BRAULA COECA - The Forgotten Pest of Beekeeping?

Fig. 1 Single Braula

THIS article was originally written for the Postal Microscopical Society and has recently been modified specifically for beekeepers. A fellow member of the Iceni Microscopy Study Group, Jeremy Quinlan, Master Beekeeper, said "You should offer it to the bee publications." He said that many beekeepers would be interested in reading it and that it would be most helpful for beekeepers wishing to increase their knowledge of Braula especially if they wish to take beekeeping examinations. For example, they must know how to recognise a Varroa mite and not to confuse it with a *Braula coeca*, an insect, to note the physical differences and describe them to the examiners. In the past I have made slides for bee examiners (in 2008) and some in 2015/16. Fig 1 shows a single adult *Braula coeca*. Fig 2 is of *Varroa destructor*, showing Varroa mites born white and going through the stages protonymph and deutonymph. The slides show the progression from a white coloured stage to a tan stage, which is the adult in the middle. They all end up tan brown as does the Braula, but I have not managed to make a sample slide of Braula going through the three instar stages yet, due to the lack of specimens. The test slides are difficult to make as most exams require that the specimens should ideally be the same colour as when they are alive. Therefore, it is necessary to make them to an archival standard, i.e. mounted in Canada balsam, gum dammar or Practamount. All these products give a useful life of 100 years without losing colour. An examiner in possession of reference slides could possibly have a career of 20-30 years, therefore he or she would be confident that the slides will remain true to colour for the whole of their career.

Braula coeca are not unique in being flightless. Many living things have adapted to survive in their specific environment. There are examples in the Falklands Islands (Becker Island) of a flightless fly, *Anatalanta aptera* (Diptera, Sphaeroceridae) which lives in tall grasses, *Poa flabellata*, commonly known as tussock grass or just tussac, which has pedestal bases surrounded by leaves up to six foot long. The sheltered areas among the tussocks are the fly's habitat on the islands.

It is known that many insects inhabit the litter among the dense pedestals of tussock grass which provides a safe habitat. Above and beyond the grassland, strong, westerly Atlantic winds buffer the Islands almost constantly, and so airborne insects have a precarious existence as they are in danger of being blown out to sea. Also, living on the islands is the Tussac moth, *Barkhausenia falklandica*[5], which walks rather than flies as it has developed very small wings. The large, predatory Darwin's black beetle, *Lissopterus quadrinotatus,* first recorded by Charles Darwin on his famous voyage with the Beagle in 1834, still exists on the Islands today. It is just one example of several flightless beetles on the Islands. The majority of other insects on the island have reduced wing sizes and rarely ever fly, one being the kelp fly (Coelopidae) which is found scuttling, rather than flying, on the shoreline. They all show very specific adaptations to survival in their unique environment.

Why am I telling you this? I would like you to imagine living on the Falklands Islands hundreds of miles away from any other living things. Nature has adapted the living creatures there over millions of years to the forms they now have, i.e. wingless, modified bodies, etc. They are no different from *Braula coeca* which live in a similarly specialised environment, a honey bee colony. They only live in one colony at a time and therefore they are in a similar predicament to the animals and insects of the Falkland Islands. They can migrate to another colony, but that hive is in the middle of an ocean as far as the Braula is concerned, i.e. the same conditions exist. There is no change in the "home" and the conditions therein. The rules of Darwinism are fully applied.

Now to details. I would like to describe the physical characteristics of *Braula coeca.* It is ellipse shaped (see Fig 3), <1.5mm long, with no working eyes and covered in spine-like hairs. They do not have the wings or halteres possessed by most flies. Braula are reddish brown in colour and are regularly wrongly described as Varroa mites due to their similar appearance. One notable difference useful as a field diagnosis to distinguish between a Varroa mite and the insect Braula, is that adult Varroa have eight legs while Braula have six legs. Furthermore, Braula has an elliptical appearance while Varroa are more compressed and oval. Despite these differences, due to their small size, both are difficult to distinguish with the unaided eye. The eggs of Braula are white, oval-shaped and have two lateral flanges. The flanges are flat and extend parallel to each other toward the long axis of the egg. The eggs range from 0.7-0.8mm in length and are about 0.3mm wide, excluding the flanges. With flanges they average 0.8mm in length and 0.4mm in width. At the larval stage they are maggot-like in appearance, with the posterior end appearing flattened, while the anterior end is pointed. The size of Braula larva makes them difficult to see with the unaided eye. A diagnosis is usually made based on comb damage. As the larvae grow, the tunnels they create in the comb increase in diameter, reaching a maximum of about 100 mm in length and an end opening of 1.5mm diameter. The changes in the diameter of the tunnels can be seen visually when the comb is held up to the light. The pupae of Braula are white/yellowish and range from 1.4-1.7mm in length and 0.5-0.75mm in width. Initially when hatched they are a white colour and take about twelve hours to change to biscuit brown, a similar length of time to Varroa pupae. The head and posterior end of the abdomen are on opposite ends of the major axis of the ellipse while the legs are along the sides at the edges of the minor axis of the ellipse. When present in an infected hive, they can be seen by the naked eye questing a ride on honeybees. Very often the queen is infested; some have as many as 25/30 or more on them at one time (see Fig 7). They cause no apparent harm to the queen or bees but their larvae spoil capped honeycomb with fine tunnels in the capping. Beekeepers wishing to sell comb honey need to put the comb in a freezer for 24 hours; this process will kill the larvae. The customers now eat faeces and dead larvae as against eating live ones!

—The tunnels in the cappings are about one third of the thickness of a pencil lead, starting at about 0.75mm in diameter and, as the larva grows, widen to about 1.5 mm in diameter wide at the open end. To collect *Braula Coeca*, the beekeeper goes to the hive and finds the infected queen (Fig. 7). The queen's legs are held in one hand by forefinger and thumb, and tobacco smoke is blown gently over the queen which narcoses the Braula with nicotine from the smoke, which then fall off. With a sheet of paper underneath, it is easy to pick them up. For later microscopic examination, place the specimens in a container of white vinegar, which allows them to remain soft. This helps if you intend to make slides, as many other chemicals will cause them to become too hard and brittle.

Most bee supplier houses used to supply tobacco for this job, but now we are discouraged from using this. We are now in the age where Braula is very rare. It is no longer found in England, Scotland, and Wales, but some are still seen on the Isle of Man, (where Varroa are now absent) and possibly some remote Scottish islands. Modern chemical treatments for Varroa have killed all the rest. Some people think there may still be infected colonies in the country in other remote areas. Wild Varroa may have killed any remaining feral colonies with Braula and we could be the last generation to see them before they are gone forever in the UK. Since the advent of Varroa in England in 1992, it became accepted wisdom that the majority of Braula infected colonies were feral colonies, and any Braula in managed colonies had been eliminated by Varroa treatments, pyrethroid-based products such as Bayvarol, Apistan etc, which are hard chemicals. However, research carried out by Professor Tom Seeley[4] in the USA suggests very strongly that the population of feral Braula colonies not only has ceased to decline, but may have recovered to its pre-Varroa numbers. We can only hope that it will be the same in the UK, so that more study can be done on this insect, but only time will tell. In my personal experience I have not seen evidence of the above in the UK but I live in hope. However, an article in BBKA News[3] (June 2020 p4) by Kevin Pope, Seasonal Bee Inspector for Dorset, records that he found Braula in eight out of 11 colonies in an apiary in May, just a few months ago! It is speculated that they could have come from Bumble bees. Perhaps this is the start of a recovery for Braula, especially as beekeepers become less dependent upon using hard chemical treatments.

My personal view is that Braula cannot over-winter on Bumble bees. The life cycle of Bumble bees means that they all end up as single over-wintering queens, which produce and add an anti-freeze, glycerol, to their blood (haemolymph[1]), enabling them to semi-hibernate at very low temperatures. These are too cold for Braula to survive in, and they would also have no food stores to feed on. However, Braula can live in the nest of a Bumble bee during warm summer temperatures. They would feed from the two types of stores found in the nest, pollen, and honey, and move from hive to hive by questing on drones from site to site during the summer. In late summer, when the colonies break up into drones and queens, Braula would be at risk. Where do they all go? Feral bees would be my first thought. I cannot think of anywhere else apart from the 250 or so races of feral bees in the wild.

We need more research to evaluate their habits and life cycle. As Braula are found worldwide, the mystery is also worldwide. It remains to be seen whether they are breeding elsewhere, on other races of bees perhaps. Braula move from honey bee hive to hive in the same way as Varroa, by questing onto bees in a phoretic stage, then into other colonies by robbing bees, drifting and swarms. By swapping frames between hives or eggs from one hive to another, the beekeeper may also inadvertently spread Braula. It may also be possible that during foraging, Braula may transfer from Bumble bee to honey bee or vice versa.

There are four known species of Braulidae, but there may be more to be discovered:

1. *Braula coeca* found mainly in Europe
2. *Braula orientalis* found mainly in Russia
3. *Braula pretoriensis* found mainly in S. Africa
4. *Braula orosi* found mainly in Asia

All are reported to be inquiline i.e. an animal that is dependent on, and living in, the home of another animal species. Unlike Varroa, which is a parasitic inquiline and a vector of harmful viruses, Braula are more or less harmless to honeybees although they are dependent on them, stealing their food and unable to survive any length of time without them. It is interesting to note that if Braula do not find a host on hatching, they die within six hours, while Varroa can survive for a whole winter in a phoretic state. They are specific only to honeybees, although they may have an alternative host and may be breeding on other bees. We just do not know at present.

Although they lay their eggs throughout the hive, only those laid on a cell filled with honey will develop. The eggs hatch/eclose. I cannot be sure whether the eggs shells eclose or not but I cannot find reports of egg shells in the larval tunnels, therefore they must eclose or similar, i.e. keeping the protein "in house" and used for the next instar. The emerged larvae feed on the wax and pollen, forming ever-lengthening tunnels in the wax cappings, increasing in diameter as the larvae grow. The larvae undergo three developmental instars. They pupate at the wide end of the tunnels and finally emerge as adults. There is now some debate as to whether the male dies and is left behind after mating with the females. At emergence, the new adult *Braula coeca* is white and changes to its characteristic reddish/brown colour in approximately twelve hours as its exoskeleton hardens. This takes almost the same length of time and ends up as the same colour as Varroa (Fig 2).

Braula development takes place entirely under the cappings of honey cells and is not associated with brood in any way. It has been recorded that egg laying to emergence of the adult takes 16 to 23 days. Any information of hatching, larval or pupal development times indicates that they seem to vary with seasonal temperatures. It is said that the adults mainly inhabit the petoile (wasp waist) area of worker bees and the heads of queens. It is believed that attachment to questing drones is the way in which adults move to colonies far away, using the fact that drones can enter any hive without being attacked. This is also known as phoresis, an interaction in which one organism attaches itself to another (the host) solely for the purpose of travel. Phoresis comes from the Greek words phoros (bearing) and phor (thief). Braula breeding takes place between late April to September which is when drones are on the wing and could possibly

Fig. 2 Varroa showing mites from white top then going clockwise through the stages; proto nymph to the adult form in the centre

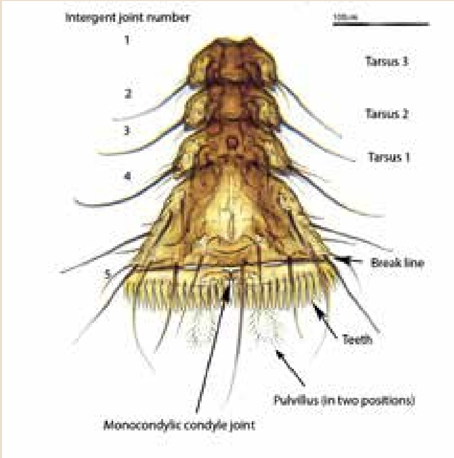

Fig. 3and 6

Fig. 3 Varroa (top) and Braula (bottom), dorsal and ventrical views

be the main transport method for them.

Braula have the ability to overwinter with honey bees, which live on their winter-stored honey and pollen in the brood frames. I have no firm information on mating, where it takes place, the number of males to females or the number of breeding cycles per female. The life of the adult is also not clear, nor are the over-wintering habits. We do know that they over-winter mainly on the queen and it is believed the extra heat in the centre of the cluster, where it is kept warmer for the queen, helps them survive. *Braula coeca* do not have fat bodies to help them generate heat. They cannot flex their flight muscles to generate heat, as they have no wings and therefore no flight muscles. They only have enough fat reserves in their bodies for their immediate needs. It is believed that if they overwintered in the supers they would die of the cold because they cannot generate heat to survive. Honeybees have fat bodies which allow them to flex their flight muscles to generate heat when cold and then when warmed up, to replace their fat bodies from the stores within the cluster.

Figs 3 and 6 show the highly adapted funnel-shaped foot of the pre-tarsus of Braula, followed by three tarsus 1, 2, 3, which means that t he foot has five intergent joints. It is clear the joints are monocondylic which allows them to swivel like a neck joint. This adaptation means Braula can place the soles of their feet anywhere on the bee and get a good grip, even when the body is round. Next note the two combs (joined in the centre like bun hair combs). The pretarsal claw of each leg is modified into a comb or ungues with 12 to 16 small spike- like teeth on each side of the mid-line of the foot. The Braula attaches to the host by drawing the ungues through the bee's hairs. Each claw has an apical brush-like pulvillus (a lobe associated with the claw) which may have sensory functions. For clarity I shall call them feather hairs. As confirmed by research by Ramirez and Malavasi (1992) [6]. A closer look shows a cut/joint - making two combs per sole (foot). A careful look at the joint shows a condyle (a rounded process otherwise called a finial) at the end of a bone forming an articulation with another bone, making a monocondylic joint. This fits into the edge of the comb allowing it to twist, as shown in Fig 4. When turned back, i.e. upwards, the anchor hairs now fit into the spaces between the combs, which, when turned up vertically, open the gap between the spiny hairs like spikes or fingers. Note the anchor hairs are coming from the break line, the centre hinge at the fifth intergent joint. Figs 3 and 6 show the anchor hairs in contact with the bottom part in Figs 4 and 5, holding the comb back. Fig 6 shows the combs extending downwards, held by hairs on the outside edges, allowing the foot to hold on to a surface covered in hairs. Fig 4a shows flat soles for walking on hard surfaces, such as bees, beehive wood, surface of the wax, etc. (horses for courses).

In the centre of the sole, two feather- type shapes (pulvillus) can be seen. These out-growths are setae or hairs more correctly known as microtrichia. It may be that they are a type of sensilla trichodia, a specialised hair (or setae) providing a wide variety of sensory information, for example heat, cold, royal jelly, queen substance, bee odour. I believe that Braula are able to inflate these pulvillae: Figs 3 and 6 show the pulvillae inflated, and to deflate them as in Fig 4, either by hydraulic action or muscular control. To walk on a hard surface, the flexible combs may go over the top and hold them down?

Many insects use their feet to receive sensory information. For example, ladybirds perceive sound through their two rear feet and honey bees taste with their feet. In honey bees their sense of smell uses receptors on their antennae. As Braula have neither functioning eyes nor antennae, they only have their feet to receive information. They use types of sensilla trichodia plus other sense hairs for location. There are no plumose (feather-like) hairs. All are setae type hairs of the message-sensing type, which gives it all the information it needs to live and breed in a beehive.

Some authors claim adult Braula inhabit the petiole (wasp waist) of worker bees, queens and drones and move to the mouthparts of the bee when it starts to feed. I have no personal evidence that this is so. I am of an age when I can remember seeing Braula on my bees, but I never saw any on the petioles. I only remember seeing them on the head or thorax of all castes. For example, when on the queen, I watched them move to the side of the queen's face and back again but at that time I had no idea (in 1995) what was happening. I now know, through my study of microscopy, that using their bristles and combs, Braula can grip any position on the queen's body whether it is a hairy surface or a non-hairy surface, using their adaptive feet. I believe that they can sense the royal jelly on the queen. I also believe that they can sense worker brood food. In the case of the queen, they lower their long legs to the lip area using their feather features, (pulvillae), on the soles of the feet to locate the remaining royal jelly on the queen's mouthparts. The Braula's mouth then follows the scent signals and feeds on the royal jelly. I have seen this happening myself. The queen reacts by extending her proboscis and then retracting it. This will renew the moisture, i.e. the royal jelly, keeping it wet. Thus, it seems the Braula taps the lip area and when the queen licks her lips, so to speak, keeping the food flowing

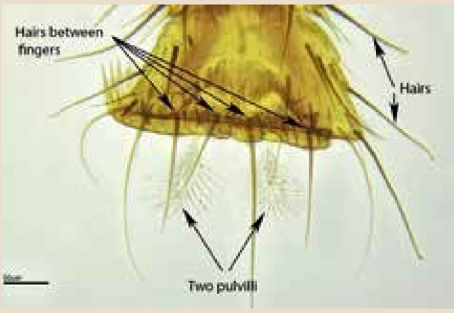

Fig.4

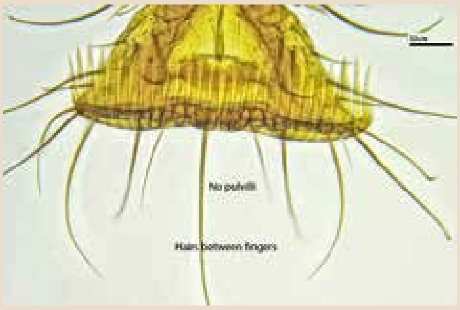

Fig 4A

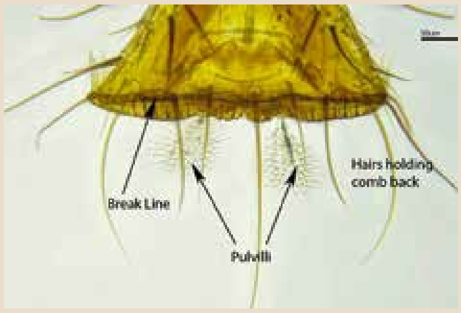

Fig.5

again, the Braula licks it off. Some authors state that Braula can induce regurgitation from honey bees by stroking the labrum, causing the bee to extend its proboscis by the reflex mechanism, and regurgitate a drop of fluid from its honey stomach. It is interesting to note that the queen's food, royal jelly, is so highly processed that the queen rarely has to pass faeces. In some old books it is called "dejectmentor". Faeces is the word used in more recent texts. Another word is defecating. I have also read that the faeces of a queen were sometimes called "squinting", a new word for me. Anyway, when the queen passes faeces it is an almost clear liquid and when she releases it, the worker bees rush in and lick it up. We used to think it was a hygienic trait, but we now know it is because the queen's faeces contain large amounts of modified queen substances, pheromones, and hormones. As Braula eat the same source of royal jelly, their urge to pass faeces is also very small. If they do defecate on the queen, the workers will clean it off enthusiastically. The reason for this low level of faeces production is because the worker bees feed royal jelly to the queen but remove all waste matter which is defecated by them by proxy, feeding the queen pure nutrients. It must be the same for Braula and in this way the queen is kept clean by the workers.

This makes the queen's food germ free, providing "royal immunity". Throughout a queen honey bee's lifetime she is attended by her worker daughters who feed and groom her. This allows possible transmission routes from the worker to the queen. In a USA- led study, queens were exchanged between colonies. After twenty-four days the queens and their foster colonies were examined for six of the most damaging pathogens to bees. Overall they found that the queen's pathogen profile did not match those of the adult workers in either the source or the foster colony, suggesting that the pathogens are not transmitted to the queen by resident workers giving "royal " immunity. BBKA News[7] 2020. If this were not so, the queen and the Braula would carry germs all over the brood frames. No such ill effects on the colony have ever been reported. It could be that feeding on royal jelly stimulates the Braula into an egg laying mode (hormones perhaps), enabling them to lay eggs. Do they have a cycle of feeding on the queen, laying eggs, resting, repeat? How times a year? Who knows?

When the Braula are on the worker bees their food is the bees' brood food, plus wax and pollen, not royal jelly. As a result, they pass near normal faeces with a high pollen content. The worker bees clean it up by mutual grooming. It may be that a high percentage of it lands on the hive floor. The larvae leave their faeces in the tunnels in the comb. In the past, microscopy has played a part in spotting pollen husks in both types of faeces, both in the tunnels and on the hive floor, which could only come from brood food not royal jelly. I could not find a report of dead males in the larvae tunnels. Maybe no one has studied this yet.

This adaptation must be a very old one indeed. For a fly to enter a nest of bees and adapt to that as its habitat millions of years ago, lose its wings, lose its eyes, and grow longer legs, with only feather type sensors on its feet providing any sensory information connecting it with its environment, is quite a unique adaptation. Braula is listed in the insect order Braulidae. Some books call it a bee louse which is incorrect. Lice are wingless obligate parasites in the insect order Phthiraptera. A louse has a stylet and can suck blood. Braula does not have a stylet, only a tongue, so it cannot be a louse.

How do they breed? No one really knows as they have not been studied as intensively as Varroa. The information below is not part of the microscopy assessment but I include it for interest and pleasure, to show you how all these things are inter-connected.

For comparison, dear readers, let me tell you how fig wasps pollinate figs. The life cycle of the fig wasp, *Blastophaga psenes,* Chalcidoidea, family Agaonidae, starts with a female, pollen-laden wasp entering the immature fig through a very small hole in the end called an ostiole (think micropyle). She forces her way in and in the process rips off her wings. When inside what is called the syconium (inverted flower), she looks for the flowers, both male and female. Remember fig flowers are on the inside with the pollen. She pollinates the female flowers with the pollen she has picked up from the host fig and this allows them to mature. She lays her eggs then dies inside the fig. As the fig matures, a wingless single male wasp emerges first. He then travels in search of female wasps for mating while still inside their galls, never leaving the fig fruit. The wingless male then digs a tunnel out of the fig through which the new females escape, picking up pollen on their way out. Once the new queens are outside, the single male quickly dies, as does the original queen still inside the ripening fig. The new females carry pollen from inside the fig to start the whole cycle over again. The figs produce chemicals (ficain), which breaks down the single male and the original dead queen to make fresh fig flesh rather like a carnivorous plant. Not wishing to put you off eating fresh figs, I need to inform you that the crunchy inside flesh is actually the ripe seeds and that figs are not full of dead wasps.

All of the above is very similar to the Varroa cycle, which could give us a clue as to how Braula breed (food for thought). Let me remind you of the Varroa life cycle. When the eggs are laid, the eight-legged protonymph is visible within the egg. It hatches within half a day of being laid. The protonymph feeds and grows, and then moults directly into the adult stage (instar 2). The final moult is directly into the adult (instar 3). Normally mites are characterised by a six-legged larval stage before the protonymph and a trionymph stage after the deutonymph, i.e. 5 instars. The shortening of the reproduction cycle in the Varroa demonstrates a high degree of specialisation to adapt, imposed by the necessity to complete reproduction during the 10-15 days that the bee brood cells are sealed. This leads us to believe that this is an adaptation formed millions of years ago with the original host *Apis cerana*, and then infested our bees, *Apis mellifera*. Both Braula and Varroa have a similar three instar cycle, each producing one male, but more females.

Fig. 7 An infected queen with many Braula on her back Image used with kind permission of Andrew Abrahams, Colonsay Black Bee Reserve

I have looked at many Braula for bursa or similar (female), or phallus for a male, but my problem is that I do not have many samples. I am down to five specimens at present. I will try to acquire some more so that they can be studied further, and I may do another paper with additional information.

It may be that Braula have a lifestyle based in the tunnels in the comb containing honey and pollen, with tunnel-bred females leaving the male to die in the tunnels after mating. Is this why it is hard to spot both males and females? I think that all three, Braula, Varroa and fig wasps, are very similar in demonstrating very specific adaptations to their habitat and life cycles. If you remove pollen from the fig wasp life cycle, they are all the same.

CONCLUSIONS

Braula probably poses a minimal threat to the beekeeping industry because no economic loss can be attributed to the damage it causes. There are very few recommendations for Braula control largely because it is not considered to be a major honey bee pest. However, they can be used like a canary in a coal mine as an environmental indicator of colony health. Braula have coexisted in partnership with bees for millions of years. Should they disappear completely it may be an indicator that there is something wrong with the colony or that something is out of balance. Personally, I hope that they make a return. The history of beekeeping has had many ups and downs over the years. In the 1940s and 1950s drones were seen as detrimental and the advice was to destroy them, but we now know better. Wasps are also considered a major threat. We know now that they are of benefit in clearing out diseases and abandoned feral colonies.

History has many examples of "home goals". The heather hills of Scotland have been the killing field of some 25,000 mountain hares[8]. Their supposed crime, now discredited, was that they were responsible for passing on louping disease via a tick, which was fatal to grouse. The loss of the hares meant that large areas of honey producing heather has been lost as Scots pines and birch seedlings have now colonised this precious bee foraging moorland[9]. At last legislation has given hares special protection under the Wildlife and Countryside Act.

Braula has its place in a modern beehive as an indicator of colony health. A healthy hive can fight off most pests. It is like the return of salmon to the Thames in London in the mid-1990s as an indicator in the improvement in water quality. Braula can do the same for us as an indicator species, especially as they occur worldwide and could give a worldwide indication. In my personal opinion we should not encourage or welcome their demise and it would be irresponsible to do so.

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(8) Scotland's hare culling; Heather Honey a Comprehensive Guide by Michael Bodger (Beecraft 2016) as quoted from Beekeepers Quarterly September 2020.
(9) Inglorious by Mark Avery (Bloomsbury 2015) as quoted from Beekeepers Quarterly September 2020
(10) Queen bee image with Braula Coeca used with kind permission of Andrew Abrahams,
Colonsay Black Bee Reserve

PS In the past the Iceni Microscopy Study Group has run courses for the BBKA microscopy exam, so far with a 100% pass rate. We could run a fresh course if ten or more people signed up. In the past few years one or two people taking the modules ask if we are running a course. This is not economical as we need a minimum of ten people to cover the rent of the hall, plus any teaching aids. The teachers only claim mileage expenses. We have a large stock of high and low powered microscopes available to loan out for participants. If any BBKA members or other beekeepers, or indeed non beekeepers, make their interest known to us, with sufficient numbers, we will run a course. Lastly, looking through a microscope can help you with all the BBKA modules e.g. it helps to understand the bees and which bit goes where (anatomy), pests and diseases. In the past I have had people travel many miles to attend the course. The course includes both technical and theoretical knowledge to make passing the exam less problematic. We promote a friendly, inclusive environment and welcome everybody, irrespective of their level of skill, to take an interest in microscopy.

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Please would you help us with our Braula survey? Details on page 57.