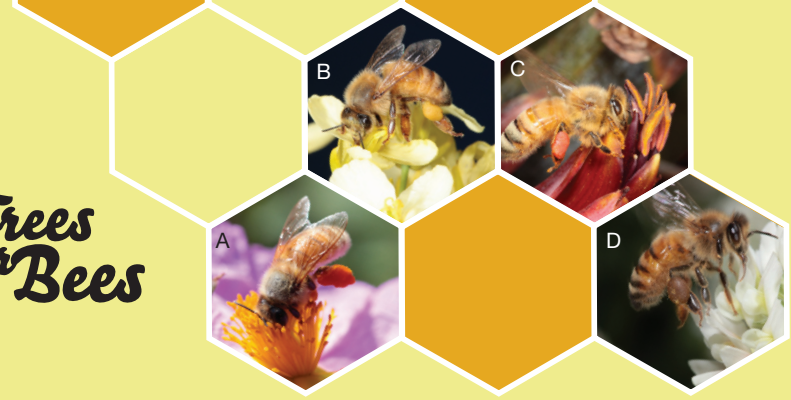

Trees For Bees

Sustainable Farming Fund
Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

Pollen pellet colour, purity & identification

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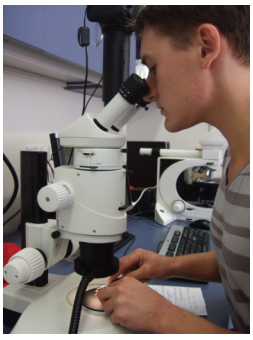
INTRODUCTION

We are investigating what plants have the best protein-rich pollen for use as bee forage in multi-purpose plantings on farms. This Sustainable Farming Fund Project "Flowers for Healthy Bees in Times of Pollen Dearth" is focused on finding plant species with high protein pollen that flower during spring build up (August to early November) and winter preparation (late February to May) in Canterbury and Gisborne.

One of the challenges in analysing pollen for protein content is to ensure that the pollen sample is purely one plant species only. We checked pollen pellets to answer these two questions:

1. Are most honey bee pollen pellets purely one plant species or are they more often mixed?
2. Can we sort pollen pellets from a hive trap into single plant species based on colour alone?



Finn Scheele preparing pollen pellets at Landcare Research

Dr. Ian Raine of GNS Science identifying pollen and checking purity

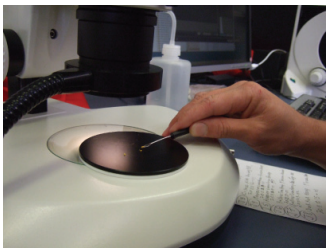
Photos at the top banner:
A. Orange pollen pellet from Rock Rose (Cistus creticus)
B. Yellow pollen pellet from Rapeseed (*Brassica napus* 'Greenlandia')
C. Orange pollen pellet from Flax (*Phomium tenax* from Sealers Creek Auckland Is.)
D. Brown pollen pellet from White Clover (*Trifolium repens*)

METHODS

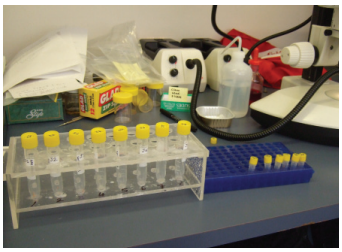
We examined pollen pellets taken from two sources: hive traps and pollen loads on bees foraging in flowers. Trap pollen was sorted by colour and analysed one pellet at a time to test for purity. We rejected mixed pollen pellets that had two colours. Bee leg pollen was analysed as a pair of pellets, tested for purity and identified by critically matching it to the pollen of the flower the bee was captured in. We made botanical vouchers of the flowers from which the bees were taken and pollen vouchers on microscope slides of pollen that we chemically cleared by acetolysis (an acid treatment). This treatment shows better detail in pollen structure to provide more accurate identification.

Pollen pellets were all photographed under consistent lighting conditions, using a ring light on the stereo microscope which eliminates the variable influence of ambient light. Camera settings and software were the same throughout, including manually setting the white balance to best achieve accurate colour. Minor variation in tone could exist due to unavoidable changes in exposure time of photographs; however the impact of this was very subtle overall.

The whole pellet was photographed displaying the external colour, followed by the squashed pellet to examine if the colour was different internally. Several factors may influence the colour of pellets, such as the moisture content, the viewing context (surrounding colours influence human perception of colour), and the lighting conditions (for both photos and pellets viewed with the naked eye). All pellets were frozen fresh and taken from freezer for photographing so the moisture content is comparable.



Close-up of a Pair of Pellets in preparation



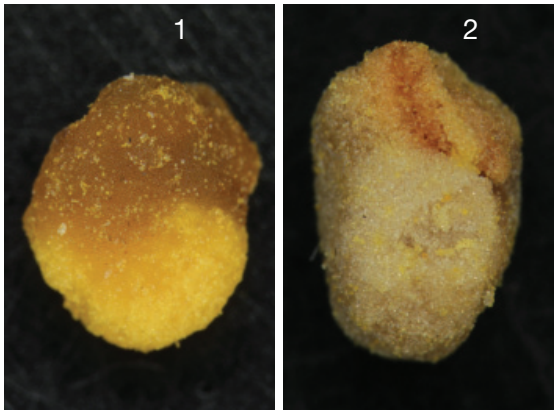
Samples for ID (left) and protein (right) taken from same pellets

PURITY

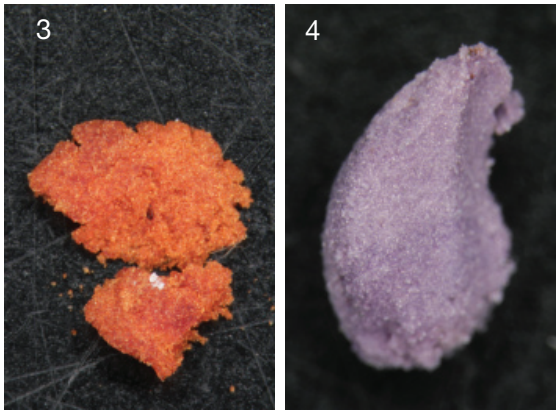
Question 1: Are most honey bee pollen pellets purely one plant species?
When sorting hive trap pollen, we found that the majority of pollen pellets are purely one colour. We rejected less than 1% of the pellets as mixed pollen with two contrasting colours (See examples in Photos 1 and 2). Some pollen colours are highly distinctive and very uncommon such as the red pollen of Horse Chestnut (PHOTO 3) and brown pollen of white clover (PHOTO D, above). Some colours are found in only a few plant species such as the purple (mauve) pollen of Phacelia (Photo 4), a colour also found in Fuchsia and some Thistles.

Hodges (1974) reports that when bees forage on two different plants 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? No this pattern is not from a dizzy bee foraging, it is derived from the mixture in the flax anther of 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.

We were not able to judge purity from pellet colour when the pollen colour of two different plant species is very similar. For example, we found a white pellet from a bee seen visiting an Ash flower that was mixed with primarily Hawthorn pollen and only some Ash (Photo 6). During work in progress we have found only one single colour mixed pellet out of over 30 pollen pellet samples examined for purity by microscopic analysis of acetolysed pollen.

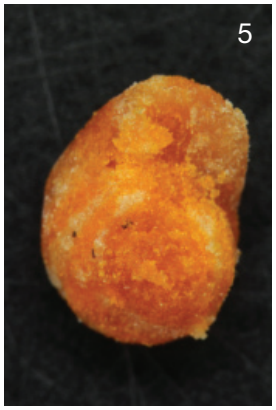


Mixed pellets with contrasting colours

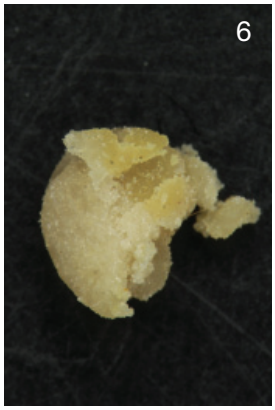


Horse chestnut (*Aesculus hippocastanum*)

Phacelia (*Phacelia tanecetifolia*)



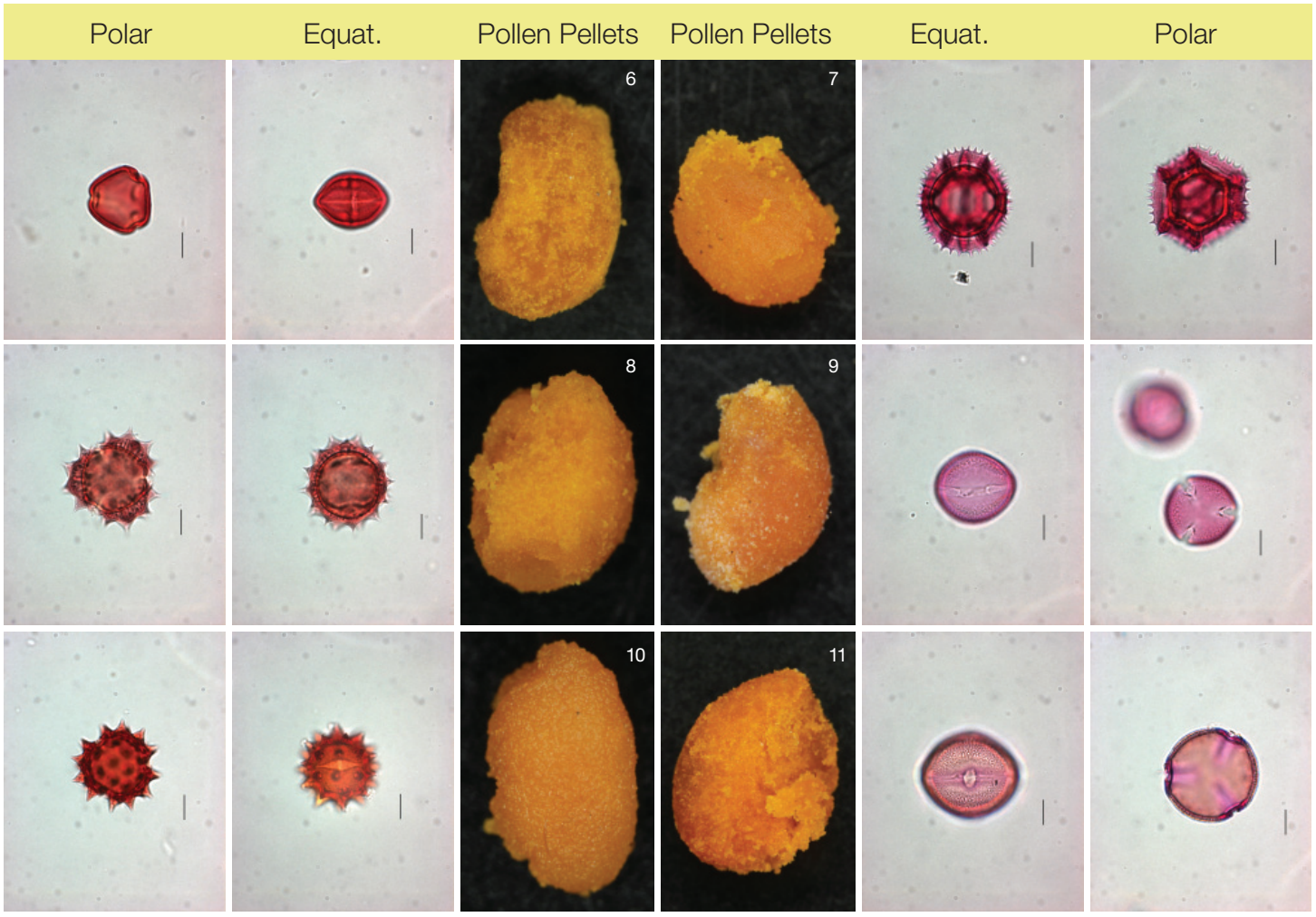
Not Mixed: Yellow and Orange pollen pellet of Flax (*Phormium cookianum* 'Tricolour')



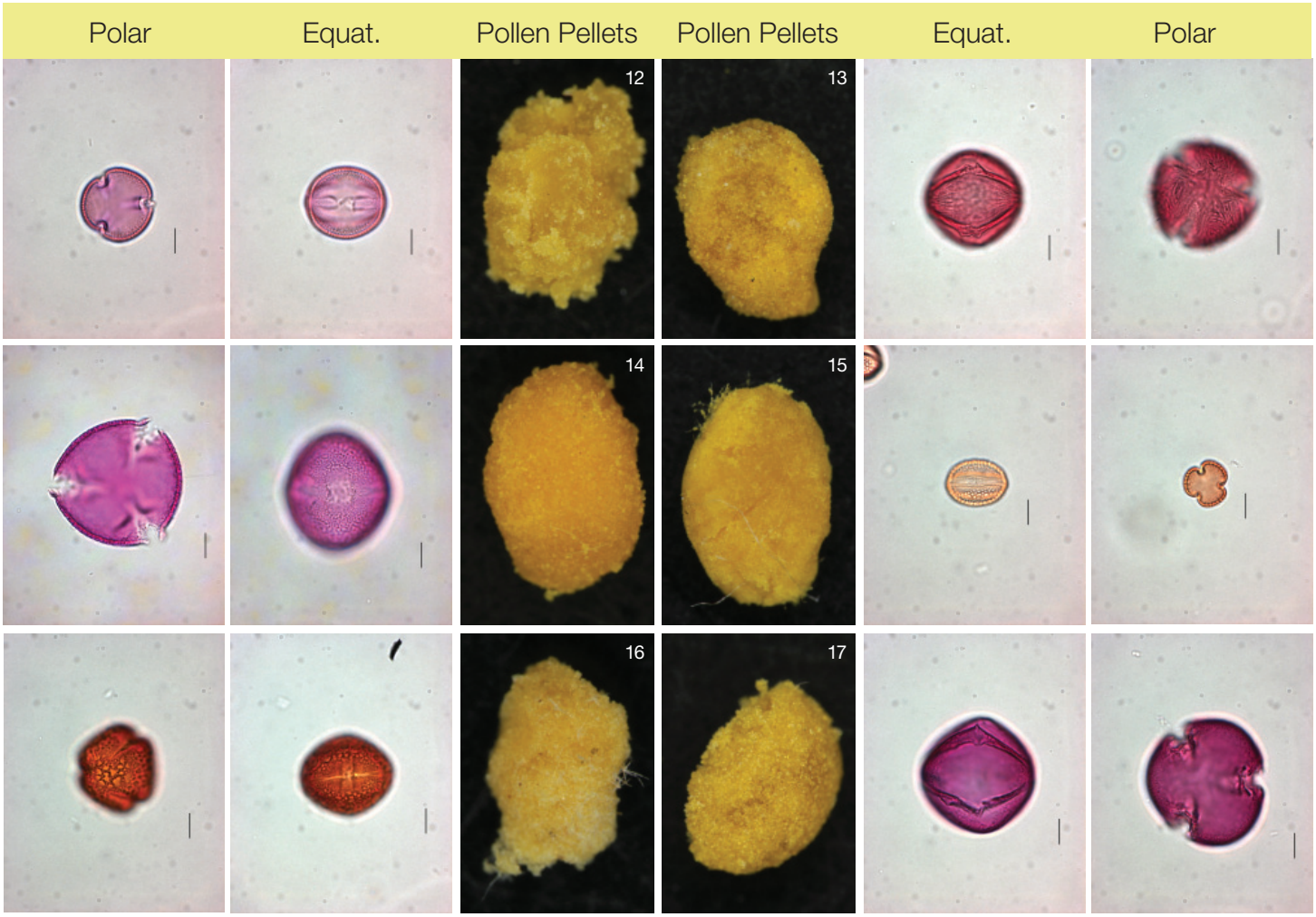
Mixed: White pollen pellet of Hawthorn (*Crataegus monogyna*) mixed with Manna Ash (*Fraxinus ornus*)

IDENTIFICATION

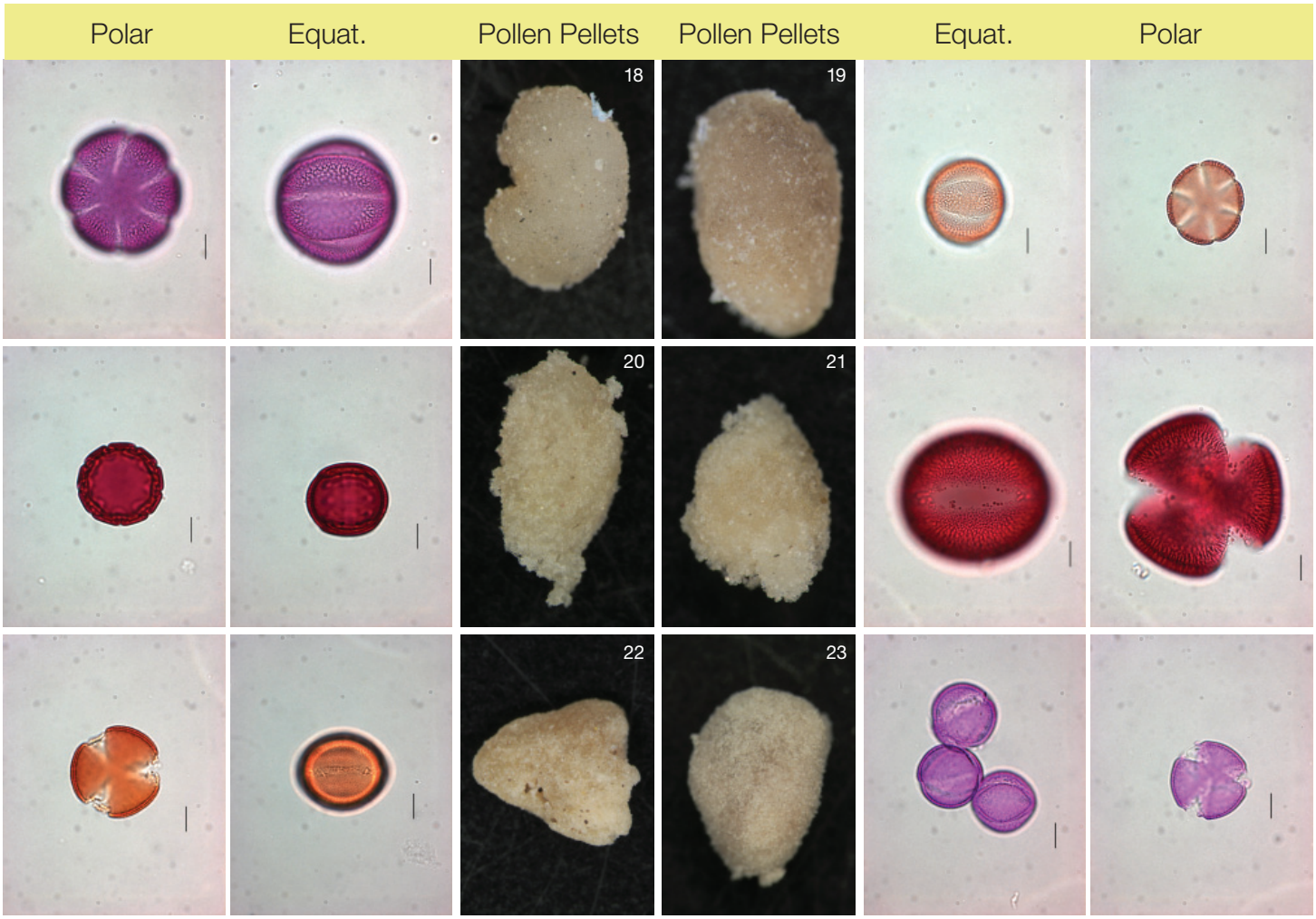
Question 2: Is it possible to sort hive trap pellets into plant species based on colour alone?
It is often thought possible to look at a pollen pellet sample from a hive trap and pick out the plant species based on colour. We asked if we could accurately sort out trap pollen by colour into categories of pure plant species? This translates into the question of how many plant species have very similar colour of pollen. We found many plant species with nearly identical colours. They are difficult to distinguish when sorting by colour. Therefore other supporting information is needed to sort pollen pellets accurately by colour. For this reason, in our study, we always analyse only one pellet at a time for protein content because we can check for purity and identity based on the acetolysed pollen from the same pellet that is sent to the GNS lab for protein analysis. (All pollen photos below are to the same scale. Scale bar = 10 microns.)



Colour A: Bright Orange
Photo 6: Mexican Orange Blossom (*Choisya ternata*); Photo 7: Dandelion (*Taraxacum officinale*);
Photo 8: Chrysanthemum (*Chrysanthemum segetum*); Photo 9: Broom (*Cytisus scoparius*); Photo 10: Daisy (*Helenium flexuosum*);
Photo 11: Rock Rose (*Cistus creticus*)



Colour B: Yellow Orange
Photo 12: Tree Peony (*Paeonia suffruticosa*); Photo 13: Flowering cherry (*Prunus* sp.);
Photo 14: Camellia (*Camellia japonica* 'Flame');
Photo 15: Willow (*Salix* sp.); Photo 16: Ivy (*Hedera helix*);
Photo 17: Black Boy Peach (*Prunus persica* 'Black Boy')

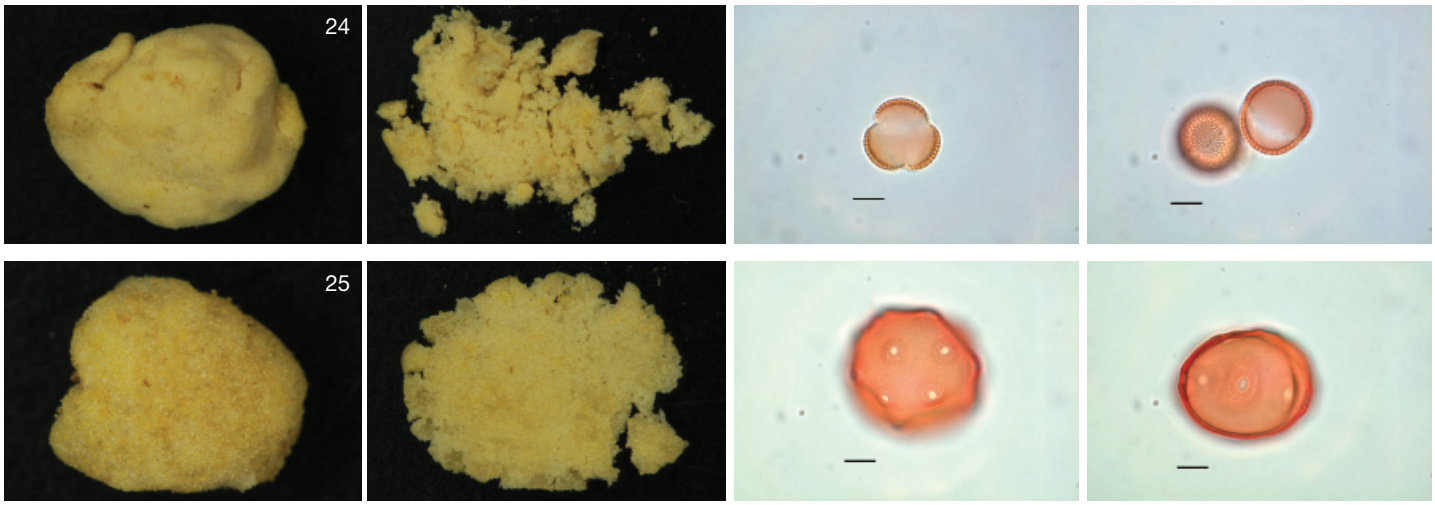


Colour C: Whitish Beige
Photo 18: Rosemary (*Rosmarinus officinalis*); Photo 19: Thyme (*Thymus vulgaris*);
Photo 20: Borage (*Borago officinalis*);
Photo 21: Silver Bush (*Convolvulus cneorum*); Photo 22: Koromiko (*Hebe* sp.);
Photo 23: Poppy (*Papaver somniferum*)

WHAT OTHER INFORMATION CAN BE USED TO HELP WITH PURITY AND IDENTIFICATION?

Experienced beekeepers can often tell the identity of a pollen pellet on a bee or from a hive trap based on their knowledge of what is flowering within a 2 to 5 km radius of their apiary at the time the pollen pellet was collected. This will work well for pollen colours that are distinctive and rare such as brown clover pollen pellets (PHOTO D) or red horse chestnut pollen pellets (PHOTO 3). In the case of purple or mauve pollen, there are three possibilities: phacelia, thistle, or fuchsia but some of these can be eliminated because they are not flowering at that time or the beekeeper knows that that plant species does not grow in the area.

Additional features of the pollen pellet can be used as well. For example, in the case of Brassica and Walnut which are a very similar yellow, the surface texture looks slightly different. When the pollen pellet is squashed Brassica is dry and crumbly (PHOTO 24) while Walnut is sticky (PHOTO 25).



SUMMARY & CONCLUSIONS

Even though we reject all obviously mixed pollen pellets (two coloured), the purity of pollen still needs to be checked under the microscope using acetolysed pollen because it is possible that a single coloured pollen pellet can consist of two or more plant species but this is quite rare. Since so many plant species share the same colour of pollen we cannot combine pellets for the protein analysis. We must analyse pollen using only one pellet at a time for hive trap pollen or using a pair of pellets from only one bee at a time. Such small samples, often less than .5 mg, are analysed by Dr. Karyne Rogers at GNS Science lab. We control for purity by examining the associated voucher of acetolysed pollen taken from the same pellet as the one sent for protein analysis.

During this examination process, the identification of pollen from samples taken from bees visiting flowers is facilitated by critically matching it to the pollen taken directly from the flowers the bee was foraging in. Hive trap pollen however, is more difficult as it requires other supporting information for pollen identification, such as knowing what species are in flower in the area at the time the hive trap pollen was taken but this is not always available especially if the bees are foraging at a great distance.

REFERENCES

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Craig JL and Stewart AM 1988. Reproductive biology of *Phormium tenax*: A honeyeater-pollinated species. New Zealand Journal of Botany 26(3): 453-463. Pollen described on Page 456.

ACKNOWLEDGMENTS

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