THE POWER OF POLLEN PROFILES

for Planting Trees for Bees

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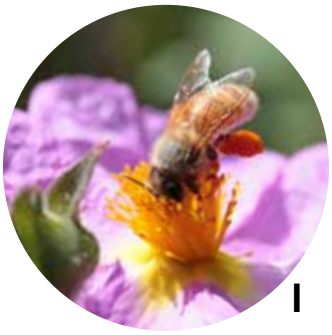
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INTRODUCTION

The Power of Pollen Profiles is that they demonstrate which pollen and nectar sources are being used by your bees at any given apiary site. Pollen profiles are valuable tools for better beekeeping. Many experienced beekeepers pay close attention to which plant species are coming into flower throughout the seasons to make sure their bees are well fed and healthy to ensure a good honey harvest. Expert beekeepers usually develop the skills to recognise the type of pollen their bees are bringing in to the hive by the colour and form of the pollen pellet loads on the bees' legs as they enter the hive. This skill requires some familiarity with the plants in flower at any given time and is best done at a local level where it is possible to readily determine the plant species because there are not too many candidate plant species to contend with in one local area. Expert beekeepers also usually know which nectar sources bees are using by recognising the colour, taste, scent, and thickness of the honey in the frame and often use back up information by obtaining a pollen profile of the honey which can be derived from various sources including by a Do It Yourself (DIY) set up.

It takes time to learn how to identify the types of flowers, pollen pellets and pollen grains but your accumulated knowledge and experience will improve your beekeeping decisions. To take the guesswork out of the learning process and speed up the accumulation of the range of plants you know, we show a way to collect data so that you can create and interpret pollen profiles yourself. Pollen profiles for pollen and nectar sources can be illustrated in several ways, for example, a pie chart, a bar chart or a simple list with proportions. These profiles depend on knowing the relative amount of each type of pollen (a quantitative analysis) but it is also possible to benefit from a simple list without knowing the proportions (a qualitative analysis). For instance, a qualitative analysis is all that is needed for using the information to find out what plant species the bees are using so that you can plant more of them to boost the floral resources at the apiary site. This is the essence of Trees for Bees. We are currently using pollen profiles to help us to create planting lists for apiary sites that will incorporate known plant species that are already growing in the area. These species are not only well-matched to the climatic and habitat conditions of the site but also known to be plants that the bees will use and even prefer. As pollen and nectar deficit periods are discovered, these gaps can be filled in with preferred bee plants that are best suited to the area. The quantity and quality of floral resources at a site can be optimised by planting more of the best candidate plant species from your local list.

Pollen Profiles for apiary sites provide important evidence by showing the relative proportions of different pollen types that bees bring into the hive either in their bee pollen pellet loads or in the nectar they store in the frames. The Pollen Profile methods that we describe can be used for many purposes besides creating superior bee planting lists. For example, they can help with investigating the presence of toxic plants in an area such as karaka or tutu, indicating the potential for a monofloral honey, determining what plants the bees were foraging on in the event of a pesticide poisoning, etc. The methods can be adapted at the local level to address many different questions about pollen and nectar foraging by bees. Methods for collecting and analysing data and building your own plant and pollen reference collection are described. We make suggestions about how to proceed along the learning curve with the aid of developing your own range of scientific evidence so that you can re-use your knowledge at other sites that you want to initiate or explore.

Photos at the top banner:

A. *Orange pollen pellet from Rock Rose (Cistus creticus)*
B. *Yellow pollen pellet from Rapeseed (Brassica napus 'Greenlandia')*
C. *Orange pollen pellet from Flax (Phomium tenax from Sealers Creek Auckland Is.)*
D. *Brown pollen pellet from White Clover (Trifolium repens)*

POLLEN PELLET SAMPLE COLLECTION

The fastest way to learn about what pollen bees are using is to watch the bees while visiting the flowers. You will see them either gathering pollen (scrabbling around the anthers) or sucking up nectar (bee tongue is out and into the nectary). To make sure the pollen pellet loads on their legs are in fact from that same flower you can take a sample of the pollen from the flower and make microscope slides of both the pollen from the bee pellet and from the flower itself. If they match which they do 95% of the time in our experience, then that is a correct identification of the pollen in the bee pellet. Techniques for making microscope slides to look at pollen and how to identify pollen are described elsewhere. The advantage of collecting bee pellets on the bee while it is visiting the flower is that if the pollen from the flower matches the pollen from the bee leg, then you have proof that the bee uses that pollen for feeding the brood. If you do not know the plant species, you can take a specimen of it and send it to an expert for identification or look it up in plant identification keys and illustrations (e.g. ask at your local nursery or regional council). The disadvantage of this method is that it is sometimes difficult and also time consuming to find and catch bees taking pollen from some plants especially native plants in the bush.

One of the fastest ways to obtain pollen that bees are using is to let the bees bring the pollen to you. Since bees forage in an area of from 1 km (300 ha) to over 5 km (a huge area) it is not possible to explore everywhere your bees might be foraging to see which plants they are using. Therefore we sometimes have collected the bees with their pollen loads at the hive entrance. This is tricky if you are not an experienced beekeeper because it could disturb the hive but some people can do it well without getting stung. However, it is much easier and more cost effective to use a hive pollen trap to collect the pellets as the bees make their way back into the hive.

We have modified a pollen trap which we obtained from Ecrotek supplies http://www.ecrotek.co.nz/superior-pollen-trap-mann-lake.html. Trevor Gillbanks of Trev's bees has modified the trap for our purposes. For our methods it is necessary that the hive pollen trap will be left on all year long without disturbing the bees or taking too much pollen from the bees so that we can sample periodically. It is important to be able to turn the hive on for a short period (one day) and keep the trap off the rest of the time. The pollen samples can be taken through as many seasons as you wish but we normally sample from early spring to early winter. We only need to collect pollen from the hive trap for one 24 hour period on a fine warm sunny day about every fortnight. It does not need to be left on any longer than one day unless there is too little pollen coming in. It does not need to be turned on any more often than a fortnight because the range of plant species in flower will not change that often as most plants flower for a week or two in general. It can be a good idea to use trap pollen from three hives per apiary because the pollen profile often differs from one hive to the next in the same apiary site. The modification that we made to the pollen trap apparatus

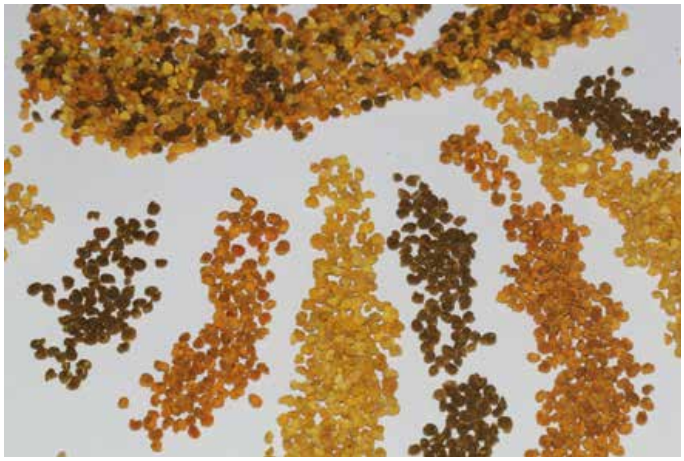
Two Pollen Traps modified by Trev's Bees with alternate entrances. For TRAP ON (left) bees can come out of tubes but will not see the tubes to go in so are forced through the trap. TRAP OFF (right) is a normal hive entrance with bees not going through the trap.

was to enlarge the entrance when the hive trap is turned off so that the bees do not have only four narrow holes to re-enter the hive. We found that bees would go through the wrong entrance when the trap was turned off because they preferred the larger entrance but this forced them to go through the trap mesh even when we had the trap turned off. To modify this entrance for the on and off modes of the hive trap we asked Trevor Gillbanks to create two different entrances that can be alternated: one for when you want to put the TRAP ON and one for when you want the put the TRAP OFF. This is best described by seeing the traps in action and is demonstrated at our Pollen Profile workshops.

Inserting the entrance for putting the pollen trap in the TRAP ON position.

Inserting the entrance for putting the pollen trap in the TRAP OFF position.

After the 24 hour period, the pollen is collected out of the drawer in the trap and put into a jar to be stored in the freezer until further analysis of the pollen can be done. A funnel can help to pour the pollen into a jar. The jar is labelled with apiary site location and date of collection. The trap drawer is cleaned by brushing it out and put back into the trap. The trap is turned off until the next fortnightly period. Each period is only approximately a fortnight. It can be plus or minus a few days if it is raining or the bees are not flying for any reason and you need to wait for a sunny day. The beekeeper will need to decide when to stop trapping pollen in the winter months as this will depend on climate. Usually the bees are not disturbed in May, June and sometimes July but this depends on your location and normal beekeeping best practices for taking care of bees in the winter.



Sorting pollen pellets into their different colours.

Horse chestnut (Aesculus hippocastanum)

Phacelia (Phacelia tanecetifolia)

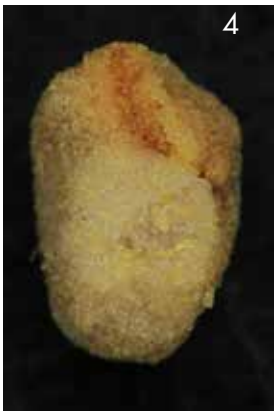
POLLEN PELLET ANALYSIS

We have two methods for analysing the pollen pellets for constructing the pollen profiles. The first, sorting pollen pellets by colour, is slow and laborious but will help you to learn the characteristics of each pollen pellet type. The second, bulk processing all the unsorted pellets for pollen identification, is fast and easy but you do not learn the pollen pellet types because you are not processing one pellet type (colour) at a time. We have worked with sorting pollen pellets by colour for many years. We encountered some difficulties with this and found it to be very time-consuming and could be prone to error. We only use it when we have enough time available and a good reason for it. We are now using method of bulk processing of unsorted pollen pellets as this gives a similar profile by a different method and we get results faster. We deliver the jar of unsorted pollen pellets to the GNS Science Pollen Lab for bulk processing to get the pollen identified and obtain a list of species and their proportions.

Although there are limitations with sorting pollen by colour it is a good way to become expert at recognising pollen pellet types on the wing for a local area so it may be well worth the time. For some very distinctive types of pollen pellets with rare colours it is easy to sort, for example horse chestnut trees have bright red pellets and phacelia has purple/mauve pellets. There are a few other species with purple pellets and so you would use information about flowering time and what is flowering at your apiary location to distinguish between them (e.g. phacelia, thistle, and fuchsia).

However, for the orange, yellow and white pollen pellet types, it can be a challenge for a number of reasons. These three colours are common to a number of species (see Page 5 and 6) and they often intergrade with each other. Furthermore, the colours of the pollen pellets can change with drying out as described by D. Hodges (1974) in her book The Pollen Loads of the Honey Bee (Bee Research Institute, Nature. 65 pages). In addition, the ambient light used when examining or sorting pollen pellets will influence the colour perception by humans and so can cause confusion. Many pollen pellets change colour with exposure to air over time by turning to dramatically different colours such as from yellow pollen in the flower to brown pollen pellets in gorse and clover and from purple pollen pellets to clear transparent pellets in Fuchsia excorticata, a native plant.

When sorting hive trap pollen, we found that the majority of pollen pellets are purely one colour. In our data over the years we have found less than 2% of the pellets as mixed pollen with two contrasting colours (See examples in Photos 3 and 4). This usually happens when there is a pollen dearth and the bee cannot find enough pollen from one plant species. Some pollen



Mixed pellets with contrasting colours

Not Mixed: Yellow and Orange pollen pellet of Flax (Phormium cookianum 'Tricolour')

Mixed: White pollen pellet of Hawthorn (Crataegus monogyna) mixed with Manna Ash (Fraxinus ornus)

colours are highly distinctive and very uncommon such as the red pollen of Horse Chestnut (Photo 1 on Page 4) and brown pollen of white clover (Photo IV on page 2). Some colours are found in only a few plant species such as the purple (mauve) pollen of Phacelia (Photo 2 on Page 4), a colour also found in Fuchsia (Photo 7 this page) and some Thistles.

Hodges (1974) reports that when bees forage on two different plants are producing a mixed pellet, the pattern of colours is usually half and half mixed because of the way that bees visit flowers in a sequence. How then do we explain the case of Flax (Photo 5) where two distinct pollen colours form a spiral pattern in the pellet? It turns out that this pattern is not from a dizzy bee foraging, it is derived from a mixture of the actual pollen in the flax anther with both orange and yellow pollen. We know this pollen is purely flax because we checked acetolysed pollen directly from the flower and matched it to that from bee legs. This mix of two colours in flax anthers is confirmed in the literature (Craig & Stewart 1988) and by direct examination of pollen in the anthers under the stereo microscope.

One of the problems we encountered when sorting pollen pellets by colour is that we were not able to judge purity from pellet colour if the pollen colour of two different plant species is very similar. For example, we found a white pellet from a bee seen visiting an Manna Ash (Fraxinus ornus) flower that was mixed with primarily Hawthorn (Crataegus) pollen with only some Ash (Photo 6). These considerations indicate that unless you check every pellet which is time consuming to judge the proportions, it is much faster to use the bulk pollen analysis method developed at the GNS Pollen Lab. This method involves mixing and homogenising all the pollen from one sample and making a pollen microscope slide for counting the proportions of each type of pollen. This is sufficient for the purpose of determining which plants bees are using for pollen so that you can plant up more of that plant species.

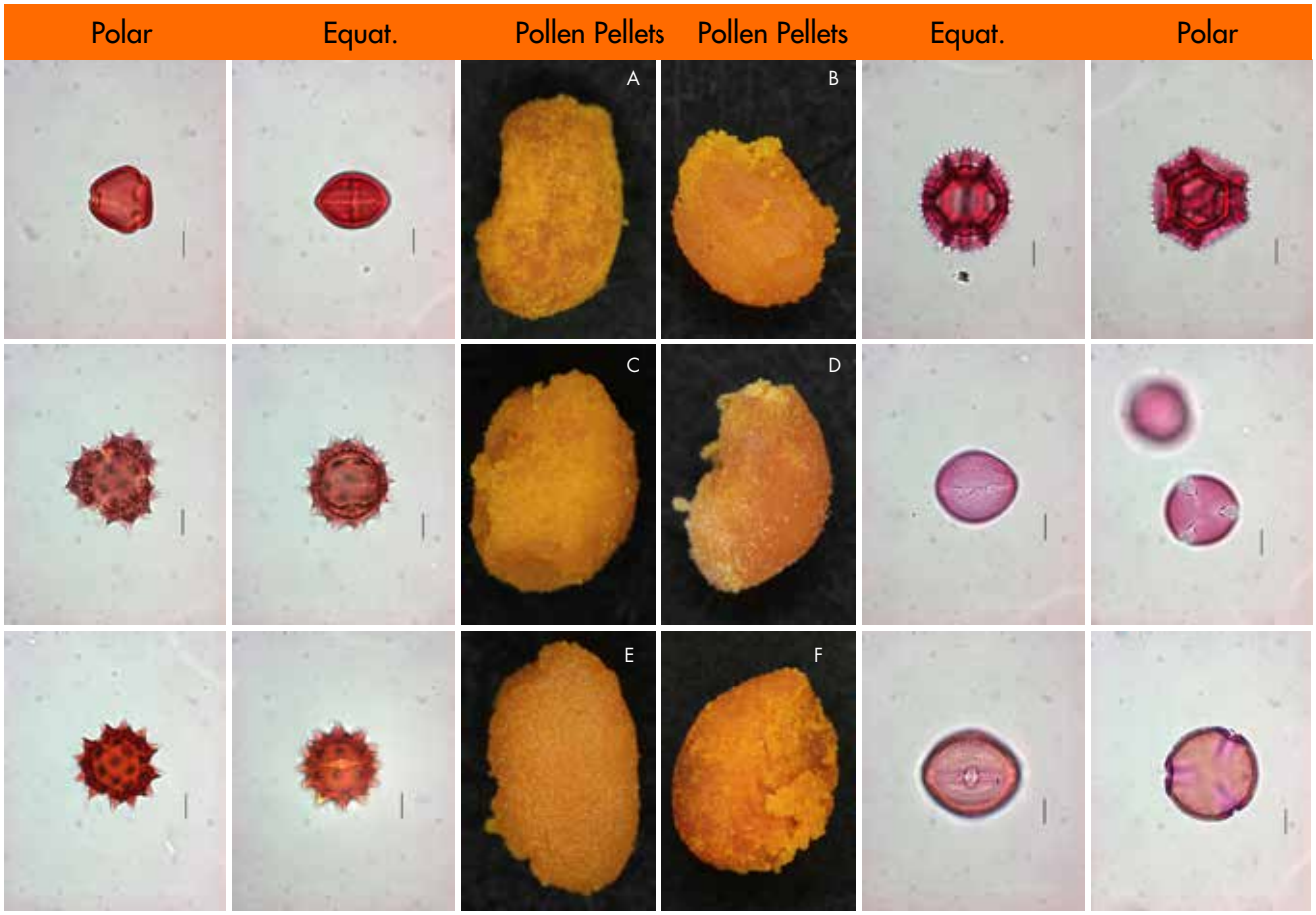
Photo 7. Fuchsia excorticata showing pink anthers with blue to mauve pollen. The petals are green at first and then turn to a purple colour later. The pollen will turn transparent upon drying.

Photo 8. These pollen pellets look like they are mixed pollen but they are all gorse pollen (Ulex europaeus) The colours change upon exposure to air and drying.

Photo 9. A set of sorted pollen from one sample from a hive trap in East Cape with Naati Beez. Measuring the volume gives the proportions of each type.

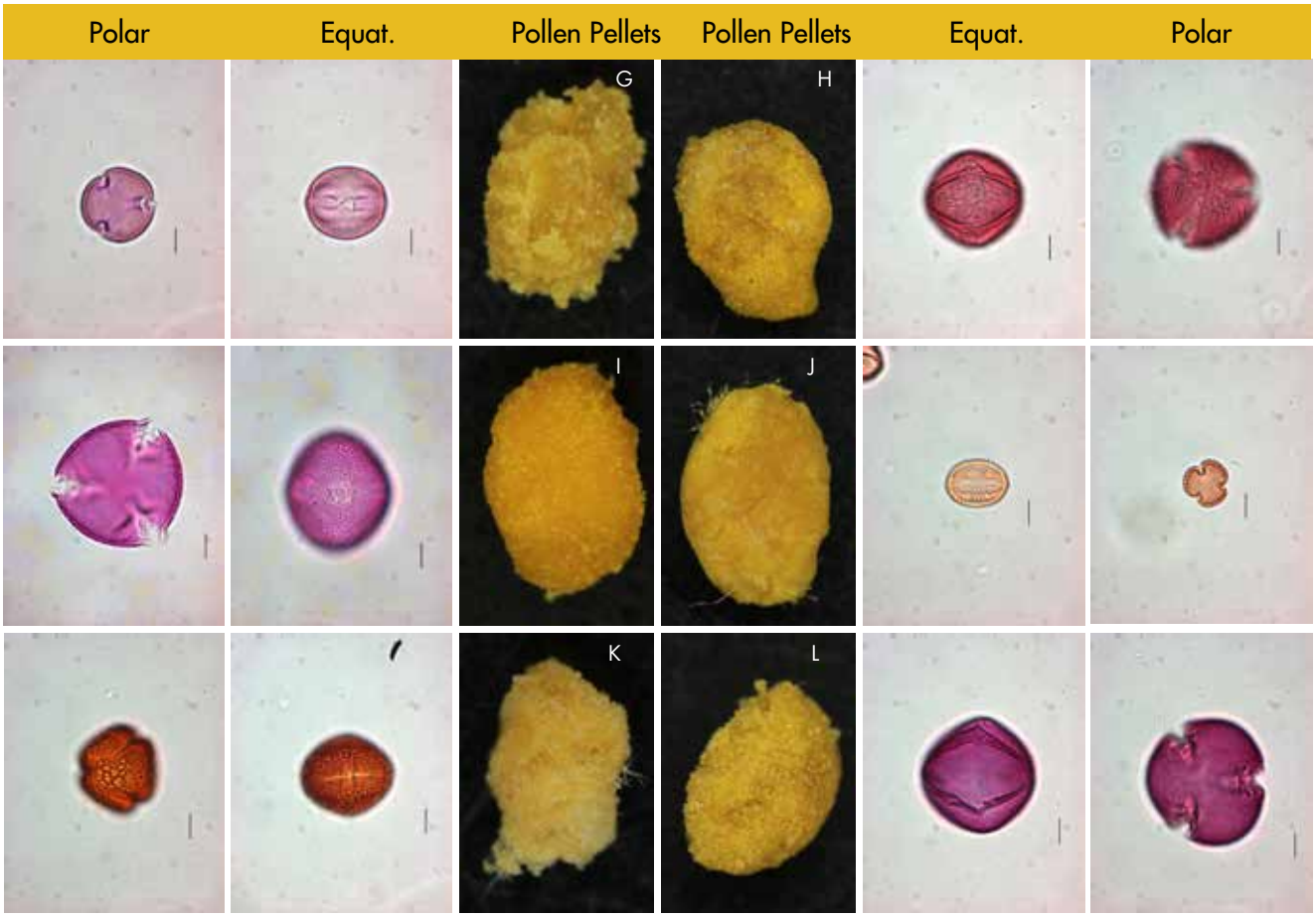
ORANGE

A: Mexican Orange Blossom (Choisya ternata);
B: Dandelion (Taraxacum officinale);
C: Chrysanthemum (Chrysanthemum segetum);
D: Broom (Cytisus scoparius);
E: Daisy (Helenium flexuosum);
F: Rock Rose (Cistus creticus)



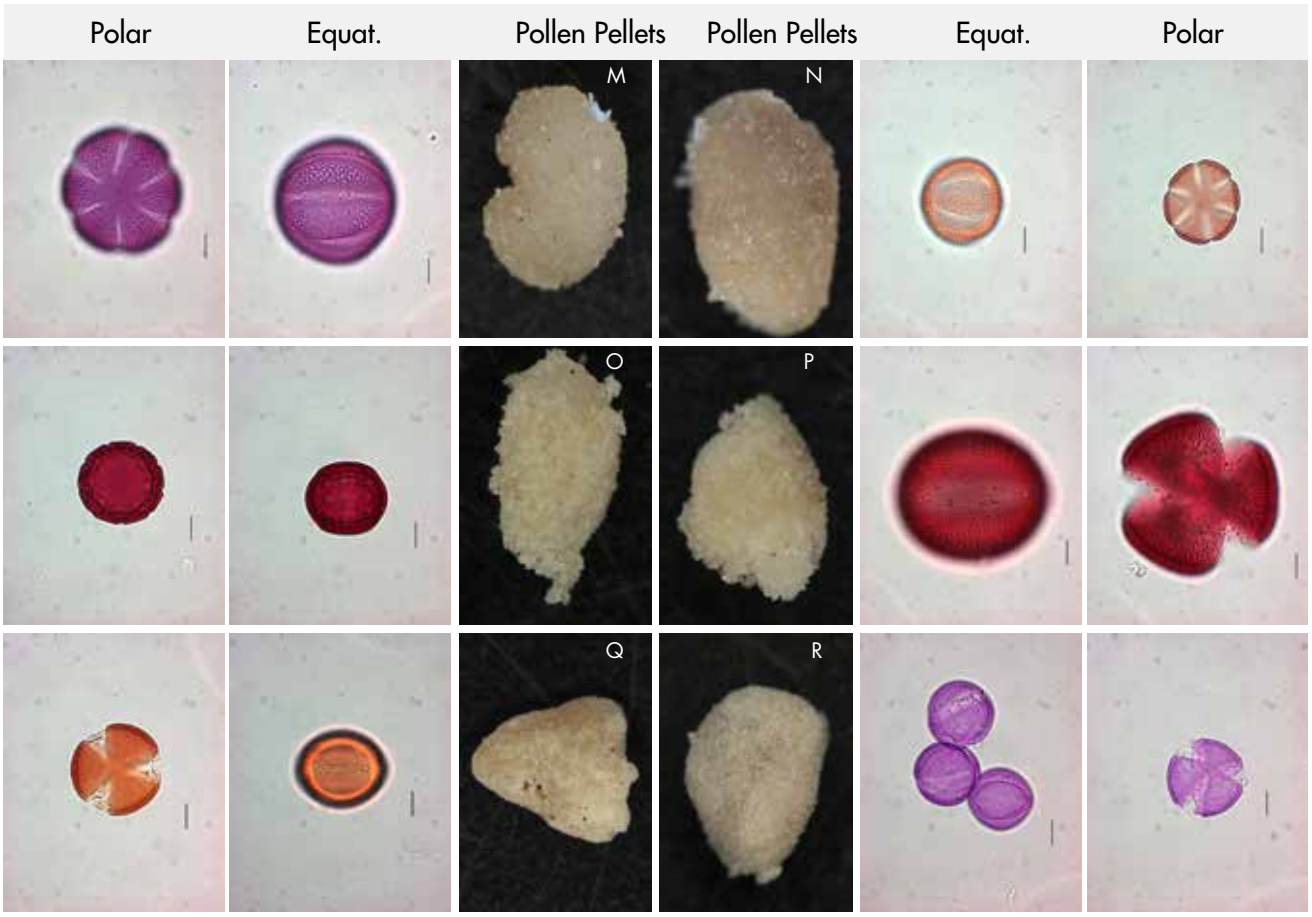
YELLOW

G: Tree Peony (Paeonia suffruticosa)
H: Flowering cherry (Prunus sp.);
I: Camellia (Camellia japonica 'Flame');
J: Willow (Salix sp.);
K: Ivy (Hedera helix);
L: Black Boy Peach (Prunus persica 'Black Boy')



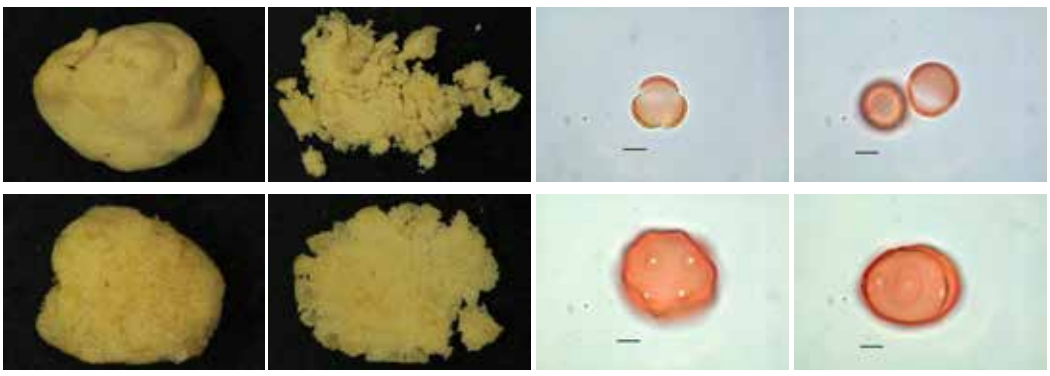
WHITE

M: Rosemary (Rosmarinus officinalis);
O: Borage (Borago officinalis);
Q: Koromiko (Hebe sp.);
N: Thyme (Thymus vulgaris);
P: Silver Bush (Convolvulus cneorum);
R: Poppy (Papaver somniferum)



These photos of pollen pellets and their corresponding photos of pollen grains demonstrate that it is not really possible to identify orange, yellow or white to beige pollen very easily. Each pollen grain is shown in both the polar and equatorial view and they have been acetolysed (treated with acid to show the features of the pollen grain more clearly). Scale bar = 10 microns. Acetolysis requires handling strong acids under a fume hood and is not part of a Do It Yourself operation at home – it requires a proper lab. However, slides can be made by placing pollen directly onto the microscope slide with or without a stain. This gives a different view but the detailed features are not as clear. Also since pollen grains can absorb water they might be swollen and enlarged so you need to be careful about judging sizes of pollen grains.

The only way to learn to identify pollen grains from the orange, yellow and white colours is to use other supporting information. This can be done if you are sorting only one hive at a time from a local area where there are not too many plants to contend with. Other supporting information will help too. Often experienced beekeepers can identify these pollen pellet colours based on years of experience based on which plants are flowering in the apiary foraging area and what time of year they are usually in flower. When in doubt compare your pollen pellet microscope slide to your reference slide of pollen taken directly from the flower of the plant species in question. Additional features of the pollen pellet can be used as well. For example, in the case of brassica and walnut (Juglans) which are a very similar yellow, the surface texture looks different. When the pollen pellets are squashed the brassica is dry and crumbly (Photo 8) while walnut is sticky (Photo 9).



Brassica pollen pellet is dry and crumbles

Walnut pollen pellet is sticky and clumps

NECTAR/HONEY SAMPLE COLLECTION AND ANALYSIS

There are two ways to determine which flowers are being used for nectar in your apiary. You can look around to see which flowers the bees are visiting while taking nectar but there is an easier way. Like the previous pollen pellet exercise, it can be much simpler to sample the nectar brought to hive by the bees to get an idea of the proportions for each plant species. To do this we take representative samples of the uncapped ripening honey in the frames which we call nectar/honey as it is only partially mature honey. We used this uncapped nectar/honey because it will be the freshest nectar in the frame and will therefore represent the fortnightly time frame that we are sampling for. There are several ways to do this but we follow the method developed by Wiremu Kaa of Naati Beez during our 2015-2016 Naati Beez SFF project in Eastcape. We took nectar/honey samples from the same three hives with pollen traps that we were studying because we wanted to see how the pollen from traps and nectar/honey compare from the same hive. Our equipment was simple: two clean kitchen tablespoons per hive that were soaked in diluted bleach or alcohol to make sure they were sterilised. We scooped up a tablespoon worth of honey from five or six different areas of one of the frames and placed the honey in a 250 ml plastic honey jar. You could take the samples from more than one frame if desired. The second spoon was used to scrape the honey off the first spoonful to get it into the jar. It is ideal to try to get about 10 grams if you want to study the colour but most of the time we took only about 2 to 3 tablespoons full depending on how much nectar/honey was available in the hive for the bees. Since this nectar/honey is not mature it will have high moisture content and so the jars need to be labelled with location and date and put into the freezer until it is time to analyse it. Otherwise it will ferment.

The analysis for these samples is the same type of analysis used for mature honey. Details of these procedures will be discussed at the Pollen Profile workshop. There are many published accounts of methods as well and references can be obtained upon request. We sent our samples for identification of pollen under the microscope by Drs. Xun Li and Ian Raine, professional palynologists at the GNS Science Pollen Lab. It is a good idea to get expert help to start out because you will then be confident in the accuracy of your pollen identifications. There are also trained pollen technicians working in the apiculture industry. Once you know the plant species that are in your nectar/honey samples, you can then build your own reference collection of pollen taken directly from the flowers of each plant species at your apiary site or elsewhere. On this basis you will rapidly build your reference collection and will become familiar with the different pollen types that your bees are bringing in for both nectar and pollen pellets. In addition, a Bee Pollen Atlas with photos is also being produced by the GNS Science Pollen Lab which will assist you if you want to Do It Yourself in the future. Since you will be dealing with a local area there will be a limited number of pollen types to become familiar with so it is quite possible to become proficient as an amateur for your local area. If you encounter any unknown pollen then they can be sent to experts for identification and added to your reference collection.

The interpretation of pollen results from the nectar/pollen samples will be interesting because there will be some plant species that bees use for both pollen (found in the pollen pellets) and honey (found in your nectar/honey sample), while others are found only as pollen pellets and some are found only in nectar. Surprisingly, there will be some plant species that are found in the nectar/honey samples that do not produce nectar at all, such as the insect pollinated poppies (Papaver), rock rose (Cistus), olive trees (Olea europea) or Plantain (Plantago) and wind pollinated plants with no nectar such as grasses (Graminaea), and conifer trees like pine (Pinus) etc. This shows that there are several ways that pollen can get into the nectar and therefore the honey. When bees suck up the nectar they will take up any particles such as pollen with it. Pollen can fall directly into the nectar in the flower from bees dislodging the pollen, wind or mechanical shaking of the flower dislodging pollen, or wind blowing pollen from elsewhere from the same or different plant species. There are also several indirect ways that pollen can get into the nectar during the ripening process inside the hive such as when bees are grooming other pollens from their bodies, or when wind blows pollen into the hive entrance.

We have two methods for analysing and interpreting the nectar/honey samples after identification of the pollen on the microscope slides. The first is qualitative where a simple list of the diversity or the range of nectar plants are summarised excluding any nectarless plants. This is all that is needed for a creating bee planting list for nectar sources. But if you wanted to know which nectar plants are preferred, or which produce the most nectar, or if you wanted to forecast the likelihood of obtaining a certain type of honey or a monofloral honey, then you would need to use a quantitative method. There are standard harmonised methods for making pollen counts and the results are expressed in two ways: (1) the relative proportion of each pollen type by percentage, and (2) the absolute pollen count of how many grains in total from each species that is found in given unit volume of nectar. This is called the APC.

If you want to know what type of honey you are likely to get then you will need to interpret both numbers – the percentage of pollen of each plant species and the the APC. It is not just the most numerous type of pollen grain that is important, it is rather the combination and proportions of all the types of pollen

grains in the sample and this defines the botanical honey type as used internationally . The percentage of pollen grains in the honey do not correspond exactly to the actual proportion of nectar contributed to the resultant honey by that individual plant species. It may be more or less pollen per unit volume of nectar in the sample but this will be characteristic for each plant species and so a correction factor is applied to the percentage number to account for this. Several factors influence the APC but the most important factors are the interaction of the insect visitors in relation to the size and shape of the flower, the distance between the anthers and the nectary in the flower, the timing of nectar production and pollen presentation and the abundance of anthers and pollen in the flower. Nectar and honey are therefore categorised into those plant species that are poor in pollen (called under-represented), average, or rich in pollen (called over-represented). Six examples of these categories from different flowers that produce monofloral honey in New Zealand are illustrated in the following pages.

Finally, one important point to consider when interpreting the pollen profiles of nectar/honey is that native bees collect pollen in a different way than honey bees and this also influences how much pollen gets into the nectar. The two main groups of native bees pack their pollen on their back legs without using nectar to moisten the pollen load. They have numerous hairs on their back legs that hold the pollen load together but the pollen is very easily dislodged when the bee is working a flower. In contrast, the honey bee moistens the pollen with regurgitated nectar and/or saliva and packs it around a single long hair in a concave pollen basket (corbicula). Pollen is not as easily dislodged from a pollen pellet that is so tightly packed. Another influencing factor is that nectar from large flowers that are adapted for bird pollination will have copious quantities of dilute nectar suited for birds. Once this nectar is concentrated by the honey bee removing the water while maturing the honey, the pollen count will be higher. These biological aspects of the flowers and their visitors are significant for interpreting the type of nectar that is coming into the hive and are consistent for the flowers of each different plant species. The correction factors are incorporated into the definitions for the different botanical honey types.

Honey bee visiting a rose flower with abundant pollen. The bee can collect an enormous pollen load very rapidly by mixing the pollen with nectar to stick it together in the pollen basket.

Close up showing how a native bee packs pollen onto its back leg. Numerous hairs hold the pollen together and no nectar is mixed into the pollen load. This bee is a species of Leioproctus taken while collecting pollen from a mānuka flower.

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CLOVER (Trifolium repens) *honey has an average representation of pollen.*

White clover has a tight packed cluster of flowers but the anthers are not exerted above the petals. These flowers are "flag" flowers with closed access, typical of many legume plants. The pollen and nectar are hidden so the bee must work the flower gain access to the rewards. The ten anthers enfolded inside the bottom petal will be exposed as the bee forces its way into the flower and this gives opportunities for the pollen to fall into the nectary. Clover honey has an average amount of pollen with about 100,000 to 160,000 P.G. per 10 gm.

THYME (Thymus vulgaris)

honey has very under-represented pollen.

Thyme flowers are tubular flowers with hidden nectar but the anthers are not hidden – they are exerted above the petals. Besides, there are only two anthers which is not much pollen. Since the bee can work the top of the flower for pollen outside the floral tube, then ,most of the pollen will be dislodged onto the ground instead of into the bottom of the floral tube. This means not so much pollen will get into the nectar. Thyme honey has very under-represented with only about 3000 to 8000 P.G./10 gm honey.

REWAREWA (Knightia excelsa)

honey has very under-represented pollen

Rewarewa flowers have a very large distance between the anthers (the yellow ends of the spikes sticking out of the cluster of flowers). The nectary is at the bottom of the red flowers inside the curled up petals. The honey bee can get to the nectary without even touching the yellow anthers and move from flower to flower easily without touching the pollen. Furthermore, the yellow pollen if it was dislodged by wind or mechanical shaking of the flowers would fall to the ground by gravity alone. The BPSC NZ Honey Profiles report shows that the relative proportion of pollen (the percentage) only needs to be 10% for rewarewa to be defined as monofloral.

POHUTUKAWA (Metrosideros excelsa) *honey has under-represented pollen*
Pohutukawa flowers have a large distance between the small numerous anthers and the yellow nectary disk. The honey bee, once it dives down into the bottom of the cluster of flowers, will be able to manoeuvre from one flower to the next without dislodging much pollen into the nectar. We do not have an APC number for this species as yet but it will be low because the BPSC NZ Honey Profiles report shows that the relative proportion of pollen (the percentage) only needs to be 20% for pohutukawa to be defined as monofloral.

KANUKA (Kunzea ericoides)
honey has highly over-represented pollen
Kanuka flowers are wide open access "dish- shaped" flowers. They are smaller than manuka flowers and the flowers are aggregated closer together in tighter clumps than in manuka. The anthers are exerted above the petals and so native bees will be brushing pollen all over the central nectary disks as it moves from one flower to the next while foraging for pollen and nectar. Kanuka is thought to have highly over-represented pollen with an Absolute Pollen Count close to or well over a million P.G./10 gm honey.

MANUKA (Leptospermum scoparium)
honey has over-represented pollen
Manuka flowers are larger than kanuka flowers and less closely aggregated into tight clumps. The anthers are shorter and not exerted above the petals. So when a native bee traverses the branch from flower to flower to collect nectar and pollen it will dislodge pollen into the nectar disk in the centre of the flower but probably not as much as in kanuka. The APC for manuka is thought to be from 500,000 P.G./10 gm honey and above but this may not be true for all varieties of manuka. More research on this is needed.

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