MELISSOPALYNOLOGY

POLLEN ANALYSIS OF HONEY

It is always useful to know what pollen is in a particular honey and whether it is correct to give it a specific floral origin. Here are two methods which a microscopist can use and are based on Rex Sawyer's book *Honey Identification* (1988) and my own experiences. The hardest part is identifying the different pollen grains and you will also need to consult Sawyer's other book *Pollen Identification for Beekeepers* (1981) and the accompanying CD-ROM spreadsheet by John Chandler and Dave Rennison for identifying grains. It also helps to get the modified version of the spreadsheet by Russell Hunt, who will send you a copy if sent evidence of purchase of Chandler and Rennison's CD-ROM. His email address is bees@russellhunt.net

Making a Pollen Slide from Honey using a CENTRIFUGE: -

It is very important to use **eye protection** for all involved, like goggles or safety glasses, when using a hand or electric centrifuge. There are some cheap hand driven centrifuges on the market with plastic cup holders, one of which recently broke when I was using it at high speed!

1. Take your honey sample and stir to mix the pollen grains. You may need to slightly warm the honey using a hotplate.
2. From this sample weigh out 10g of honey into a 50ml beaker.
3. Stir in 25-30ml of water until the honey is dissolved. You may need to warm this slightly.
4. Split the solution equally into two 15ml centrifuge tubes.
5. Centrifuge for 5 minutes at about 2,000 to 2,500 revolutions per minute. This can be longer if you have an electronic centrifuge. I find a hand centrifuge is adequate, although hard work! If you do not have the use of a centrifuge you can use the sedimentation method as below.
6. You should now have a sediment at the bottom of each tube. Carefully take out most of the liquid from each tube using a pipette. Half fill one tube with clean water and carefully shake to disperse the sediment which you should now pour into the other tube and shake. Mark this tube. Fill the other tube with water to balance.
7. Centrifuge again for at least 3 minutes.
8. From the marked tube with the sediment remove most of the liquid using a pipette. Now stir up the sediment with the end of the pipette and with slight pressure on the bulb draw up all the sediment into the bottom part of the pipette.
9. Put the sediment onto the middle of a glass slide and gently spread it over an area slightly less than that of the cover slip you will use. It is important for measuring purposes to use all the sediment on one slide rather than try to make several or discard some sediment. You can use a template under the glass slide to show the exact middle.
10. Place the slide carefully onto a hotplate to dry.
11. Warm some glycerine jelly, which can be ready stained, and, using a thin glass rod, add a very small drop to the middle of the slide. The amount of jelly to use comes with experience and is normally less than you think, as you just want it to fill the space under the cover slip.
12. Very slowly and carefully lower your cover slip, from one side first, to minimise air bubbles. A small drop of Isopropanol under the cover slip can

help in this respect. The jelly should spread out but may need gentle pressure with the handle of a scalpel. Ideally, the grains should be evenly distributed.

13. Put the slide back on the hotplate for about 10 minutes for any stain to act and bubbles to disperse.
14. You can now examine the slide carefully, but I would advise not using a x40 objective until it has hardened in a few days, as it could move the cover slip.
15. When it has set you can carefully wipe off any surplus jelly with cold water, then dry and seal the edges of the cover slip with varnish or clear nail varnish.

Making a Pollen Slide from Honey using the SEDIMENTATION method: -

1. Dissolve 10g of honey into a honey jar of at least 250ml capacity.
2. Leave in a draught-free place for 24 hours. Draughts cause convection currents which may disturb the sediment.
3. Remove all but about 10ml of the liquid. Swirl this around to mix the sediment and pour it into a test-tube. Leave for another 24 hours.
4. Remove the liquid and continue as in 8 above.

Total Pollen Count for Type: -

This is the number of 1000s of pollen grains in a 10g sample of honey and gives valuable information about the previous history and composition of the honey and helps in the overall assessment of a honey sample.

Obviously, you cannot count every single pollen grain on your slide, so here is a formula to give you a rough estimate: -

((C/F)xA)/1000

C = Coverslip area in square mm
F = Field of view area in square mm
A = Average number of grains in 10 random views

C The area of a 19mm round coverslip is
3.14159 x 9.5 x 9.5 = **283.53 sq mm**

F The field of view of a particular compound microscope using x400 magnification could be a 500 microns diameter circle whose
area = 3.14159 x 250 x 250 = 196,349.375 sq microns or **0.196349 sq mm**

A The average number of grains in 10 random views of a particular slide could be **32.8**. Of course, allowances can be made if the slide has patches where pollen grains have clumped together or are unevenly distributed.

TOTAL POLLEN COUNT = **(283.53 / 0.196349) x 32.8) / 1000 = 47.36**

47.36 indicates a normal floral honey from pollen-free combs as shown below.

Here are the range of counts, some do overlap: -

0-1 Pressure-filtered honey with diatom remains and mineral flakes.

10-20	Honey from under-represented sources, I.E. produce little pollen. Sugar feeding. Adulteration with high-frucose syrup. Honeydew honey.
20-80	**Normal floral honey from pollen-free combs.**
100-500	Honey from over-represented sources or from combs containing pollen stores.
10-250	European heather honey.
1000-50000	Pressed floral honey.

Pollen Coefficients: -

To compensate for the fact that some plants have more nectar than others for the same amount of pollen grains each plant is given a Pollen Coefficient. The Pollen Coefficient is measured as 1000 grains in 10g of honey. The Pollen Coefficient should be divided into the Pollen Percentage. Here is an abridged table of Pollen Coefficients for mainly UK honey: -

5	Labiates (Rosemary, Thyme, etc.)
10	*Cirsium* type (Thistles)
	Erica spp. (Heaths, Bell Heathers)
	Helianthus annuus (Sunflower)
	Tilia (Lime Tree, Basswood)
25	*Ligustrum* (Privet)
	Lotus (Birdsfoot Trefoil)
	***Prunus/Pyrus* type (Fruit Blossom)**
	Trifolium pratense (Red Clover)
35	***Vicia faba* (Field/Broad Bean)**
50	*Ilex* spp. (Holly, Gallberry)
	Rubus spp. (Blackberry, Raspberry)
	Trifolium repens (White Clover)
	Unidentified, sporadic or unlisted pollen grains
75	*Eucalyptus*
	Melilotus (Melilot or Sweet Clover)
150	***Brassica* (Oil-Seed Rape type)**
250	*Echium* spp.(Viper's Bugloss, Blueweed)
	Leptospermum scoparium (Manuka)
1000	*Castanea sativa* (Sweet Chestnut)
	Eucryphia lucida (Leatherwood)
5000	*Myosotis* (Forget-me-not)

Predominant Pollens: -

Predominant pollen	> 45%
Secondary pollen	16% to 45%
Important minor pollen	3% to 15%
Minor pollen	< 3%

A honey can be given the name of the predominant pollen(s) if above 45% for relative percentage nectar.

Pollen Grain Features: -

Refer to Sawyer's books for the list of pollen grain features to identify the grains by size, shape, aperture numbers, aperture type, surface, exine, section and other structural features. Use the CD-ROM to search for possible grains.

Calculation Spreadsheet: -

Build the spreadsheet below, which is self explanatory, and fill in 10 random views of your honey pollen slide. Fill in the pollen coefficient for each different grain to give the relative percentage nectar for each different pollen grain. To have a predominant pollen, so you can name say 'Oil Seed Rape Honey', you need a relative percentage nectar above 45%, otherwise you can only say for example 'Multi Floral Honey'.

You can re-use the spreadsheet, but you may have to adjust some of the columns and formulae.

My spreadsheet is set up so you only have to fill in the yellow boxes for each sample.
Main formulae as follows: -
B(column)13(row) =SUM(B3:B12)/10
B14 =SUM(B13/H13)*100
B16 =SUM(B14/B15)
B17 =SUM(B16/H16)*100
B20 =SUM((B18/B19)*H13)/1000

ANALYSIS OF POLLEN IN HONEY	1	2	3	4	5	6	Totals
Pollen Names	OSR	Field Bean	Prunus	Yew	Dandelion	Others	
Field Counts 1	13.00	2.00	8.00	1.00	1.00	7.00	32.00
2	17.00	2.00	9.00	1.00	0.00	0.00	29.00
3	25.00	2.00	5.00	1.00	0.00	3.00	36.00
4	25.00	2.00	7.00	1.00	0.00	0.00	35.00
5	24.00	1.00	2.00	1.00	0.00	1.00	29.00
6	42.00	2.00	8.00	0.00	0.00	3.00	55.00
7	13.00	4.00	5.00	0.00	0.00	2.00	24.00
8	29.00	0.00	3.00	1.00	0.00	0.00	33.00
9	9.00	3.00	6.00	0.00	0.00	1.00	19.00
10	24.00	3.00	7.00	0.00	0.00	2.00	36.00
Averages	22.10	2.10	6.00	0.60	0.10	1.90	32.80
Pollen %	67.38	6.40	18.29	1.83	0.30	5.79	100.00
Pollen Coefficient	150.00	35.00	50.00	50.00	50.00	50.00	
Relative Nectar	0.45	0.18	0.37	0.04	0.01	0.12	1.16
Relative % Nectar	38.84	15.82	31.63	3.16	0.53	10.02	100.00
Coverslip area sq mm	283.53						
Field of View area sq mm (x400)	0.196349						
General Assessment: -	47.36						
Normal floral honey from pollen-free combs.							
No predominant pollen.							
Mainly Rape, Prunus and Field Bean.							
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John Rhodes