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Pollination of *Brassica campestris* (Cruciferae) by *Andrena savignyi* (Andrenidae: Hymenoptera): Female vs. Male Pollination

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Abstract

Female and male solitary bees usually differ in their behavioral and morphological attributes and consequently in their pollination effectiveness. The current study was carried out at the research farm of The Islamia University of Bahawalpur, Pakistan to compare the foraging behavior and pollination efficiency of female and male *Andrena savignyi* Spinola, 1838 on *Brassica campestris.* The impact of different environmental factors (temperature, relative humidity, light intensity and wind speed) on foraging behavior was also studied. *Andrena savignyi* was the most abundant floral visitor of *B. campestris* and comprised 52.17% of the floral visitors. Female individuals fed on both nectar and pollen while male fed on nectar. Visitation frequency, visitation rate, pollen harvest and pollen deposition of females were significantly higher than that of males because of their larger size and more dry weight. The maximum abundance of females was recorded at 12:00 pm followed by a sharp decline until 4:00 pm whereas males attained their maximum abundance at 2:00 pm. The female pollinated flowers resulted in greater pod weight, pod length, number of seeds per pod, seed weight per pod and germination percentage than the males. Our results suggest that females of *A. savignyi* deliver better pollination of *B. campestris* than males in terms of its reproductive success and germination percentage. Future studies should emphasis on exploring the biology and ecology of *A. savignyi* with special focus of its artificial nesting.

Introduction

Bees (Apoidea: Hymenoptera) are typically considered the most important pollinators of cultivated plant species (Delaplane et al., 2000). They use nectar and pollen for meeting their energy requirements and raising brood (Bohart & Nye, 1956; Roswell et al., 2019; Rodney & Purdy, 2020). The pollination efficiency of bees is the function of their foraging behavior and body morphology (Ohara & Higashi, 1994; Kudo, 2003). Both the foraging behavior and body morphology not only vary among species but also within the sexes of a particular species (Akram et al., 2019; Tang et al., 2019).

Many studies have investigated the foraging behavior, life history and pollination efficiency of female solitary bees but how males forage and pollinate the crops largely remains unclear. Females of solitary bees are considered as the better foragers than the males (O'Toole & Raw, 1991). Female bees feed nectar from flowers to meet their energy requirements used for construction, maintenance and protection of their nests. Besides this they gather abundant pollen grains for their young ones (Ostevik et al., 2010; Smith et al., 2019). They usually have high visitation frequency, better capability of utilizing anthesis timing, shorter foraging range and ability to deposit more viable pollen grains than the males (Ne'eman et al., 2006, Akram et al., 2019).

Female bees are sometimes ineffective pollinators of some plant species as they harvest more but deposit less pollen grains on the stigma. This is mainly due to deposition of heterospecific pollen grains as an outcome of intensive feeding on multiple plant species (Thomson & Thomson, 1992).

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Moreover, pollen deposition on stigma depends on pollen carrying method as well as behavior on flower of a certain bee species. Reason being females of all the bee species do not necessarily deposit more pollen grains on stigma (Young et al., 2007). The males on the other hand, spend most of the time in searching for suitable mates to a longer distance (Willmer & Stone, 2004; Roswell et al., 2019). Although they fulfil their energy requirements from the nectar however, they can also carry some pollen grains. Maybe because of this, they may transport pollen grains long distances (Ne'eman, et al., 2006).

Some recent studies have shown that the females of *Andrena emeishanica, Anthophora plumipes*, *Habropoda tarsta*, *Eucera nigrilabris* and *Megachile cephalotes* are better pollinators than the males in terms of foraging behavior (Ne'eman et al., 2006; Pascarella, 2010; Cane et al., 2011; Akram et al., 2019; Smith et al., 2019; Tang et al., 2019) and pollination effectiveness (Akram et al., 2019; Tang et al., 2019).

Keeping in view the inadequate information on foraging behavior and effectiveness of male and female counterparts especially under sub-tropical climatic conditions of Indian subcontinent, we focused soil nesting *Andrena savignyi*, the most abundant floral visitor of *Brassica campestris* in the study area. Both the sexes in genus *Andrena* differ in their morphological features responsible for the pollination success i.e., females are larger than males and have some other pollen transport areas besides tibial scopa (Tang et al., 2019). Therefore, the present study was carried out to study whether females are better in (i) foraging behavior (visitation frequency, visitation rate and stay time), (ii) pollination facilitating morphological features (body length and proboscis length) and (iii) pollination effectiveness (pollen harvest, deposition and plant reproductive success) than the males of *A. savignyi*. The response of native bees to local environmental factors is unknown and as the environment at the study site is substantially different from the conditions elsewhere. The effect of environmental factors i.e., ambient temperature, relative humidity, light intensity and wind velocity -at the time of different observation hours across different observation dates- on visitation frequency, visitation rate and stay time of both the sexes was also explored.

Material and Methods

Study area and plant species

The study was carried out at the Research Farm of The Islamia University of Bahawalpur (29.376830 N 71.762234 E; 181 meters above sea level), Punjab, Pakistan. The climate of the district Bahawalpur is subtropical, blessed with cold winters and hot summers. The mean daily minimum and maximum temperatures are 18.8 °C and 33.5 °C in winter and summer, respectively and the average annual rainfall is 83-218 mm (Ahmad et al., 2019).

The experimental plant species was field mustard, *Brassica campestris* L. var. toria (Cruciferae), sown in an area of half an acer. About 80% pollination of *Brassica* spp. are usually performed by insects (Chhuneja et al., 2007). The yellow flowers attract variety of insect orders i.e., Hymenoptera, Lepidoptera, Diptera, Neuroptera, Thysanoptera and Coleoptera (Williams, 1978; Ali et al., 2011). The study was lasted from last week of December 2020 to last week of March 2021 (i.e., from initiation of flowering until harvest).

Foraging behavior

First, in order to assess the pollinators profile of *B. campestris,* we conducted a three days-long survey of floral visitors in District Bahawalpur. For this purpose, three widely isolated (>10km) *B. campestris* fields of at least two acers each were selected. Pollinators were collected for two hours (10:00 to 12:00) in each field and one field was surveyed in each day. A hand collection net was used for the collection of floral visitors. We regarded pollinator abundance as number of individuals in each species. The collected floral visitors – other than *Andrena savignyi* – were identified to family level by using taxonomic keys proposed by Borror et al. (1981). We focused on *A. savignyi* for further studies as both the male and female individuals were frequently available throughout flowering season*.* The sex of *A. savignyi* were distinguished on the wing i.e., females have scopa on hind legs while it is absent in males.

The foraging behavior was recorded in terms of visitation frequency, visitation rate and stay time. We defined 'visitation frequency' as the number of individuals visited/ m^2/120 seconds, 'visitation rate' as the number of flowers visited by an individual in 120 seconds and 'stay time' as the time spent by an individual on a flower during a single visit. Only one foraging behavior was focused in a single day. The visitation frequency, visitation rate and stay time of *A. savignyi* was recorded at 09:00, 10:00, 12:00, 14:00, and 16:00 hours with three days interval throughout the flowering period. For measuring visitation frequency, visitation rate and stay time ten observations were made at each 09:00, 10:00, 12:00, 14:00, and 16:00 hours (total 50 observations/census). In case of visitation frequency, ten plants were randomly selected (at least 20 feet apart) from the field margins. A stopwatch was used to record these observations. A digital thermo-hygrometer, an anemometer and a lux meter were used before each observation to record temperature, relative humidity, wind speed and light intensity, respectively.

Morphological features

Ten individuals of each sex were caught and killed for measuring their body and proboscis length with the help of a digital vernier caliper. The dry weight of female and male *A. savignyi* specimens was measured by using an electronic weighing balance accurate to 0.001g (n = 10 for each sex) (Gilbert, 2011).

Pollination effectiveness

Pollination effectiveness of both the sexes was measured in terms of pollen deposition on stigma in a single visit, pollen load carried by an individual bee during peak activity hours and reproductive success of *B. campestris* as a result of their single visits. To measure pollen deposition, 30 floral buds were caged with nylon mesh bags 24 hours before their opening. Each bud was caged on a separate plant. The flowers were then un-caged during the peak activity timing of *A. savignyi* i.e., 11:00 to 2:00 hours. Once a flower had been visited by a female or a male bee, the stigma was removed using a sharp blade. A stereoscopic microscope with 40× magnification was used to count pollen grains (Ali et al., 2011). Fifteen such observations each were made for female and male *A. savignyi*. To measure pollen Load, fifteen specimens of each sex were collected and killed during peak activity timing. The pollen grains were removed from their body by placing them in a glass vial containing 70% ethanol. Once the pollen grains were obtained within the solution, a 10,000µl sample was taken. Ten subsamples of 10µl each were then taken and the pollen grains counted by using a stereoscopic microscope with 40× magnification. Finally, the average number of pollen grains for the ten subsamples was multiplied by the total 10,000µl, and the result divided among the volume of each subsample i.e., 10µl (Canto-Aguilar & Parra-Tabla, 2000).

To see the effectiveness of female and male *A. savignyi* in terms of plant reproductive success, some other floral buds were caged with nylon mesh bags 24 hours before they opened. Each bud was caged on a separate plant. They were un-caged during peak activity timing of *A. savignyi* and re-caged once a single visit had been made by a female or male bees. Twenty-two such observations were made for each sex. The resultant pod weight (g), pod length (cm), number of seeds/pod and seed weight/pod (g) was recorded as the measures of pollination effectiveness. The seeds obtained from pods were subjected to germination tests, carried out in separate Petri dishes containing filter paper. Each Petri dish was moisturized everyday with distilled water for three days. Seeds were considered germinated when 2-3mm long radicles emerged. Twenty-two open-pollinated (unrestricted insect visitation) and 22 caged (no insect visitation) flowers were also maintained on 44 different plants.

Data analysis

To compare the foraging behavior of both the sexes, we used Generalized Linear Mixed Model (GLMM). The dependent variables included visitation frequency, visitation rate and stay time whereas the predictor variables were the environmental factors (i.e., ambient temperature, relative humidity, light intensity and wind speed) and sex was the fixed factor. Separate GLMMs were run for visitation frequency, visitation rate and stay time. We used Poisson distributions with linear-log link functions.

The means of pollen deposition, pollen harvest, body length, proboscis length and dry weight of female and male bees were compared using t-test as the data followed normal distribution (alpha 0.05). One-way ANOVA was applied to see the significance in pod weight, pod length, number of seeds/pod, seed weight/pod and germination percentage among female, male and open-pollinated pods. Means were compared by using Tukey's post hoc test at alpha 0.05.

In order to know how much variation in foraging behavior (the dependent variables) of *A. savignyi* is explained by environment factors (the predictors) we performed multiple linear regression analysis separately for male and female. The foraging behavior included stay time, visitation rate and visitation frequency while environmental predictors included temperature, relative humidity, wind speed and light intensity. The linear regression's F-test was applied with null hypothesis that model explains zero variance in dependent variables. In order to see the significant environmental predictors of foraging behavior, their regression coefficients were compared at alpha 0.05 by using t-statistics. All the analyses were performed in XLSTAT (XLSTAT, 2021).

Results

A total of 991 individuals of 29 species i.e., 9 bees, 3 wasps, 1 ant (Order, Hymenoptera), 10 flies (Order, Diptera) and 6 butterflies (Order, Lepidoptera) visited the flowers of *Brassica campestris*. Hymenopterans were the most abundant (92.23%) followed by Dipterans (6.36%) and Lepidopterans (1.41%). Among bees, *Andrena savignyi* was the most frequent floral visitors of *B. campestris* and comprised 52.17% of the floral visitors followed by *Apis dorsata* (23.01%), *A. florea* (13.97%) and *Lasioglossum albescens* (1.01%).

Female to male population ratio of *A. savignyi* was 2:1 i.e., 349 and 168 individuals, respectively. The foraging activity of both female and male *A. savignyi* started somewhere between 9:00 to 10:00 am. The maximum abundance of females was recorded at 12:00 pm followed by a sharp decline until 4:00 pm. Males attained their peak abundance at 2:00 pm (Fig 1).

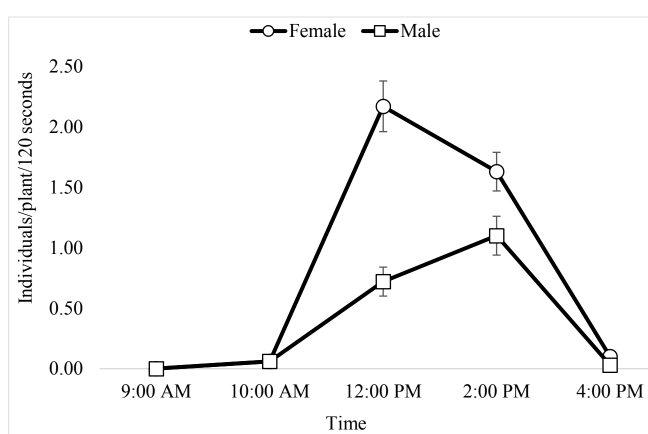

Fig 1. Diurnal fluctuations in abundance of female and male *A. savignyi*.

Females landed directly on the anthers and their thorax and legs came in contact with anthers while males landed on the petals and took up nectar by extending their proboscis. Although males had dense hairs on the thorax but they rarely came in direct contact with anthers (Fig 2). The comparison of foraging behavior of female and male *A. savignyi* is presented in Table 1. The results of GLMM showed that females had significantly higher visitation frequency and visitation rate while lower stay time than the males. The results of t-test showed that females harvested and deposited more pollen grains than the males, potentially due to the presence of scopa on the hind legs of females (Fig 2).

Fig 2. Hind legs of *A. savignyi*: (a) female with load of pollen grains, (b) male.

The comparison of morphological features of female and male *A. savignyi* is presented in Table 2. There was a significant difference between female and male *A. savignyi* in terms of body length ($p = <0.0001$) and dry weight ($p = <0.0001$). Female individuals have longer body and more dry weight than males. There was no significant difference between female and male *A. savignyi* in terms of proboscis length ($p = 0.433$).

Table 1. Results of Generalized Linear Mixed Model.

	Visitation frequency[1]	Visitation rate[2]	Stay time[3]
Female	2.44 ± 0.14	16.04 ± 0.32	3.06 ± 0.15
Male	1.88 ± 0.14	12.58 ± 0.43	4.01 ± 0.26
Wald Chi-square	8.62	58.41	20.46
df	1	1	1
p	0.003	< 0.0001	< 0.0001

[1]individuals/m²/120 seconds, [2]flowers/120 seconds and [3]seconds/flower

The results of multiple linear regression revealed that the model had very low R^2 values therefore did not explain much of the variation in foraging behavior (visitation frequency, visitation rate and stay time) on account of environmental factors (temperature, relative humidity, wind speed and light intensity). However, the models were significant except for the stay time of female *A. savignyi*. The results of t-statistics revealed that visitation frequency of females significantly increased with increase in temperature, relative humidity and light intensity. Visitation frequency of males on the other hand significantly increased with increase in temperature and light intensity. Visitation rate of females increased with increase in wind speed and light intensity while the visitation rate of males increased with increase in temperature and wind speed. None of the environmental factors affected the stay time of females. Stay time of males decreased with increase in light intensity (Table 3).

Table 2. Comparison between female and male morphological features and pollination effectiveness.

	Body length (mm)	Proboscis length (mm)	Dry weight (g)	Pollen deposition	Pollen harvest
Female	14.55 ± 0.024	3.10 ± 0.10	0.023 ± 0.001	351.33 ± 17.91	64853.33 ± 2371.78
Male	11.15 ± 0.028	3.00 ± 0.075	0.015 ± 0.001	180.93 ± 9.62	3893.33 ± 78.20
Results of t-test					
t-observed	9.216	0.802	9.258	8.381	25.688
t-critical	2.101	2.101	2.101	2.048	2.048
p-value	<0.0001	0.433	<0.0001	<0.0001	<0.0001
df	18	18	18	28	28

Table 3. Multiple regression analysis between foraging behavior of female and male *A. savignyi* and environmental factors (T = Temperature, R.H = Relative humidity, W.S = Wind speed and L.I = Light intensity).

Variables	Gender	Model	F	p-value	R^2
Visitation Frequency	Female	Y = -5.21+0.12 (T)* +0.03 (R.H)* -0.04 (W.S) +0.01 (L.I)*	16.202	<0.0001	0.180
	Male	Y = -2.82+0.07 (T)* +0.02 (R.H) -0.02 (W.S) +0.00 (L.I)*	9.367	<0.0001	0.113
Visitation Rate	Female	Y = -0.78+0.24 (T) +0.07 (R.H) +0.30 (W.S)* +0.01 (L.I)*	7.764	<0.0001	0.137
	Male	Y = -4.85+0.98 (T)* -0.08 (R.H) +0.35 (W.S)* -0.01 (L.I)	7.213	<0.0001	0.159
Stay Time	Female	Y = 7.80-0.11 (T) -0.02 (R.H) -0.06 (W.S) -0.00 (L.I)	1.831	0.125	0.037
	Male	Y = 11.80-0.14 (T) +0.02 (R.H) -0.04 (W.S) -0.01 (L.I)*	5.682	0.000	0.128

*t-statistics predicts significance at alpha 0.05

Female bees produced higher pod weight (0.07g), pod length (4.84cm), number of seeds per pod (12.05), seed weight per pod (0.030g) and germination percentage (45.70%) than the males (Table 4). The alternate floral resources of *A. savignyi* are presented in Table 5. The maximum abundance was recorded on *Raphanus sativus* and *Brassica napus*.

Table 4. Visitation of *A. savignyi* on alternate floral resources at Bahawalpur, Punjab, Pakistan.

Plant local name	Scientific name	*Andrena savignyi* individuals
False flax	*Camelina sativa*	1
Rapeseed	*Brassica napus*	12
Cauliflower	*Brassica oleracea*	7
Radish	*Raphanus sativus*	23
Turnip	*Brassica rapa*	6

Discussion

In the present study, 29 insect species in three orders were observed foraging on *Brassica campestris* in our experimental plot. Pollinator composition and abundance vary with geographical area, time and latitude (Ollerton & Louise, 2002). Due to generalized floral structure of *Brassica* spp., a large group of floral visitors are attracted i.e., bees, flies, butterflies and wasps (Kunin, 1993; Mussury & Fernandes, 2000). Williams (1980; 1985) reported that the flowers of *Brassica* spp. produce abundant pollen and nectar that makes it attractive to bees.

Among bees, *Andrena savignyi* were the most frequent floral visitors of *B. campestris*. Several factors determine the population abundance of a certain bee species in an ecosystem i.e., temperature fluctuations (Bennett et al., 2015), agricultural intensification (Connolly, 2013; Woodcock et al., 2017), habitat loss (Potts et al., 2010; Belsky & Joshi, 2019) and natural enemies (predators, parasites and diseases) (Evison et al., 2012).

Table 5. Pod weight, pod length, number of seeds/pod, seed weight/pod and germination percentage resulting from single visit by female and male *A. savignyi*.

	Pod Weight (g)	Pod Length (cm)	No. of Seeds/Pod	Seed Weight/Pod (g)	Germination Percentage
Female	0.07 ± 0.01 b	4.84 ± 0.23 b	12.05 ± 1.19 b	0.030 ± 0.003 b	45.70 ± 9.82 b
Male	0.01 ± 0.00 c	0.75 ± 0.31 c	0.95 ± 0.89 c	0.001 ± 0.001 c	0.01 ± 0.00 c
Open	0.13 ± 0.01 a	6.18 ± 0.28 a	15.90 ± 0.93 a	0.045 ± 0.003 a	78.58 ± 7.37 a
Results of ANOVA					
F	103	106	59	60	31
df	2	2	2	2	2
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Female *A. savignyi* showed higher visitation frequency and visitation rate than the males. Females have more energy requirements than the males as they have to build their nests, lay eggs, collect nectar and pollen for their immatures and defend and maintain their nests (Eickwort, 1975; Peterson & Roitberg, 2006; Danforth et al., 2019). Consequently, they have to uptake more nectar than the males (Roswell et al., 2019). Tang et al. (2019) reported that the males of *Andrena emeishanica* visited a smaller number of flowers than the females.

In the present study, male *A. savignyi* showed higher stay time than the females. The female solitary bees spend less time on the flower because they have to visit several flowers for both nectar and pollen to fed their young ones. Whereas the higher stay time in males is might be due to sole feeding on nectar to fulfil their own energy requirements (Peterson & Roitberg, 2006; Roswell et al., 2019). Bee species which are intensive foragers (having high foraging rates) usually work on rapid pace but stay for a shorter period of time on flowers than species with less foraging rates (Sajjad et al., 2008; Ali et al., 2011; Ali et al., 2014; Ali et al., 2016; Zameer et al., 2017, Farooqi et al., 2021).

Female individuals of *A. savignyi* have longer body and heavier dry weight than males. Large-bodied pollinators generally harvest and deposit more pollen grains per single visit than small-bodied ones (Cruden & Miller-Ward, 1981; Willmer & Finlayson, 2014). The body size is positively related to pollen load capacity and heterospecific pollen deposition (Ramalho et al., 1998; Greenleaf et al., 2007). In our study, females of *A. savignyi* harvested and deposited more pollen grains than males. The *Andrena* females have large hind tibiae with obvious scopa and about 90% of the pollen grains are collected on the hind legs (Tang et al., 2019). In general, females collect both nectar and pollen grains whereas males prefer feeding on nectar and hardly collect pollen grains (Thorp, 1979; Ostevik et al., 2010). Tang et al. (2019) found that females of *Andrena* sp. usually collected ample quantity of pollen grains on their hind legs but contrarily, they deposited less on stigma whereas males harvested less but deposited more. The pollen transfer efficiency of males or females depends on how untidily the pollen grains are attached with the body.

In the present study, female bees produced higher pod weight, pod length, number of seeds per pod, seed weight per pod and germination percentage than the males. Female solitary bees are more efficient pollinators than males (Michener, 2000; Ne'eman et al., 2006; Akram et al., 2019; Tang et al., 2019). Female bees move readily among flowers, carry abundant outcrossing pollen grains and therefore can have high single visit efficiency than the males (Akram et al., 2019).

In this study, the visitation frequency of females and males was significantly affected with temperature, relative humidity and light intensity. Visitation rate of female was affected with wind speed and light intensity whereas in case of males, it was affected with temperature and wind speed. The comparison of female and male solitary bees in their foraging behavior towards different environmental factors is less understood. Our results are in line with Wang et al. (2009) who found that visitation rate of bees (*Andrena parvula, Anthophora melanognatha, Megachile abluta, M. spissula* and *Xylocopa valga*) increased with the increase in temperature and light intensity but decreased with increase in relative humidity. Moreover, visitation frequency increased with increase in temperature, relative humidity and light intensity.

While observing association between foraging behavior and environmental factors, biotic factors are often ignored. The environmental factors also influence the vegetative and reproductive growth of plants. During vegetative development of *Brassica* spp. high mean temperature reduces the floral abundance (Morrison & Stewart, 2002) which ultimately affects pollinators' abundance (Nayak et al., 2015).

Conclusion

It is concluded that females of *A. savignyi* exhibited better foraging behavior in terms of visitation frequency and visitation rate than males. They also proved to be the effective pollinators, as compared to males, in terms of pollen harvest, pollen deposition and plant reproductive success. Therefore, in terms of conservation strategy, special focus should be given to females of ground nesting bees as they are effective pollinators than the males.

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