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PROTOCOL TO DETECT AND MONITOR POLLINATOR COMMUNITIES

GUIDANCE FOR PRACTITIONERS

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POLLINATION SERVICES FOR SUSTAINABLE AGRICULTURE

PROTOCOL TO DETECT AND MONITOR POLLINATOR COMMUNITIES

GUIDANCE FOR PRACTITIONERS

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This publication provides guidance on using a common methodology for monitoring pollinator diversity and abundance, as part of the GEF supported Project "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach" implemented in seven countries - Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa. The project is coordinated by the Food and Agriculture Organization of the United Nations (FAO) with implementation support from the United Nations Environment Programme (UNEP).

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PREFACE

In agro-ecosystems, pollinators are essential for orchard, oilseed crop, horticultural and forage production, as well as the production of seed for many root and fibre crops. Pollinators such as bees, birds and bats affect 35 percent of the world's crop production, increasing outputs of 87 of the leading food crops worldwide, plus many plant-derived medicines in the world's pharmacies.

At the same time as the role of pollinators is gaining increasing attention, evidence points to potentially serious decline in populations of important pollinators on local scales. For example, surveys of the Himalayan cliff bee have shown significant declines in the number of colonies or total loss across a 15-year period. In Europe and North America, species of bumblebees have been well documented to be severely declining. In Brazil, 2 species of native bees are officially listed as endangered.

Considering the urgent need to address the issue of the worldwide decline in pollinator diversity, the Conference of the Parties to the Convention Biological Diversity established an International Initiative for the Conservation and Sustainable Use of Pollinators (also known as the International Pollinators Initiative-IPI) in 2000. First amongst the aims of the International Pollinators Initiative is to "monitor pollinator decline, its causes and its impact on pollination services".

Yet, fifteen years after the creation of the International Pollinator Initiative, changes in the trends and distributions of most pollinator taxa and pollination failures remain poorly described. The need for a global collaboration that pools case study evidence from a multitude of ecosystems and contributes to a monitoring system that returns consistent, scientifically sound information to policy-makers, remains a high priority on the pollinator conservation agenda.

Within the context of its lead role in the implementation of the International Pollinator Initiative, FAO established a Global Action on Pollination Services for Sustainable Agriculture. FAO also developed a global project, supported by the Global Environment Facility (GEF) through the United Nations Environment Programme (UNEP) entitled "Conservation and management of pollinators for sustainable agriculture, through an ecosystem approach".

Seven countries (Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa) worked together with FAO to identify and carry out targeted activities that can address threats to pollinators in agricultural landscapes. The outcomes of the Global Pollination Project are an expanded global understanding, capacity and awareness of the conservation and sustainable use of pollinators.

As a contribution to the IPI, and as part of the Global Pollination Project to expand global understanding, FAO and its partners collaborated with the San Francisco State University to develop a protocol for monitoring bee pollinator populations in crop production landscapes. Field testing and adaptation of the monitoring methodology was made possible through the work of the partners of the Global Pollination Project. Given the importance of pollinators for crop production, it is hoped that this bee monitoring protocol can provide options for local implementation for a variety of groups including researchers, extension agents, farmers, students and others.

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Acknowledgement is given to all of the contributors to the Handy Bee Manual (see References, and http://bees.tennessee.edu/publications/HandyBeeManual.pdf), a multi-collaborator online manual of best practices for working with bees. Most of the techniques for collecting and processing bees came directly from contributions to that manual. We would also like to thank the GEF/UNEP/FAO Global Pollination Project partners in the seven countries, who tested and used the methodology described in this protocol, for their collaboration.

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SECTION 1
INTRODUCTION AND CONCEPTUAL FRAMEWORK

Pollination is a poorly considered aspect of world food security – yet terrestrial plants (including crops) require pollination for seed and fruit production. Approximately one third of that pollination comes from pollen transfer mediated by insects (Klein *et al.,* 2007). Low or absent populations of pollinators reduce production of food, timber, and fiber plants, creating additional pressure on the supply of foods that is limiting in many parts of the world. Aspects of pollination have been widely studied as part of basic ecological and agricultural research, but until recently it has simply been assumed that pollinators do not limit any aspect of agricultural production. Consequently, understanding of pollinator status and the interaction between native and managed populations of pollinators with landscape management and agricultural practices has been largely anecdotal, resulting in a current inability to make substantive statements about the health, trends or status of pollinators in agricultural regions.

Pollinators make significant contributions to world agriculture (Klein *et al.,* 2007). In 2005, the total economic value of pollination worldwide represented about 9.5 percent of the value of the world agricultural production used for food. In addition, the crops that depend on pollination services tend to be higher value crops. They average values of around €761 per tonne whereas those crops that do not depend on animal pollination average €151 per tonne, five times less than the animal pollinated crops (Gallai *et al.*, 2009).

The value of pollinators results from their contribution to both yield and quality of fruit and seed production. For example, strawberries that do not receive sufficient pollination result in smaller, deformed fruits which in many markets would be discarded rather than sold (Klatt *et al.,* 2014). Runner beans that do not receive full pollination result in sickle shaped pods rather than full, straight pods and cannot be sold for export (Vaissière, Freitas and Gemmill-Herren, 2011). In addition, there is clearly both global and local economic impact of insufficient pollination. For some species, it is not just the presence of pollinators but the composition of the pollinator community that can affect yield. Several crop species require

specific types of pollinators. For example, honey bees cannot pollinate tomatoes as these require buzz pollination (Buchmann, 1983). There is also evidence that the presence of native bees can increase the efficiency of honey bees (Greenleaf and Kremen, 2006; Garibaldi *et al.*, 2013).

Given the importance of pollinators, there is the need for a global monitoring program to track trends in pollinator diversity and abundance, for pollinator services. The lack of a monitoring program means that it is not known whether there is a crisis in pollination, nor can the priority focus for conservation measures be identified.

A global monitoring programme needs a common methodology that can be applied throughout the world, under a wide diversity of local circumstances and conditions. As with all monitoring programmes, it is not feasible to count everything; a suitable indicator and sampling methodology needs to be agreed that gives a reasonable estimate of trends applicable to the target taxa, in this case pollinators. It is possible to develop a means of surveying the most important group of pollinators: bees. Thus, the purpose of this document is to present bee monitoring protocols with options for local implementation as guidance for a variety of groups including researchers, extension agents, farmers, students and others, and to provide some resources for analyzing those data.

SECTION 2
PROCESS TO DEVELOP A PROTOCOL

To develop a pollinator monitoring protocol, one needs to evaluate alternative methods for sampling that minimize variance within samples while accurately measuring changes in a pollinator community. The protocol must also determine where and how many sites are needed to detect changes and evaluate the cost of the different methods. In addition, a good monitoring protocol will have clearly defined questions and be repeatable.

To develop a monitoring protocol, two key questions must be answered: 1) How many samples are needed to track changes?; and 2) Is the monitoring program adequately precise? Three factors will influence the sample size: variability in the samples (counts), precision and trend. The variability in counts is described using the coefficient of variation (CV). The CV is calculated by dividing the standard deviation among samples by the mean among samples. When CVs are high, trends are more difficult to extract from year to year because there is considerable background noise in the data. This means that more samples will be needed to detect a trend of a given magnitude and, therefore, costs of sampling will be higher. The source of the background noise in the data is not relevant, but in all cases, the CV should be lowered where possible and strategies for doing that can include options like standardizing sampling on a time of year or a particular habitat.

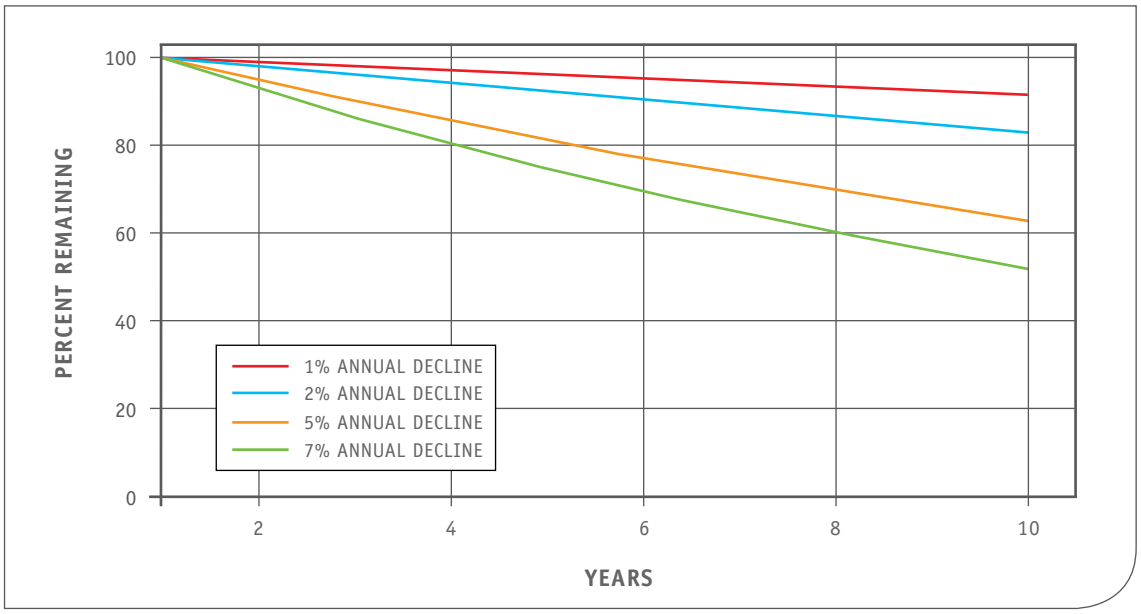
Good sampling programs have a high probability of detecting change if one is occurring and can detect significant changes in populations over time. For monitoring, it is often more important to react when a trend is suspected than to be certain a trend exists. This means that when evaluating the data, one should err on the side of detecting a trend that is not there, rather than missing a true trend. Statistical tests designate the probability of incorrectly rejecting a true trend or hypothesis as the experiment-wise error rate which is symbolized as alpha (α). In this case, the precision of a test can be set to have an $\alpha = 0.2$ rather than the more common $\alpha = 0.05$. This will increase the probability that changes that are present will be detected.

A program needs to be able to detect significant change. The amount of change detected is called the effect size. At a minimum, a monitoring program should pick up a 50 percent change over a set period of time. Even small annual shifts in the number of individuals or species in a habitat can add up to significant changes over a ten-year period (Figure 2.1).

It is important to determine the amount of change and the period over which changes are to be detected as both variables can significantly change the number of sites needed to sample adequately. The longer a researcher samples, the fewer sites they will need (Figure 2.2).

Figure 2.1

CUMULATIVE EFFECT OVER TEN YEARS OF DIFFERENT PERCENTAGE ANNUAL DECLINE ON TOTAL REMAINING POPULATION SIZE



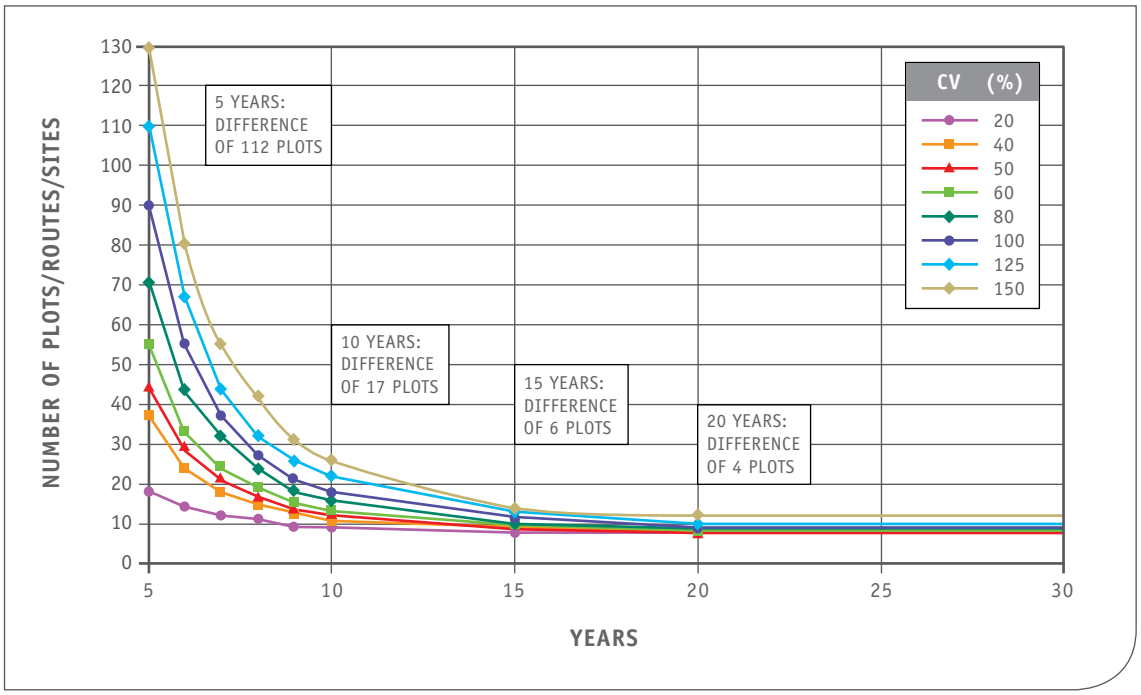
Source: G. Lebuhn and E. Connor

Using data from a variety of sites, LeBuhn *et al.* (2012) found that using the protocol described below, studies will have 80 percent power to detect even a 7 percent decline in species richness within two years with 100 sites and a 2 percent decline in species richness within five years with 25 to 50 sites. LeBuhn *et al.* (2012) used population data from the literature and from existing studies of bees to estimate the degree of variability in counts of bees across years. These data sets originated from three continents (North America, South America and Europe) and included a diversity of counting techniques, yet the results indicated that the degree of

year-to-year fluctuations across these studies were the same. Consequently, the development of the protocol assumes that fluctuations in counts of bees within the areas studied will also be similar to the populations used in LeBuhn *et al.* (2012). Data suggests these estimates represent reasonable approximations of the variation that would be detected in Africa and Asia (LeBuhn *et al.*,2016). This is a testable assumption and after five years of data gathering, the degree of variation from studies can be computed.

Figure 2.2

MINIMUM NUMBER OF PLOTS NEEDED TO OBTAIN AT LEAST 90 PERCENT POWER TO DETECT AN EXISTING DECLINE OF 3 PERCENT WITH THREE COUNTS PER YEAR, GIVEN COEFFICIENT OF VARIATION AND NUMBER OF YEARS, AND ASSUMING AN α = 0.2, TREND CV = 1, 2-TAILED TEST, EXPONENTIAL GROWTH AND WHOLE NUMBER ROUNDING



Source: USGS Patuxent Wildlife Research Center. Managers' Monitoring Manual

SECTION 3
PROTOCOL AIM AND STRUCTURE

The protocol thus developed, in view of the points made above, aims to apply a standard sampling design to assess the degree to which species richness or abundance are changing in a given area over time, and which can be used at local, national, regional or even continental scales.

The protocol can be implemented to simply detect changes in species richness and abundance of pollinators. However, this protocol can also be used to test a variety of hypotheses when used in a single or in a variety of areas. If all samples are placed in a single type of area (no different treatments), then the protocol will detect changes in that area's pollinator community. When implemented across treatments such as in agricultural versus wild areas, it can be used to look for differences in the trend of pollinators in those different regions.

The benefits of following the monitoring protocol described below depend on the length of time that sampling continues. In three to five years, this type of monitoring will be able to detect changes in abundance and total species. Maps of distributions and changes in distributions can be developed and the sampling will generate a considerable number of specimens that can be used in natural history collections, genetic and taxonomic studies or for teaching. These data can also provide the start for a public database of bee biodiversity (e.g. GBIF, Discover Life, and see Carvalheiro *et al.* 2016). Over the longer term, 5-20 years, this type of monitoring will be able to detect shifts in individual species abundances, regional trends and the ability to detect large scale pollinator crashes in any year. Standardizing permits the methods to be used to monitor bees across localities, regions, even countries, allowing comparisons among sites. The data can be used to detect patterns of distribution, abundance, composition and fluctuation. The sampling will generate enough data for both community and biogeographic analyses.

SECTION 4
GENERAL CONSIDERATIONS FOR EXPERIMENTAL DESIGN AND STUDY SITE SELECTION

To develop a national monitoring protocol, it may make sense to overlay sample sites on an already established network such as agricultural experiment stations, national parks or forests, a regional school system or farmer field schools. Involving local managers or farmers in the study will have the added benefit of increasing dissemination of the results, as well as raising awareness of pollinator and pollination management-related issues. The protocol is simple enough for high school students to implement.

Box 1

STEPS FOR MONITORING SURVEY

Step 1: Site selection

- Select area to be studied
- Determine number and placement of plots

Step 2: Site sampling (bee collection)

- Collection in the field
- Preparing and identifying the bee specimens

Step 3: Data collection and recording

- Enter specimen data into database
- Verify data accuracy and quality

Step 4: Data analysis and synthesis

This protocol targets sampling bees in a region covering approximately 10-200 Km2, although it can be applied over larger or smaller geographic regions. The specific goal is to detect changes in native bee populations and to establish the ability for communities, countries and regions to track changes in the common species of bee pollinators as well as detecting shifts in the number of species present and the aggregated number of all individuals.

4.1 STUDY AREA SELECTION

The researcher or participant will determine the specific study area. Within that study area it must be decided if the entire area is going to be sampled or only parts.

Some examples of partial surveys would be a survey of the bees:

- In orchards
- In natural areas
- In urban areas
- Along roadsides
- In fields in valley lowlands
- In bean or canola fields and the natural areas adjacent to those fields.

All are legitimate targets for investigations; however, statements about trends in bees will be limited to only those targeted areas. For example, if the research surveys orchards and find declines in most of the bees there, the researcher cannot say that bees are declining throughout the region or in the county, they can *only* say that they are declining in orchards in the area being studied.

4.2 NUMBER OF STUDY PLOTS

To detect a 3-5 percent annual change in bee species richness or abundance over a five year period, approximately 25 study plots are needed (LeBuhn *et al.*, 2012). The number of plots needed will decrease if sampling will take place for longer than five years and will increase if one: 1) wishes to detect smaller annual changes; 2) has a more variable fauna; or 3) wishes to sample for a shorter period of time.

4.3 PLACEMENT OF STUDY PLOTS

There are three options for placing study plots:

- Place plots wherever a researcher likes.
- Place plots randomly.
- Place pots systematically.

The consequences of placing plots wherever a researcher likes rather than randomly or systematically is that one can only talk about trends in bees on those chosen sites, but one cannot extrapolate to locations outside of the those sites. So, if a researcher is sampling a set

of orchards and simply chose the ones that were easiest to access and found that bees had declined in those orchards they may say: “Bees have declined at my sampling locations”. They may not say: “Bees have declined in orchards in this region”. Under this choice, the researcher has severely limited their ability to talk about declines in bees.

To be able to make a statement based on more robust results, a better option is to select a set of random sites. For example, if one choses a set of random apple orchards in a region or a set of orchards in specific habitat types, one can speak to declines in bees more broadly. Thus, the consequences of placing plots randomly or systematically are the same. As the sites were not pre-selected based on a specific criteria, one may therefore say that the sites are representative of the region as a whole and can make statements like: “From our surveys, we have shown that bees are declining in this region’s orchards.” This results in a much stronger and definitive statement from which management decisions can be based.

Many textbooks and web sites cover how to choose random or systematic locations for a study area. Refer to them and then show the protocol to a statistician for review. It is far, far better to have errors detected prior to collecting data rather than after.

SECTION 5
PROTOCOL FOR SAMPLING AT PLOTS

The protocol uses bowl or pan traps (LeBuhn *et al.,* 2012). These are inexpensive, easy to use and easy to standardize. There are a variety of other methods for sampling bees. Some examples would be netting, trap nests, Malaise traps. These methods have similar success at sampling but tend to be more difficult to use, expensive and difficult to standardize (LeBuhn *et al.,* 2012). Those other methods can all be used as ancillary data, if the researcher likes, but be careful to only use them away from the standardized pan traps so they do not reduce the specimens collected by the main survey.

Bowl traps are small plastic bowls or cups, coloured white, fluorescent blue or fluorescent yellow. The bowls are filled with water mixed with a small amount of detergent, which acts as a surfactant. Bees are attracted to the colours that mimic the colour of flowers, so they land in the water and drown. Bees do not see red but do see UV (fluorescent) and are highly attracted to UV colours when combined with yellow and blue. Normally, a bee landing on water would float on the surface tension but, by adding detergent to the water, the soap diminishes the surface tension enough that they sink.

There have been a number of studies in both Europe and North American indicating that bees readily fly to bowl or pan traps. However, in areas of tropical rainforest where many of the bees are canopy feeders and vegetation elsewhere can be quite dense, it may be useful to do preliminary testing to assess whether using bowls is an efficient way of collecting - and perhaps assess other survey techniques such as netting or Malaise traps that might be appropriate for these environments prior to the establishment of a survey system.

5.1 USING BOWL TRAPS

At an individual study location, bowl traps are placed in a line or transect. Twenty-four bowls are used, eight of each colour, and the colours are alternated throughout the transect. Bowls

are placed on the ground (or elevated as mentioned below) 5 m apart and are located such that each individual bowl is not hidden by vegetation, but left out in the open where a bee can spot it. If bowls are placed too close together, they interfere with each other so, the 5 m distances is critical. While a transect that follows a straight line is useful, it is not a requirement, and transects can bend and wiggle around structures (such as clumps of bushes and trees, roads, and dense vegetation). Alternatively, bowls can be placed in the pattern of an "X".

To set out a transect of bowls, the researcher needs a container filled with water (Figure 5.1) to which a large squirt of dishwashing liquid or laundry soap has been added. Any type of dish or laundry detergent can be used - except those with citrus scent added. Citrus will decrease the number of bees caught. Laundry detergent is generally better than dish soap as it does not create many suds on top of the water.

Bee capture rates are the same no matter what size bowls are used; the standard is to use a 66 ml portion bowl (3.25 ounces) of the type often used in take-away containers at restaurants. This size has the advantage that it increases efficiency as it allows a researcher to hold all 24 bowls in one hand. Then, as the researcher travels down along the transect, s/he can get a bowl ready using their thumb and forefinger while pouring the water with the other hand (thus without having to put the bowls on the ground). This makes setting up the transect go quickly. Also, the smaller sizes have an advantage in that they do not require the researcher to carry as much water.

Figure 5.1

RE-PURPOSED CONTAINER FOR HOLDING SOAPY WATER SOLUTION USED TO FILL SAMPLING BOWLS IN THE FIELD

© G. Lebuhn

5.2 LENGTH OF SAMPLE

Bowls can either be left out for any 24 hour period of good weather or placed out prior to when bees are active in the morning and collected after they have become inactive in the afternoon. If bowls are left out for less then 24 hours, note the length of time that they were out sampling. These data can then be used to statistically correct for differences in sampling across a field season.

5.3 WEATHER

If the weather is rainy, extremely windy or very cloudy, then catch will be minimal. Consequently, bowl surveys should not be run on those days. Conditions are good for doing surveys if rain does not threaten and if it is sunny or only partially cloudy. Rain can be tolerated during the night or, if for only a brief period during the day. However, if it rains heavily it will splash the collected bees out of the bowl. One option might be to put a tiny overflow hole in the top of the pan trap to let water out.

5.4 HANDLING LOSS OF BOWL TRAPS

Within a sample day and site, the loss of individual traps is not a problem if only one or two are lost. Each time the researcher does a survey they will need to record the number of bowls that remained full of water throughout the survey period. So, if the researcher started with 24 bowls and during the course of doing the survey one bowl was destroyed by a goat, one bowl was stepped on, one bowl couldn't be found, and one bowl was found to not have water in the morning then the researcher would record the presence of 20 active bowls in the survey notes, and not 24. At the end of the collection process, during the analysis of the data, the researcher will divide the numbers of bees the researcher captured by the number of bowls available.

5.5 REMOVING BEES FROM BOWL TRAPS

Retrieving bowls from a transect and shifting the bees to a container takes about the same amount of time as setting out the bowls. At each bowl, remove all moths, butterflies, slugs, and very large bodied non-hymenoptera (e.g. grasshoppers and crickets) to a different container if you are saving them. These groups tend to contaminate the other specimens when placed in alcohol. Following

their removal, the remaining specimens can be moved along with the water in the bowl into an aquarium net, sieve, or tea strainer. It is very important to choose a strainer with extremely fine mesh in order to catch the smallest of bees, some of which may only be 2-4 mm. Aquarium nets like those used for brine shrimp nets and tea strainers tend to have the finest meshes.

Bowls from one transect or plot can be pooled rather than keeping individual trap data separate, as handling time increases greatly when collecting from individual bowls. The catch should be stored in 70 percent alcohol in containers or sample bags (for the purpose of this protocol, the term "sample bags" will be used to indicate a either a container or sample bag) that can be tightly sealed so no alcohol will escape. Sample bags with plastic zippers will *not* work for this. If using a net, it can help to use a spoon to gather the specimens from the net and then transfer them to the sample bags. Alternatively, the researcher can pick out the mass of insects in the net or strainer with their fingers and move it into an individual sample bag. However, the researcher may use a larger sized sample bag along with a small tea strainer; giving the strainer a sharp rap after placing it in the bag will thereby dislodge all the insects at once.

Insects captured in white pan trap

An alternative to sample bags is to move the catch into small numbered squares of cloth that are rolled up and rubber banded together. Once back from the field, put each cloth into plastic "zippered" bags and freeze until the specimens are ready to be pinned. This will decrease the probability that samples will dry out because of leakage in the sample bag.

TIP

Often alcohol needs to be diluted to achieve the right percentage (70 percent). The label on bottles of alcohol should have information on the alcohol percentage. Note that most inexpensive stores sell isopropyl that is only 50 percent alcohol. If the bottle is labeled with the percent alcohol in terms of "proof", you will need to have at least 140 proof. Proof is a simple doubling of the percentage. Therefore, 100 proof is 50 percent alcohol and 190 proof is 95 percent alcohol. To dilute from 100 percent alcohol to 70 percent, choose a convenient sized container, such as a quarter liter bottle, then fill it approximately 70 percent full with alcohol and the rest with tap water. This measurement does not need to be exact, but as close as possible.

Each bag or cloth should have a tag inside listing the sample location and date written on paper with pencil. Do not trust any kind of writing to stay on the outside of a sample bag, as they inevitably get wet with alcohol or water and the writing will become illegible.

5.6 FIELD DATASHEETS

Sample site data sheets should be prepared prior to sampling and brought to the field. The first time any site is sampled, the data fields the researcher needs to fill out to document the characteristics of each of transect (the researcher only need to do this once) include: transect name, latitude, longitude and a description of each site. If there are other characteristics of the site of interest such as presence of grazing or a particular plant species, which should be measured at the site to characterize it, they should be added to this data sheet.

Each time the site is sampled, the researcher should write down the number of bowls missed or destroyed on the data sheet. The data fields the researcher will need to fill out **each time** include: site name, date, time bowls set out, time bowls retrieved, name of the person who set out and retrieved the bowls, and number of bowls that retained water during the time they were in the field. This data sheet can also include other variables of interest that may change with each time the plot is sampled such as the amount of floral resources available.

TIP

It is a good practice to count and record the number of insects in a bag in the field on the data sheet. If one returns to the laboratory with two unlabeled bags, it can help to identify which bag came from which site.

A sampling data sheet can be found in Annex A. A field trip (and laboratory) checklist is provided in Annex B.

EFFICIENCY TIPS

It is helpful to create the sets of bowls the day before setting them out. In particular, it is very handy to have an empty, divided flat like those found holding plant starts at a local nursery, as this holds the separate sets of bowls quite nicely (Figure 5.2). Red or pink wire flags can be very useful for re-finding the transects. In areas with few landmarks, it is particularly useful to use GPS. By using GPS to locate the transects as the researcher positions the bowls, the researcher can then use the "goto" feature of the GPS unit to track back the transect locations that same evening or the next day. This is particularly useful when working in an area with few landmarks.

Figure 5.2

PLANT FLAT USED TO HOLD SETS OF BOWLS

5.7 TRANSPORTING BEE SPECIMENS IN ALCOHOL

When traveling with, or shipping, bags of specimens, the researcher should partially drain the alcohol out of the bags to diminish the possibility of leaking while in transit without affecting their preservation. Be sure to properly close the sample bags. Put all the sample bags into a "zipper" storage bag and then, into another larger "zipper" storage bag to make sure nothing leaks. Some paper towels in the outer bag will provide added insurance.

Box 2

SUMMARY OF STEPS FOR SETTING OUT BOWLS AT SITE

- Put one heavy squirt of dish washing liquid in a 5 litre jug of water.
- Place bowls level on the ground.
- Fill each bowl with soapy water about three quarters more full.
- Set bowls out in transects with 24 bowls spaced 5 meters apart (can be measured by pacing) alternating blue, yellow and white bowls.
- Avoid putting bowls in any heavy shade, as few to no bees will come to those bowls. There do not have to be flowers nearby to have bees come to bowls, as often there are bees scouting over flowerless areas.
- Collect bowls after 24 hours and place specimens in sample bags with alcohol. If you are not going to process the specimens within one to two days, store these samples in a freezer.

SECTION 6
PROCESSING SPECIMENS

Once the specimens have been collected from the field, they need to be processed for identification and this information must be entered into a database. The entire process has several steps: washing and drying the bees, sorting, pinning and labeling and identifying. Each step is reviewed in the sections below.

6.1 WASHING AND DRYING BEES

Pinning bees directly from water or alcohol usually results in matted hairs and altered colours, along with a good coating of pollen, scales and other detritus picked up from the sample. Washing and processing bees using the process listed below will result in high quality specimens that can exceed the quality found when hand-collected.

There are two main approaches to washing bees, using either a strainer or a bee washer to accomplish the task. Both are explained below and videos are available at https://www.youtube.com/watch?v=A2y-ind12Cc.

Strainer Washing

To begin, fill the specimen sample bags with water and then move contents into the strainer (Figure 6.1). Transfer the specimens from the strainer into a plastic container with a lid (it is helpful if the container you make a small hole in the lid to let out the foam). Add warm water and dish washing liquid, and **very** vigorously shake the specimens around with for **60 seconds**. Place specimens back into the strainer and rinse under tap water until no more suds are present. Break the force of the water with a hand or spoon to protect the specimens. Tap off loose water and use a cloth towel to blot out as much excess water as possible on the bottom of the strainer or net. Next, squirt 95 percent alcohol onto the specimens, dip the strainer into a bowl of alcohol, or drop them into a jar of alcohol and blot again. Then, move the specimens onto a set of three to six paper towels and fold the paper towels over the specimens and roll them around with a finger, pencil or tweezers and refold a few times to

remove the bulk of the alcohol. At this point the researcher can fold corners of the paper towel up and shake the specimens around inside to further dry them. When the specimen's wings are no longer stuck together or folded up on themselves and all bee hair is fluffy, the specimens do not need to be shaken anymore. Now the researcher can dry them and pin them.

It helps to hold the corners *and* the towel area between the corners, or the specimens will jump out while the researcher is shaking them.

After the specimens have been dipped in alcohol the researcher can leave them lying on the paper towel for up to 45 minutes before further fluffing them.

The best looking bees are those that are cleaned within 24 hours of capture.

TIP

Tea strainers work well because of their fine mesh, aquarium nets designed for brine shrimp also have sufficiently small mesh, but it is more difficult to remove specimens because of the flexibility of the netting.

Figure 6.1
TEA STRAINER

Washing using a magnetic stirrer

Rather than cleaning bees by swirling them around in a jar by hand, a researcher can use a magnetic stirrer, the same as used in all chemical laboratories. A small magnet is turned inside a jar or cup by a magnetic plate. The water, soap, and bees are swirled around as gently as the researcher wishes. This method does the best job of removing pollen, nectar and other detritus on specimens, simply because the researcher can leave it washing for quite a while without a time penalty.

6.2 DRYING COLLECTED BEES

It is important to dry specimens as it makes it easier for identification. On bees with long hair, it is useful to use some form of drying system to speed the process. A video that demonstrates how to dry bees can be seen at https://www.youtube.com/watch?v=935jlJep6go.

To dry bees, you will need a small clear glass half liter jar that has a canning jar lid of the kind with a removable central metal disk, a piece of window screen that will be used to replace the center of the canning jar lid, and a hair dryer (Figure 6.2).

Figure 6.2

GLASS JAR MODIFIED FOR DRYING BEES WITH FIBERGLASS SCREEN IN LID

The basic idea is to move the bees around while blowing air on them so that the hair that has been matted by getting wet returns to its original condition. To do this, follow the same procedure as listed under the strainer section (above) but just quickly blot of the specimens on the paper towels to get the bulk of the alcohol off. Then, use a funnel to move the specimens from the paper towel into the jar. Put the lid back on the jar with the screen in the middle; make

sure the screen is snug around the entire lid. It may help to add small rolled up bits of paper towel in with the specimens. Place the jar on its side on the folded hand towel and place hair dryer pointing into the jar as close as possible, without causing the hair dryer to turn off (usually about 3 cm). This can be hand held or set up in a wide variety of ways so that the researcher does not need to hold the blower. While drying, shake the specimens back and forth vigorously, hitting the sides on the towel periodically to dislodge them if they stick to the glass. Specimens, when wet, are very flexible and tough, so they can sustain a moderate amount of bumping around.

Once the specimens are all loose, shift the jar slightly downward so that the specimens slide towards the screen and whirl around in the dryer's wind; continue shaking the specimens. Small short-haired specimens are done once their wings are flexed away from their body and their hairs are not matted. Bumblebees and long-haired specimens take longer. Depending upon the hair dryer and the bees, this may take anywhere from 1.5 to 3 minutes. Alternative methods for drying bees and working with large volumes of bees can be found in Annex C.

TIP

Fiberglass screening is an appropriate material to use as the insert in the jar, but metal screen can also work. One advantage to a loose fiberglass screen is that it can be cut with scissors to fit the jar.

6.3 PINNING COLLECTED BEES

Specimens need to be pinned to be identified by experts. If the research group has expertise with the bees in the area, doing batch processing where common, easily identified bees are not pinned individually can circumvent this process. This type of processing is covered in Annex D. Here, it is assumed that all bees will be pinned for identification.

To mount bees, it is recommended to glue, and not pierce, bees to the pin. Start by taking the dried specimens and placing them on a foam pad such that they are either upside down or on their sides. For pinning use Number 2 or Number 3 pins. Even large bees can be glued to pins (the larger the bee, the more glue required) rather than pinned through their bodies.

Gluing, while not traditional for larger specimens, has several advantages over pinning:

- It does not permanently damage the integument.
- It permits more of the specimen's scutum to be visible.
- It permits the processing of dried specimens without rehydrating (and further damaging them).
- It is much faster.

The glues used should not be permanent and can either be white glue, tacky glue (a form of white glue with greater immediate adhesive power) or school glue gel.

To glue an insect to a pin, take an insect specimen tray or even a piece of paper, drop a series of pins into the bottom, and then run a line of glue along the top of one edge of the side of the tray or paper. Pick up a pin with the fingers or reverse tweezers and dip one side of the pin into the glue at the proper height. The proper height means there must be room above the bee for someone to easily pick up the pin without snapping off the antennae and there must be room below the pin to hold one or more labels. Use more glue for a large specimen, less for a smaller one (Figure 6.3).

Figure 6.3

GLUING PIN TO THE SIDE OF A BEE SPECIMEN

Take the pin and place the glued side onto either the side or the underside of the bee. Museums generally require that bees only be glued on the right side. When gluing to the underside, the optimal place to glue the specimen would be to the thorax or, even better, the joint between the thorax and the abdomen (see figures in Annex G). The angle of the legs often dictates where the pin will most easily go.

After allowing the pins to lay on the specimens for a few minutes the specimens can be moved and pinned into temporary storage boxes (usually these are foam lined cardboard boxes, either made in the lab or purchased, see Annex D for more information). To move the pin, simply press the tip of the pin with the finger into the foam and the head to the pin will elevate allowing the researcher to pick up the specimen. Also note that for very small specimens the glue is of sufficient immediate strength that the pin need only be touched to the specimen and then moved to its resting place in the box.

6.4 LABELING SPECIMENS

There are a number of ways to generate labels for specimens. Most people use word processing programs (font size 4 usually), others use spreadsheet or database, or proprietary programs. Here are the commonalities necessary for a good label:

- It should have a unique specimen identification number that links the researcher to the database record for that specimen.
- The paper used should be thick (35-65# cardstock is good) and should be acid-free or archival in quality (regular paper deteriorates over long periods of time).
- The size should be such that the label does not extend greatly to either side of the specimen and therefore take up too much shelf space (20 mm x 8 mm is a good size).
- The label should have country, region, city (or location or park), collection date (with the month written in roman numerals or text, but *not* abbreviated as the month's number), latitude and longitude, name of collector.
- Optional would be a scannable bar or matrix code that permits the specimen number to be scanned in directly.

Each specimen should then be labeled with collection information. Labels should be placed close to the specimen, but not so close that the information on them cannot be read when the specimen is tipped at an angle and enough room should be left on the pin to add an identification label and potentially other labels.

After cutting out the labels lay them out on the board and then spear them with the pinned specimens. The body of the specimen should be parallel to the long axis of the label (Figure 6.4). Once labeled, the specimens can be sorted.

Figure 6.4

LABELS ON BEE SPECIMEN

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SECTION 7
IDENTIFYING AND MAINTAINING SPECIMENS

Identifying bees requires the engagement of a specialist or multiple specialists. For most regions of the world, keys are not available for identifying many if not most genera. The reason for this is three-fold:

1. Differentiating the species within certain groups of bees is extremely difficult without a good set of identification papers, access to a good collection of correctly identified bees and plenty of experience. There are no regions of the world with a complete set of keys for identification.
2. The taxonomy, names and status of bees are incomplete. Many bee species have yet to be found and named.
3. There are very few taxonomists and insect collectors working on these issues. The renewed collection of bees throughout the world and taxonomic studies of those specimens should be encouraged.

This means that most groups will either want to send their bees off for identification elsewhere, participate in a regional identification center, or have someone on staff that has spent several years training with experts on bee identification of regional bees and has access to a collection of correctly identified regional bees.

A good introduction to the identification and families of bees is available at: http://www.yorku.ca/bugsrus/resources/keys/BFoW/Images/Introduction/Introduction.html.

Once labeled, specimens can be easily grouped and moved. Most people working on bees first sort to the morpho-species (simply sorting into groups by what they look like without knowing their names) level, or sort by genus. This accelerates the identification process, particularly if most of the species are unfamiliar to the person doing identifications. Here, the large foam boards described in Annex C work well to organize the specimens.

As the identification expert looks at specimens, they can pin individual species into trays or simply pin them into boxes with identification labels (also called determination labels),

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with all species identified as the same species or group grouped in rows or sections. A new row or section is started for each new taxa which, makes it easy to see the groupings. It is easiest to have a determination label precede the row or section of specimens so that the person doing the data entry can clearly see the name of the species they will be entering.

Another useful and time saving technique is to orient female specimens vertically in the box or tray, and males horizontally. In that way, the person doing the identifications does not have to write out another label for a different sex, the person doing the data entry need only read the species name, and the person doing the checking afterward can quickly and visually determine if the sex information has been entered correctly.

MAINTAINING SPECIMENS

Specimens will degrade if exposed to UV light, pests, and excess humidity. Using air conditioners in humid environments can control humidity. Keeping specimens in closed cabinets and boxes can control the light (note that UV light can penetrate though most glass and is also generated by indoor fluorescent lighting). Pests can be controlled by periodically freezing the collection (three days at -10 ^{o}C) and by keeping the specimens in tightly fitting museum drawers and cabinets or by keeping them in smaller cardboard specimen boxes but enclosed in large re-sealable plastic bags (such as storage bags). Usually freezing specimens once a year is sufficient, alternatively, specimens can be inspected every three months for signs of beetle infestation (dropping and “dirt” under specimens are a clear sign) and freeze only the affected units.

SECTION 8
DATA ENTRY AND DATABASE MAINTENANCE

For large collections, the data from the specimens should be entered into a database system. However, if more convenient, data can be entered initially into excel and then uploaded into a database system. In both cases, the data should be accompanied by detailed metadata that describes the project and each variable in detail. An excellent guide to data management can be found at https://www.dataone.org/sites/all/documents/DataONE_BP_Primer_020212.pdf.

There are many ways to put together a database system (Annex A). For any database, there is a core set of data fields that need to be present somewhere in the system. These are outlined below, as well as some general guidance about managing such systems.

CORE FIELDS		OPTIONAL FIELDS
• Country • Region • Locality • Site • Latitude • Longitude • Treatment 1 • Treatment 2 • Method • Unique number for the collecting event • Date and time when the trap or collecting event started • Date and time when the trap or collecting event was picked up or stopped • Unique number for the individual specimen	• Order • Family • Genus • Subgenus • Species • Count • Name of collector • Name of person who identified the specimen • Date the identification took place • Name of person who entered the record • Date the data were entered • "Notes" (for recording weather, conditions, problems encountered, flowers blooming etc.)	• Habitat • Physiographic province • Flower information if the researcher is collecting specimens at flowers

Additional columns can be added to the datasheet, to reflect treatments or categories that are pertinent to the individual's study, such as the type of agricultural field, size of patch, season sampled or whatever other variables that have been chosen to test.

SOME DATABASE SUGGESTIONS

After the data are entered, the researcher needs to examine the box of specimens and compare it to the database records as a double check. Often, errors occur when people enter their data. It is also useful to set up a query in the database or spreadsheet program to do a count of the different kinds of species in the database. By doing this, the computer will count as a different "species" entries that have an extra space, subtle spelling mistakes, and capitalization problems.

Data should be backed up and the backup copy should be stored at a different location.

VALIDATION AND DOUBLE CHECKING

The establishment of a solid and statistically valid, survey program for bees is not a simple matter. Throughout the process there are numerous opportunities to make mistakes, use incorrect assumptions, use inappropriate statistical techniques, make identification errors, and make inappropriate changes to the project over the years that reduce, or even eliminate, the usefulness of the data. So, to prevent this, it is recommended that a person familiar with bees and statistical design of monitoring programs helps the researcher establish the initial program, and review it after Year 1, Year 3 and Year 5 to make sure that errors are caught and the project is successful.

SECTION 9
DATA ANALYSIS

There are numerous appropriate and even more numerous inappropriate ways to analyze these data. If sites have been chosen at random, sign tests, simple regression, route regression, estimating equations and graphs can be used to detect trends across time. A sample R code for analyzing data using a sign test, a paired t-test or a linear regression, is provided in Annex E.

When other factors such as landscape change or habitat types are of interest, ANOVA, multivariate techniques, ordination and other analyses are potentially useful ways of analyzing the data. The decision of which technique to use will depend on the types of independent variables the researcher wishes to include in the analysis as well as the structure of the data. However, as when designing the initial surveys, it is recommended that each group consults with a statistician prior to analyzing their data, as no simple one-size fits analysis will be appropriate for everyone.

Data from different studies that use this protocol can be combined into a meta-analysis to examine trends at larger scales (see Freitas *et al.*, 2016).

SECTION 10
GENERAL CONCLUSIONS

While managed honey bees are the dominant pollinator in many agricultural systems, other bee species make significant contributions to a broad array of crops and are essential for the health of non-cultivated plant species. Monitoring the health of the broad community is essential for agricultural and other ecosystems.

The protocol is broadly applicable across habitats and regions (LeBuhn *et al.*, 2016). The protocol was designed with the ideal of catalyzing a standardized monitoring methodology across the globe to better document the patterns of our key pollinators (i.e. bees). It is hoped that this protocol will be implemented broadly as the more sites collecting data in this standardized way, the greater our power to understand whether pollinator populations are declining.

ANNEX A
SAMPLING DATA SHEET

SITE NAME _____

DATE BOWLS PUT OUT ___/___ /2015

DATE BOWLS PICKED UP ___/___ /2015

TIME BOWLS PUT OUT ____:____ AM

TIME BOWLS PICKED UP ____:____ AM

COLLECTORS _____ NUMBER OF BOWLS MISSING _____

NUMBER OF INSECTS COLLECTED _____

NOTES _____

_____

_____

_____

SITE NAME _____

DATE BOWLS PUT OUT ___/___ /2015

DATE BOWLS PICKED UP ___/___ /2015

TIME BOWLS PUT OUT ____:____ AM

TIME BOWLS PICKED UP ____:____ AM

COLLECTORS _____ NUMBER OF BOWLS MISSING _____

NUMBER OF INSECTS COLLECTED _____

NOTES _____

_____

_____

_____

Metadata fields and descriptions

DESCRIPTION OF STUDY	Describe your study here in a paragraph.
SPECIFIC SITE DESCRIPTIONS	Add any details about each of your sites here. This can take up several lines if needed.
WHO TO CONTACT ABOUT STUDY	Name and email address.
VERSION OF DATA	Any time data gets changed we should change the version number of the data set and record what was changed.
MISSING VALUES	Identify how missing values are coded. We suggest they be signified with a '.' Leave blank if those data were not collected. If you collected on a date but found no pollinators, enter the country, site, appropriate treatments, method, time and collection date. Put NONE for the Order, Family, Genus and Species and '0' for the count.
DATA FOR EACH SPECIMEN:	
Country	The country where the samples were collected. Write the full name.
Region	The region where the sites were placed. Write the full name.
Locality	The locality where the samples were collected. Only use if needed.
Site	The name of the site where you collected (on a separate metadata page, you should provide the latitude and longitude in digital degrees for each site and any details about it).
Latitude	
Longitude	
Treatment 1	Add as many treatment columns as needed. You also do not need to have treatment columns if you did not have treatments. This could be region, temperature, or crop type (e.g. *Phaseolus*…) or something else important to your study (e.g. bowl colour, sample location - in crop or on edge, etc.). On your metadata page, identify all the possible options for treatment levels.
Treatment 2	You can add as many treatment columns as you need. If the previous one is crop type (e.g. *Phaseolus*…), this one might be organic versus non-organic. On your metadata, identify all the possible options for treatment levels.
Method	Identify how pollinators were collected. Please only use the terms "pan traps", "sweep nets" or "visual observation" so that there is a standard across all data sets. If you sampled in a different way, a new standard will need to be set.
Unique number for the collecting event	A unique number that relates all the bees collected at the same time and place. This can also reflect that the bees were collected on the same bowl or same species of plant.
Date and time when the trap or collecting event started	Time of day and date collecting started (use 24 hour time). Please use the format dd.mm.year.hh:mm.
Date and time when the trap or collecting event was picked up or stopped	Time of day and date collecting completed (use 24 hour time). Please use the format dd.mm.year.hh:mm.
Unique number for the individual specimen	A unique number that will correspond to a label on the specimen.

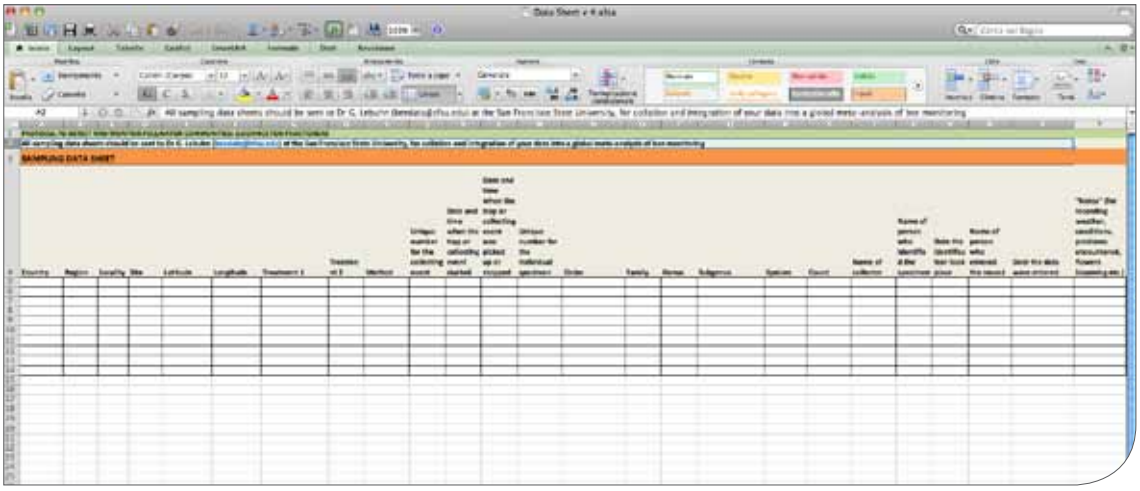
Order	Order of the species (if data were lumped taxonomically give as much detail as available). If not known, type 'unknown'.
Family	Family of the species. If not known, type 'unknown'.
Genus	Genus name. If not known, type 'unknown'.
Subgenus	Subgenus name, If not known, type 'unknown'. If this is not normally collected, please feel free to ignore.
Species	Species name. If not known, type 'unknown'.
Count	Number of individuals of that species. If nothing was collected on that date, put zero here. May not be needed if individual specimens are given unique numbers.
Name of collector	Identity of the collector of the specimen.
Name of person who identified the specimen	Identity of the expert who identified the specimens collected.
Date the identification took place	Date in dd.mm.year.
Name of person who entered the record	Identity of the person entering the data.
Date the data were entered	Date in dd.mm.year.
"Notes" (for recording weather, conditions, problems encountered, flowers blooming etc.)	
OPTIONAL FIELDS:	
Habitat	
Physiographic province	
Flower information if the researcher is collecting specimens at flowers	

Visualization of sampling data sheet – mandatory fields

A sampling data sheet can be found at: http://www.fao.org/pollination. This sampling sheet was developed as a companion tool to this protocol, and consists of three work sheets: (a) meta data; (b) sample data; and (c) a description of the data required for the sample data work sheet.

Figure A1

SAMPLING DATA SHEET



ANNEX B
FIELD TRIP AND LABORATORY CHECKLISTS

FIELD TRIP CHECKLIST

- Bowls
- Plastic spoon
- Mesh filter (e.g. tea strainer)
- Detergent
- Dishwashing liquid
- Alcohol
- Sample plastic bags (must seal tightly)
- 1-Litre jug
- 20-Litre water jug
- Location Log
- Data sheets
- Blank paper
- Permanent ink pen
- Pencils
- Clipboard
- Maps
- GPS Unit
- Batteries
- Charger
- Hand lens
- Two-way radios
- Sun glasses
- Hat
- Toilet paper
- Matches
- Cell phone
- Collecting permits
- Plant ID material
- Technical pens
- Boots
- Sun screen
- DEET insect repellent
- Drinking water bottle
- Backpack
- Hip pack
- Camera
- Watch
- First aid kit

LAB CHECKLIST

- Scissors
- Tweezers
- Determination ("Det") Labels
- Paper triangles
- Humidors
- Enamel sorting pan
- Hair dryer
- Pinning board
- Bee washer jar
- Empty bee boxes
- Pins
- Glue

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ANNEX C
ALTERNATIVE METHODS FOR DRYING BEES

Using Compressed Air

It was found that using compressed air results in the quickest drying of wet bees. When using compressed air, be aware that there can be moisture in the air lines. Run the air wide open for a few seconds to get rid of any loose moisture. Also be aware that at high pressure, compressed air can blow apart specimens, particularly their abdomens. Direct the air stream to the side of the jar and let it swirl the specimens around in a vortex (if the pressure is too high or they are bouncing violently around, the researcher can rip some abdomens off). Small specimens with short hair take less than one minute to dry. Bumblebees take about two minutes to have all the hair on their thorax fluff up.

Making and using an autobeedryer

If the researcher is involved in collecting and processing many specimens, s/he may want to invest in the creation of an autobeedryer. A slideshow and video that demonstrate how to make such a device can be seen at:

http://www.slideshare.net/sdroege/how-to-create-an-autobeedryer

http://www.youtube.com/watch?v=935jlJep6go

Upright blow dryer bee dryer

Another system that is fairly compact and easy to transport to the field is the upright blow dryer. It can be built out of a piece of 1 x 4 lumber and a few small pieces of PVC. The blower is set upright and blows air through the tube placed on top of the dryer and dries the bees. The specific design of the wooden frame depends upon the size and shape of the particular blow drier that is used. A frame can be built around the dryer, making sure it can slide in and out of the frame. Use a blow dryer that has a “cool” temperature setting. “Warm” or “hot” will bake the bees and make them brittle (although switching to “warm” air for a few minutes can accelerate the drying process for *Bombus* and other large hairy bees).

Figure C1

ASSEMBLED BEE DRYER MADE WITH A HAIR BLOW DRYER AND CLOSEUP OF THE PLASTIC CONTAINER THAT IS INSERTED IN THE TOP OF THE BEE DRYER

To dry the bees, the researcher can use a clear plastic or PVC tube that fits into the larger piece glued on top of the dryer. The clear tube allows the researcher watch the bees bounce around (Figure C1). Glue or use electrical tape to attach fine netting at the bottom of the tube; close the top with another piece of netting and a rubber band. After washing and partially drying the bees, drop the wet bees in the plastic tube, set it in the large PVC tube holder on top of the dryer and turn it on. By the time the researcher has washed the next batch of bees and prepped them, the bees should be dry.

Cleaning bees that have become moldy

To remove mold, cut a piece of foam board (like the foam the researcher will find in a standard insect box) to fit snugly in a small plastic food storage container. Wedge this into the bottom of the container, stick pinned specimens (with the labels removed) into the foam, and add warm, soapy water to submerge the bees. With the top on, gently shake the container for about five minutes, then drain it and repeat. Next, fill the container with 70 percent ethanol and shake for five minutes. Do two additional alcohol rinses, then removed the foam board from the container and use a hair dryer to dry and fluff the bees. The bees emerge from this treatment with most of their body parts intact. Some pollen is removed from scopas. Most of the fungus is removed, but

some may still cling to hairy places and the tight spaces between body segments. The researcher may be able to use a soft watercolour paintbrush to dislodge more of the fungus during one or more of the rinses. Be careful that the foam board does not break free and float, causing the specimens to become pressed up against the top of the container.

Re-hydrating bees that have been pinned

At times, there is a need to re-hydrate collected bees in order to remove them from the pin, or to pull the tongue or genitalia (note that pulling open the jaws on specimens is difficult after they have dried, even with extensive re-hydration). Place bees into a rehydration container, a humidor or a covered Petri dish with a moist paper towel inside. It can take anywhere from a few hours to several days for larger specimens to relax. To prevent mold, add a few drops of ethyl acetate, a few mothballs, or a large dose of alcohol in the water. The longer the bee has been pinned the longer it takes to relax and the more fragile it becomes.

Alternative bee storage boxes

If you have a large volume of bees being processed, you can use other types of cardboard boxes with lids, such as boxes used for pizza, as an inexpensive alternative to traditional field boxes. They can be inexpensive, save shelf space and hold more specimens than traditional boxes. However, the researcher will need to purchase material separately and assemble the box, as they are not as sturdy as other boxes and pest insects may have greater access. In many countries, blank pizza boxes can be ordered online. Restaurants may also be willing to donate cardboard boxes. Cross-linked polyethylene foam as a pinning base within the boxes seems to have superior pin holding properties to that of ethafoam, but either could be used. Ordering foam in bulk quantity, directly from a manufacturer, can lower costs. The manufacturer could be asked to cut the foam to 3/8" (about 1 cm) in thickness and ship as 2'x4' (about 0.6 m x 1.2 m) sheets.

Foam boards for labeling and sorting

Labels can be added to a pinned specimen using the traditional pinning block, but a much faster method is to use closed cell or cross-linked polyethylene foam to set the label height (note that styrofoam or polystyrene does not supply enough support for a paper label). To make a foam pinning board, simply glue a piece of foam to a thin board (plywood works well, regular wood often adsorbs too much moisture from the glue and will warp…at least for a time) or a piece of thick plastic. White glues work quite well. Be sure to place another board on top of just glued foam and add weight to that board to increase the adhesion of the glue while drying. After the

glue has dried (approximately two days) the researcher can trim the edges with a saw to make things neat and tidy. The United States Geological Survey laboratory uses these boards for a variety of activities and finds that boards of the following dimensions are most useful: 50 cm x 30 cm, 25 cm x 25 cm, 20 cm x 12 cm. The thickness of the foam should be approximately the height of the labels, but better too thick than too thin.

ANNEX D
BATCH PROCESSING OF COMMON BEES

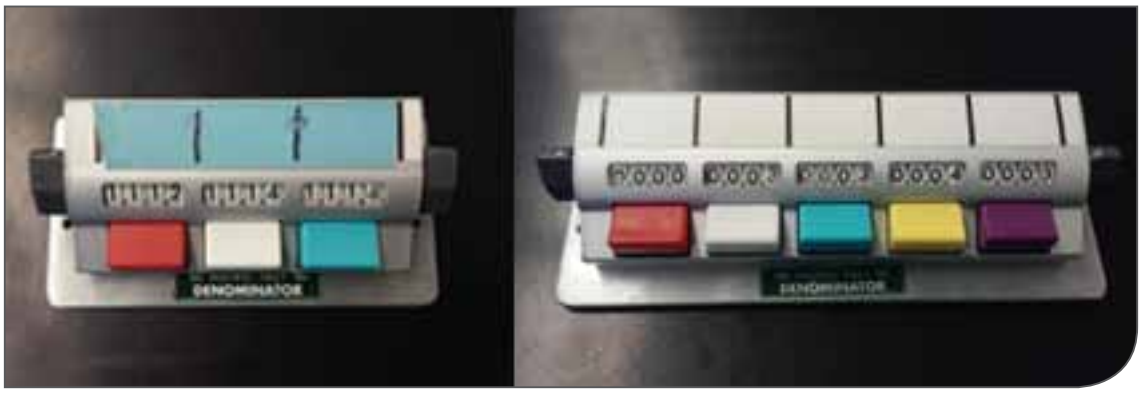
Bee identification is difficult in most circumstances, particularly at the beginning of a project - and will require sending specimens to an identification center or a series of experts. However, the researcher will find that at the sites there will be a few species of bees that are easy to identify, and which are very abundant. Consequently, there is no real need to actually pin these specimens and a great deal of savings can occur if they are processed immediately without first pinning them.

To most efficiently accomplish this, the researcher will first need to assign a site or batch number to the group of bees they are working on. Additionally, using a set of precise flat-tipped, self-closing, reverse tweezers will speed the process as these self-closing tweezers greatly ease the manipulation of dried specimens. And finally, it will also be of use to have a counter of some kind available – this can be a one as illustrated in Figure D1, or, alternatively, a web version.

If the bees are not pinned and the researcher wishes to work with a batch of specimens, the researcher will want to put the specimens in a shallow box, tray, or Petri dish so the researcher can hold the tray in one hand and manipulate the specimens with the tweezers in the other.

Figure D1

COUNTERS FOR KEEPING TRACK OF NUMBERS OF BEES OF DIFFERENT TAXA



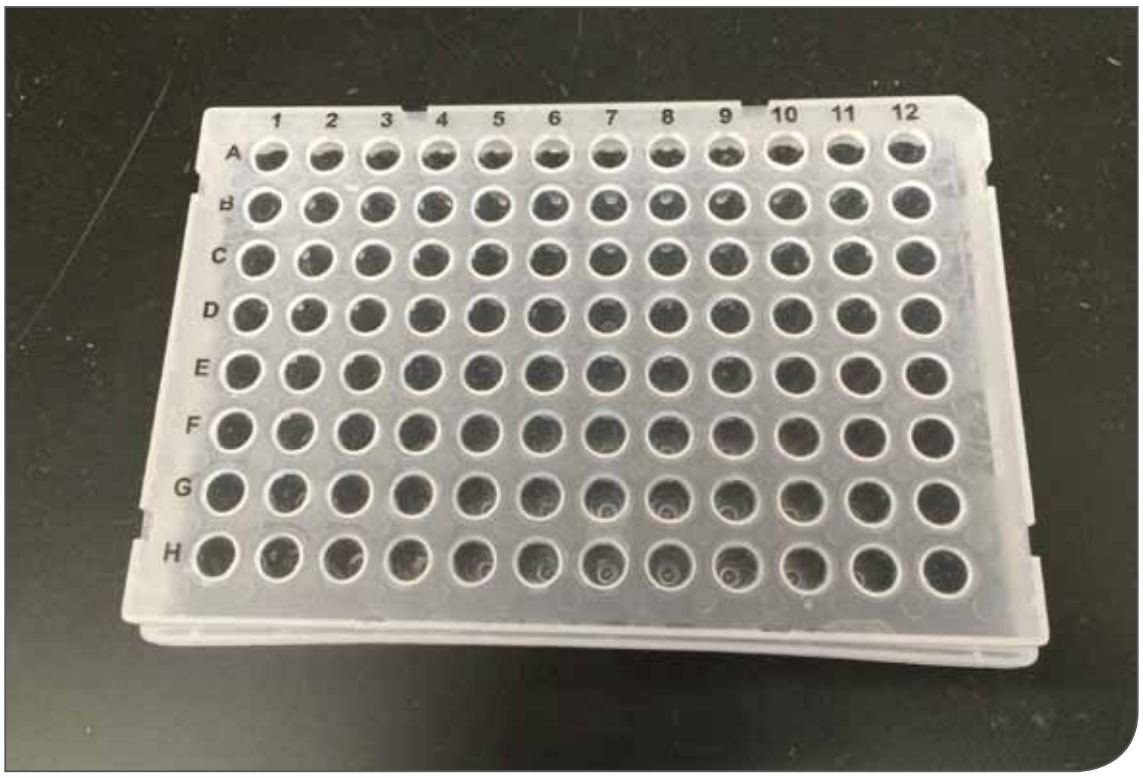
This will allow the researcher to manipulate the tray under the microscope and use the tweezers to pull out and identify specimens. Set the focal length of the microscope such that the focal length is above the level of the box. This allows one to remove a specimen from the box and set the box down, to hold and manipulate the specimen under the scope but over the box.

Note that there are groups of bees and wasps that look quite similar to one another. During the start of any survey or when less experienced sorters are working with specimens, no wasps should be discarded until it can be identified for certain that they may actually be bees.

In addition to bees, many other flies, beetles, wasps and other insects are caught in bowl traps. While this protocol deals with bees, the specimens of other species should be saved for other taxonomists or researchers. The most efficient means of saving the surplus would be to keep them in the same Petri dish as the unpinned bees.

Specimens that the researcher can identify can be put aside either in a sorting tray (Figure D2) or into another tray of their own choosing. Individual species are assigned to each one of the counter buttons. The different sexes would also be assigned different buttons. As the

Figure D2
SORTING TRAY



researcher continues to count specimens, they click the counter for each species/sex. For those species the researcher is unsure of, or for some reason want to pin, the researcher would place those directly onto a foam pad for pinning or put them into another box for pinning later. Warning: if the researcher is processing specimens immediately after they have been washed and dried then they will find that the large specimens are too heavy to stay glued to a pin and will pull away. The researcher can pin them in the traditional manner or wait until they are dried in a couple of weeks.

After the specimens have been sorted then transfer the counts for each species/sex from the bee counter into a spreadsheet[1] along with a count of the number of unidentified specimens that will be pinned or pinned later.

The species that have been identified can be pooled together and placed into a Petri dish or other container along with their identification tag. As long as these specimens are kept out of the light, checked regularly for pests, and kept at low humidity then they will remain archived for future researcher's use. Such archived species should be kept indefinitely. If the researcher lives in a high humidity area then specimens should be kept in an air-conditioned room at all times during the humid seasons and monitored closely for mold.

In general, the process outlined above greatly speeds up the processing of specimens, lowers the costs necessary for pins, insect trays, insect drawers, insect cabinets, and large amounts of humidity-controlled storage space.

[1] To record data during the processing of bees, an Excel spreadsheet (designed as a companion to this protocol) is available at http://www.fao.org/pollination. Alternatively, a new data entry format can be constructed, as suits the researcher's preference.

ANNEX E
SAMPLE R CODE

This is presented through a hypothetical data set (BeeSpecies.txt) that could be used to compare species richness. This data set is too small to be an accurate measure of trend. For simplicity, only three sites and three years were included.

E.1 DATA SET FILE FORMAT

#BeeSpecies.txt (This is the filename for this file)

LOCATION	HABITAT	SPECIES	ABUNDANCE	YEAR
site1	agricultural	41	150	2001
site1	agricultural	44	138	2006
site1	agricultural	38	120	2011
site2	rural	36	95	2001
site2	rural	35	88	2006
site2	rural	32	90	2011
site3	suburban	38	105	2001
site3	suburban	37	100	2006
site3	suburban	31	80	2011

E.2 SAMPLE CODE FOR STATISTICAL ANALYSES

 # Computing summary statistics

```
y = c(41,44,38,36,35,32)
ysum = sum(y)
ysq = sum(y^2)
n = length(y)
ymean = ysum/n
yvar = (ysq - ysum^2/n)/(n-1)
mean(y)
var(y)
```

Does species richness appear to increase or decrease with time?

The code below produces a scatter plot of species richness vs. year using different colours to indicate the type of habitat of each observation.

```
# Trend in pollinator species (linear regression)

d = read.table('beeSpecies.txt', header=TRUE)
y = d['species']
x = d['year']
plot(x, y, xlab='Year', ylab='Species number', type='n')
i = d['habitat'] == 'agricultural'
points(x[i], y[i], col='black')
i = d['habitat'] == 'suburban'
points(x[i], y[i], col='red')
i = d['habitat'] == 'rural'
points(x[i], y[i], col='blue')
legend(x='topleft', title='Bee research', fill=c('black','red','blue'),
legend=c('Agricultural','Suburban','Rural'), horiz=FALSE, bty='n')
```

To use a sign test to compare trends.

```
# We can perform the sign test with the sign.test function:

sign.test<-function(x=0,y=NULL,alternative="two.sided"){

        n<-sum((x-y)!=0)
        T<-sum(x<y)

        if (alternative=="less") {
                p.value<-pbinom(T,n,0.5)}
        if (alternative=="greater"){
                p.value<- 1-pbinom(T-1,n,0.5)}
        if (alternative=="two.sided"){
                p.value<-2*min(1-pbinom(T-1,n,0.5),pbinom(T,n,0.5))}

list(n=n,alternative=alternative,T=T,p.value=p.value)}

#data set
counts.in.2001 <- c(41,36,38)
counts.in.2011 <- c(38,32,31)
```

```
#When calculating the differences, be careful which variable is labeled "y"
#and which variable labeled "x":
sign.test(x=counts.in.2011, y=counts.in.2001, alternative="less")
```

To use a paired t-test to compare the value of each sample between years.

```
#We can perform a paired t-test with the function t.test

t.test(counts.in.2011,counts.in.2001,paired=TRUE)
```

To use a linear regression model to estimate the intercept and slope parameters associated with each sample location and to superimpose the estimated regression lines on the scatter plot.

```
# We can fit a regression model
species = d[,'species']
year = d[,'year']/100
habitat = as.factor(d[,'habitat'])
fit = lm(species~year*habitat)
```

To examine the residuals, the code below produces a scatter plot of the model's residuals vs. year and superimposes a horizontal dotted line at zero (= no residual error).

```
# We can compute regression line for each habitat from estimates of model parameters
plot(year, species, main="Habitat",
   xlab="Year ", ylab="Species ", pch=19)
beta = coef(fit)
a = beta[1]
b = beta[2]
abline(a, b, col='black')  # regression line for Agricultural
a = beta[1] + beta[3]
b = beta[2] + beta[5]
abline(a, b, col='red')  # regression line for Urban
a = beta[1] + beta[4]
b = beta[2] + beta[6]
abline(a, b, col='blue')  # regression line for Rural

# We can plot residuals of model's fit
plot(year, fit$residuals, main="Residual Plot")
abline(h=0, lty=2)
```

ANNEX F
GLOSSARY OF BEE TAXONOMIC TERMS

ANGULATE forming an angle rather than a curve

AREOLATE an area dissected by reticulating raised lines forming clear and strongly defined cells

ANTERIOR toward the head or on the head side of a segment being described

APEX end of any structure

APICAL near or at the apex or end of any structure

APPRESSED tight and flat against the body of the bee, usually used to describe hair

ARCUATE curved like a bow

AREOLATE integumental (skin) sculpture pattern: divided into a number of small irregular spaces, very similar if not used interchangeably with reticulate

AROLIA the pad between the claws found at the ends of some bees legs

BANDS usually referring to bands of hair or bands of colour that traverse across an abdominal segment from side to side

BASAD (BASALLY) toward the base

BASE (BASAL AREA) on whatever part being described, this would be the section or the area at or near to the point of attachment, or nearest the main body of the bee, the opposite end of which would be the apical area

BASITARSUS the segment of the tarsus that is the nearest to the bee's body....usually the largest of all the tarsal segments

BASITIBIAL PLATE a small plate or saclike projection at the base of the hind tibia (like a bee knee pad)

BIFID cleft or divided into 2 parts; forked

CARINA a clearly defined ridge or keel, not necessarily high or acute, usually appears on bees as simply a raised line

CARINATE keeled; having keels or carinae

CAUDAD towards the tail, or on the tail side of a segment being described

CHEEKS the lateral part of the head beyond the compound eyes, includes the gena and the subgena

CLYPEUS a section of the face below the antennae, demarcated by the epistomal sutures

CONICALLY cone shaped, with a flat base, tapering to what is usually a blunt or rounded top

CONVEX the outer curved surface of a segment of a sphere, as opposed to concave

CORBICULA a hairless area or patch surrounded by longer hairs used to hold and transport pollen. Bumblebees and honey bees have this on their tibia, while Andrena have a patch on the sides of their propodeum

COSTA wing vein

COXAE the basal segment of the leg

CUBITAL wing vein

DENTICLE a small tooth-like projection

DISC a generic term for the middle surface of a plate (usually in reference to an abdominal segment) as opposed to what might be going on along the sides

DISTAL away from the body or a description of a place on a segment that is furthest from the place of attachment with the body of the bee

DORSUM in general, the upper surface

ECHINATE thickly set with short, stout spines or prickles

EMARGINATE a notched or cut out place in an edge or margin, can be dramatic or simply a subtle inward departure from the general curve or line of the margin or structure being described

FASCIAE a transverse band or broad line, in bees often created by a band of light coloured hairs on the abdomen

FERRUGINOUS rusty, red–brown, orange-brown

FLAGELLUM the third and remaining part of the antenna beyond the pedicel and scape, containing most of the antennal segments

FORE usually refers to the first pair of legs, the ones closest to the head

FOVEAE a depressed region of cuticle, in bees this depressed area is usually only very slightly hollow and usually on the face

FULVOUS a brownish yellow-tawny colour to orange brown

FUSCOUS dark brown, approaching black; a plain mixture of brown and red

GENA the cheek or the region below the eye seen when viewing the head from the side and holding the head so that the flat of the face is at right angles to the line of sight

GLABROUS a surface without any hairs

GLOSSA part of the tongue

GRADULUS a line that runs from side to side on abdominal segments of some bees that is formed by the step between two regions that differ in height, often that difference is only apparent upon very close inspection

HYALINE transparent, glassy

HYPOEPIMERAL area on the thorax

HYPOSTOMA the notched region underneath the head and behind the mandible that holds the folded tongue

IMBRICATE lined with microscopic inscribed lines that form a fish scale like pattern

IMPRESSED AREA almost always refers to the rear part of the upper abdominal segments, these areas often being very slightly (often very difficult to detect) lower than the front part of the segment

IMPUNCTATE not punctate or marked with punctures or pits

INFUSCATED smoky gray-brown, with a blackish tinge

INNER usually refers to legs and refers to the part that faces the body

INTEGUM the outer layer of the bee; the skin or cuticle

INTERCUBITAL wing vein

INTERSTITIAL when describing veins it refers to the end of one approximating the beginning of another, as in a grid intersection

LABRUM abutting the clypeus in front of the month

MACULA a spot or mark

MACULATIONS spotted or made up of several marks

MALAR SPACE the shortest distance between the base of the mandible and the margin of the compound eye often completely absent in bees

MANDIBLES bee teeth, so to speak, usually crossed and folded in front of the mouth

MARGINAL CELL a wing cell located on the edge (margin) of the wing

MESALLY (MEDIALLY) pertaining to, situated on, in or along the middle of the body or segment

MESEPISTURNUM, MESOPLEURA, OR MESOTHORAX the second or middle segment of the thorax bearing the middle legs and the forewings, the pronotum is the first segment

METAPLEURA thorax segment bearing the hind legs and hind wings

NOTAULICES a pair of lines on some bees that appear on either side of the scutum near the base of the wings

OCELLI the 3 simple eyes or lenses that sit at the top of the head of bees

OCHRACEOUS pale yellow

PAPILLAE (PAPILATE) very tiny short hard cone-like projections usually in bees only found on the wing or legs and often having small hairs arising from the top

OUTER usually refers to legs and specifically to the surfaces facing away from the body

PECTINATE comb-like, having large comb-like teeth that are clearly separate from one another

PETIOLATE having a stalk

PICEOUS glossy brownish black in colour, pitch like

PLEURA the lateral or side areas of the thorax, excluding the lateral surfaces of the propodeum

PLUMOSE feather-like

POLLEX a thumb; the stout fixed spur at the inside of the tip of the tibia

POSTERIOR toward the tail end or on the tail end of a segment being described

PREAPICAL referring to a section of a bee that is just physically found just before the outermost (or apical) end of the section or segment

PRONOTUM a collar-like segment on the thorax and directly behind the head; extends down the sides of the thorax toward the first pair of legs

PROPODEUM the last segment of a bees thorax (although not evident, it is in fact considered anatomically part of the abdomen)

PROTHORACIC of or pertaining to the prothorax

PROTUBERANT rising or produced above the surface or the general level, often used as a term to define a single or pair of small bumps

PROXIMAL that part nearest the body

PUBESCENT downy; clothed with soft, short, fine, loosely set hair

PYGIDIAL PLATE unusually flat area (a plate) surrounded by a ridge or line and sometimes sticking well off of the end of the bee. If present, found on the sixth upper abdominal segment in females, seventh in males

REPOSE in a retracted physical state

REFLEXED bent up or away

RETICULATE made up of a network of lines that creates a set of netlike cells, similar to areolate except perhaps more of a regular network of cells - undoubtedly both have been used to describe the same patterns at times

RUGOSE a wrinkled set of bumps that are rough and raised well above the surface

SCAPE the first or basal segment of the antenna

SCOPA a brush; a fringe of long dense and sometimes modified hairs designed to hold pollen

SCUTELLUM shield shaped plate behind scutum

SCUTUM the large segment on top of the thorax located between the wings and behind the head

SERRATE notched on the edge, like a serrated knife

SETOSE covered with setae or stiff short hairs

SINUATE the margin with wavy and strong indentations

SPATULATE shaped like a spatula

SPICULE small needlelike spine

SPINOSE armed with thorny spines, more elongate than echinate

STERNA the plates on the underside of the abdomen

STIGMA a thickened coloured spot or cell in the forewing just behind the costal cell

STRIAE a set of parallel lines (usually raised) and can be thick or thin

SUBAPICAL located just behind the apex of the segment or body part

SUBCONTIGUOUS not quite contiguous or touching

SUBEQUAL similar but not necessarily exactly equal in size, form or length

SUBMARGINAL CELLS one or more cells of the wing lying immediately behind the marginal cells

SUBRUGOSE a bit bumpy but not forming an extensive set of wrinkled bumps

SULCUS groove; more of an elongate hole or puncture in the skin of the bee

SUPRA above, beyond or over

SUPRACLYPEAL AREA the region of the head between the antennal sockets and clypeus, demarcated on the sides by the subantennal sutures

SUTURE a groove marking the line of fusion of two distinct plates on the body or face of a bee

TARSUS the leg segments at the end of the bee's leg, attached to the tibia

TEGULAE the usually oval, small shield like structure carried at the extreme base of the wing where it attaches to the body

TERGUM the segments on the top side of the abdomen

TESSELLATE small very fine lines that make up a network of squares like a chessboard on the surface of the skin; can often be very faint markings that appear like fingerprints on the shiny surface of the skin

TESTACEOUS brownish-yellow

TIBIA segment of the leg, between the femur and the tarsus

TOMENTUM a form of pubescence composed of short matted, woolly hair

TOMENTOSE covered with tomentum

TRANSVERSE across the width of the body or segment rather than the length, in other words at right angles to the head to abdomen axis of the body

TROCHANTERS segment of the insect leg between the coxa and the femur

TRUNCATE cut off squarely at tip

TUBERCLE a small knoblike or rounded protuberance

UNDULATE wavy

VENTER the undersurface of a section of a bee or bee part, usually the abdomen

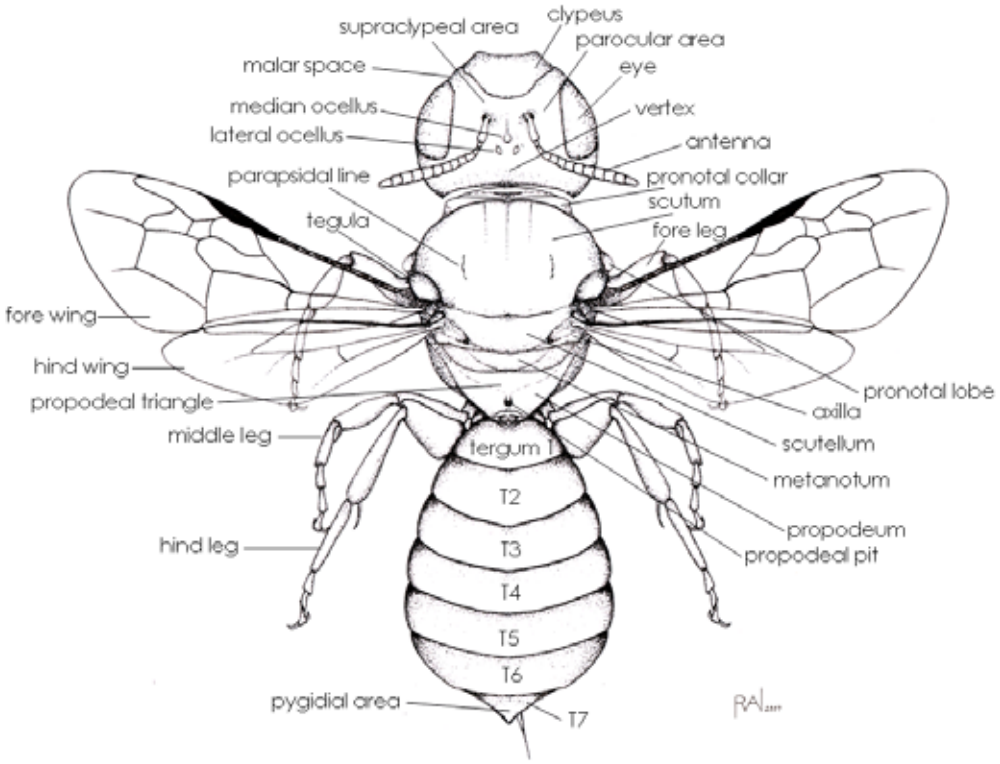
VENTRAL pertaining the undersurface of the abdomen

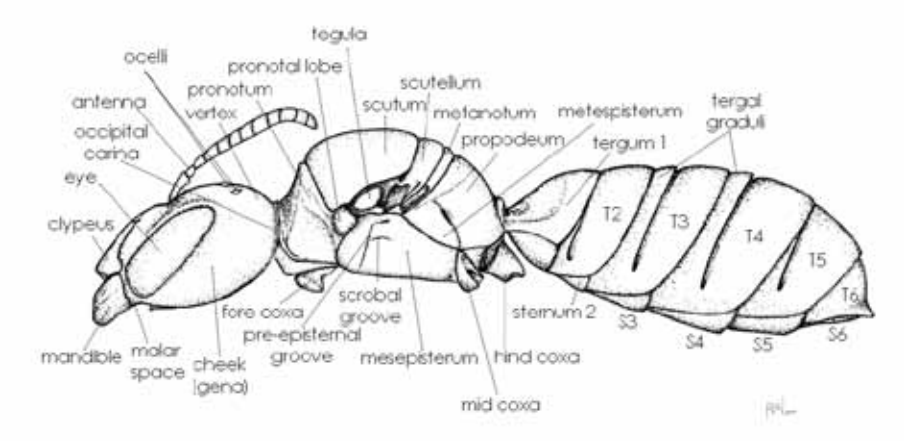
VERTEX the top of the head

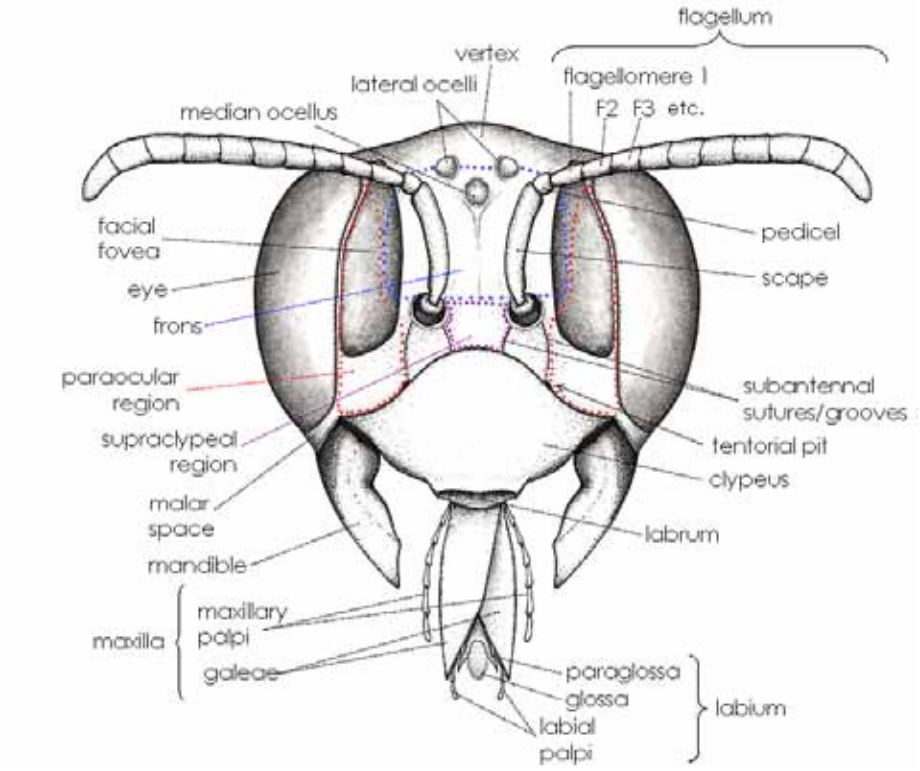
VIOLACEOUS violet coloured

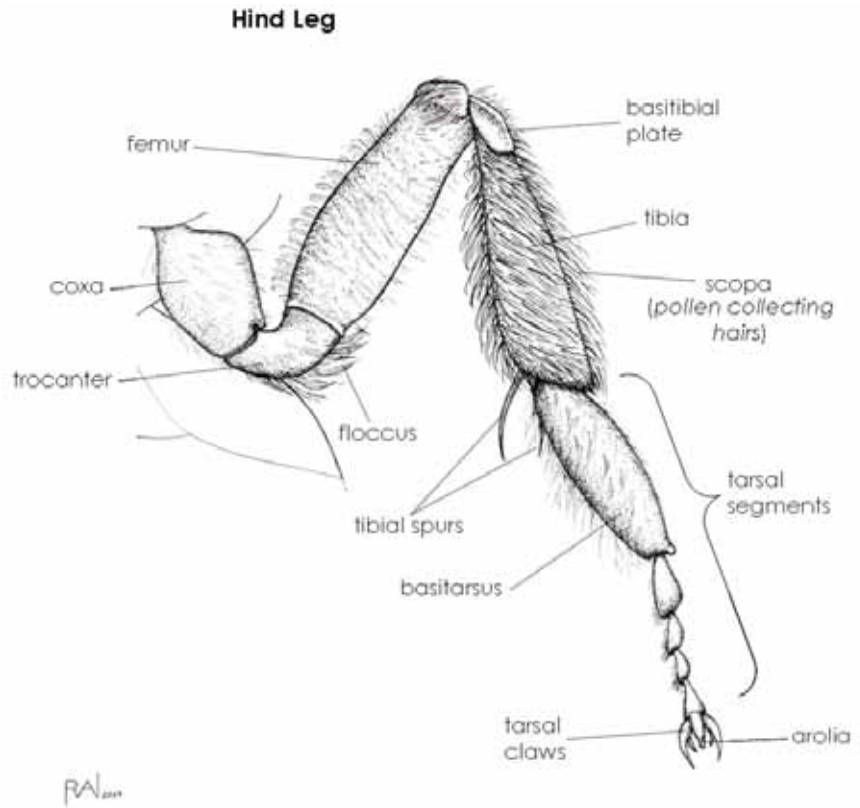
ANNEX G
BEE BODY PART FIGURES

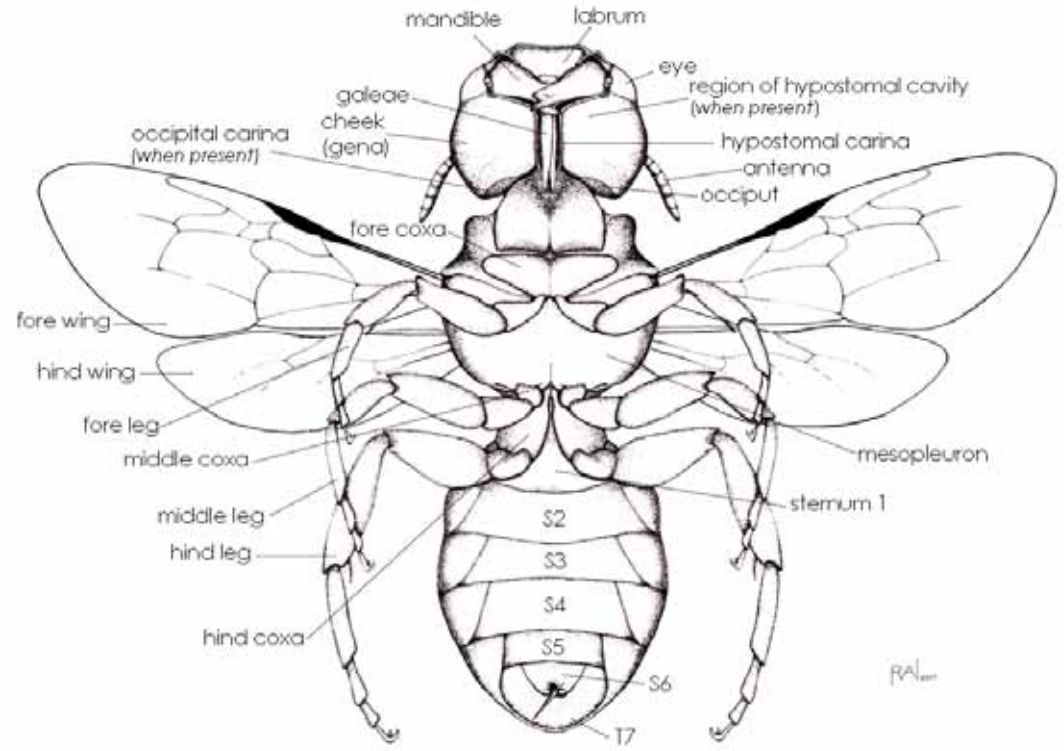
Drawn by Rebekah Nelson

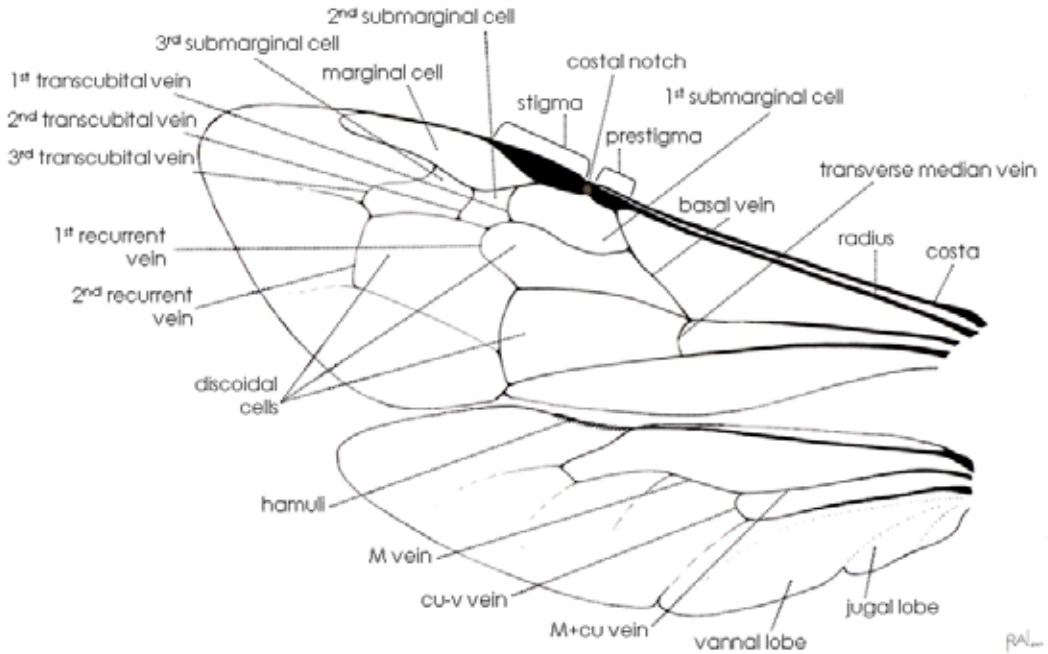





flagellum
vertex
lateral ocelli
flagellomere I
F2 F3 etc.
median ocellus
facial fovea
pedicel
eye
scape
frons
paraocular region
subantennal sutures/grooves
supraclypeal region
tentorial pit
clypeus
malar space
labrum
mandible
maxilla
maxillary palpi
galeae
paraglossa
glossa
labial palpi
labium


Hind Leg
basitibial plate
femur
tibia
coxa
scopa (pollen collecting hairs)
trocanter
floccus
tarsal segments
tibial spurs
basitarsus
tarsal claws
arolia


mandible
labrum
eye
region of hypostomal cavity
(when present)
galeae
cheek
(gena)
occipital carina
(when present)
hypostomal carina
antenna
occiput
fore coxa
fore wing
hind wing
fore leg
middle coxa
middle leg
hind leg
hind coxa
mesopleuron
sternum 1
S2
S3
S4
S5
S6
T7


2nd submarginal cell
3rd submarginal cell
marginal cell
costal notch
1st transcubital vein
2nd transcubital vein
3rd transcubital vein
stigma
1st submarginal cell
prestigma
transverse median vein
basal vein
1st recurrent vein
radius
costa
2nd recurrent vein
discoidal cells
hamuli
M vein
cu-v vein
jugal lobe
M+cu vein
vannal lobe

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As a contribution to the International Pollinator Initiative, and through the GEF/UNEP/FAO Global Pollination Project, FAO collaborated with the San Francisco State University to develop a protocol for monitoring bee pollinator populations in crop production landscapes. Practical guidance is provided for using a common methodology to monitor pollinator diversity and abundance. The publication provides options for local implementation for a variety of groups including researchers, extension agents, farmers, students and others.

GLOBAL ACTION ON **POLLINATION SERVICES**
FOR **SUSTAINABLE AGRICULTURE**

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