SEM Diaries - 8

Obtaining and identifying specimens, and first steps in colouring

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Fig. 1: A pollen beetle, as imaged in the SEM prior to post-processing

ne of the joys (or should that be hazards?) of having an interest in weird and wonderful creatures, is that friends occasionally turn up with a container housing a live or dead specimen of some insect or other creature, and expect an identification. I have certainly had my fair share of such requests, as I recounted in my article "Beware Friends Bearing Gifts" in BP 94, page 29. I have

had to break the news to one friend that her priceless cabinet was infested with furniture beetle (woodworm) and to another that the gift from his girlfriend was, in fact, *Pthirus pubis*.

Well, last autumn, a friend recounted to me that the inside cill of his kitchen window was strewn with little black flies. Could he bring me a sample to identify? Obviously I said yes, and about 100 specimens duly arrived in a little plastic box, which I left on the hall table for a day or two. Next time I looked, many of the creatures were waving their legs in the air. It was as if they had come out of hibernation!

Despite having a copy of the Collins Field Guide to Insects, by Chinery [1], I failed to identify these "flies", but I did get round to imaging them on the SEM, with the results shown in Figures 1 and 2. I gave my micrographs file names starting with "small fly" since I had no more accurate identification.

Now, the reason for this rambling introduction is that in September of last year I attended a one-day course on colouring electron micrographs, at the John Innes Centre in Norwich. This was



Fig. 2: Face view of pollen beetle showing mouthparts

organised by the Royal Microscopical Society and presented by Steve Gschmeissner [2].

All electron micrographs straight from the SEM (or TEM) are, of necessity, monochrome, since the wavelengths of the electron beam are much shorter than those of visible light. Many practitioners like to colour their micrographs artificially in Photoshop or a similar programme, especially if intended for publication. The colouring might be carried out in order to reflect closely the colouration of the subject in real life, or alternatively may be totally artificial but designed to differentiate between different parts of the specimen's anatomy or structure. The product of my attendance is illustrated in Figure 3, overleaf. Given that this creature is totally black in real life you will deduce that I opted for the use of colour to differentiate structures (eyes, legs, elytra etc) rather than natural colouring!

When I sought advice on work in progress from the lecturer he saw my file name and said "that's not a fly, its a beetle!". Of course, any idiot could see that! He was also able to explain that it was a pollen beetle. These are often carried into the house on bunches of cut flowers. They then fly towards the light, where their path is blocked by the window. I did feel a bit of a fool, but in my defence I am a physicist, not a biologist (but I cannot keep on using this excuse).

Use of the SEM over the Winter, has been quite light. This is partly because I have been experimenting with fluorescence following Mike Gibson's prompt in the last BP, but also this is a "quiet" time of year for specimens. Insects and spiders, my main interests (despite my chronic inability to identify insects correctly) are generally in short supply, since they tend to hibernate or over-winter as eggs etc. Similarly, pollen is seasonal, unless you have pot plants indoors (which enabled me to



Fig. 3: Micrograph of pollen beetle, coloured to indicate anatomical features

image the anthers of my spider plant, as described last time).

Why not image preserved specimens, you may wonder. Well, obviously I have done a fair bit of that, but I do find that even when kept in 70% alcohol they do not necessarily maintain their natural shape or condition. I guess I could so some colouring in of existing micrographs in the winter evenings - but, to be honest I dread the idea of making selections around the hairs on many of my favourite images. Besides, BP seems to take up a lot of my spare time, especially when I have insufficient material, strange to relate. Like most people, and especially those interested in natural history, I am really looking forward to the spring.

Back in September of last year I held an "SEM warming party". This consisted of a barbeque with around 25 guests. I left trusted guests in charge of the BBQ itself and gave a number of demonstrations of the instrument to smaller groups. Among these was the deputy head of one of the local boarding schools, and the 10 year old daughter of one of my friends. Even before I could raise the subject, the deputy head suggested that my lab would be of interest to some of her senior boys. The following Monday, the 10 year old was excitedly telling her science teacher all about her experience. I have also been in touch with the science teachers from the other two secondary schools in the area to offer demonstrations. I am not quite sure what

I might be letting myself in for, but I do remember the buzz I got in first receiving a demonstration of an SEM, way back in 1968. I might be finding out fairly soon, though. The father of the 10 year old has passed on my details, and I have been warned to expect a call from the head of the Junior Department of his daughter's school shortly.

Despite my enthusiasm for demonstrating my SEM, my initial reaction on receiving that news was "Oh dear, what on earth can I show them that is suitable. It is probably best not to go into the copulatory habits of spiders". I think I could do worse than contact the Royal Microscopical Society to obtain more details of their kits aimed at older primary school pupils.

In the first week of March, I had a visit from Don, the director of the company who supplied my SEM and who maintains it for me, to install a "backscattered electron detector". This was a significant event, both in cost and technological enhancement, and it will deserve several columns in the next "Diaries". Sadly, things did not go quite to plan! I had in my minds eye a picture of a small plate of semiconductor material clipped around the nose of my electron gun, with some cables discreetly wired through the wall of the chamber.

Imagine my surprise, then, when Don turned up with a lead-screw arrangement 18" long to mount onto the main port on the right of my chamber, a large power supply and a smaller amplifier box.

Despite my having allocated that port, in my dreams, to a completely different accessory, Don proceeded to assemble the device to the SEM, and all went well until it came to installing the drivers on the Windows XP® PC that controls the SEM. A call to the suppliers elicited the fact that they had not realised that Don would be installing the software on such an old operating system, but they did send a patch which "sort of worked". However, we eventually decided to remove the detector and think again about the actual type to install. Don left at 18:40 with a 4 ½ hour drive home ahead of him. Watch this space!

References

- Chinery, M., Insects of Britain and Western Europe. Collins Pocket Guide, reprinted 1993.
- See Steve's website for many beautiful images. http://www.theworldcloseup.com/