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# A study on the long-term variation in pollen foraging by *Apis cerana* (Fabr.) [Apidae: Insecta]

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**Abstract**
Pollen is the vital source of nutrition for the bees in their larval stage which in turn determines their overall fitness. Pollen traps established at bee hive entrance during two sampling period i.e. morning (0900-1100 hrs) and midday (1100-1300 hrs) at Baruipur in 2015 was used to determine the amount of pollen collected by *Apis cerana*. A total of 28 different pollen types collected by the bees demonstrated the occurrence of pollen rich flora in the surroundings of study site. Significant difference in temperature between two sampling periods seems to affect the foraging behaviour of bees. The dry weight, amount and type of pollen grains thus collected were also noteworthy amongst other unknown relevant parameters. Predominance of *Pisum sativum* and *Litchi chinensis* pollen types in the morning and *Rosa* and *Brassica* pollen types in the midday samples highlighted the significance of such flowering plants as pollen sources during specific hours of the day.

**Keywords:** *Apis cerana*, pollen traps, *Pisum sativum*, *Litchi chinensis*, *Rosa*, *Brassica*, pollen foraging

## 1. Introduction
Pollen which constitutes an indispensible source of nutrition for the newly emerged bees [1, 2] is required predominantly for brood rearing [3]. The quality and quantity of such pollen as fed to the larvae, in turn, enhances their fitness and survival abilities [4]. Nurse bees indiscriminately feed royal jelly to young developing larvae [5]. However on approaching pupation such larvae are subsequently fed with worker jelly, a mixture of hypopharyngeal gland secretions, honey and pollen [6].

Besides the quality and quantity of pollen, the distance of flowering plants from their colony and their attractiveness as enhanced by the crowding of flowers [7] are known to increase the foraging tendencies of honey bees towards such profitable resource [8]. Tropical regions are credited with high density of flowering plant species and any study on the foraging behaviour of their potential pollinators would probably contribute towards better understanding of plant-pollinator interaction in a particular area. In Asia *Apis cerana* (Fabr.) is considered to be an excellent pollinator due to its efficiency in collecting pollen from a broad medley of flowering species [9]. Thus the present study was designed to highlight the changes in the foraging pattern of *Apis cerana* throughout the entire study period. Construction of pollen traps at the entrance of two bee colonies served as a useful tool for the above observation on honey bees.

## 2. Materials and methods
The present study was conducted during 2015 for 24 days altogether at different places in Baruipur (22.35°N, 88.44° E), (situated 20 km from Kolkata), South-24 Parganas, West Bengal, India. All observations were conducted on sunny days. Specially designed pollen traps were set up to capture pollen [10, 11, 12, 13] foraged by the *Apis cerana* workers during two sampling periods i.e. morning (0900-1100 hrs) and midday (1100-1300 hrs). Such traps were established at the entrance of two bee colonies (hives) supporting about 10,000 honey bees. Weather proof pollen traps were installed in order to keep pollen moisture free especially during the humid months [13]. Temperature was measured for each pollen sampling period during the entire study period.

Pollen thus collected was dried for 24 hrs at 70°C to obtain total pollen dry weight. Pollen samples were subsequently diluted, homogenised in acetic acid and subjected to acetolysis [14] followed by mounting in silicone oil for identification under microscope. The different plant species were determined by counting and identifying the pollen grains. The representation of different pollen types from such sample was determined by random counting of 100 pollen grains from atleast two slides under light microscope with the help of Sedgewick-Rafter counter chamber. Equatorial diameter of pollen was determined with the help of stage micrometer. Pollen identification was done using reference slides of known pollen types by the assistance of an expert along with specific morphological identification keys for different plant families as available with him. Paired t-test was used to determine whether the temperature recorded during morning and midday sampling periods was significantly different.

## 3. Results
A total of 28 pollen types were identified representing 20 angiosperm plant families (Table 1-4) during the entire study period. Additionally, a few unidentified pollen types were also present in the sample.

**Table 1:** Hive stored pollen grains collected in the months of January-March 2015

| Percentage of pollen types | Range of equatorial diameter of the pollen grains (in µm) (mean) | Probable plant source |
|---|---|---|
| Predominant pollen (>45%) | 32 -42 (37) | *Pisum sativum, Brassica nigra, Brassica campestris,Mangifera indica, Butea monosperma, Brassica oleracea, Lycopersicon esculentum* |
| Secondary pollen (16 to 45%) | 18 – 41 (29.5) | *Rosa* sp, *Psidium guajava, Solanum melongena, Mitragyna parvifolia* |
| Important minor pollen (3 to 15%) | 43 – 50 (46.5) | Unknown |
| Minor pollen (<3%) | 50 | Unknown |

**Table 2:** Hive stored pollen grains collected in the months of April-June 2015

| Percentage of pollen types | Range of equatorial diameter of the pollen grains (in µm) (mean) | Probable plant source |
|---|---|---|
| Predominant pollen (>45%) | 32 – 48 (40) | *Litchi chinensis, Terminalia arjuna, Butea monosperma, Helianthus* sp, *Allium cepa* |
| Secondary pollen (15 to 42%) | 25 – 45 (35) | *Hibiscus esculentus, Azadirachta indica* |
| Important minor pollen (3 to 15%) | 45 – 55 (50) | Unidentified |
| Minor pollen (<3%) | 42 – 62 (52) | Unidentified |

**Table 3:** Hive stored pollen grains collected in the months of July-September 2015

| Percentage of pollen types | Range of equatorial diameter of the pollen grains (in µm) (mean) | Probable plant sources |
|---|---|---|
| Predominant pollen (<45%) | 28 – 42 (35) | *Cocos nucifera, Eucalyptus* sp,*Syzygium cumini* |
| Secondary pollen (16 to 50%) | 32 – 48 (40) | *Zea mays* |
| Important minor pollen (3 to 10%) | 44 – 50 (47) | Unidentified |
| Minor pollen (3%) | 40 | *Magnolia grandiflora* |

**Table 4:** Hive stored pollen grains collected in the months of October-December 2015

| Percentage of pollen types | Range of equatorial diameter of the pollen grains (in µm) | Probable plant sources |
|---|---|---|
| Predominant pollen (45%) | 34 – 42 (38) | *Musa paradisica*, Papaya, *Lycopersicon esculentum, Rosa* sp, *Dillenia indica, Aegle marmelos* |
| Secondary pollen (16 to 48%) | 35 – 49 (42) | *Cassia* sp, *Carica* sp, *Dalbergia sissoo* |
| Important minor pollen (3 to12%) | 44 – 46 (45) | Unknown |
| Minor pollen (>3%) | 38 – 50 (44) | Unidentified |

Pollen was classified into four types [15]. Predominant pollen (>45%), Secondary pollen (16 - 45%), Important minor pollen (3 - 15%) and Minor pollen (<3%). *Pisum sativum* pollen was mostly dominant in the month of January while *Brassica nigra* and *Brassica campestris* pollen were dominant in February. *Mangifera indica* pollen was found abundant in March (Table 1), while *Litchi chinensis* pollen was observed mostly during April. *Terminalia arjuna* pollen was dominant in May in contrast to *Butea monosperma* being mostly abundant in June (Table 2). *Cocos nucifera* and *Eucalyptus* pollen types were found dominantly in July and August respectively. *Syzygium cumini* pollen was mostly observed in September (Table 3) while *Musa paradisica* and papaya pollen type were abundant in October. *Lycopersicon esculentum* pollen was dominant in November while *Rosa* pollen type was mostly observed in the month of December (Table 4).

Thus the composition of pollen types differed significantly within the trapping period of 24 days. The dry weight of pollen, amount of pollen collected in the trap and number of pollen types identified in samples differed considerably between two bee colonies (Table 5, 6) during the sampling period.

**Table 5:** Mean Temperature (°C) recorded along with dry weight (gms), number and different pollen types collected at two bee hive entrance during morning sampling period

| Date | Temperature (°C) | Colony One | | | Colony Two | | |
|---|---|---|---|---|---|---|---|
| | | Pollen collected (gms) | Pollen counted | Pollen types | Pollen collected (gms) | Pollen counted | Pollen types |
| 10.01.15 | 15 | 0.4 | 605 | 10 | 0.2 | 420 | 12 |
| 24.01.15 | 13 | 0.2 | 432 | 12 | 0.3 | 450 | 9 |
| 10.02.15 | 15 | 0.3 | 525 | 8 | 0.5 | 610 | 11 |
| 20.02.15 | 14 | 0.5 | 460 | 10 | 0.4 | 502 | 7 |
| 12.03.15 | 20 | 0.4 | 340 | 7 | 0.1 | 230 | 5 |
| 20.03.15 | 22 | 0.3 | 437 | 11 | 0.3 | 470 | 8 |
| 13.04.15 | 21 | 0.6 | 502 | 9 | 0.7 | 640 | 10 |
| 22.04.15 | 22 | 0.2 | 320 | 6 | 0.5 | 440 | 7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 14.05.15 | 24 | 0.1 | 250 | 8 | 0.2 | 310 | 9 |
| 24.05.15 | 26 | 0.4 | 420 | 10 | 0.6 | 440 | 10 |
| 11.06.15 | 25 | 0.3 | 230 | 7 | 0.4 | 340 | 8 |
| 23.06.15 | 27 | 1.5 | 470 | 9 | 1.2 | 270 | 7 |
| 10.07.15 | 26 | 2.2 | 390 | 11 | 1.4 | 356 | 10 |
| 22.07.15 | 27 | 2.0 | 370 | 10 | 1.7 | 360 | 8 |
| 12.08.15 | 22 | 1.8 | 270 | 8 | 1.9 | 270 | 11 |
| 25.08.15 | 27 | 0.6 | 456 | 12 | 1.2 | 440 | 9 |
| 10.09.15 | 22 | 1.5 | 340 | 9 | 1.8 | 405 | 6 |
| 22.09.15 | 24 | 0.8 | 270 | 7 | 1.1 | 310 | 8 |
| 14.10.15 | 24 | 2.1 | 540 | 10 | 1.9 | 480 | 11 |
| 24.10.15 | 22 | 1.9 | 457 | 8 | 1.6 | 434 | 10 |
| 10.11.15 | 20 | 2.5 | 480 | 11 | 2.2 | 390 | 7 |
| 24.11.15 | 18 | 1.8 | 510 | 9 | 0.9 | 402 | 9 |
| 12.12.15 | 14 | 2.3 | 470 | 10 | 1.7 | 440 | 11 |
| 26.12.15 | 11 | 1.9 | 530 | 12 | 1.3 | 340 | 8 |

**Table 6:** Mean Temperature (°C) recorded along with dry weight (gms), number and different pollen types collected at two bee hive entrance during midday sampling period

| Date | Temperature (°C) | Colony One | | | Colony Two | | |
|---|---|---|---|---|---|---|---|
| | | Pollen collected (gms) | Pollen counted | Pollen types | Pollen collected (gms) | Pollen counted | Pollen types |
| 10.01.15 | 25 | 1.7 | 650 | 10 | 1.5 | 580 | 8 |
| 24.01.15 | 26 | 2.2 | 620 | 9 | 3.1 | 780 | 11 |
| 10.02.15 | 27 | 1.9 | 720 | 7 | 3.5 | 820 | 10 |
| 20.02.15 | 28 | 0.8 | 670 | 11 | 2.7 | 775 | 9 |
| 12.03.15 | 33 | 2.7 | 790 | 10 | 1.1 | 567 | 7 |
| 20.03.15 | 32 | 3.8 | 782 | 8 | 0.9 | 690 | 10 |
| 13.04.15 | 35 | 2.5 | 667 | 12 | 1.8 | 540 | 11 |
| 22.04.15 | 33 | 3.8 | 850 | 11 | 2.7 | 785 | 10 |
| 14.05.15 | 34 | 0.7 | 765 | 9 | 1.8 | 770 | 8 |
| 24.05.15 | 35 | 3.7 | 825 | 8 | 2.8 | 745 | 9 |
| 11.06.15 | 32 | 1.3 | 558 | 9 | 2.2 | 637 | 7 |
| 23.06.15 | 34 | 2.6 | 610 | 7 | 1.7 | 537 | 8 |
| 10.07.15 | 35 | 0.9 | 545 | 8 | 1.2 | 612 | 9 |
| 22.07.15 | 33 | 1.9 | 670 | 10 | 2.3 | 735 | 6 |
| 12.08.15 | 32 | 3.2 | 770 | 9 | 2.9 | 690 | 10 |
| 25.08.15 | 35 | 2.8 | 634 | 10 | 1.4 | 578 | 8 |
| 10.09.15 | 30 | 2.3 | 575 | 8 | 1.5 | 756 | 11 |
| 22.09.15 | 34 | 1.8 | 678 | 9 | 2.0 | 654 | 9 |
| 14.10.15 | 30 | 4.1 | 825 | 10 | 2.5 | 637 | 8 |
| 24.10.15 | 31 | 2.7 | 690 | 8 | 3.2 | 658 | 10 |
| 10.11.15 | 30 | 1.5 | 580 | 10 | 2.1 | 723 | 8 |
| 24.11.15 | 28 | 1.1 | 490 | 8 | 0.9 | 470 | 7 |
| 12.12.15 | 25 | 1.9 | 582 | 9 | 3.3 | 675 | 10 |
| 26.12.15 | 23 | 2.0 | 635 | 10 | 1.7 | 587 | 9 |

Moreover, a significantly cooler weather was experienced during the morning as compared to the midday sampling period (d.f = 23; t = 21.27; P<0.0001). *Litchi chinensis* and *Pisum sativum* pollen were especially dominant in morning samples (Table 7). In contrast *Brassica* and *Rosa* pollen types were most abundant in midday samples from both hives during the study (Table 7).

**Table 7:** Pollen types identified in pollen samples during two sampling period

| Family | Pollen type | Sampling period | Total pollen grains counted for each pollen type | Number of pollen sample containing pollen type |
|---|---|---|---|---|
| Fabaceae | *Pisum sativum* | morning | 1270 | 7 |
| | | midday | 565 | 4 |
| Cruciferae | *Brassica nigra* | morning | 560 | 3 |
| | | midday | 1156 | 7 |
| Cruciferae | *Brassica campestris* | morning | 980 | 6 |
| | | midday | 1120 | 8 |
| Aracardiaceae | *Mangifera indica* | morning | 1033 | 7 |
| | | midday | 960 | 6 |
| Fabaceae | *Butea monosperma* | morning | 640 | 4 |
| | | midday | 340 | 3 |

| | | | | |
|---|---|---|---|---|
| Cruciferae | *Brassica oleracea* | morning | 170 | 2 |
| | | midday | 345 | 4 |
| Rubiaceae | *Mitragyna parvifolia* | morning | 250 | 3 |
| | | midday | 375 | 4 |
| Solanaceae | *Solanum melongena* | morning | 510 | 5 |
| | | midday | 450 | 5 |
| Fabaceae | *Cassia* sp | morning | 175 | 2 |
| | | midday | 470 | 5 |
| Sapindaceae | *Litchi chinensis* | morning | 1256 | 10 |
| | | midday | 560 | 6 |
| Combretaceae | *Terminalia arjuna* | morning | 634 | 6 |
| | | midday | 332 | 4 |
| Liliaceae | *Allium cepa* | morning | 228 | 3 |
| | | midday | 520 | 5 |
| Meliaceae | *Azadirachta indica* | morning | 178 | 2 |
| | | midday | 475 | 4 |
| Compositae | *Helianthus annuus* | morning | 240 | 3 |
| | | midday | 434 | 5 |
| Arecaceae | *Cocos nucifera* | morning | 680 | 7 |
| | | midday | 786 | 8 |
| Myrtaceae | *Eucalyptus* sp | morning | 532 | 5 |
| | | midday | 786 | 8 |
| Myrtaceae | *Syzygium cumini* | morning | 561 | 5 |
| | | midday | 998 | 8 |
| Graminae | *Zea mays* | morning | 231 | 3 |
| | | midday | 354 | 4 |
| Magnoliaceae | *Magnolia grandiflora* | morning | 235 | 3 |
| | | midday | 775 | 7 |
| Malvaceae | *Hibiscus esculentus* | morning | 780 | 6 |
| | | midday | 1024 | 8 |
| Musaceae | *Musa paradisica* | morning | 135 | 2 |
| | | midday | 456 | 5 |
| Solanaceae | *Lycopersicon esculentum* | morning | 325 | 4 |
| | | midday | 458 | 6 |
| Rosaceae | *Rosa* sp | morning | 421 | 6 |
| | | midday | 1237 | 10 |
| Myrtaceae | *Psidium guajava* | morning | 435 | 5 |
| | | midday | 768 | 8 |
| Dilleniaceae | *Dillenia indica* | morning | 431 | 4 |
| | | midday | 876 | 7 |
| Rutaceae | *Aegle marmelos* | morning | 213 | 3 |
| | | midday | 437 | 5 |
| Fabaceae | *Dalbergia sissoo* | morning | 225 | 3 |
| | | midday | 432 | 6 |
| Caricaceae | Papaya | morning | 241 | 4 |
| | | midday | 878 | 8 |

## 4. Discussion

Insects appear committed towards serving the ecosystem by engaging themselves in the act of pollination. In contrast they are benefitted with nectar during such a selfless act of cross pollination [16]. Such an intimate relationship between flowering plants and their pollinators thereby represents a stepping stone towards better understanding of different events of organic evolution [17, 18]. Plants have uniquely devised a mechanism for presenting the pollen in doses either by packaging or by dispensing [19]. Such models thereby represent a significant event towards better elucidating the pollen presentation theory [20, 21, 22, 23, 24, 25, 26] which in turn enhances the fitness of such plants [19].

Earlier, studies on pollen presentation by plants and pollen foraging by bees have employed specially designed pollen traps fitted at the entrance of bee colonies [27, 11, 12, 13, 28]. Although such traps are known to increase the amount of pollen collected by such colonies [29] but the movement of foragers inside and outside the hive occur at a reduced rate [12]. However such traps are immensely beneficial for the beekeepers involved in collecting surplus load of pollen carried in by the forager during summer. Such pollen is subsequently utilized by the bees in spring to contribute towards brood rearing [13].

In Asia *Apis cerana* are the most efficient pollinators of cultivated tropical plant species [30, 31, 32, 33, 34]. Pollen foraging in *A. cerana* begins much earlier in the day and is also associated with their greater number of visits to the same habitat as compared to other species [35, 30, 31, 33, 36, 34]. Pollen grains thus collected by them are digested under the influence of the secretions from the hypopharyngeal gland [37]. An enzymatic study on the role of hypopharyngeal gland reveals the interplay of several enzymes in intracellular digestion of pollen grains [38].

Importantly, the tropical region is bestowed with a remarkable variety of flowering plant species. Thus the present study would further enrich the information on pollen spectra as available from India, especially West Bengal [39, 40, 41]. Interestingly, seasonality in pollen samples isolated at two bee hives entrance was also observed in the present and earlier studies [42, 43, 44]. In contrast to wide variation in the diameter of European pollen type (5-200 μm) as reported [1], the large diversity of pollen with a narrow range of diameter as observed in this study is fascinating. The determination of the amount of pollen deposited in hive and amount of pollen trapped at the entrance of colonies emphasized on the availability of pollen rich flora in the specified area throughout the year [28]. Similar observation in this study could probably help in increasing the prospects of bee keeping industry in the study area.

A significant difference in temperature during morning and midday pollen trapping period as observed in this study was probably associated with alteration in foraging ability of bees [45]. Such difference in foraging behaviour could probably be responsible for an alteration in dry weight and different types of pollen collected at the colony entrances in this study. Amount of pollen also varied considerably between the morning and midday trapping periods probably due to their tendency to return early to hive before collecting a full load of pollen under varied meteorological conditions [10]. A variation in size of pollen pellet could be attributed to a number of factors i.e. time and meteorological conditions of day along with plant species most frequently being foraged [10]. Foraging activity of *Apis cerana* was lowest at 0800 hrs while it reached its peak at 1400 hrs [46].

Interestingly, honeybees are blessed with ability to remember the time of day when resources are at its peak [47, 48, 49]. In the present study *Pisum sativum* pollen was most dominant in the morning samples. In accordance with this fabaceous crops were probably referred to as early morning crops due to their maximum pollen presentation at 0800 hours [50]. *Litchi chinensis* pollen type was the next most prevalent in the above sample. Thus previous observation [51] on honey bee foraging from this exclusive pollen source between 0800-1100 hours is noteworthy. On the contrary, midday sample was dominated by *Rosa* and *Brassica* type pollen. Predominance of *Rosa* pollen type in such samples was probably associated with occurrence such plant species in the study area. Inspite of several complexities associated with pollination by insects on *Brassica* [52], bees have intelligently devised a pollen transferring devise for such plants [53]. Similar pollination service of *Apis mellifera* as recorded on *Brassica* has also been reported from the Himalayan region [53].

Notably, the bees are considered as the most efficient biotic pollinators [54, 9, 55] and further investigation on them would probably help in enhancing the prospects of cultivated plants which rely on them. Our study on the long-term variation in foraging preferences of *Apis cerana* could probably serve as key towards better understanding of their pollination biology.

## 5. Conclusion

Seasonal variation in the pollen collected by *Apis cerana* worker was observed throughout the study. Pollen traps placed at the entrance of two bee colonies helped to ascertain the amount, dry weight and type of pollen foraged by the bees. Significant difference in temperature between the morning and midday sampling period affected the foraging behaviour of honeybees. This in turn led to a variation in the dry weight, amount and pollen type as collected by bees at two different times of the day. *Pisum sativum* and *Litchi chinensis* pollen types dominated the morning collections while *Rosa* and *Brassica* type pollen was predominant in the midday samples.

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