or nectar foragers) (Hagbery and Nieh 2012).

After initial species identification in the field, bees were sacrificed with ethyl acetate and the two hind legs were clipped. Both legs and the remaining bodies (hereafter referred as "body") were stored

separately in ethanol (70 %) in two individual Eppendorf tubes until pollen analysis.

All individuals were again microscopically checked for species identity and sex using the identification key of Mauss (1994). Using a calliper, we also measured the length of the extended proboscis (including glossa) of a randomly chosen sub-sample from each sex and species ($n=26$; $n_{total}=$ 104), which is known to correlate with the corolla length of the visited flowers and can cause specific foraging patterns (Inouye 1980).

2.2. Pollen samples

Pollen loads of the bodies (pollination-active pollen) were quantified and qualified from homogenous pollen solutions. Pollen grains were washed off the bodies through vortexing in 3 mL HPLC water–SDS solution (5 %). Pollen grains already washed off in the ethanol storage solution were pelleted by centrifugation (15,000 rpm, 15 min) and added to the pollen suspension to avoid pollen loss. All pollen suspensions were subsequently centrifuged (15,000 rpm, 15 min) and re-suspended in 100 μL HPLC water–SDS solution to obtain a standardized concentration for pollen counts. Though pollen grains occasionally may be damaged from the centrifugation, deformed or fragmented pollen grains were rare throughout all samples.

For high pollen concentrations the pollen solutions were further diluted until reliable pollen counts and type assignments could be done. Pollen loads per bee were microscopically quantified using a Fuchs-Rosenthal counting chamber at ×100 magnification and correcting for the used dilution.

Since we could not identify the individual plant species for every pollen grain with certainty, each grain was assigned to clearly distinguishable categories ("pollen types") based on size, shape and structure. New categories were established for any so far undetected pollen type, such that most pollen grains per sample were assigned to a type. Deformed pollen grains or grain fragments that could not be reliably assigned to any category were excluded from any further analysis. Using Von der Ohe and Von der Ohe (2003) some pollen types could be roughly matched to the typical pollen appearance of some plants flowering in the area: A: *Echium vulgare*, B: *Rubus* spp./*Rosa* spp., D: *Trifolium repens*; E:

Carduus spp./*Cirsium* spp., F: *Trifolium pratense*, H: *Lotus corniculatus* and K: *Leontodon* spp. Other (infrequent) pollen types could not be associated to a forage resource.

2.3. Data analysis and statistics

The composition of an individual bees' pollen load is not only determined by the bees' behaviour and/or morphological characteristics but also by the availability of pollen in the foraging area. Measuring the total amount of pollen available for pollination in a given area is difficult at best. However, the total amount of pollen found on the bodies of bees foraging in a specific area may provide a useful proxy representing those flowers actually visited by bees. It may also reflect the quantity of pollen that is typically deposited during a flower visit on a bee's body and remain there available for pollination during subsequent flower visits.

We, therefore, processed the recorded pollen loads of each species and sex (i.e. four different groups: *B. lapidarius* males, *B. lapidarius* workers, *B. terrestris* males and *B. terrestris* workers) in two different ways. First we used the overall sum of pollen grains of each pollen type within each of the four groups to subsequently ranked these cumulative pollen numbers descending from the most to least frequent pollen type. We used this ranked pollen spectrum as a proxy for the overall pollen availability in the landscape (hereafter referred to as "expected").

Secondly, we individually ranked the pollen types found on each bee, again descending from the most to least frequent pollen types on that specific bee. After the individual ranking we calculated the mean and total number of pollen grains in each rank (hereafter referred to as "observed"). As the most frequently present pollen type can vary from bee to bee, rank sums and means per group are typically composed of a mixture of several pollen types. The pollen type composition of the first rank was used to compare the foraging preferences of males and workers, if any.

In case of non-preferential foraging, i.e. bees visiting flowers at random, we expect the individual pollen load composition (observed) to follow closely the pollen composition of the area (expected). Here the most frequently available pollen type should

consistently dominate the individual pollen loads. Alternatively, preferential foraging is expected to result in marked differences between individual pollen loads and the pollen distribution in the landscape. Also pollen load compositions between individual bees are expected to vary more or less widely rather than uniformly following the expected distribution. To test for preferential flower visitation we, therefore, compared the expected distribution of pollen types to the observed cumulative pollen loads in each group.

All statistical analyses were performed using the statistical software GenStat for Windows (14th Edition). We used a linear mixed model (LMM) fitted by restricted maximum likelihood (REML) suited for highly unbalanced datasets. For comparisons of the pollen loads among species and sexes we used a crossed fixed model (species × sex × pollen type) and a nested random model (bee ID). We also used REML to test whether the individual ranked pollen distribution is reflected by the ranked overall distribution of pollen types using species × sex × ranking mode (i.e. ranked expected vs. ranked observed) as fixed model. Where necessary, parameters were transformed using $\log_{10}(x+1)$ to achieve variance homogeneity and normal distribution.

3. RESULTS

We analysed pollen loads on the body surface (pollination active pollen) of males ($n_{\male\text{lap}}=40$ and $n_{\male\text{terr}}=53$) and workers ($n_{\female\text{lap}}=46$ and $n_{\female\text{terr}}=48$) of *B. lapidarius* ("lap") and *B. terrestris* ("terr"). Overall ten pollen types could be clearly distinguished with six (*B. lapidarius*) and eight pollen types (*B. terrestris*) present on more than 10 % of the individuals (pollen type frequencies per bee ranged 0–95 % (♂)/0–100 % (♀) and 0–92 % (♂)/0–98 % (♀) in *B. lapidarius* and *B. terrestris*, respectively).

3.1. Pollen quantity

The estimated total numbers of pollen grains on the individual bees' bodies significantly varied among pollen types (PT), species (S) and between sexes (SX) ($F^{\text{PT}}_{9,\,1,169.5}=285.23$,

$p<0.001$; $F^{\text{S}}_{1,181.3}=27.35$, $p<0.001$; $F^{\text{SX}}_{1,247.3}=285.2$, $P<0.001$). Overall workers of both species carried significantly more pollen grains on their body surface than the males did (*B. lapidarius*: pollen quantity $(QN)_{\female\text{lap}}=40,784.78\pm3,809.96$ (SE) vs. $QN_{\male\text{lap}}=10,335.00\pm1,561.10$ (SE); *B. terrestris*: $QN_{\female\text{terr}}=57,900.00\pm8,841.71$ (SE) vs. $QN_{\male\text{terr}}=13,049.06\pm2,888.18$ (SE) ($F^{\text{S*SX}}_{1,246.2}=4.01$, $P<0.05$).

3.2. Pollen load composition and flower constancy

In both bumblebee species ranking of the individual pollen loads revealed that one pollen type per individual dominated ("major") the total pollen load composition within each sex (major$_{\male\text{lap}}=67.7$ %±0.02 (SE) vs. major$_{\female\text{lap}}=75.3$ %±0.02 (SE); major$_{\male\text{terr}}=62.3$ %±0.02 (SE) vs. major$_{\female\text{terr}}=72.7$ %±0.03 (SE)) (Figure 1a, b).

Comparing the ranked pollen distribution found on the bees (observed, O) with the approximated pollen spectrum provided by the landscape (expected, E), we found that the pollen counts in the "majoring"—rank (sum of observed rank 1) were significantly higher as compared to the most frequent pollen type in the landscape (sum of expected rank 1; $F^{\text{O-E}}_{1,108.0}=5.75$, $P<0.018$). Likewise, other pollen types were less frequently found than expected from overall pollen availability (Figure 2). This effect was consistent across both species and sex ($F^{\text{S}}_{1,108.0}=1.87$, $P=0.18$; $F^{\text{SX}}_{2,108.0}=1.50$, $P=0.23$; $F^{\text{S*SX*O-E}}_{2,108.0}=0.09$, $P=0.91$).

Analyzing the composition of the "majoring"—rank we found significant differences in the majored pollen types between workers and males ($F^{\text{SX*PT}}_{91,669.5}=10.92$, $P<0.001$; Figure 3a, b). Workers of *B. lapidarius* predominantly majored pollen type A (60.9 % of all individuals) which was only majored by 7.5 % of the males. The males almost equally majored on pollen type B and E (42.5 and 40 % of all individuals), which were only majored by 6.5 % (B) and 19.6 % (E) of the workers. Similarly, workers and males in *B. terrestris*
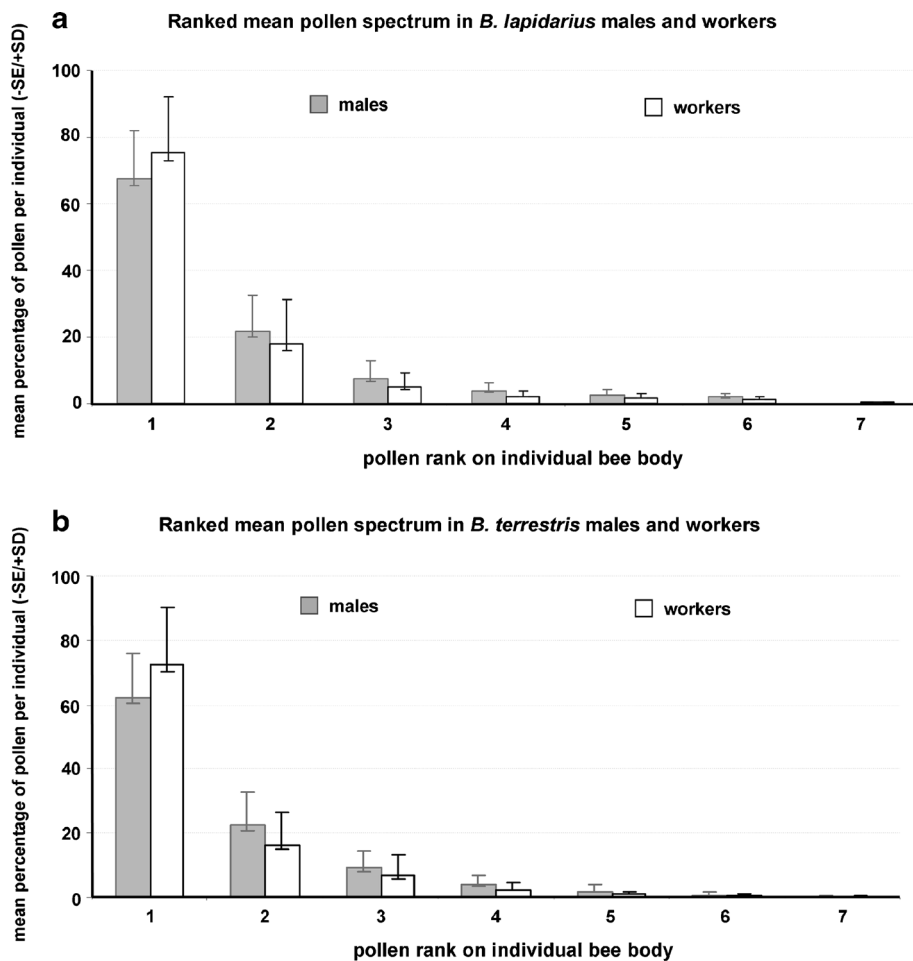
Figure 1. Means of the pollen ranks on the bodies of individual bees (− standard error/+ standard deviation) in males and workers of **a** *B. lapidarius* and **b** *B. terrestris*. Pollen categories were ranked according to their individual frequency. In both sexes one pollen type per individual was overrepresented (>60 %) in comparison to the other types found on the body typical for "majoring" and "minoring".

show a diametrically opposed majoring frequency on pollen types A and E, the two most frequently majored pollen types for both sexes (majorA+E$_{\female terr}$=91.7 %; majorA+E$_{\male terr}$= 88.7 %). Workers mostly majored on pollen type A (64.6 %; males, 15.1 %), whereas 73.6 % of the males majored on pollen type E (workers, 27.1 %; Figure 3a, b).

The proboscis length (PL) were in both species were significantly longer in males than in workers (*B. lapidarius*: PL$_\male$=5.2 mm±0.07 (SE) vs. PL$_\female$=4.7 mm±0.07 (SE); *B. terrestris*: PL$_\male$=5.9 mm±0.07 (SE) vs. PL$_\female$=5.2 mm±

0.12 (SE); $F^S_{1,100.0}$=48.68, P=0.18; $F^{SX}_{1,100.0}$= 44.41, $P<0.001$). *B. terrestris* workers had similar tongue lengths as *B. lapidarius* males ($F^{S*SX}_{1,100.0}$=0.58, $P<0.45$).

4. DISCUSSION

Our results provide the one of the first indications that bumblebee males may well be important pollinators and considerably contribute quantitatively and qualitatively to the colony pollination service. The efficiency of bee-mediated pollen transfer is independent of the
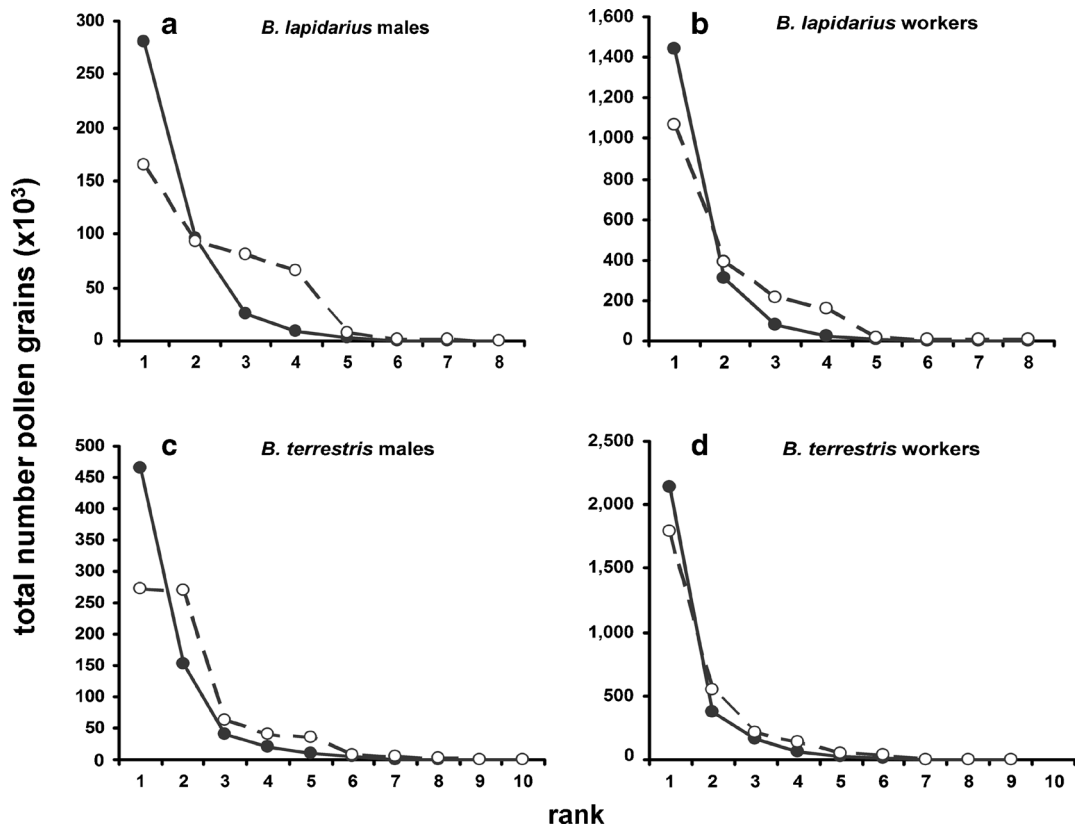
Figure 2. Ranked observed (*filled circles*) versus ranked expected (*open circles*) total numbers of pollen grains per species and sex. In all cases rank 1 of the observed pollen counts ("majored" pollen type per bee) were significantly higher than the most frequently available pollen type in the landscape (expected), whereas individual pollen counts for the lower ranks were consistently underrepresented with respect to potentially available pollen. This indicates preferential flower choice (majoring) independent of pollen availability in the foraging area in both workers and males.

bees foraging specialization (Heinrich 1976). We therefore indiscriminately sampled workers independent of their foraging specialization (nectar, pollen and both; Hagbery and Nieh 2012) as well as flower-visiting males allowing for a comprehensive assessment of pollen flow through bumblebees.

We could show that bumblebee males carry substantial numbers of pollen on their body surface, potentially available for pollination. In contrast to our initial hypothesis, however, males carried less pollen on their bodies than workers did. This is counterintuitive given their weak grooming behaviour in comparison to foragers but might be explained by a lower frequency of flower visits compared to workers.

Workers visit flowers to forage pollen and nectar for the demands in the colony, hence collecting a multiple of their own energy demands (Heinrich 1979b). Males, in contrast, visit flowers only for their own energy demands and typically spend most of the day patrolling (Svensson 1979; Goulson 2003). They preferentially forage early in the morning and late in the afternoon (Svensson 1979; Alcock, et al. 1978; Eickwort and Ginsberg 1980), which may result in fewer flower visits per day than for foragers. Nevertheless, a mean of over 10,000 pollen grains per individual male should be sufficient to facilitate pollination in most plants. Carré, et al. (1994) examined pollen numbers on head and thorax of *B. terrestris* workers finding

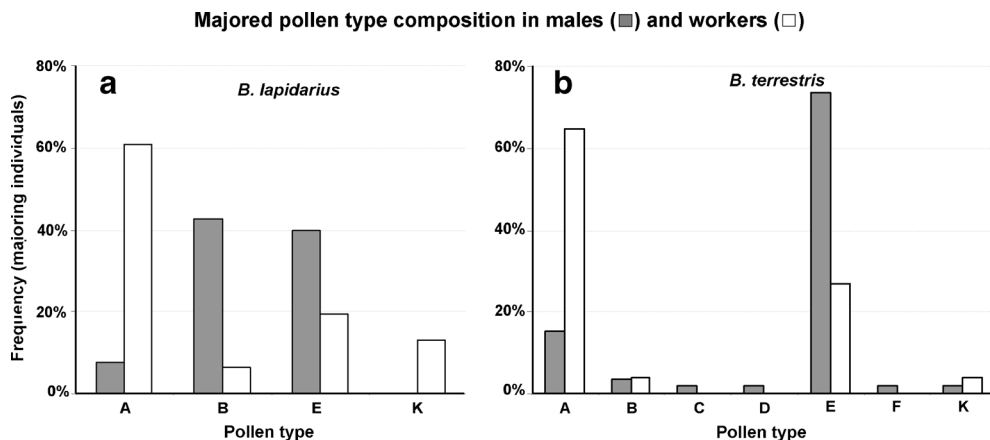**Majored pollen type composition in males (■) and workers (□)**

Figure 3. Pollen type composition of the 1. rank (most abundant pollen per individual) per sex in **a** *B. lapidarius* and **b** *B. terrestris*. There is a highly significant difference in pollen type representation between sexes in both species indicating that males may complement the pollination potential of workers broadening a colonies overall pollination service.

only 1,620±300 to 6,300±400 grains after ten visits of *Vicia faba* flowers. In other pollinator–plant systems even lower numbers of loose pollen grains per pollinator seem to suffice for pollination (*Bombus*–*Pontederia*, 28.5–74.5; *Apis*–*Pontederia*, 14.0–49.1; *Melissodes*–*Pontederia*; 30.4–52.0 (Wolfe and Barrett 1989); specialist bees–*Clarkia*, 335±73; generalist bees–*Clarkia*, 81±29 (Moeller 2005)).

Our results strongly indicate that males of both species did not visit flowers at random but displayed the typical "majoring" and "minoring" described for workers (e.g. Heinrich 1979a). Though based on pollen types, which may not necessarily represent a flower species, our approach provided sufficient resolution to clearly demonstrate the well-known flower constancy in workers. From this, methodological constraints artificially causing the demonstrated effects in males but not in workers are highly unlikely. Also, our results are in line with reports on selective, female-like foraging behaviour of male orchid bees (Ackerman, et al. 1982).

Finding preferential foraging in both workers and males is not surprising, since the visitation of rewarding flowers should be highly adaptive for both sexes as it optimizes the net energy gain per time unit (Bertsch 1984; Real 1991;

Waddington 2001), reduces the risks of predation (Cartar 1991; Dukas 2001; Abbott 2006) and infection (Durrer and Schmid-Hempel 1994).

The ability to learn and memorize reward-linked floral traits (e.g. Menzel 2001; Raine and Chittka 2007; Raine and Chittka 2008) and its efficient exploitation (e.g. Lihoreau, et al. 2011; 2012) has been impressively demonstrated in bumblebee workers (Laloi, et al. 1999). Although the cognitive abilities of bumblebee males remain to be studied our results suggest that the cognitive mechanisms driving flower choice may be very similar in males and workers. Indeed, the learning capacity of honeybee (*A. mellifera*) drones (Benatar, et al. 1995) was shown to be similar to that of workers despite drones, being fed by workers in the colony, are not foraging on flowers.

Alcock, et al. (1978) pointed out, males of several Apoidea species are attracted by flowers that are also highly attractive to females (including gynes) and visits to particular flowers may enhance their chances to encounter a mate by visiting these flowers. However, with an elaborate and costly pre-mating behaviour in form of patrolling (Alcock, et al. 1978; Eickwort and Ginsberg 1980) it is unlikely that flowers are chosen to increase queen encounter rates.

A more likely factor driving the difference in the pollen spectra of males and workers may be the differences in nutritional demands. Whereas workers are attracted by flowers providing either nectar or pollen or both (e.g. Waddington 2001), it would be adaptive for males to only prefer rich nectar providing flowers, since they do not feed on pollen (Ranta and Lundberg 1981). Though flower rewards were not measured, this difference may well be the driving force behind our findings, where males obviously preferred different flowers than workers as inferred from the pollen spectra differing significantly between both sexes.

Proboscis lengths differences have repeatedly shown to affect the exploitation of tube-shaped flowers (i.e. Red Clover) both between bumblebee species (e.g. Inouye 1980) and even within a colony (tongue length correlating with worker body size: Harder 1985; size-specific foraging performance: Spaethe and Weidenmüller 2002). In our dataset proboscis length of *B. lapidarius* males and *B. terrestris* workers were very similar. If tongue-length alone would determine flower choice, one should expect overlapping pollen compositions of these two groups. This is not what we found. In an area rich in easily exploited flowers, proboscis length is likely to be only of minor importance.

The pollen dispersal by male bumblebees might resemble that of other freely dispersing pollinators like butterflies, moth or hoverflies. Like adult bumblebee males, they lead a solitary life and mostly visit flowers for nectar and consume no or only little pollen (Kevan and Baker 1983). Detailed information about pollen flow through non-bee pollinators is limited (Courtney, et al. 1982; Nilsson, et al. 1987; Chase, et al. 1996) but the importance of these pollinators has repeatedly been emphasized (Weiss, 2001; Biesmeijer, et al. 2006, Garibaldi, et al. 2013). In fact, Herrera (1987) found pollinator effectiveness of some Lepidopteran pollinators higher than that of honeybees and surpassed only by a number of solitary bee species. Butterflies also facilitated more outcrossing on *Lavandula latifolia* as compared to bee pollinators due to longer inter-flower flights (Herrera 1987). Some studies also emphasize the potential importance of butterflies and moth for long-distance pollination, especially if pollen remains on the insect over a long period of time (Courtney, et al. 1982; Chase, et al. 1996). Further studies assessing the time pollen remains available for pollination on bumblebee males' bodies over time and the inter-floral movement patterns of foraging bumblebee males during this time will be able to conclusively address the spatial scale and effectiveness of pollen dispersal through male bumblebees.

In conclusion, our data suggests that bumblebee males are behaviourally and morphologically well equipped to substantially contribute to pollination. In light of the extended population-level flight ranges and different flower choices compared to workers, they may well complement the pollination activities of the workers and hence expand the pollination service of the bumblebee colony as a whole. An increase in crop pollination efficiency with increased wild bee diversity as compared to managed pollinators alone (Klein, et al. 2003; Garibaldi, et al. 2013) strikingly illustrates the importance of a (behaviourally) diverse pollinator assemblage for ecosystem function. Bumblebee males certainly add to the diversity of flower visitors and contribute in specific ways to insect-mediated pollen flow.

Given these promising findings and the numerous still open questions it is clearly most timely to invest more research on the role of male bees as pollinators and sex-specific aspects of pollination efficiency in general.

ACKNOWLEDGMENTS

Le potentiel de pollinisation des bourdons mâles, *Bombus* **spp. (Hymenoptera: Apidae) butinant pour eux-mêmes**

Bourdon / mâle / charge pollinique / rôle pollinisateur d'une colonie/fidélité florale

Das Bestäubungspotential von freifliegenden Hummelmännchen (*Bombus* spp., Hymenoptera: Apidae)

Hummelmännchen / Pollenladungen / Bestäubungsservice / Blütenstetigkeit

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