Crop productivity profoundly depends upon efficient pollination. Pollinator diversity and foraging behaviour patterns of pollinators ensure efficient pollination. Therefore, we conducted an observational study on the diversity of insect pollinators of black mustard (*Brassica nigra*). We also compared the pollination efficiency of its two primary pollinators, i.e. *Apis dorsata laboriosa* and *Apis cerana indica*. The study was conducted in Uttarakhand, India, during the peak flowering season (February and March). The flower visitation rate of *A. cerana* (9.87 ± 2.45 flowers/min) was significantly higher (*P* < 0.0001) than that of *A. laboriosa* (7.52 ± 2.08 flowers/min). Time spent per flower and time spent per flight were higher for *A. laboriosa* than *A. cerana*. Also, *A. cerana* started its diurnal activity earlier (08:00 h) and ceased later (17:30 h) compared to *A. laboriosa* (09:00 h and 17:00 h respectively). A brief decline was observed in the activity of *A. cerana* during the peak activity of *A. laboriosa*. This is possibly due to asymmetric interspecific competition, which might lead *A. cerana* to shift its peak activity earlier (11:30 to 12:00 h) than *A. laboriosa* (13:00–13:30 h). The pollen load and area of corbiculae of *A. laboriosa* were more than that of *A. cerana*. Our results suggest that *A. cerana* is a more efficient pollinator of black mustard than *A. laboriosa*.

**Keywords:** Black mustard, foraging behaviour, honey bee, pollination efficiency, sympatric species.

ANIMALS forage to sustain their lives. Similarly, honey bees gather food (pollen and nectar) for feeding broods in their colony. While foraging, a bee visits several flowers and pollinates them. Around one-third of the world’s crop rely upon bee pollination, a vital prerequisite for forming fruits and seeds. It helps to sustain biodiversity and improve crop productivity. However, wild and domestic bees are continuously declining globally and India is no exception. This is due to excessive non-selective pesticides (mainly neonicotinoids), habitat loss by deforestation and infestation by parasitic mites. It leads to a continuous decline in the productivity of insect-pollinated crops in India. Correspondingly, insect-pollinated crop productivity has also reduced in the Western Himalayas, where a reduction in wild nesting of *Apis laboriosa* has been reported. *Apis dorsata subspecies laboriosa*, also known as the Himalayan cliff bee, is distributed across higher elevations (above 1000 m) of the Himalayan region, with lower elevations dominated by *A. dorsata*. *Apis laboriosa* and *Apis cerana* occur in sympathy in the study site. The former builds nests under open, high, vertical cliffs providing essential pollination to apples, bottlebrushes and other crops, whereas the latter is reared in the wall hives of traditional Garhwali homes for its honey.

Agriculture in the Himalayan region acts as a primary source of livelihood for the indigenous people. The traditional agricultural practices such as mixed cropping maximize the agricultural output with limited resources. In the central Himalaya, mixed cropping of wheat (*Triticum aestivum*) and black mustard (*Brassica nigra*) was found to be the most energy-efficient, with maximum output-input ratio, this enhances black mustard’s significance as a crop along with its cultural and medicinal values. Black mustard is also considered as a good model crop for studying pollination because of its short life cycle and enormous nectar and pollen production. It belongs to family Brassicaceae, which includes plants that are generalists in pollinator preference through visual (bright petals) and chemical (prominent scent) attractants. These adaptations lure diverse insect fauna on which they are predominantly dependent for their pollination. Numerous exploratory and caging (covering flowers to inhibit insect pollination) experimental studies proved that insect pollination improves the yield of Brassicaceae crops increasing the number of pods, seed set, weight of seeds and seed germination. In previous studies, honey bees were found to be dominant insect pollinators of Brassicaceae and other crops. Notably, only...
a few studies elaborate upon the pollination efficiency of *A. cerana*, and none elucidates the foraging patterns and pollination efficiency of *A. laboriosa*.

Efficient pollination depends on various factors such as body size, morphology, behaviour and physiology of pollinators and plant species\(^6,21\). In general, bees are well equipped as efficient pollinators with structural and behavioural adaptations\(^21\) such as flower constancy – consistently foraging a particular plant in a specific foraging trip to ensure cross-pollination\(^22\). To compare pollination efficiency, flower visitation rate of insects, time spent per flower and time spent per flight (inter-flower flight time) are crucial parameters. Several studies have reported a positive correlation between visitation rate and pollination efficiency\(^19,23,24\). Therefore, in addition to the pollinator diversity of black mustard, we also compared the pollination efficiency of wild *A. laboriosa* and reared *A. cerana* in the high-altitude temperate climate of western Himalaya. We considered four foraging parameters: (i) Flower visitation rate: the number of flower visits per minute. (ii) Time spent per flower: the duration between landing on and departure from a flower, and time spent per flight: duration between departure and landing from one flower to another. (iii) Diurnal foraging activity patterns. (iv) Pollen load and pollen carrying capacity – area of the corbiculae. Additionally, to compare diurnal foraging patterns of these two species, we explain the interspecific competition and foraging resource utilization through temporal resource partitioning.

**Materials and methods**

The study was conducted during the peak flowering season of black mustard from mid-February to April 2020, in Chamoli and Rudraprayag districts of Uttarakhand, India (Figure 2, prepared using ArcMap 10.5). Majority of the observations were taken in the Hudu (30°30’14.63”N, 79°9’5.24”E; 1800 m asl) and Usada (30°30’37.64”N, 79°9’18.92”E; elevation 1600 m asl) villages. Black mustard was sown in late October 2019 and harvested in May 2020. Wheat and black mustard were mixed cropped where ocular observations and discussion with farmers confirmed that both crops shared, almost equal proportions on the ground. Daily observations were collected from 07:00 to 18:00 h (all in IST – India Standard Time) during the flowering of black mustard, in 18 randomly selected fields. To avoid bias, we limited our sampling to sunny days as honey bees are in active on rainy days\(^25\). Temperature (10–14°C), humidity (58%) and wind velocity (8 km/h) were nearly uniform throughout the sampling days.

We examined diversity and abundance of the pollinators by observing insects visiting black mustard flowers. The interactions were considered as pollination only if the insect’s body touched the stigma. Counts for insect pollinators were conducted using 500 m transect in the fields. These data were used to describe the pollinator’s species diversity and species evenness using Shannon diversity index \((H’)\) and Shannon evenness index \((J’)\) respectively, as follows\(^26\)

\[
\text{Shannon diversity index } (H’) = \sum_{i=1}^{S} pi \ln pi,
\]

\[
\text{Shannon evenness index } (J’) = \frac{H’}{\ln S},
\]

where *S* is the total number of species and *pi* is the proportion of the total individuals of species *i*.

---

**Figure 1.** Honey bees pollinating black mustard: (a) *Apis cerana* and (b) *Apis laboriosa*.

**Figure 2.** Map showing the study sites.
To estimate the time expenditure while foraging, a particular bee was followed for recording its activity. When a bee moved from one flower to another, the time spent per flower and time spent per flight were recorded. In total, 212 A. cerana and 242 A. laboriosa were observed for studying these parameters. The observations were made for half an hour, after every 30 min, from 0900 to 1700 h each day. These recordings were collected using a digital timer with an accuracy of microseconds, which was also used for calculating visitation rate of the bees by counting the number of flowers visited per minute.

To assess diurnal activity, observations were made from 0700 to 1800 h each day, for a total of 11 days. The number of individuals was noted by visual counts in a plots of size 2 × 1 sq. m, after 30 min (ref. 23). The plot size was calibrated to efficiently assess the maximum area without disturbing the bees. Since measurement of foraging activity at the nest of A. laboriosa was not feasible on the high-rock cliffs, individual counts using the plots were preferred.

The pollen load and corbiculae area were determined to evaluate the pollen carrying capacity of honey bees. Their pollen collection depends on stored pollen in the hive, the pollen load might not reflect the true pollen carrying potential. Therefore, area of the corbiculae was used to compare the pollen carrying capacity as bees with larger corbiculae carry more pollen27. Only the bees with enormous pollen loads were collected and preserved. These pollen loads were carefully removed using fine forceps from 18 A. cerana and 19 A. laboriosa individuals, and measured using an analytic weighing machine with an accuracy of 0.0001 g. For measuring area of the corbiculae, 14 A. cerana and 15 A. laboriosa were photographed using microscopic digital photography. These images were analysed using ImageJ version 1.8.0 software (Figure 3).

Results

Black mustard attracts diverse insect fauna for its pollination, consisting of 18 species belonging to 15 genera, 10 families, and 4 orders (Figure 4 and Supplementary material, Appendix 1). A. cerana (68.5%) and A. laboriosa (22.7%) dominate in abundance, followed by the other dipterans and lepidopterans. The Shannon diversity index of insect pollinators of black mustard is 1.02, whereas the Shannon evenness index is 0.35.

Independent T-test reveals that flower visitation rate of A. cerana (9.87 ± 2.45 flowers/min, n = 212) was significantly higher (Figure 5a and Table 1) than A. laboriosa (7.52 ± 2.08 flowers/min, n = 252; t = 11.025, df = 416.54, P-value <0.0001).

The data on time spent per flower and time spent per flight were positively skewed. So we performed Welch’s T-test after log transformation of the raw data28. Since the mean of log values is the same as the geometric mean, we interpreted the statistical test results in terms of the median, as it is close to the geometric mean. Figure 5b depicts these results in the form of a boxplot. The test revealed that A. laboriosa spent significantly more time (t = 5.941, P-value <0.0001) per flight than A. cerana (Table 1).

Since A. cerana was higher in abundance than A. laboriosa, a normalized scale was used for their comparison.

Figure 3. Measurement of corbicula area from microscopic digital images using ImageJ software.
The size difference in visitation rate was 19.30. Similarly, 

$$n - \frac{t}{- \text{v}}$$

ing commencement, cessation and duration; pollen load ($n$ is the number of specimens used), and area of corbiculae ($n$ is the number of specimens used) of Apis cerana and Apis laboriosa.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A. cerana</th>
<th>A. laboriosa</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower visitation rate</td>
<td>9.87 ± 2.45 flowers/min ($n = 212$)</td>
<td>7.52 ± 2.08 flowers/minute ($n = 252$)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time spent per flower</td>
<td>4.08 (2.65–6.18) sec ($n = 212$)</td>
<td>4.78 (2.92–7.77) sec ($n = 252$)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Time spent per flight</td>
<td>0.844 (0.634–1.21) sec ($n = 212$)</td>
<td>1.08 (0.7761.74) sec ($n = 252$)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Commencement of foraging activity</td>
<td>08:00 h</td>
<td>09:00 h</td>
<td></td>
</tr>
<tr>
<td>Cessation of foraging activity</td>
<td>17:30 h</td>
<td>17:00 h</td>
<td></td>
</tr>
<tr>
<td>Foraging duration</td>
<td>9 h 30 mins</td>
<td>8 h</td>
<td></td>
</tr>
<tr>
<td>Pollen load/bee</td>
<td>4.17 ± 0.907 mg ($n = 18$)</td>
<td>10.9 ± 2.94 mg ($n = 19$)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Area of corbiculae</td>
<td>1.89 ± 0.118 mm$^2$ ($n = 13$)</td>
<td>4.10 ± 0.153 mm$^2$ ($n = 18$)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$$X_{\text{normalized}} = \frac{(X - X_{\text{minimum}})}{(X_{\text{maximum}} - X_{\text{minimum}})}.$$  

Figure 6 depicts the diurnal foraging activity of honey bees. A. cerana started its activity earlier in the morning (08:00–08:30 h) followed by the progression and achieving peak activity at 11:00 h–11:30 h. A drop in the foraging activity was observed just after its peak (just before peak activity of A. laboriosa), followed by progression and finally cessation around 17:30–18:00 h. A. laboriosa started its activity later in the morning (08:30–09:00 h) increased steadily and peaked during 13:00–13:30 h, followed by a sharp drop (between 13:30 h and 14:00 h) until its cessation around 16:30 h (Figure 6 and Supplementary material, Appendix 2). The total foraging duration of A. cerana was 9 h 30 min and for A. laboriosa it was 8 h.

The pollen load of A. laboriosa was significantly heavier ($t = 9.4766$, $P$-value $<$0.0001) than A. cerana. Similarly, area of the corbiculae for A. laboriosa was larger ($t = 46.069$, $P$-value $<$0.0001) than A. cerana (Table 1).

**Discussion**

Black mustard is pollinated by diverse insect fauna, viz. Gomphrys rhamni, Pieris brassicae, Aglais cashmirensis, Bombus festivus, Andrena spp., etc. with A. cerana and A. laboriosa being the major pollinators. Some studies also suggest that A. cerana has been traditionally reared for a long time$^{3,1}$ and A. laboriosa is the most abundant wild hymenopteran insect in some parts of Western Himalayas$^{25}$. Since A. cerana and A. laboriosa dominated in the study area, the species diversity and evenness indices were markedly low compared to previous studies. However, insect pollinators significantly contribute to species richness, as reported in other studies$^{14–16,18,23,29}$.

In previous studies, the visitation rate of A. cerana on Brassicaceae and apple flower was either significantly higher$^{17,19}$ or the same$^{24}$ as the coexisting Apis mellifera. But, when A. mellifera was nearly tenfold less abundant than A. cerana, the size difference in visitation rate was much higher$^{17}$ compared to studies where sympatric species were nearly equally abundant$^{19,30}$. The latter scenario is in agreement with our results. This implies that the
visitation rate of *A. cerana* is negatively affected by competition from sympatric species, which needs validation through species exclusion experiments.

So far, no study has elucidated the foraging time expenditure of pollinators through direct recording of time spent per flower. Nevertheless, bees minimize flight time by visiting nearby flowers in a particular direction for maximizing their foraging efficiency\(^{19,20,31}\). The more time an insect utilizes being engaged with flowers rather than in flight, the more would be the foraging efficiency. Though *A. laboriosa* spent significantly more time per flower as well as per flight, the relative size difference was more in the case of time spent per flight. This further adds to the pollination efficiency of *A. cerana* compared to *A. laboriosa*.

The total foraging duration for *A. cerana* was more than that of *A. laboriosa* where *A. cerana* started before and ceased later, supported by former studies where it co-existed with *A. mellifera*, in temperate climate\(^{19,20,31}\). Generally, in sympatric species of social bees and ants, the more aggressive species arrives later at the foraging site\(^{22}\). The reason could be small colony size due to low population density and distant foraging resources\(^{33}\). The physiology of honey bees might also play a crucial role in the diurnal foraging patterns; for instance, cavity-enclosed nesting honey bees can better thermoregulate in low temperatures than the open nesting bees, enabling them to forage in low ambient temperatures in the early morning and late evening hours\(^{34}\). Furthermore, learning and memory also influence the foraging patterns in honey bees for effective exploitation of resources as they adjust their foraging timings according to resource availability and presence of competitors\(^{35}\). In this study, the less aggressive *A. cerana* anticipates its foraging time in response to the more aggressive *A. laboriosa*. These rationales explain the late commencement and early cessation in the foraging activity of *A. laboriosa*. The early commencement of *A. cerana* provides pollination to black mustard during the morning hours when another principal pollinator (*A. laboriosa*) is unavailable. The overall foraging patterns of *A. cerana* and *A. laboriosa* depict asymmetric interference competition. In the field, *A. cerana* was unable to displace *A. laboriosa* from a preoccupied flower, whereas the inverse was not true; an arriving *A. laboriosa* mostly displaced *A. cerana* from a preoccupied flower. This further adds to the understanding of aggressive dominance hierarchy in foraging of these two species. To facilitate their coexistence, behavioural temporal niche partitioning occurs, which can be inferred from their pattern and peak foraging activity time. The foraging pattern and peak activity explain the temporal niche partitioning for avoiding interspecific competition. Temporal resource partitioning of floral resources by honey bees\(^{34,36,37}\), stingless bees\(^{32,36,38}\) and bumble bees\(^{39}\) has been reported by numerous studies, where sympatric species exhibited similar foraging patterns and behaviour as in the present study. These studies reported a similar shift in foraging activity of less aggressive species and also heterospecific dislocation behaviour by aggressive species\(^{32,37}\). The underlying mechanisms involved in temporal resource partitioning are not yet well understood. However, few studies delve into these mechanisms and reveal that interference maybe reduced by communication among nestmates\(^{32}\), or by learning adaptations\(^{35}\), or both. However, more studies are needed for clarity. Few bee species also evolve as nocturnal and forage during the night to minimize the interference competition\(^{38}\).

In Bengaluru, India, *A. dorsata* foraging was reported during the night in low-light conditions (full moon, street light)\(^{34}\). However, in the present study, *A. laboriosa* was not found foraging during night, which might be due to limited artificial light availability in the study area.

Since *A. laboriosa* has a larger body size, the area of the corbicula and pollen loads were greater than that of *A. cerana*. However, all other parameters support *A. cerana* being efficient in pollination. The high carrying capacity of *A. laboriosa* indicates that the species hoards pollen to carry back to distant nests and this could be the reason that it spends more time per flower.

As can be incurred from the above-mentioned facts, four points, *A. cerana* is more efficient in pollinating black mustard than *A. laboriosa*. Further experiment-based studies are required, especially for poorly explored *A. laboriosa* to elaborate on different aspects of foraging. Nevertheless, the natural mutualism between black mustard and *A. cerana* can be utilized positively to increase the overall yield by encouraging *A. cerana* beekeeping closer to black mustard crop fields. Based on these scientific revelations, the local beekeepers can be trained to strengthen their resilience for socio-economic and climate change effects. The concerned authorities can incentivize the traditional beekeeping techniques and organize community outreach programmes to nurture environment-friendly bee keeping. This would be a source of livelihood to subsistence farmers of the Himalaya, who endure the massive thrust of climate change and natural disasters that follow.

**Figure 6.** Graph representing diurnal foraging activity of *A. cerana* and *A. laboriosa* by plotting mean and local regression fit line of the normalized values against hours of the day. The points and error bars depict the mean and standard error respectively.
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