



INDIAN COUNCIL OF AGRICULTURAL RESEARCH, NEW DELHI



# INSECT POLLINATION MANUAL



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NICRA Project on  
**Effects of Climate Change on Pollinator Populations**

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## CONTENTS

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	<b>Particulars</b>	<b>Page No.</b>
1	Introduction	01
2	Flowering Phenology	02
3	Floral Biology	05
4	Flower visitors	15
5.	Synchrony between Flowering Phenology and Pollinator Populations	21
6	Pollinator exclusion experiments	21
7	Factors affecting flowering phenology and pollinator activity	22
8	Economic valuation of pollination service	23
9	Conserving pollinator populations	23
10	Further Reading	25
11	Appendices	
i	Illustrated key to Families, Subfamilies and common Genera of bees	27
ii	Key questions for Research in Pollination Ecology	39

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## 1. INTRODUCTION

It has been established that pollinating agents are essential for survival and reproduction of several wild plant species (Kearns et al., 1998) and in the recent years, there has been an increasing recognition of the importance of pollination, mostly by insects, in crop plants (Klein et al., 2007). The major pollinator dependent crops are fruit and vegetable crops, spices and plantation crops and pulses and oilseed crops. It has been estimated that the total annual economic value of crop pollination worldwide is about € 153 billion (Gallai et al., 2009). Klein *et al.* (2007) found that 87 of the world's leading food crops depend upon animal pollination, representing 35 percent of global food production. The area covered by pollinator-dependent crops has increased by more than 300 percent during the past 50 years (Aizen *et al.* 2008; Aizen and Harder 2009).

Losey and Vaughan (2006) also emphasized that flower-visiting insects provide an important ecosystem function to global crop production through their pollination services. The rapid spread of human habitation is affecting the available natural habitats through urbanization and other land-use practices, putting pressure on ecosystem services delivered by wild pollinators. At the same time, the demand for pollination in agricultural production will have to keep pace to sustain food production.

Plant-pollinator interaction in both wild and cultivated plant species is under threat (Biesmeijer et al., 2006) as a result of indiscriminate pesticide use (Kremen *et al.* 2002), habitat fragmentation (Mustajarvi *et al.* 2001; Aguilar *et al.* 2006) and intensified cultivation practices (Tscharrntke *et al.* 2005; Ricketts *et al.* 2008). Though climate change is projected to be a further cause of concern to pollination services (Memmott *et al.* 2007; Schweiger *et al.* 2010; Hegland *et al.* 2009), empirical studies explicitly linking the effects of climate change with plant-pollinator interactions are scarce and those on crop pollination practically non-existent (Visser and Both. 2005). In the fifth meeting of the Conference of Parties of the CBD held at Sao Paulo, in 1995, a decision was taken to establish an International Pollinators Initiative. The action plan for the IPI included six major areas of concern: 1. Reducing taxonomic impediment on pollinators;

2. Monitoring decline of pollinators; 3. Identifying the causes of pollinator decline; 4. Quantifying the economic value of pollinators to agriculture; 5. Conservation of pollinator diversity and 6. Sustainable use of pollinators.

India, being a signatory for the CBD, is obliged to take up studies on the importance of pollinators, which constitutes an important component of development. Unfortunately, we do not even have the basic information on the species of pollinators involved in the production of several crops. Therefore it is important to promote efforts to monitor the populations of various flower visitors and establish the relation between the flower visitors and their role as pollinators and in turn their contribution to increased productivity. The data collected over a period of time would enable us to correlate the various biotic and abiotic factors and to develop strategies to conserve pollinator populations.

In this context, the present manual is developed to provide a) standard protocols for pollination studies, b) methodology for assessment of pollinator populations and c) analytical techniques. Illustrated keys are provided for identification of bees up to families and genera that occur in India. The emphasis is mostly on bees, the more frequent flower visitors, though syrphids and other insects are also important.

## **2. FLOWERING PHENOLOGY**

Phenology is the timing of physiological stages such as growth and reproduction; accordingly, flowering phenology refers to the seasonal timing of flowering. Temperature, moisture and photoperiod are the three known factors that affect the phenology (growth and reproduction) of both plants and their pollinators (Partenen et al., 1998). Timing of flowering helps in maintaining reproductive isolation and in reducing competition for pollinators. Hence, in any pollination ecology study, it is important to record observations on flowering phenology of the crop/plant in question.

Methods for studying flowering phenology: There are two important components of flowering phenology viz., **flowering frequency** at the population level, and **flowering intensity** at the individual plant level:

**Flowering frequency** refers to the proportion of individuals in a population that are in flowering at any given time. To quantify this, a set of randomly selected individuals (~50) be tracked with time and from date of first flowering of any individual, number of plants in flowering be recorded each day till all of them stop flowering. From this, frequency or proportion of plants that are in flowering ( $P_i$ ) on any given day be arrived at as  $P_{id}$ . The  $P_{id}$  may be plotted on a graph along days (Fig.1).

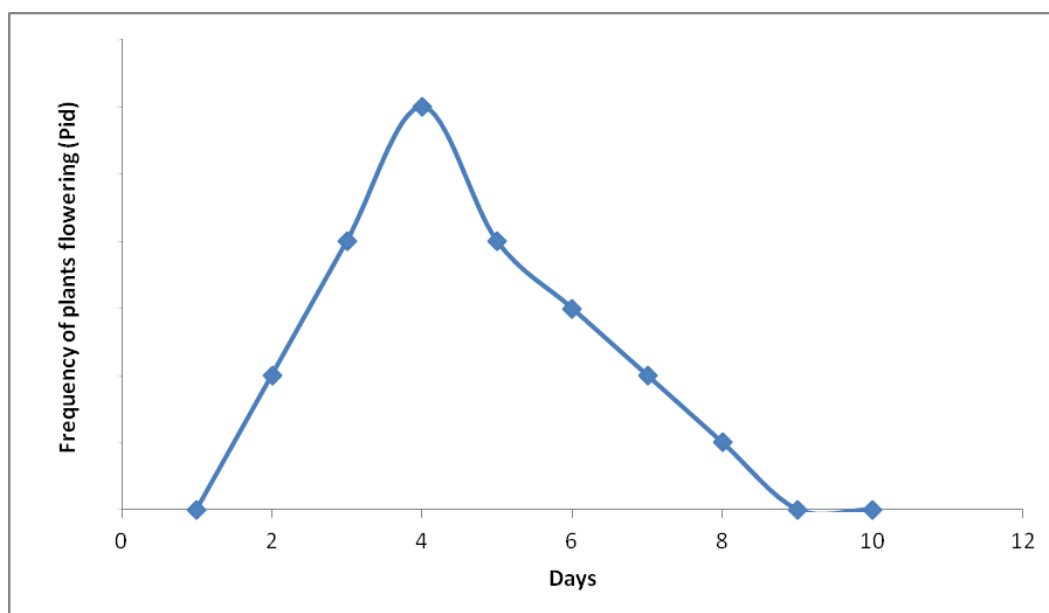


Fig. 1 Number of plants flowering in a population

**Flowering intensity** refers to the intensity of flowering of an individual plant. To quantify this, number of receptive flowers (or in bloom) be recorded at regular intervals (~each day) on a set of labelled branches or inflorescences ( $n = \sim 5$ ) on a set of randomly selected plants ( $n = \sim 5$ ). From such data available on about 20 – 25 inflorescences, the intensity of flowering be computed as the average number of flowers opening per inflorescence each day (**fid**). This data may also be plotted against days (Fig. 2).

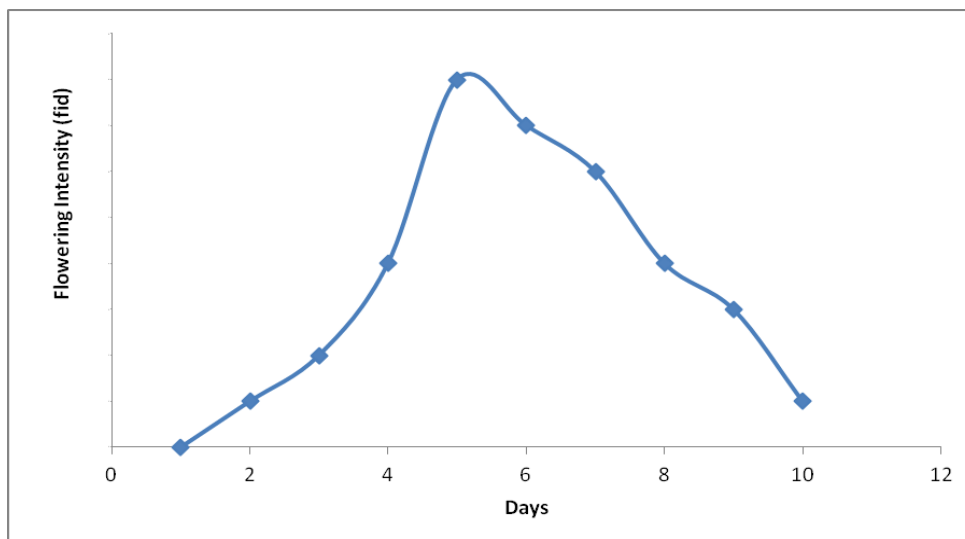


Fig. 2 Mean number of flowers opening per plant per day

Flowering Phenology (FP) may be computed as a product of  $Pid$  and  $fid$  and represented in a graph as shown below (Fig. 3). This can also be normalized for the product to compare among different plots and experiments.

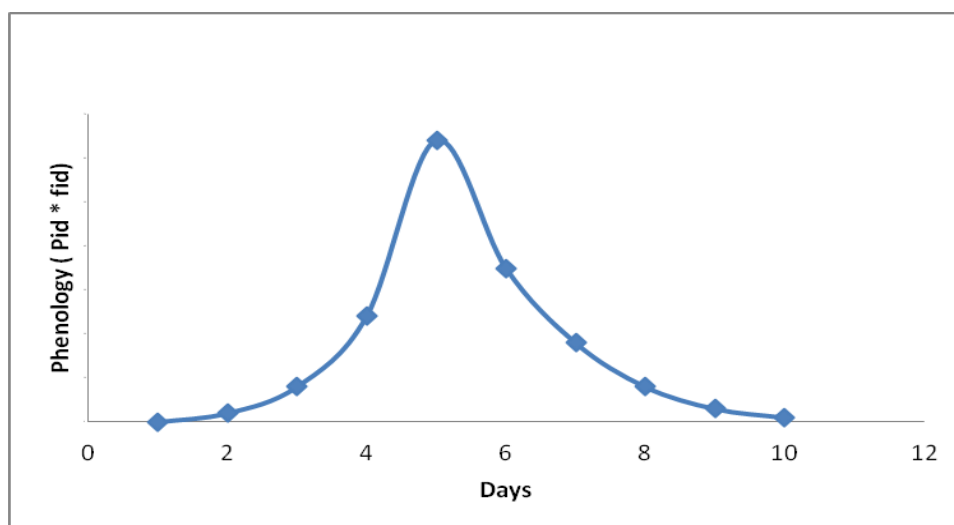


Fig. 3 Flowering phenology

### Additional Suggestions:

If the plant species is a perennial tree, plants/trees should be selected randomly and should be labelled properly for easy recognition, and from a sub set of them, 50 branches or 50 inflorescences may be tagged for recording observations. In some annuals, where the flower size is small and the numbers per plant large,

several micro-plots of  $1\text{m}^2$  may be randomly selected for recording observations. Often it may also be important to record the number of new flowers, number of old flowers and number of flowers that have either set fruits or dropped, on each day of observation.

Observations on flowering phenology may also include flora available in the vicinity of the target crop. Such long term observations on flowering phenology would provide information on temporal and quantitative variations in resource availability for flower visitors.

In studies involving effects of abiotic factors on flowering phenology, it is important to record observations on temperature, humidity and rainfall of the location. For studying the effects of climatic factors, the target crop can be cultivated in different altitudes and/or latitudes with differences in weather factors like temperature and rainfall and observations recorded and phenologies compared.

Observations recorded on phenology can be used to develop flowering curves, to understand the duration of flowering and realise the total number of days the resources remain available for flower visitors.

### **3. FLORAL BIOLOGY**

Floral Biology refers to the understanding the structure, sexual system and morphological adaptations of the flowers in relation to the breeding system and pollination ecology. Thus, a careful study of floral biology of a plant is important for discerning the mode of pollination (self or cross), types of pollinators and mechanisms of pollination involved.

#### Sexual systems and pollination

While most flowers have four whorls, two each of the covering layers (calyx and corolla) and reproductive organs (androecium and gynoecium), modifications are not uncommon. Accordingly, flowers can be unisexual (with separate male and



female flowers) or bisexual (hermaphrodite). Unisexual (male and female) flowers may be borne on the same plant as in ridge gourd (Monoecious) or on different plants as in papaya (Dioecious). Such plants necessarily depend on an agent for transfer of pollen. The agent can be wind, water, an insect, a bird or an animal. While self pollination is a common feature in bisexual flowers, several of these avoid self pollination through various mechanisms:

- a) **Dichogamy:** This is a condition wherein functionality of male and female organs are temporally separated. Accordingly anther dehiscence precedes stigma receptivity (Protandry) as in coconut, or follows stigma receptivity (Protogyny) as in bananas. Both the situations ensure cross pollination. The extent of such temporal isolation between the functioning of two sexes could be a few hours to even days (Fig. 4).

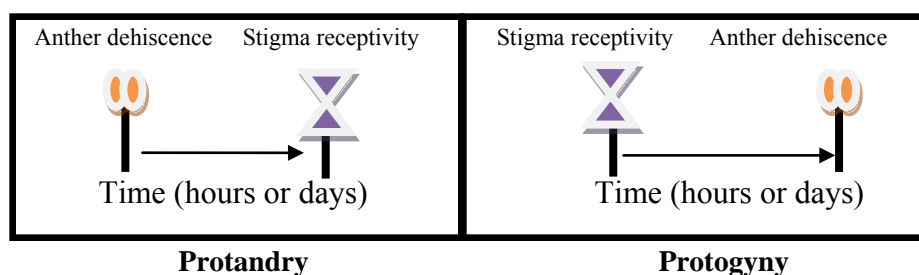


Fig. 4 Temporal difference in anther dehiscence and stigma receptivity

- b) **Herkogamy:** This is a condition wherein the male and female organs, though present in the same flower, are separated spatially or by physical barriers. The stigma may be placed above the anthers, so that the pollen grains of the flower have a very less probability of reaching the stigma of the same flower as in cardamom (Approach Herkogamy) or the stigma may be placed much below the anthers as in some species of orchids (Reverse Herkogamy). Occasionally these spatial isolation is also augmented by physical barriers as in *Calotropis*.

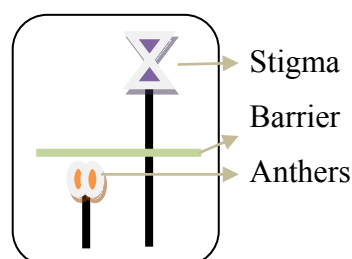


Fig. 5 Physical barrier for preventing self-pollination in hermaphrodite flowers

- c) **Self-incompatibility:** This is a condition wherein pollen grains of a flower may not be compatible for fertilizing the ovules of the same flower or that of the same plant (sporophytic self incompatibility). In some situations a set of pollen grains of a specific genetic composition are incapable of fertilizing the ovules of a specific genetic constitution. This situation termed as gametophytic self incompatibility, also leads to cross pollination as specific pollen grains only could be successful in fertilizing the flowers as in apple.

#### *Flower Polymorphism and Pollination:*

In some species of plants, two or three types of flowers are found (polymorphic flowers) on different plants in a population. Usually the difference will be in the length of stamens, relative to the position of stigma, when they are referred to as heterostylous. Heterostylous plants having two flower morphs are termed distylous. In one morph (termed *pin* or *longistylous* flower) the stamens are short and the pistils are long; in the second morph (termed *thrum* or *brevistylous* flower) the stamens are long and the pistils are short; the length of the pistil in one morph equals the length of the stamens in the second morph, and vice versa.

Heterostylous plants having three flower morphs are termed tristylous. Each morph has two types of stamens. In one morph, the pistil is short, and the stamens are long and intermediate; in the second morph, the pistil is intermediate, and the stamens are short and long; in the third morph, the pistil is long, and the stamens are short and intermediate.

#### *Studying floral biology*

Pollination mechanism of a given species can be better interpreted through a detailed study of its floral biology. For this a systematic recording of the following parameters is recommended:

- a. Date of bud initiation and days taken for the full bud development
- b. Time of anthesis (opening of the flower)
- c. General structure of the flower
  - i. Number of anthers and position of anthers (in relation to stigma and other floral whorls)
  - ii. Length of style and position of stigma ( in relation to anthers)
  - iii. Number of ovules
- d. Number of pollen grains per anther and per flower
- e. Time of anther dehiscence
- f. Pollen viability
- g. Stigma receptivity
- h. Location of nectar source/ nectar glands
- i. Nectar quality - % sugar
- j. Nectar quantity
- k. Longevity of flowers
- l. Per cent fruit set in general

#### *Methodology involved*

- a. **Date of flower bud initiation:** Tag 100 plants or shoots and observe as frequently as possible to record developing flowering shoots or formation of buds. Once bud initiation has started, tag 100 freshly formed buds to record the date of their opening and sequence of opening on the inflorescence. Other details needed for recording the flower phenology are provided in a separate section.
- b. **Time of anthesis:** Anthesis, the process where anthers dehisce rendering the pollen grains available for cross pollination, can be observed by the physical process where pollen is sprinkled or sprayed as yellow dust around by the anthers. This stage determines the temporal window when the pollen donation occurs.

#### Additional suggestions

For recording this, a reasonable number of buds (preferably 100 buds) may be tagged and monitored for the time of opening. Flowers open very early in the morning, or in the evening or at night depending upon the species. The procedure may be repeated several times on different days during the flowering period. When the buds are opening, it is important to record the

exact time of anthesis for the species being studied and of the time when they are ready for insect visitation. The colour of the flowers and their attractiveness could help assessing the diurnal or nocturnal nature of flower visitors.

It is also important to record the nature of pollen grains – whether they are powdery, dust like or sticky, which is an indication whether the flowers are wind pollinated or insect pollinated. Usually pollen grains are sticky in insect pollinated flowers.

- c. **Flower structure:** For most species, the structure of the flower may be available in the literature. However, it is useful to make a detailed study on the flower structure to get first hand information. While observing the flower structure, it is important to record if there are any special modifications to attract flower visitors – like landing platforms for bees, nectar guides, etc. Measure the length of corolla tube (in tubular flowers). Count the number of anthers, length of anther filament, variation in length of anther filaments, size of anther lobes, variation in anther lobe size if any, and position of anthers in relation to the position of stigma. Also record the length of style and position of stigma. Make a detailed sketch of the flower structure.
- d. **Number of pollen grains:** A minimum of five completely matured anthers just before dehiscence are transferred on to a cavity slide with a drop of water and crushed well with a spatula to get all the pollen grains out to the water. The contents are then transferred on to a glass vial and the volume be made up to (5 ml or 10 ml) using of 70% ethyl alcohol. From this, a known amount, about 1 ml is transferred to a cavity slide and the pollen grains are counted using a haemocytometer or a binocular microscope fitted with a grid. The total pollen grains in the vial and hence for all the collected anthers can be determined using the counts. From this, the number of pollen grains per anther, total pollen grains per flower and per plant can also be computed.

- e. **Pollen viability:** Pollen viability refers to the profile of the germination percent of the pollen grains with time. Generally, the pollen viability would be maximum at anthesis or dehiscence and decreases thereafter. Understanding the pattern of this decreasing viability (and hence the period up to which the pollen grains retain their viability) is essential both for assessing the time available for the transfer of the pollen grains on to the stigma, and also for breeders to achieve effective crossing in their breeding programmes.

*Methodology:* Pollen viability may be assessed either *in vitro* or *in vivo*. In the former, the pollen grain are grown in an artificial medium to record the percent of them germinating while in the latter, ability of pollen to grow and fertilize on the flowers would be used as an index.

- a. ***In vitro* analysis:** For determining temporal profile of pollen viability, pollen grains are sampled from the flowers at periodic intervals during the day commencing from just prior to the time of dehiscence till the flower withers off or till the pollen collectors cease to visit the flowers. Occasionally, in some species, inflorescences can be collected and kept at the lab in a beaker containing water and used for periodic sampling. While the interval of sampling may be decided based on the length to which the flowers remain attractive, as a thumb rule, a minimum of 5 to 6 time periods be considered from opening to drying of the flowers. Once the pollen grains are collected they are tested for germination as given below.

*Germination tests:* i) One simple methods to check viability of pollen grains would be to place a known number of pollen grains on a cavity slide containing sucrose solution and recording their germination. However, the per cent sucrose solution ideal for germination varies with the species and needs to be determined in advance. This can be done by using 1, 2, 5, 10, 20, 30 and 50% sucrose solution. The tests

can be replicated several times to find out the ideal concentration to be used.

The cavity slides containing pollen grains in the sucrose solution should be placed in a petriplate with a wet filter paper to maintain humidity. Observations should be recorded on the number of pollen grains with pollen tubes after six, eight, ten and 12 hours.

ii) Brewbaker – Kwack Medium can also be used instead of sucrose solution. It is prepared by dissolving

10% sucrose (to be adjusted according to species) + 100 mg/l Boric acid + 300 mg/l Calcium nitrate + 200 mg/l (Magnesium sulphate heptahydrate) + 100 mg/l  $\text{KNO}_3$  (Potassium nitrate) in distilled  $\text{H}_2\text{O}$ .

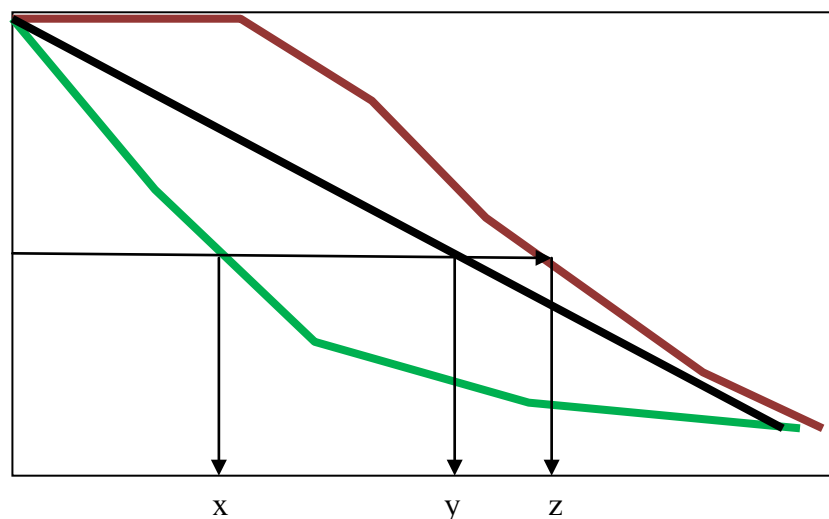


Fig. 6 The viability profiles and time to which the pollen grain remain viable may vary among species. Accordingly the 50 % viability period may be estimated (as shown by arrows as example for three species x, y and z)

The percent germination thus assessed be plotted on a graph along time and the time taken for 50 % of the viability loss be estimated as the pollen viability (Fig. 6). The critical percent of viability accepted may vary depending up on the needs. For example for breeders, it is preferred that the percent viability is about 90 % w hen artificial pollination is attempted.

**b) *In Vivo* analysis:** A set of 25 flower buds may be randomly selected and each bud is covered with a butter paper packet. After anthesis, a known number of pollen grains collected from another flower are placed on the stigma of each flower. Five of these flowers are collected after two hours and brought to the lab, stigmas extracted along with the style, placed on a slide with a drop of glycerine and carefully dissected to observe for germinating pollen grains. The number of pollen germinated and the length of pollen tubes could be recorded and the per cent germination may be worked out. The procedure may be repeated every two hours to determine the duration for which the pollen grains remain viable after dehiscence.

Pollen viability can also be assessed *in vivo* by the extent of fruit set by artificially pollinating flowers at different intervals. For this a set of 25 flowers are artificially pollinated, at hourly or two hourly intervals and prevented from any insect visitation. Monitoring fruit set in these flowers offer a clear picture on how long the pollen will remain viable after dehiscence.

- f. Stigma receptivity: The above mentioned *in vivo* and *in vitro* studies on pollen viability, done at intervals of every two hours will also give information about, when stigma receptivity will be maximum.
- g. Nectar and nectar quality: It is important to locate the nectar glands in the flower and estimate the quality and quantity of nectar produced from the point of view of insect visitors. The nectar quality may be determined using a hand held refractometer. One drop of nectar may be removed from the flower using a micro-syringe and put on the platform of the refractometer and readings on TSS may be recorded. The sugar concentration in nectar should be measured in different times of a day (morning, afternoon and evening; or at intervals of two or three hours) and also under different weather conditions (sunny, cloudy, rainy). The observations should be repeated at least five times with ten replications each time to get the mean and standard deviation.

- h. Nectar quantity: Total quantity of nectar produced in a flower can be measured by closing a set of flowers using paper packets to prevent flower visitors for a known length of time (2, 4, 6 hours) and the nectar can be removed completely using a capillary tube or a micro-syringe and measured in  $\mu\text{l}$ .

Nectar measurement should be done before and after insect visitation to estimate the quantity of nectar taken by an individual flower visitor. This data can be used to compute the number of flowers a single insect has to visit to fill its crop.

- i. Longevity of flowers: The duration for which a single flower remains open helps in understanding the pattern of nectar/pollen availability for flower visitors. A set of 100 flowers may be tagged in the bud stage and monitored from the day and time of anthesis till the petals drop or the flowers wither. Depending on the species, the flower longevity may range from a few hours to several days. If the longevity is more than a day, it is also important to record whether on all the days the flower keeps producing nectar and remain attractive to flower visitors and whether there will be any change in the colour of petals which can be correlated with successful pollination.
- j. Per cent fruit set: Before commencing any studies on pollination biology of the plant species, it is necessary to have an idea about the fruit set. Twenty five to 100 flowers may be randomly selected and all flowers (or 100 flowers, if the number of flowers is very large) may be tagged and followed till fruit set, fruit retention and harvest.

It should be noted that 100% fruit set in any plant/crop is not possible. For each species there is an optimum level of pollination. For example, a ten year old mango tree, produces about 1000 fruits in a season, which is about 200 kg. The fruit set in mango ranges from 0.1% to about 3.5 per cent depending on the variety. Supposing with a fruit set of 0.1% the tree is producing 200 kg of fruits, imagine the fate of the plant if more than 10 per cent of the flowers set to fruits and if all the fruits are retained. Identifying



and studying the behaviour of pollinators in mango would help us to enhance the fruit set considerably but not anywhere close to 100%. If the fruit set is increased abnormally, it may result in smaller fruits or the branches may break due to the weight of fruits or sometimes the plant may die due to exhaustion. So, there is an optimum fruit set for every species that depends on optimum pollination.

*Optimum pollination:* Pollination that leads to maximum fruit or seed set leading to maximum sexual reproductive output given the current available resources over the lifetime of the plant. In the case of crops, this refers to the agricultural output that depends upon pollination, and it takes into account the production objectives in relation to the market and the sustainability of the crop management. To define pollination deficits, it is necessary to define (and understand) how to attain optimum pollination levels.

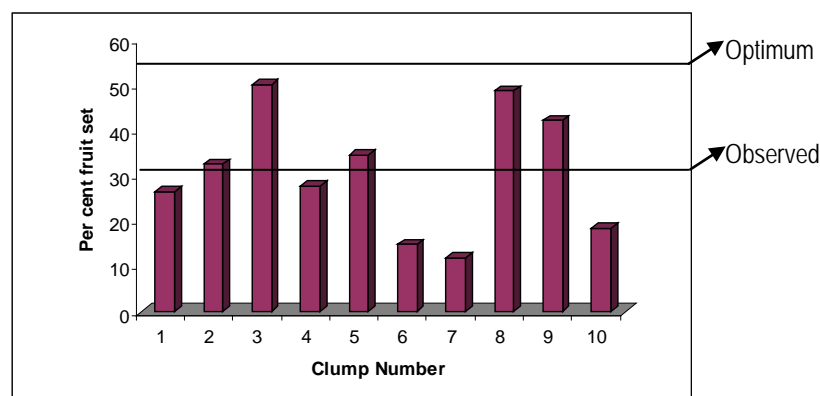


Fig. 7 Observed and optimum fruit set in cardamom indicate pollinator deficit

*Pollination deficit:* Quantitative or qualitative inadequate pollen receipt which decreases the sexual reproductive output of plants (from Wilcock and Neiland (2002) who defined the concept of pollination failure).

#### 4. FLOWER VISITORS

***Identification of flower visitors:*** Most flower visitors can be identified to the species or at least to the genus level using the key provided in Appendix 1.

**Diversity and abundance of flower visitors:** All flower visitors need to be collected and various methods of collection may be adopted including sweep net sampling, transect sampling, pan trap sampling, using malaise traps, interception traps, bee bowls, etc.

**Sweep net sampling:** A standard insect net is to be used. Each sample should be of at least ten full sweeps (to and fro) on each sampling day.

**Transect sampling:** In a field with the crop in bloom, transect sampling can be done to record the activity of pollinators. Depending on the size of the field, 10 or 15 or 25 m long transects can be made. In each transect of the predecided length, one has to walk slowly, to cover the entire distance in a time span of five or ten minutes. While covering a transect, the species of flower visitors and the numbers of each species can be recorded. On a given sampling day a number of such transect samples can be taken to determine the diversity and abundance of flower visitors.

**Pan traps:** Most insects get attracted to yellow colour and yellow pan traps can be used to catch many flying insects. Yellow pan traps are shallow trays painted yellow using either enamel yellow paint or fluorescent yellow. The tray should be half filled with water and a few drops of liquid soap may be added. The soap helps in making the insects that fall into the trap to drown. The traps should be placed on the ground in the field.



Fig. 8 Yellow pan trap

Several traps may be placed separated with a distance of at least ten meters and each should be numbered. The pan traps can be kept for 24 hours before recording observations on flower visitors.



Fig. 9 Bee bowls

Bee bowls: Using bee bowls is a standard methodology for monitoring pollinator populations developed by the FAO. These can be prepared using small ice cream cups painted on the inside with fluorescent yellow, white and blue paints. The bowls are to be filled half 5% soap solution. In any plot 24 bowls (8 of each colour) are to be placed five meters apart randomly and numbered. The bowls can be placed on the ground or if possible at about canopy height. For

example, if you are planning to collect flower visitors of brinjal, about 90 cm long PVC pipes of 6 cm diameter can be fixed in the field on which the bee bowls can be placed. The bee bowls can be kept in the field for 10 hours (morning till evening) or for 24 hours on the day of sampling.

While collecting specimens from the pan trap or the bee bowls, you need to carry a tea strainer or a sieve and specimen tubes (as many as the number of traps). You also should carry a larger bottle or beaker to drain the soap water through the strainer. The specimens from the strainer should be transferred to a separate specimen tube or a paper packet, with proper label including the date, crop, location and trap number.

After returning to the lab, the specimens should be washed with running water, dried on filter paper and then mounted using appropriate insect pins and labelled.



Fig. 10 Malaise trap and interception trap

Malaise traps and interception traps:

These are traps for flying insects for unbiased sampling. These can be used for additional collection of flower visitors active in the field.

***Diversity Index***: Index of diversity can be determined with the data on number of species of pollinators and numbers of each species collected or recorded on a crop or location. The diversity indices can be compared between locations, between crops, between different days of flowering, etc. More commonly Shannon-Weaner diversity index and Berger-Parker dominance index are used for

comparisons. The Shannon-Wiener Index of Diversity can be calculated using the formula:

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

Where H' is the Shannon-Wiener Index of diversity

$p_i$  is the proportion of the  $i^{\text{th}}$  species

Here is an example:

Suppose the flowers of coffee are visited by six species of bees and the observations record a total of 412 visits including the frequency of visits by each species.

<b>Pollinator species</b>	<b>Frequency of visits</b>	<b><math>p_i</math></b>	<b><math>\ln p_i</math></b>	<b><math>P_i \cdot \ln p_i</math></b>
Species 1	24	0.058	-2.847	-0.165
Species 2	3	0.007	-4.962	-0.035
Species 3	291	0.706	-0.348	-0.246
Species 4	41	0.1	-2.303	-0.230
Species 5	40	0.097	-2.333	-0.226
Species 6	13	0.032	-3.442	-0.110
<b>Sum=</b>	<b>412</b>	<b>1</b>		<b>-1.012</b>

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

So,

$$H' = -(-1.012) = 1.012$$

The Berger-Parker Index gives an idea of about the most dominant species of pollinator when several species visit the flowers of a particular crop. Based on this most potential pollinator may be identified. This index is calculated using the formula,

$$d = N_{\max} / N$$

where  $d$  is the index of dominance,  $N_{\max}$  is the highest number of individuals represented by a species and  $N$  is the total number of individuals in a sample.

In addition there are several other methods by which diversity index can be determined (Southwood and Henderson, 2000).

*Procedure for recording diversity and abundance of flower visitors:*

All insects visiting the flowers should be recorded in different times of the day.

*Ad-libitum* sampling may be followed. If the species being observed is known to get insect visitation during the day time, the entire day may be divided into several observation time units of ten minutes every two hours starting at 5 or 6 am and continued till 6 or 7 pm. Observations can be recorded in different ways.

1. At each observation time unit, at least five or ten flowers should be constantly observed for ten minutes for all the insect visitors. Observations should be recorded on the species, numbers of each species, whether the insect collects pollen or nectar or just hovers over the flower and leaves.
2. If the plot is long or large, transect sampling can be done.
3. A passive method of recording the activity of flower visitors in the field is by using bee bowls or yellow pan traps.
4. Bee bowls can be placed continuously or run at weekly intervals depending on the plant species to see the fluctuations in the populations of insects caught.

***Time activity budget for pollinators:*** Once a most frequent flower visitor is recognised detailed observations on the behaviour of the selected species can be taken up. These studies would include the foraging behaviour of the pollinator by developing a repertoire of activities and recording the relative time spent by an individual on all these activities. To make such detailed observations it is

necessary to mark the individual insects uniquely so that each one can be observed for their behaviour.

Marking bees: Individual bees can be marked using quick drying paints. Quick drying paints are available in hobby stores, where it is sold for children interested in model aircrafts and automobiles. The paints are non poisonous and dry quickly. A foraging bee should be collected with an insect net and held tightly so that its dorsal aspect of thorax is clearly visible through the mesh of the net. An insect pin head may be dipped in the desired paint and a spot may be placed on the thorax through one of the perforations. After marking, the bee may be left to stay within the net for a few minutes, so that it becomes normally and during this time, the colour, shape and position of the mark should be noted in a field note book giving it an unique number. It is almost impossible to mark any two bees the same way and hundreds of bees can be marked with six or eight shades of colours. Once the bee is released, the bee resumes its foraging, though very rarely it may fly away.

Bees can also be marked using fluorescent powders. Fluorescent powder of a given colour can be dusted on foraging bees. This way it may not be possible to identify the individual but one can recognise the marked bee and record observations on patch fidelity.

Preparing a repertoire of behaviours exhibited by foraging bees: A list of all the behaviours exhibited is to be made. The list may include the following: foraging (visiting flowers), flying (between flowers or plants), sitting, grooming, loading pollen to carbigulum, etc. Time that a marked bee spends on each of these activities should recorded.

***Foraging behaviour:*** Individual marked foragers can be followed to observe their foraging behaviour. Following observations may be recorded:

1. Time spent by individual foragers per flower
2. Number of flowers visited per unit time
3. Number of flowers visited per plant
4. Number of plants visited in a single bout

5. Movement pattern: whether the forager moves up an inflorescence or it moves down in plants with vertical inflorescences; whether it visits nearest neighbouring flowers; whether it visits flowers in a plant at random
6. Time spent in flight between flowers and between plants,
7. Number of trips made by a bee, etc.

Time spent may be recorded using a stop watch.

It is also necessary to have as many replications as possible on all these behaviours. Observations should also be recorded in different times of the day, under different weather conditions; at different flower densities, etc.

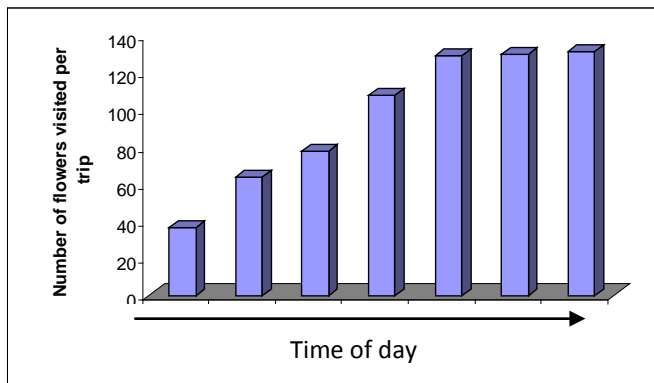


Fig. 11 Number of flowers visited in different times of a day

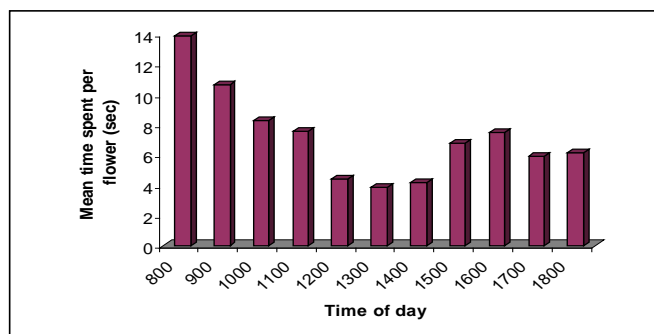


Fig. 12 Time spent per flower by a single bee

Time spent for various activities may vary in different times of a day. For example, a bee may spend more time per flower and visit less number of flowers in the morning hours compared to afternoon or evening hours (Fig. 11). This is directly related to the quantity of nectar available per flower per visit, which will probably be due to competition between foraging bees.

Observations on the rewards collected by individual foragers should be recorded.

## 5. SYNCHRONY BETWEEN FLOWERING AND POLLINATOR ACTIVITY

When the crop or plant species is flowering, the pollinator should be active, in other words, flowering and pollinator activity should be synchronous. Even if there is a slight shift in either of flowering time or pollinator

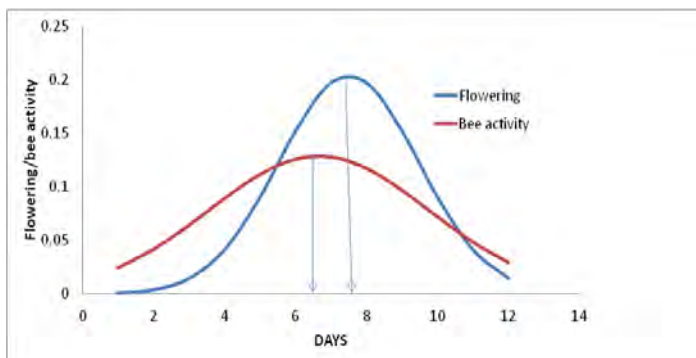


Fig. 13. Extent of overlap in flowering and bee activity

availability time, there can be significant effects on the fruit set and yield of the crop. Hence it is necessary to plot the two over time (Fig. 13) and see to what extent there is an overlap between the two and if there is

difference, whether some corrective measures can be taken up.

## 6. POLLINATOR EXCLUSION EXPERIMENTS

As mentioned earlier, a plant species may be self compatible, might be self pollinated but still might benefit from visitation by bees. Many species of plants which are believed to be self pollinated have been known to set higher percentage of seeds when bees visited flowers. This means there can be a certain percentage of cross pollination in all species of plants, excluding cereals. Hence, the first thing to determine is the extent of cross pollination in the target plant species. This can be done through some simple experiments.

If the flowers are large enough, individual flower buds can be closed using paper packets or muslin cloth bags to prevent the visitation by any flower visitor when the flower remains open. A set of 50 flower buds may be closed this way and another set of 50 buds may tagged on the same day and left for open pollination. Recording the number of fruit/pod/seed set in these two sets gives an indication about the percent self pollination and the extent of cross pollination in the species



If the flowers are small or very small and are in clusters, the entire branch may be enclosed in a cloth sleeve to prevent flower visitors. Care should be taken to remove all open flowers and set fruits/pods and retain only buds in the branches before enclosing. At least ten such branches should be maintained and an equal number of branches with only buds should also be tagged for recording per cent fruit set in the two treatments.

If the plants are small, entire plant may be enclosed in a field cage at flower bud initiation. Ten or 20 plants may be caged like this and a similar number of plants may be tagged and left for open pollination. Per cent fruit set in both may be recorded.

## **7. FACTORS AFFECTING FLOWERING PHENOLOGY AND POLLINATOR ACTIVITY**

Both plant and its pollinators are greatly influenced by environmental factors. Temperature affects the flowering phenology of a plant species and also the activity of the pollinators. In addition to temperature, humidity, wind speed, rainfall, light intensity, cloudiness, sunshine, etc. may affect the flowering behaviour of the plant and also the foraging activity of the pollinator. Hence, it is necessary to record most or all of these parameters which may help in correlating the different abiotic factors with both flowering and pollinator activity.

There can also be difference between locations, altitudes and terrain of the fields where the study is undertaken.

Another important factor that affect the pollinator activity can be competition. If in the vicinity of the target crop, there is another species that is profusely flowering and offering more quantity and better quality nectar, the target crop may not get any visits by the pollinator species.

## 8. ECONOMIC EVALUATION OF POLLINATION SERVICE

Pollination is often considered as a free natural ecosystem service. As said earlier it is taken for granted. In fact, if the pollinators were not there, we would not have had many of the fruits, vegetables, pulses and oilseeds that we consider as important in our daily diet for maintenance of our nutrition and health. On which ever crop we work on, it is important to determine the value of the pollination service. It is important to express the increased fruitset or yield of the target crop in terms of monetary benefit derived. For example, arabica coffee, though can set berries through self pollination, when bee visits are allowed the berry set will almost double. Which means if an hectare of coffee which has adequate bee population, produces 1000 kgs, at the rate of Rs. 3000 per bag of 40 kg, bees would be responsible for nearly Rs. 32,000/- of the total income from one hectare. Unfortunately we do not have such data for many of the common crops that are insect pollinated.

## 9. CONSERVING POLLINATOR POPULATIONS

The crop plant species which need pollination service will be in flower only in certain months or seasons of the year. During other parts of the year, it is necessary to understand on what plants the pollinators forage. Hence, it is necessary to develop a floral calendar for the given region. If the target crops are closer to natural habitats like forests the plant species closer to the cropped area should be identified and the flowering phenology of each species should be recorded. In addition, the common weeds and other species of plants in the vicinity of the crop are also to be identified and their flowering times recorded. All these information can be used to analyse their importance in conserving populations of pollinators in the fields.

Nesting sites: Pollinators, whether they are social bees or solitary bees, require nesting places. In pollination studies it is also essential to locate the nesting sites of the common pollinator species and make efforts to conserve them. *Apis cerana* nests in existing cavities like tree hollows or burrows in

soil. Non-Apis bees which are solitary, require entirely different kinds of nesting sites. Leaf cutter bees nest in existing holes in plants or in the soil, digger bees nest in soil and several other bees like the *Ceratina* spp. bore into the cut ends of dry twigs for nesting. Out of ignorance, these nesting sites are disturbed and the populations of pollinators decline. Instead, attempts can be made to increase their populations by providing artificial nesting sites.

Trap nests: Artificial nesting sites are also called trap nests. These can be prepared by drilling holes of 2, 4, 5, 8 and 10 mm dia into wooden pieces. Ideally any soft wood 4" thick, 4" wide and 12 or 24" long can be used. In these pieces, as many holes as possible may be drilled using the corresponding drill bits and an electric drill. The trap nests can be placed before flowering of the crop so that the leaf cutter bees or other solitary bees start nesting.

## 10. FURTHER READING

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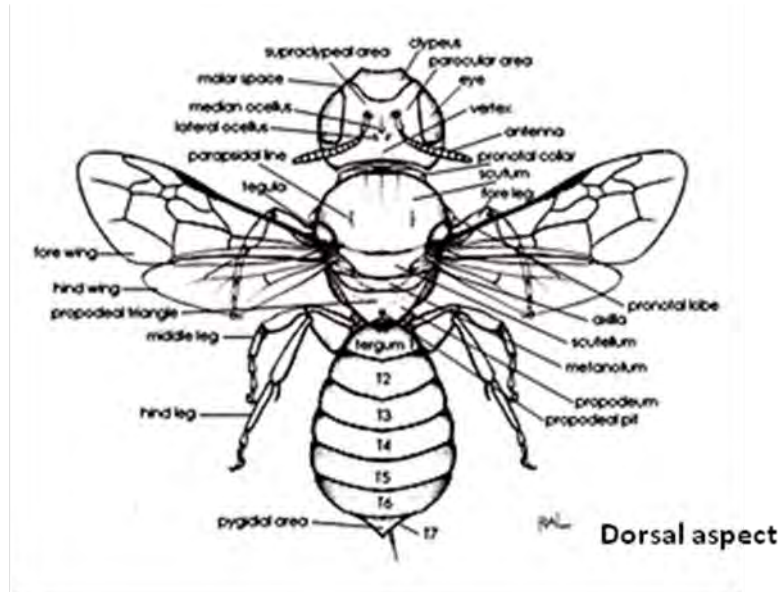
## **Appendix I**

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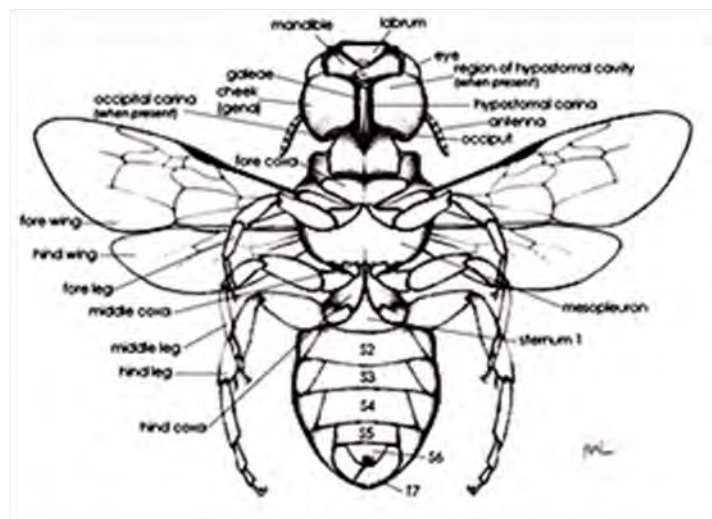
### **Key to Families, Subfamilies, Tribes & Genera of Bees**

Appendix 1

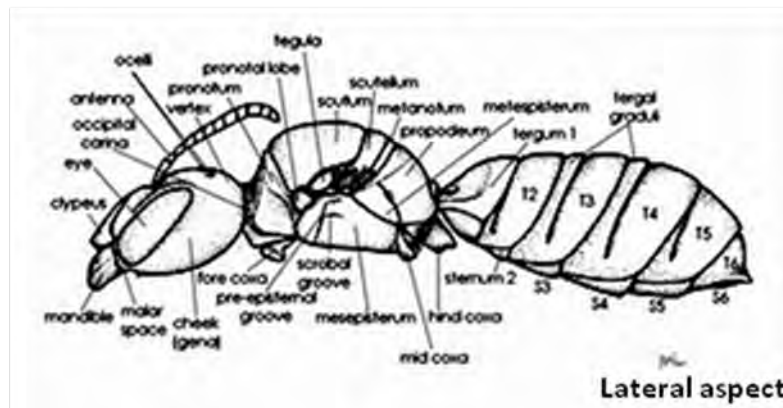
Characters used in identification of bees



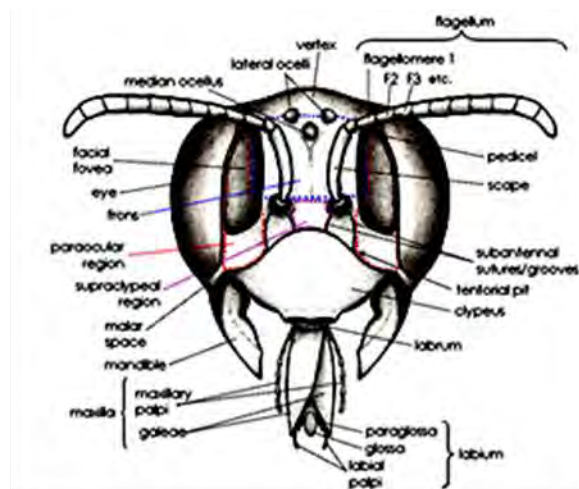
Dorsal aspect



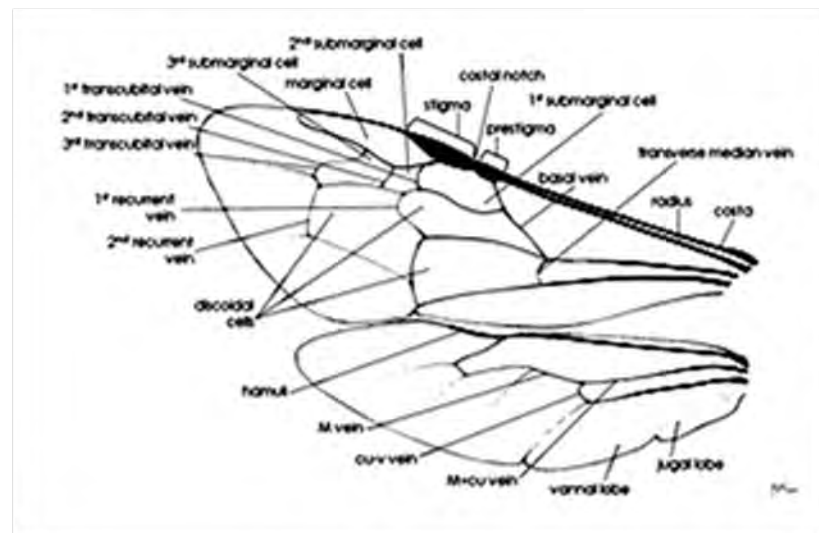
Ventral aspect



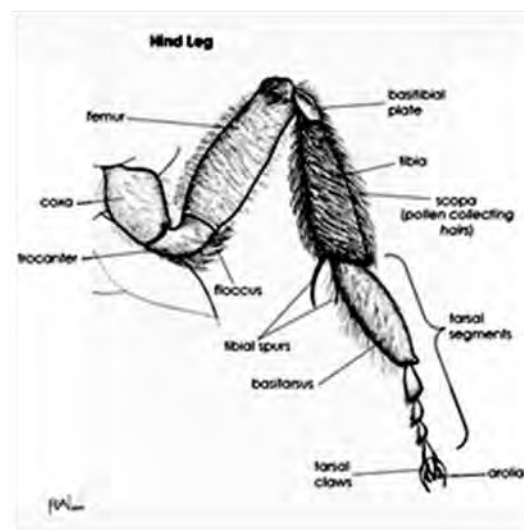
Lateral aspect



Head Front View



Wings

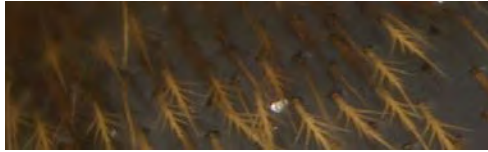


Hind leg



### A. Illustrated key to Apoidea

1. Body hairs branched; first segment of hind tarsus wider than remaining segments .....Apiformes



- Body hairs not branched; first segment of hind tarsus as wide as remaining segments .....Spheciformes



### B. Key to Families of Apiformes

1. Forewings with three submarginal cells; scopa on hind leg .....2



- Forewings with two submarginal cells; scopa on ventral aspect of abdomen .....Megachilidae



2. Facial fovea present; two subantennal suture below each antenna .....Andrenidae



- Facial fovea absent; one subantennal suture below each antenna .....3

3. Episternal groove present .....4
- Episternal groove absent .....Apidae
4. Glossa pointed; basal vein strongly arcuate .....Halictidae
- Glossa bilobed or broadly truncate; basal vein gently arcuate .....Colletidae



**C. Key to Genera of Colletidae**

1. Body densely hairy; female with scopa on underside of hind femur; wings with three submarginal cells; second recurrent vein sigmoid with posterior half arcuate distad; without any maculations on face .....*Colletes* Latreille



- Body with hairs short and relatively sparse; scopa lacking; wings with two submarginal cells; second recurrent vein straight; small black bees, yellow or white maculations on face .....*Hylaeus* Fabricius

**D. Key to Subfamilies of Halictidae**

- 1 Episternal groove distinct and directed strongly downward below scrobal groove .....2



- Episternal groove below scrobal groove absent or represented by weak depression .....**Nomiinae**

- 2 Prepygidial fimbria of female divided by longitudinal median zone .....**Halictinae**



- Prepygidial fimbria of female not divided medially .....**Nomioidinae**

**E. Key to Genera of Nomiinae**

- 1 Tegula large, extending well behind level of scutoscuteellar suture; Preoccipital carina present .....***Pseudapis* Kirby**



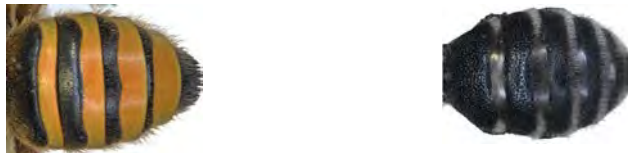
- Tegula small, not reaching level of scutoscuteellar suture; preoccipital carina absent .....2

- 2 Submarginal cells two; male antenna with last two segments enlarged and flattened .....***Steganomus* Ritsema**



- Submarginal cells three; male antenna usually not enlarged and flattened at apex .....3

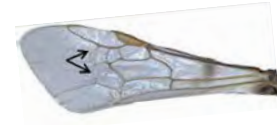
- 3 Marginal zone of T2 to T4 and often T1 except near bases hairless, impunctate, usually opaque white, yellow, green, or blue, although some times concolorous with rest of metasoma .....***Nomia* Latreile**



- Marginal zone T1 to T4 variable, often hairy, punctate; the zones never white, yellow, green or blue .....***Lipotriches* Gerstaecker**

**F. Key to Genera of Halictini**

- 1 Distal cross veins of the forewing as strong as first submarginal cross vein



.....2

- Third and often second submarginal crossvein and second recurrent vein weaker than nearby veins

.....*Lasioglossum* Curtis

- 2 Scopa present; T5 of female with longitudinal median finely punctate and hairy specialized area; female clypeus with truncate margin

.....*Halictus* Latreille

- Scopa absent; T5 of female without longitudinal median specialized area; clypeus with straight margin

.....3

- 3 First two flagellar segments of male distinctly broader than long; body with moderate to fine punctation

.....*Eupetersia* Blüthgen



- Second flagellar segments of the male longer than broad; body and especially propodeum coarsely pitted

.....*Sphecodes* Latreille

**G. Key to Tribes of Subfamily Megachilinae**

- 1 Pygidial plate of male present, that of female represented by apical spine; outer surface fore & mid tibiae, with coarse tubercles not ending in bristles

.....**Lithurgini**  
Genus *Lithurgus* Berthold

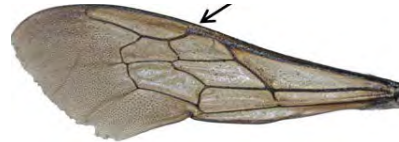
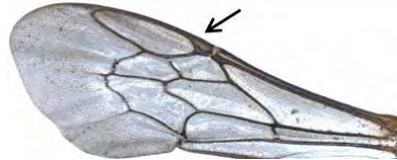


- Pygidial plate absent; outer surface of tibiae without tubercles

.....**2**

- 2 Stigma less than twice as long as broad; prestigma short, less than twice as long as broad; female claws cleft or with an inner tooth; body with yellow or white integumental markings

**Anthidini**



- Stigma over twice as long as broad; prestigma much more than twice as long as broad; claws of female usually simple; body almost without yellow or white integumental marks

.....**3**

- 3 Arolia present; body sometimes metallic green, blue, or brassy

.....**Osmiini**

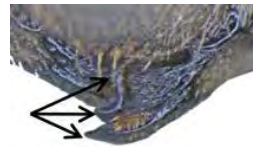
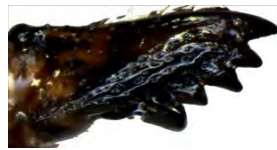


- Arolia absent; body non metallic or nearly so

.....**Megachilini**

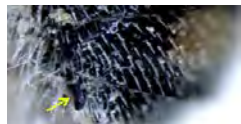
**H. Key to Genaea of Anthidini**

- 1 Female Mandibles with 5 to 18, sharp teeth separated by acute notch; maxillary palp minute, two segmented .....***Anthidium Fabricius***



- Female Mandibles with 3 to 4 rounded teeth; maxillary palp three to four segmented .....**2**

- 2 Female T6 with one or more tubercles on lateral side .....**3**



- Female T6 without tubercles on lateral side .....***Anthidiellum Cockerell***

- 3 Face with three longitudinal ridges or carinae, two of them juxtantennal carinae; mesepisternum in front of middle coxa with strong vertical ridge; scutellum produced as two broad, flat lobes overhanging matanotum and propodeum .....***Euaspi Gerstaecker***



- Face without longitudinal median ridges, juxtantennal carina present although sometimes weak; mesepisternum in front of middle coxa without vertical ridge; scutellum rounded .....***Eoanthidium Popov***

**I. Key to Genera of Osmiini**

- 1 Parapsidial line punctiform or rearily short linear, one fifth as long as tegula or usually less; body commonly with some greenish or bluish metallic color .....***Osmia Panzer***



- Parapsidial line linear, at least one fourth as long as tegula; body nonmetallic .....***Heriades Spinola***

**J. Key to Genera of Megachilini**

- 1 Scopa present on S2 to S4 or S6 metasoma not tapering throughout its length; axilla rounded **Megachile Latreille**



- Scopa absent on S2 to S4 or S6 metasoma tapering from near base to narrow, often acutely pointed, apex ; axilla almost always produced posteriorly spine **Coelioxys Latreille**

**K. Key to Subfamilies ad Tribes of Apidae**

- 1 Female scopa when present, forming a corbicula on posterior tibiae; pygidial and basitibial plates absent; jugal lobe of hind wing absent .....**Apinae, Bombini**  
Genus **Bombus Latreille**



- Female scopa when present, not forming a corbicula; pygidial and basitibial plates frequently present; jugal lobe of hind wings almost always present .....**2**

- 2 Stigma large; wings distally pubescent; flagellar segment 1 shorter than 2 & 3 together ; arolia present; usually slender, < 12 mm .....**3**

- Stigma usually absent; wings distally strongly papillate; flagellar segment 1 as long as broad, longer than 2 and 3 taken together; arolia absent; usually robust forms, more than 13mm long **..Xylocopinae, Xylocopini**  
Genus **Xylocopa Latreille**



- 3 Pygidial plate present, in females and most males; epistomal suture not as above; clypeus strongly protuberant .....**5**



- Pygidial plate absent; clypeus not or weakly protuberant .....**4**

- 4 Submarginal cells 2; clypeus typically slightly

constricted at level of tentorial pits, near middle of clypeus; apical metasomal terga of female depressed

**..Xylocopinae, Allodapini**  
Genus *Braunsapis* Michener



- Submarginal cells three; clypeus not constricted at level of tentorial pits, above middle of clypeus; apical metasomal terga strongly convex, not depressed

**...Xylocopinae, Ceratinini**  
Genus *Ceratina* Latreille

- 5 Labrum longer than broad or rarely about as long as broad; scopae absent; body rather short haired, or if long haired, then metasoma with patterns of white, appressed hair-spots or bands; distal parts of wings, beyond venation, hairless and coarsely papillate

**.....Apinae, Melictini**  
Genus *Thyreus* Panzer



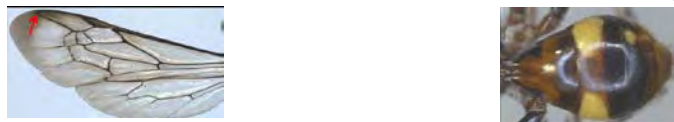
- Labrum broad than median length; scopae present; body with long hairs, and metasoma without spots or appressed white hair; distal parts of wings, beyond venation, with hairs, not or weakly papillate



.....6

- 6 Apex of marginal cell sharply pointed; metasomal terga without distinct apical hair bands; S6 of female subtruncate or feebly emerginate; body frequently with white or yellow markings

**.....Nomadinae, Nomadini**  
Genus *Nomada* Scopoli



- Apex of marginal cell rounded; metasomal terga with strong apical hair bands; S6 of female not emerginate; body frequently without pale markings

.....7

- 7 Paraglossa as long as first two segments of labial palpi taken together; stigma large; antenna of males elongate

**.....Apinae, Eucerini**  
Genus *Tetralonia* Spinola



- Paraglossa much shorter than first two segments of labial palpi taken together; stigma small, antenna of males not elongate

**.....Apinae, Anthophorini**

**L. Key to Genera of Anthophorini**



- 1 First recurrent vein joining second submarginal cell near middle ; third submarginal cell sub-quadrat, with front and rear margins of about equal length, and basal and distal margins of about equal length

.....2



- First recurrent vein terminating at or near apex of second submarginal cell , third submarginal cell with front margin much shorter than rear margin

.....**Habropoda Smith**

- 2 Arolia present; body nonmetallic

.....**Anthophora Latreille**



- Arolia absent; body metallic white, blue, green and black markings

.....**Amegilla Fries**

## **Appendix II**

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**Key Questions for Research in Pollination Ecology**  
**From Mayer *et al.*, 2011**

## Appendix II

### I Questions on Plant Sexual Reproduction

1. Why are some plant species capable of autonomous pollination – and what are the consequences regarding inbreeding depression and population viability?
2. Does pollen limitation affect breeding systems, genetic diversity and speciation rates and, if so, how?
3. What is the relative contribution of abiotic versus biotic factors in shaping the evolution of flowers?
4. Is male reproductive success more variable than female reproductive success?
5. Is there sexual selection in plants?
6. What is the role of hybridization in plant speciation?

### II Questions on pollen and stigma biology

7. How can we assess pollen viability and stigma receptivity under field conditions?
8. How often is “stigma clogging” (incompatible pollen physically blocking the stigma) an important effect in nature?
9. What influences the pollen availability of a population?
10. What proportion of pollen grains from a plant are viable?
11. How much viable pollen is transferred to flowers?
12. What is the lifespan of pollen grains?

### III Questions on abiotic pollination

13. How many animal-pollinated plant species have cryptic or partial wind pollination?
14. When, where and how did evolutionary shifts from biotic to abiotic pollination systems, and vice versa, occur?
15. How are biophysical mechanisms involved in abiotic pollination?
16. What are the relative proportions of biotic vs. abiotic pollination services in crop and wild plants?
17. How frequent is pollen limitation in wind pollinated plants?

### IV Questions on evolution of animal-mediated pollination

18. What roles do pollinators play in the processes that lead to plant speciation?
19. What role does coevolution play in plant diversification?
20. How, and how frequently, do positive correlations emerge between floral specialisation and species diversity within a clade?
21. How and why does specialisation in pollination systems evolve and what are the driving factors?
22. Why does floral deception evolve?

23. How do plants avoid heterospecific pollen interference given that generalist pollinators visit other sympatric flowering plants?
24. How frequently do floral traits converge on traditional pollination syndromes?
25. What are the ultimate factors determining taxonomic diversity of visitors to a plant species?
26. How is floral diversity generated, or why are there so many kinds of flowers?

#### **V Questions on interactions of plants, pollinators and floral antagonists**

27. What is the relative importance of selection on floral traits and mating systems by mutualists (pollinators) vs. antagonists (florivores, nectar robbers, pathogens, seed predators, pollinating herbivores)?
28. How does community context alter the outcome of floral interactions with pollinators and antagonists?
29. How do floral traits mediate interactions with pollinators and floral antagonists, and how do pollinators and floral antagonists alter these traits?
30. How do pathogens transmitted at flowers affect population dynamics and evolution of plants and pollinators?

#### **VI Questions on pollinator behaviour**

31. What influences the pattern of movement of pollinators across landscapes?
32. How does pollinator behaviour affect pollen dispersal, gene flow, pollination, and plant reproductive success?
33. Are pollinator preferences mainly driven by learned or innate behaviour?
34. How and to what extent do pollinator species differ from one another in their cognitive abilities?
35. What factors determine the foraging choices of pollinators, and to what extent do these approximate optimal choices?
36. How and at which distances do floral traits influence foraging choices of pollinators?
37. How does competition for pollinator services influence patterns of gene dispersal?

#### **VII Questions on taxonomy**

38. How do we solve the taxonomic impediment?
39. Which molecular methods are useful to evaluate inter- and intraspecific diversity of pollinators?
40. What are the ecological, social and economic impacts resulting from an inability to identify and manage pollinators?
41. How many species of pollinators have been described and how many undiscovered species remain to be described?

### **VIII Questions on plant-pollinator assemblages**

42. To what extent are local plant communities dependent upon pollinators?
43. To what extent is functional replacement of different species of pollinators possible?
44. What is the relationship between pollinator diversity and plant diversity?
45. How do pollinator population fluctuations affect pollination?
46. What are the proximate, ecological determinants of pollination system specialisation or generalisation?
47. What ecological and evolutionary processes determine the structure of interactions in a network?
48. What proportion of pollination is undertaken by the different functional groups of pollinators in a community?
49. To what extent are pollinator life cycles synchronised to the phenologies of their forage plants?
50. What factors influence the composition of pollinator species at the community level?
51. How commonly do pollinators compete for floral resources?
52. How do plants of different species interact through competition or facilitation via common pollinators?

### **IX Questions on geographical trends of pollinator diversity**

53. How does the diversity of pollinators vary geographically at the level of species and functional groups?
54. How and why do plant and pollinator specialisation, ecological redundancy, and other network characteristics vary geographically?
55. What are the geographic units of functional relevance to pollinator diversity, e.g. local, landscape, regional, or continental?
56. How common are geographical mosaics of plant-pollinator interactions?
57. What is the scale of the biotic homogenisation of pollinators, as observed in Europe?

### **X Questions on drivers of pollinator loss**

58. Besides habitat destruction, pollution, invasive species, and climate change – how can we best identify future risks to plant-pollinator interactions?
59. Which pollinator taxa and functional groups are in decline?
60. What are the geographical patterns of decline?
61. What are the rates of decline?
62. What is the relative importance of the various drivers of pollinator decline?
63. How do drivers of loss interact, and how do they vary in space and time?
64. Do drivers of pollinator decline also drive loss of pollination services and, if so, what is the rate and shape of change?
65. How do we quantify drivers of decline experimentally and at the landscape scale?

66. What features of the life histories of pollinators (e.g. haplodiploidy in bees, or specialist larval food plants in Lepidoptera) make them more susceptible to extirpation?

### **XI Questions on pollination as an ecosystem service**

67. What are the most important pollinators of crops and wild plants?  
 68. How widespread are pollination deficits in crops and wild plants?  
 69. What is the economic value of pollination services?  
 70. How will we supply the growing demand from agriculture for pollination services?  
 71. What is the role of pollination in global food security?  
 72. What other ecosystem services would be affected by the loss of pollinators?

### **XII Questions on managing pollination services**

73. How do we match the right pollinators to individual crops?  
 74. When do we need to use managed pollinators?  
 75. What alternative species of pollinators can we manage for pollination services?  
 76. How can beekeepers, and providers of other pollinators, be paid for delivering pollination services?  
 77. How can the potentially conflicting demands for pollinator diversity conservation and crop pollination be reconciled?

### **XIII Questions on conservation**

78. What conservation laws, policy requirements and adaptation of existing agri-environmental funding schemes are needed to sustainably conserve pollination services, as well as plant and pollinator diversity?  
 79. How can we best monitor and document the status, threats and pressures on pollinators including effects on plants and biodiversity as a whole?  
 80. What essential modifications in land use management and practices are needed to halt and reverse plant and pollinator declines?  
 81. How can we ensure adequate prioritisation, sufficient action and implementation?

### **XIV Questions on implementation of plant-pollinator interaction conservation**

82. How can we promote strategic networking on pollination issues in a broad, integrated, and interdisciplinary approach?  
 83. How can we effectively raise awareness among society about plants, pollinators and pollination services?  
 84. What kinds of training, education and capacities are needed to protect the diversity of pollinators and their food plants?

85. How can we better employ plants and their pollinators as educational tools for public awareness?
86. How can pollination ecologists learn from other fields to communicate effectively about pollinators with policy makers and the general public?