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Pollen deposition by *Bombus terrestris* L, between male-fertile and male-sterile plants in *Vicia faba* L

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Summary — We have studied pollen transfer by *Bombus terrestris* from a male-fertile line (D-27) to a male-sterile line (INRA Ad 23) of *Vicia faba*. Studies on visitation sequences were conducted with single workers visiting caged plants. The average pollen production was 24 200 ± 2 000 grains per flower, and the pollen carried on the head and thorax of a worker after visiting 10 male-fertile flowers ranged between 1 620 ± 300 and 6 300 ± 400 grains. Of 21 workers, only 11 foraged on male-sterile flowers. The total amount of pollen deposited on stigmas by a single worker was on average 55 grains. A total of 67% visited flowers received no grains at all on the stigma. The size of non-null stigma loads ranged from 1 to 152 grains, but frequency of small deposits (1–10 grains) was high (78%). Runs varied greatly in visitation sequence length and pollen deposition patterns on successively visited flowers, which were not always monotonically decreasing. Statistical analysis by Monte-Carlo tests has provided a good method for studying sequences.

***Bombus terrestris* / *Vicia faba* / pollination / pollen transfer / Monte-carlo simulation test**

INTRODUCTION

In entomophilous plants, a successful use of male-sterility to produce hybrid seeds depends on the effective transfer of pollen from the hermaphrodite male-fertile (MF) line to the male-sterile (MS) line. The lack of pollen in the MS flowers is often associated with unfavourable traits, such as reduced nectar production (Mesquida and Renard, 1978), altered aroma production (Erickson and Peterson, 1979; Erickson *et al*, 1979) or modified floral morphology (Shroff, 1980). These factors, which affect the foraging behaviour and pollen transfer by pollinators in commercial seed production, have to be studied and integrated at the start of the selection process (Erickson, 1983). Despite the large amount of research following the discovery of 2 kinds of cytoplasmic inherited male-sterility (CMS) in the faba bean

Vicia faba L, 'PBI 447' in 1956 and 'REN 350' in 1967 (Berthelem and Le Guen, 1974), commercial production of F1 hybrids is not yet possible. A better understanding of CMS could lead to an economical programme towards F1 hybrid seeds (Bond, 1989). The main problem is that male-sterility is phenotypically unstable (Le Guen and Berthelem, 1983). The availability of a spring genotype (line INRA Ad 23) submitted in 1991 as female parent in a 3-way hybrid allowed the first detailed investigations of pollen transfer (Le Guen and Duc, 1992). Much of faba bean pollination depends on wild vectors (Stoddard and Bond, 1987). Pollen deposition on MS flowers is permitted by the movement between lines of long-tongued bumble bees and solitary bees (Taséi, 1976). According to the same author, *Bombus terrestris* L bites through the corolla for nectar and does not pollinate the flowers in 90–95% of instances (called 'negative visits'). In controlled conditions in the glasshouse, workers of *B terrestris* could effect frontal visits on *V faba* flowers for pollen collecting (Carré *et al,* 1993). The current availability of *B terrestris* colonies made this species convenient for a first pollination experiment.

Studies on sequential visits of pollinators were conducted essentially in natural populations to examine plant/pollinator coevolution more closely (Price and Waser, 1982; Waser and Price, 1983; Thomson, 1986; Campbell, 1991). Some authors have analyzed the sequences by the mean of linear (Price and Waser, 1982) or exponential (Lertzman and Gass, 1983; Campbell, 1985; Thomson, 1986) regression, or methods based upon autocorrelation indices (Thomson, 1986).

In this work, we devised a standard method to study sequences of pollen carryover and pollen deposition on MS flowers by individual bumble bees. This was conducted under cages maintained in a glasshouse. We used Monte-Carlo procedures for the statistical analyses of the sequences. These methods were first introduced by Barnard (1963) in a general context, and applied more recently to ecological data, especially to spatial pattern data analysis (Besag and Diggle, 1977; Vaillant and Badenhausser, 1989). Such procedures are based upon the simulations of the hypothesis to be tested. They can perform valid tests for a large variety of hypotheses because the knowledge of the exact distribution function of the statistic is not needed.

MATERIALS AND METHODS

Plants

The pollen donor line D-27 was a self-fertile spring line belonging to the *equina* group; the recipient line INRA Ad 23 was a spring genotype of the *equina* group with stable CMS. The plants were grown individually during the spring of 1990 in 4-l pots, in insect-proof conditions. Fully opened flowers in which the standard was reflexed (Kambal et al, 1976) were used for the pollination trials.

Insect pollinators

B terrestris colonies were reared in glasshouse with plants of *Lupinus, Medicago, Melilotus* spp and supplied with powdered pollen. Previous analysis of the powdered pollen has shown that it was free of *V faba* pollen. In addition, as other *Fabacae* pollens are quite different in size and morphology from *V faba*, no confusion was possible when transferred pollen was counted. Pollen-collecting workers were caught on available flowers, isolated in plastic screen tubes and kept in the dark for 30 min, until the introduction into the first pollination cage.

Experimental device

The experimental device was made up of 6 nylon mesh cages (0.60 x 0.50 x 1.50 m) joined side

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by side and separated from each other by a slid-
ing Plexiglas plate. The cages were set up in an
insect-proof greenhouse. A pollen donor MF plant
was introduced in the first cage, while next cages
contained one MS plant each. Pollination trials
were performed between 9.00 h GMT and 14.00 h
GMT. In front of each cage there was a nylon
screen, which was removable by the mean of
Velcro strips and allowed handling of the plants
and bumble bees. For each run, the tube con-
taining one worker was opened in front of the
lowest node of MF plant in the first cage. Gen-
erally, *B terrestris* workers moved upward on ver-
tical inflorescence so that multiple visits could be
avoided without manual intervention. When a
worker had visited 10 MF flowers, the sliding plate
was taken off and the worker was induced to
move to the 2nd cage to continue the foraging
sequence on the MS flowers. Unless the worker
stopped foraging, cage changes were made suc-
cessively up to the 5th MS plant. After each posi-
tive visit of a flower, marks of acrylic paint were
applied on standard petal according to a sequen-
tial code. The ovary and style were cut out from
each coded flower and frozen at –20°C in a micro-
centrifuge tube until further examination. Among
the 21 workers that foraged on MF flowers, 11
foraged on MS flowers. The total sequence dura-
tion varied between 4 and 20 min and duration
of a visit on one flower was quite similar to that
observed by Taséi (1976).

Pollen quantification

Pollen production of the MF line D-27

Pollen production of D-27 flowers was estimated
for 3 flowering nodes: node 5; node 10; and node
15. Two flowers per node were sampled on 5
plants. The pollen production of anthers from
pointed buds (stage just before anthesis) was
estimated according to the method adapted from
Kambal *et al* (1976). To get a homogeneous sus-
pension, anthers were put in a microcentrifuge
tube containing 0.5 ml H_2O with Triton 1% W/V
and agar 0.1% W/V. After stiring with a vortex
mixer for 60 s, 3 subsamples of 150 µl each were
immediately deposed in a Nageotte haemo-
cytometer. Counts were realized under a micro-
scope (x 400) in 10 cells defined through an ocu-
lar grid, each cell volume being 1.4 µl.

Pollen carried by bumble bees

For each trial, after visiting 10 MF flowers of line
D-27, *B terrestris* workers were immediately cap-
tured in small tubes containing ethylacetate. The
quantification of pollen carryover was limited to
that of the head and thorax which are the parts of
the body in most contact with the stigmas, espe-
cially the ventral surface of the head (proboscidial
fossa) according to Spencer-Booth (1965) or ven-
tral face of thorax (Kubisova and Haslbachova,
1990). These parts were washed 3 times in a
microcentrifuge tube, each wash containing 1 ml
H_2O with Triton 1% W/V with a vortex mixer for
60 s. Preliminary trials have shown that 95% of
total pollen on these body parts were removed
after 3 washings. After centrifugation at 15 000
rpm for 5 min, the supernatant was removed and
pollen was stirred as in the previous paragraph.
Counts were made under a microscope (x 400) in
all cells of a Nageotte haemocytometer.

Pollen deposition on stigma of MS plants

For each coded flower, the style was cut at the
distal end of the ovary. The brush of long hairs
just below the stigma and the stigma were stained
separately with Alexander's stain (1969) which
allows identification of the origin of pollen grains;
D-27 pollen grains with red-stained cytoplasm
were easily recognizable from green-stained
aborted pollen of MS line. The samples of anthers
from Ad-23 MS plants contained only aborted
pollen. Fertile grains adhering to each stigma or
brush were counted under a microscope (x 400).

Statistical analysis by simple Monte-Carlo tests

A precise description and discussion on the prop-
erties of Monte-Carlo tests can be found in Besag
and Diggle (1977). Let a simple null hypothesis $\mathcal{H}_0$
be tested by means of a set of data that gives a
calculated value u_1 of a statistic u. If this hypoth-
esis is simulated $s - 1$ times, we have a random
sample of u from the null distribution: $u_2, ..., u_s$. If
we order these statistics, we can use the rank of
u_1, say $r(u_1)$, the calculated value of u from the
data, to construct an exact test of $\mathcal{H}_0$. If it is a 1-
tailed significance test of size α, level of signifi-
cance will be $s\alpha$, while if it is a 2-tailed test it will

be $s\alpha/2$. The type I error is α and the power of the test is good if s is large enough, *ie* $s\alpha \geq 5$ (Hope, 1968). No distributional theory is needed. Only the hypothesis to be tested has to be defined in order to choose the right criteria and to simulate.

The hypotheses we have tested on pollen deposition sequences were:

– $\mathcal{H}_1$, is the hypothesis that pollen grains were distributed independently from one flower to the next. If we have a set of count data $\{x_1, x_2, ..., x_n\}$ consisting of number of pollen grains deposited on n sequentially visited flowers, we simulated $\mathcal{H}_1$ by the random permutation of the data: a possible set would have been $\{x_4, x_7, ..., x_n\}$. For each simulation under $\mathcal{H}_1$ and for the observed data, we calculated the correlation between x_i and x_j where $j = i + 1$ was the flower visit order. We did not need to know the exact distribution of this statistic in the context of Monte-Carlo test.

– $\mathcal{H}_2$ is the hypothesis of independence between the number of grains deposited per flower and the order of visit. The simulation was the same, but the criterion calculated was the correlation ρ_2 between the number of pollen grains and the flower sequence number.

– In a similar way, we tested the hypothesis $\mathcal{H}_0$ of pure random distribution of the total number of pollen grains in the n flowers:

$$N = \sum_{i=1}^{n} x_i$$

This hypothesis was simulated by the redistribution of the N grains independently of each other in n flowers. This hypothesis tests if grains are deposited in aggregates or regularly or at random. The statistic chosen was the index of dispersion (I_D = variance/mean) (Pielou, 1969) which tests $\mathcal{H}_0$ against regularity or against aggregation.

We have developed programs for Monte-Carlo procedures using the Splus language (Becker *et al*, 1988), which run under the UNIX operating system (Sun 4/75). The number of simulations made was related to the computational effort: $s = 100$ for $\mathcal{H}_0$ and $s = 199$ for hypotheses $\mathcal{H}_1$ and $\mathcal{H}_2$. We applied a 2-tailed test of the hypothesis at level $\alpha = 0.05$; the hypothesis was rejected against its alternatives if u_1 was among the 5 lowest indices or the 5 largest indices when $s = 199$, and among the 3 lowest indices or the 3 largest indices when $s = 100$.

RESULTS

Pollen production and pollen carryover

Pollen production per flower of line D-27 was 23 600 ± 1 300 (mean ± SE), 22 500 ± 1 000 and 26 400 ± 1 500 respectively for nodes 5, 10 and 15. After a visitation sequence of 10 flowers on D-27 plant, pollen carried on the head and thorax of *B terrestris* workers ranged between 1 620 ± 300 and 6 300 ± 400 grains (mean ± SE = 3 150 ± 430). The residual pollen grains carried in the hair coat of foragers after visiting 10 flowers of the MF line represented only 1.3% of the pollen production of the visited flowers.

Variation in pollen grain deposition during visitation sequences

Description

A great variation between runs was observed (table I); the mean number of visited flowers with or without pollen was 18, and sequences ranged between 2 and 51 flowers. The total number of grains deposited per run on successive stigmas averaged 55, which represented only 1.7% of the total amount of pollen pool on the insect head and thorax. The whole deposit on the pistil (stigma and brush) represented 5% of the total (table I). The minimum total deposit per run was 8 and the maximum was 240. The high maximum was due to a single deposit of 152 grains on the second visited flower. Visited flowers that received at least 1 grain on their stigma represented only 33% and the number of grains deposited on a stigma averaged only 3 grains. Compared with these figures, the density of aborted grains from MS line Ad23 ranging between 100 and 500 was very high. The frequency distribution of the size of non-null deposit on stigmas is presented

Table I. Descriptive statistics of pollen deposit from 11 runs.

| Variable	Minimum	Maximum	Mean	SE
No flowers visited	2	51	18	18
Stigma				
No grains deposited per run	8	240	55	66
No grains deposited per flower	0	152	3	11
No grains per non-null deposit	1	152	9	19
Stigma and brush				
No grains deposited per run	27	595	156	170
No grains deposited per flower	0	340	8	27
No grains per non-null deposit	1	340	14	35

The figures were rounded in view of the SE values.

in figure 1, which shows that deposition of a single grain was a frequent event and that variation of pollination intensity was great. Such characteristics are available for the total number of grains deposited on both the stigma and the whole pistil (stigmatic surface and brush of stylar hairs) (table I). Further analyses were only made on grains deposited on stigmas because this repre-sents the effective site of pollen deposition for fertilization.

Pollen deposition and its relation to visitation order

Pollen deposition was studied by the test of the hypothesis $\mathcal{H}_1$ of independence between the number of grains deposited on
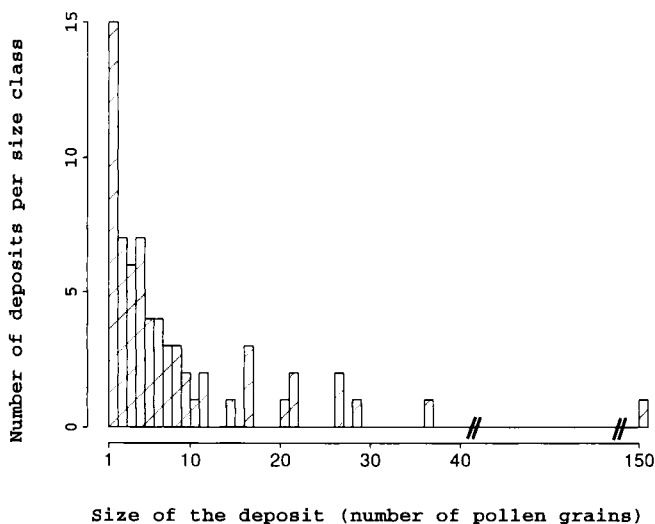
Fig 1. Frequency distribution of pollen grains on stigmas.

a stigma and the number deposited on the next visited stigma. A 2-tailed test was applied for the correlation criterion ρ_1; if it was among the 5 lowest indices, the correlation was negative, ie there was an alternation of high and low number of grains in successively visited flowers, which was higher than it would have been if grains were deposited independently of the visitation order. Alternatively, when the indice was among the 5 largest indices, successive flowers were correlated (positively or negatively).

The test of the hypothesis $\mathcal{H}_2$ gave complementary information. It tested whether the number of grains deposited on a stigma was a function of flower position. A correlation value ρ_2 among the 5 lowest indices meant that there was a significant decrease in grain numbers from one flower to the next, and a value among the 5 largest indices revealed that deposit increased from one flower to the next.

The results in table II give the order of the statistics ρ_1, ρ_2 for $s = 199$ simulations. These are for runs for which the number of visited flowers was at least 9 flowers.

Variability was important from one run to another. Independence between deposits on each successively visited flower occurred in half of the cases as shown by the tests which were not significant for runs 2, 7, 10. Independence between pollen deposits and visitation order was also observed for run 5 but it was associated with regularity between 2 successive flowers: each deposit was followed by a null deposit. A decrease of grain deposit was observed for the 3 other runs (runs 1, 3, 4). The results for 6 of the runs are illustrated in figure 2, which also indicates that the first flower generally did not receive any pollen grain.

Pollen grains distribution per visitation

The hypothesis $\mathcal{H}_0$ is more restrictive than the previous ones and tested the random
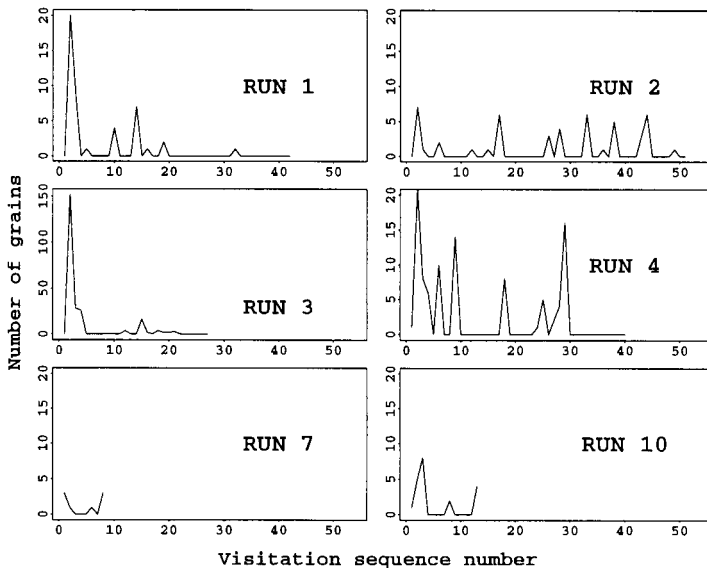

Fig 2. Description of pollen deposition on stigmas for 6 runs; relation between size of pollen deposit and visitation order.

Table II. Tests on pollen deposition sequences, ranks of the Monte-Carlo test criterion for hypothesis $\mathcal{H}_1$ and $\mathcal{H}_2$ (s = 199 simulations), and for hypothesis $\mathcal{H}_0$ (s = 100 simulations).

Run	$\mathcal{H}_1: \rho_1 =$ $cov(x_i,x_j)$	$\mathcal{H}_2: \rho_2 =$ $cov(x_i,i)$	$\mathcal{H}_0: I_D$
1	192	1*	101*
2	61	107	101*
3	198*	1*	101*
4	155	2*	101*
5	1*	51	101*
7	128	84	77
10	182	41	101*

* Significant rank at 5% level.

distribution of pollen grains on stigma against regularity or aggregation. The runs were the same as above. The results are presented in table II; in most cases, pollen was not distributed at random but in aggregates. Visited flowers received deposits that were more different than if the grains had been deposited at random. A random distribution of pollen grains was observed for only one run (run 7), which carried a very low number of grains.

DISCUSSION

We noticed that compared with pollen availability, the pool of pollen carried on the body of bumble bees was small. Similarly, the quantity of pollen deposited on stigma was small compared with the pool of pollen on the body of bumble bees. The lines we had previously observed revealed a great variability in pollen production as was also shown by Kambal *et al* (1976) with lines that were much less productive (from 1 300 to 2 800 grains per flower) than ours. The first type of loss was discussed by Young

and Stanton (1990) on wild radish for which removed pollen per visit represented 52% of the available quantity and was related to the accessibility of pollen in flowers. Harder (1990) has shown that in lupin, whose flower morphology is quite similar to *V faba*, this type of loss represents only 19%. In addition, in *V faba* some of the sticky pollen contained within a pocket in the keel petal frequently fell during the foraging activity of the bumble bee, as reported by the same author in *Erythronium americanum* where this 'passive loss' was, on average, 14% of available pollen. In addition, pollination by pollen foragers may be less efficient than by nectar foragers, as demonstrated in cotton, for which changes of flowers, plants, rows and lines occurred more frequently in nectar collecting bees (Vaissière, 1991). The second route to pollen loss could be explained by the intensity and frequency of grooming for corbicular packing, named 'active loss' by Harder and Thomson (1989). For *Apis mellifera* less than 0.007% of the pollen harvested by a forager gets lodged in 'safe site' where it cannot be easily groomed off during transport (Buchmann *et al*, 1990).

The main result concerning pollen deposition during visitation sequences was the great variability between runs in terms of visitation sequence length. This reveals an important stochastic component in pollen transfer from preferential sites on the bumble bee body to the stigma. Grooming has been implicated in emergence and deposition of grains and can influence the extent of pollen transport (Thomson, 1986). Effects of variance in pollen deposition have been explored by Galen and Rotenberry (1988) in natural populations. Compared with similar experiments between fertile lines of *V faba*, the sizes of sequences were shorter and visitation often stopped after unsuccessful visits of the first MS flower. In addition to the attractiveness of the MS line (nectar and pollen), mechanical resistance to tripping

could affect the visitation of MS flowers, especially those which have not yet been visited, as reported by Taséi (1976). Genotypic differences were also mentioned by Kreitner and Sorenson (1985), who suggested that both cell turgidity and morphology of the intermeshing ridges on the keel petals control tripping in *Medicago sativa*. A learning component for workers without previous foraging experience (Laverty, 1980) could also explain the frequency of short sequences and small deposits on the first visited flower of MS line.

The statistical method we have presented and adapted from spatial data analysis has provided useful results in situations where classical tests could not have been used. For example, the distributional function of I_D is constrained to approximations that are valid only when counts or means are greater than a given value (Kathirgamatamby, 1953). In addition, Monte-Carlo tests allow the testing of well-identified hypothesis which can be complex. The only necessary condition is that the hypothesis can be simulated. In quantitative behaviour analysis, Monte-Carlo tests should be of great interest because samples are generally too small for classical parametric and non-parametric tests, and because hypotheses are not simple. The only problem is the high computational effort due to simulations.

The relationship between pollination intensity and seed production is not well known for MS line of *V faba*. A pollen response curve exists for cotton (Vaissière, 1991), but this is not frequent in other hybrid seed production. Mesquida and Renard (1983) have shown that the fertilization of *Brassica napus* L is related to pollen quantity, which must be greater than ovule number. In our experiment, the size of deposit generally varied from 1 to 5 grains and could result in a lack or absence of seed set. Moreover, seed production depends not only on the quantity of pollen reaching of stigma but also on pollen 'quality' (Waser, 1983) defined in term of success during 'post-pollination' events, such as rate of germination, ovule fertilization and seed maturation (Waser and Price, 1991). Viability of deposited pollen grains was not considered here and could be reduced by the cleaning and pollen-packing behaviour of pollinators (Parker and Hatley, 1979). According to Mesquida (personal communication) the germination rate of *B napus* pollen may be reduced by 20–45% just after its collection by honey bees. Further experiments will take into account the possible beneficial effect of multiple visits which could increase the size of pollen deposit, as reported by Young and Stanton (1990) on wild radish. Moreover, alternative behaviour between MF and MS lines is a key factor in pollen deposition in hybrid seed production in *V faba*. Taséi (1976) demonstrated that this behaviour varied between bee species. In this case, changes of lines could have been over-estimated because of the rate of fertility restoration (7–20%) of the MS line this author used. Other experiments on long-tongued bee species have to be conducted in our standard conditions to determine their potential efficiency in pollen transfer and compare results in the field and in enclosures.

ACKNOWLEDGMENT

Résumé — Dépôt de pollen par *Bombus terrestris* L, d'une lignée mâle fertile à une lignée mâle stérile de *Vicia faba*. L'étude portait sur le transfert de pollen et plus particulièrement sur les séquences de dépôts de pollen par *Bombus terrestris*, d'une lignée mâle fertile (D-27) à une lignée mâle stérile (INRA Ad 23) de *Vicia faba*. Le

dispositif expérimental constitué de plantes sous des cages permettait de contrôler, pour chaque ouvrière, le nombre de fleurs fertiles visitées et de noter l'ordre de visite des fleurs stériles. Le nombre de grains de pollen fertile était évalué après coloration d'Alexander. Les analyses statistiques réalisées par des procédures de Monte-Carlo ont montré leur intérêt pour l'étude des problèmes d'autocorrélation entre fleurs et de distribution du pollen. Ces procédures initialement employées pour l'étude des structures spatiales sont basées sur la simulation de l'hypothèse à tester. Elles pourraient présenter un grand intérêt pour l'analyse quantitative de données comportementales, car elles ne reposent pas sur une approche distributionnelle des tests. La production de grains par fleur de la lignée fertile était comprise entre 22 500 et 26 400 grains selon l'étage floral. La quantité de pollen transporté sur la tête et le thorax d'une ouvrière après la visite de 10 de ces fleurs variait entre 1 600 et 6 300 grains. Seules 11 ouvrières sur 21 ont accepté de visiter les fleurs stériles, ce qui pourrait s'expliquer par leur résistance mécanique au déclenchement et par l'absence d'apprentissage des ouvrières. Ceci est confirmé par le nombre élevé de petites séquences de butinage et par les faibles dépôts sur la première fleur stérile rencontrée (fig 2). La quantité de pollen déposé sur le stigmate des fleurs visitées par une ouvrière était faible (de 8 à 240 grains) (tableau I) comparée à la production des fleurs et au pollen transporté. Ceci pourrait être dû à la chute de paquets de grains pendant la récolte de pollen, à l'accessibilité du pollen dans la fleur, à l'intensité et à la fréquence du brossage pendant le butinage. Aucun grain de pollen n'a été déposé sur le stigmate de 67% des fleurs visitées. Quant aux dépôts non nuls, leur taille a varié de 1 à 152 grains (tableau I) et 78% de ces dépôts comportaient entre 1 et 10 grains (fig 1). La distribution du nombre de grains par dépôt a été de type agrégatif (tableau II)

et la surdispersion maximale entre 2 fleurs visitées consécutivement. La taille des séquences de butinage (de 2 à 51 fleurs), ainsi que la distribution des grains selon l'ordre de visite étaient très variables d'une ouvrière à l'autre (fig 2). Dans la moitié des cas, la taille des dépôts était indépendante de l'ordre de visite (tableau II). Pour les autres séquences, elle diminuait en fonction de l'ordre de visite et un phénomène de régularité était souvent observé aux petites échelles. Bien qu'on ne connaisse pas la relation entre la quantité de pollen déposé et la production de graines chez *V faba*, il est probable que de si faibles dépôts conduisent à des problèmes de fécondation. L'effet bénéfique de visites multiples est possible mais le comportement d'alternance des ouvrières entre les lignées fertile et stérile est également à considérer.

Bombus terrestris / Vicia faba / pollinisation / transfert pollen / test simulation Monte-Carlo

Zusammenfassung — Pollenübertragung zwischen fertilen und männlichen Pflanzen der Pferdebohne (Vicia faba) durch Hummeln (Bombus terrestris). Es wurde die Pollenübertragung von einer männlich-fertilen Sorte (D-27) von *Vicia faba* auf eine männlich-sterile Sorte (INRA Ad 23) durch *Bombus terrestris* in Form der Sequenz der Pollenablagerung auf nacheinander folgende Blütenbesuche untersucht. Die Versuchsanordnung bestand aus Pflanzen in Käfigen, bei denen die Zahl der von jeder Arbeitsbiene besuchten männlich-fertilen Blüten und die Reihenfolge der von männlich-sterilen Blüten kontrolliert und vermerkt werden konnten. Die Pollenkörner der Linie D-27 wurden nach Färbung entsprechend dem Verfahren nach Alexander in einem Hämozytometer gezählt. Die statistische Analyse nach der Methode der Monte-Carlo-Simulation erwies sich als gutes Verfahren

zur Prüfung der Hypothese einer Autokorrelation zwischen den Pollenablagerungen in einer Sequenz. Diese Verfahren, angepaßt nach Analysen der Daten von räumlichen Mustern, beruhen auf der Simulation der zu prüfenden Hypothese (welche komplex sein kann). Sie verzichten auf eine komplizierte Verteilungstheorie und sollten für quantitative Analysen des Verhaltens von Interesse sein. Die mittlere Pollenproduktion der männlich-fertilen Linie schwankte je nach Lage im Blütenstand zwischen 22500 ± 1000 und 26400 ± 1500 Körner. Nach dem Besuch von 10 männlich-fertilen Blüten wurden an Kopf und Thorax der Arbeiterinnen zwischen 1600 ± 300 und 6300 ± 400 Körner gezählt. Von 21 Arbeiterinnen ließen sich nur 11 dazu bringen, männlich-fertile Blüten zu besuchen. Mechanischer Widerstand beim 'Tripping' männlich-steriler Blüten sowie der Mangel an früherer Sammelerfahrung der Arbeiterinnen an dieser Linie könnten sowohl dieses Ergebnis wie die große Häufigkeit kurzer Sammelfolgen und geringer Ablagerungen an der zuerst besuchten Blüte erklären (Abb 2). Die Gesamtmenge des von einer Arbeiterin auf dem Stigma abgelagerten Pollens schwankte zwischen 8 und 240 Körnern (Mittel 240, Tabelle I). Im Vergleich zur Menge des zur Verfügung stehenden Pollens und zu dem an den Hummeln haftenden Pollen, war die Menge des auf dem Stigma deponierten Pollens gering. Der Abfall des klebrigen Pollens während des 'Tripping', die Erreichbarkeit des Pollens in der Blüte sowie Intensität und Häufigkeit des Auskämmens aus dem Haarkleid während des Sammelns könnten die Menge des aufgenommenen sowie des auf dem Stigma abgelagerten Pollens beeinflussen. Bei 67% der besuchten Blüten wurde überhaupt kein Pollen auf die Stigmen übertragen; bei den übrigen Blüten wurden zwischen 1 bis 152 Körner gezählt (Tabelle I). Die Verteilung der von Null abweichenden Ablagerung erwies sich als nicht zufällig (Tabelle II), sondern es zeigte sich eine Über-Dispersion, besonders bei zwei nacheinander besuchten Blüten. Es bestand eine große Variabilität zwischen der Größe der Besuchssequenz (zwischen zwei und 51 Blüten) und dem Muster der Pollenablagerung auf nacheinander besuchten Blüten (Abb 2). In der Hälfte der Fälle zeigte sich eine Unabhängigkeit zwischen den Ablagerungen auf besuchten Blüten (Tabelle II). In den übrigen Fällen nahm die Größe der Ablagerungen in der Reihenfolge der Besuche ab, aber häufig war auch eine Gleichmäßigkeit zu beobachten. Die Variabilität weist auf eine bedeutende stochastische Komponente bei der Übertragung des Pollens von dem Insekt auf das Stigma hin. Trotz des Fehlens von Kenntnissen über den Zusammenhang zwischen der Pollenablagerung und dem Samenansatz bei *V faba* wird angenommen, daß auch die geringe Pollenmenge auf dem Stigma die Befruchtung beeinflussen kann. Wiederholte Besuche können die Menge des Pollens auf dem Stigma vergrößern, andererseits könnte sie durch das Fehlen alternativen Sammelns zwischen männlich-fertilen und männlich-sterilen Linien begrenzt werden.

Bombus terrestris / Vicia faba / Bestäubung / Pollenübertragung / Monte Carlo-Simulationstest

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