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For the past five years the Laboratory of Apiculture and Social Insects at the University of Sussex has been carrying out many research projects collectively called the Sussex Plan for Honey Bee Health and Well Being. These four articles summarize Sussex Plan research in two main areas: diseases and foraging. The disease work includes research on the control of varroa and on hygienic behaviour; a defence mechanism against brood diseases. The foraging work includes research that investigates honey bee foraging by decoding waggle dances, and on making urban gardens and parks more bee-friendly. In addition, the Sussex Plan is researching neonicotinoid insecticides and foraging in farm land.

The Sussex Plan has been carried out by Professor Ratnieks and his team, which has included 35 volunteers, overseas visitors, undergraduate students, postgraduate students, technicians and postdoctoral researchers. The BBKA have contributed half of the funding for one student, Gianluigi Bigio, who recently completed his PhD on hygienic behaviour.

Varroa: Towards Integrated Control

Varroa mites are a major challenge to honey bee health. For many years varroa could be controlled using Apistan strips. However, many varroa are now resistant to Apistan. There are many alternative control methods. How effective are they and do they harm the bees? LASI research shows that oxalic acid is highly effective in killing varroa, especially when applied via the sublimation method, which can kill 97%. Applying oxalic acid in solution via spraying or dribbling can be effective at killing varroa, but also harms the colonies. Oxalic acid only kills mites sitting on the bodies of the adult bees and so is most effective just before or after Christmas when most hives are broodless. Hygienic behaviour can greatly reduce the build-up of the varroa population inside a colony, and in combination with annual oxalic acid treatment should keep varroa numbers to harmless levels.

The mite, *Varroa destructor*, is a significant problem for honey bee, *Apis mellifera*, colonies. Varroa is native to the Asian honey bee, *Apis cerana*. Varroa does little damage to *A. cerana* as the female mites only breed in capped drone cells, resulting in low mite populations. But in *A. mellifera* the mites can also breed in worker cells and mite populations build to high levels and damage the bees directly. Varroa also vector honey bee viral diseases and this is probably their greatest harm. The main aim of LASI's varroa research is to determine the effectiveness of different control methods used by beekeepers. We have studied three methods in detail.

Beekeepers have long used oxalic acid and much research has been done on it. However, no project had compared the effectiveness of different doses and application methods. In an experiment using 110 hives we did this. Hives were treated in early January when they were broodless, as oxalic acid kills only varroa sitting on adult bees not those in brood cells. We determined the proportion of mites killed by washing the mites off of a sample of approximately 300 worker bees immediately before and ten days after treatment.

Our results showed that applying oxalic acid directly as a gas via sublimation was superior to application as a solution via spraying or dribbling. Sublimation gave greater varroa kill at lower oxalic acid doses and gave no increase in bee mortality either soon after application or four months later. In fact, colonies treated via sublimation had more brood than untreated control colonies. The

sublimation method is also quick and easy because it is applied via the hive entrance. One year later, we retested the sublimation method and obtained the same result. Just 2.25g of oxalic acid per hive kills 97% of the varroa.

The second method we tested was drone brood trapping. Varroa are particularly attracted to drone brood. Our results show that half a frame of drone comb placed into a hive in early spring, when the first drones are being reared, can trap about half the varroa. The third method is via hygienic behaviour. We compared the build-up of the varroa population in hives following oxalic acid treatment. In the 42 study hives, varroa populations increased from as few as seven times to as many as 74 times in one year. Build up was significantly lower in highly hygienic colonies (average 19 times) than non-hygienic colonies (average 45 times).

Overall, our results show that annual treatment with oxalic acid just before or after Christmas, when most hives are broodless, is probably enough to control the varroa population, especially when colonies are also hygienic. Drone brood trapping is less effective. Because only half the mites are killed, one round of mite breeding is enough to restore the population given that a female mite breeding in a worker cell normally has one or two daughters. Killing 97% of the mites using oxalic acid would seem to be about twice as effective. Actually, it is five times as effective as it will take five varroa doublings to build back to the level before treatment. ■

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Treating a hive in winter with oxalic acid using the sublimation method. The oxalic acid is turned into a gas using a heated tool which is inserted into the hive entrance. For the purpose of the photo the tool has not been inserted and the oxalic acid vapour can be seen (photo by H AlToufalia).

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These projects have all been completed and written up for publication. The first is about to be published:

- AlToufalia H, Amiri E, Scandian L, Kryger P, Ratnieks FLW. Towards integrated control of Varroa: Effect of variation in hygienic behaviour among honey bee colonies on mite population increase and deformed wing virus. *J Apicult Res* (accepted).

Helping Bees in Urban Gardens and Parks

by Francis LW Ratnieks

In Britain there are about 30,000 beekeepers but millions of gardeners, not to mention other urban landowners including the parks departments of local councils. Helping bees and insects in their own gardens is probably the most widespread opportunity the public can have to help bees. Garden flowers vary approximately one hundred-fold in their attractiveness to honey bees and other flower-visiting insects. Varieties that are attractive to bees and other insects are just as pretty to look at and as easy to grow as the 'unattractive' varieties, so an attractive garden for both bees and humans can be achieved at zero additional cost. All that the public needs to do is to choose those plant varieties that bees like. It is quite easy to determine the good and not so good varieties simply by counting insects on flowers and comparing visitor numbers. Wild flowers can also be encouraged in urban areas by, for example, reducing grass cutting, which can encourage what is already there to bloom.



Southover Grange Garden is a beautiful park in the Sussex town of Lewes. In August 2012 the garden had a wonderful display of attractive flowers. However, most of the 79 flower varieties being grown were visited little, 47%, or not at all, 30%, by bees and other insects. Only 4% were highly attractive. This is probably typical of many gardens.

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Bees are almost entirely dependent on flowers for food. One way of helping bees is to increase flower numbers. Garden flowers are visited by many bee species including honey bees. Honey bees routinely forage several kilometres from the hive, and maximally at ten to twelve kilometres. As a result, a garden with attractive flowers has the potential to provide food for many hives. Garden flowers should be especially valuable in the summer, which is the season when honey bees fly the furthest to forage, which indicates that it is hard to find high quality flower patches.

Helping bees in gardens seems simple: just plant more flowers – but which ones? Ornamental garden flowers vary greatly in their attractiveness to bees and other insects. Lists of bee-friendly plants are available, but in a survey we made of these lists we found that they have various shortcomings. Unsurprisingly, given that there are tens of thousands of garden flower varieties, lists are incomplete. In addition, they oversimplify. Asters are often included in lists, but there are many varieties. We surveyed over two hundred aster varieties at the national collection at Picton Garden in Worcestershire. We found that most varieties actually attracted few bees and other insects. In short, different varieties of asters range from excellent to useless when it comes to attracting bees.

Perhaps the biggest shortcoming is simply that it is not clear how the lists were made. In particular, what data were used to compile them. To put the process on a firmer scientific footing we planted special beds of 32 summer flowering garden flower varieties on the university campus, and counted the insects throughout the bloom period for two years. Overall, 29% of the insects attracted were honey bees. The most attractive varieties had one hundred times as many visiting

insects as the least attractive. This is an important result as it shows that it is possible to make a garden more bee-friendly at zero additional cost simply by choosing different plant varieties, as every variety we used was attractive to the human eye, and easy to obtain and grow. We found a similar situation in a local park, as only three of the 79 flower varieties being grown were highly attractive to insects.

Helping bees in parks and gardens is not just about planting attractive varieties. It is also possible to help what is already there. One unsung hero is ivy. Ivy has the least colourful flowers possible, green and lacking petals, but they produce pollen and nectar. By analysing pollen loads, we found that 90% of the autumn pollen collected by honey bees was ivy. Towns also contain grassy areas. In a Brighton park, the Saltdean Oval, we monitored wild flowers and insects in half of the park where the council had reduced grass cutting. A huge range of wild flowers, which had been living there all along, could now grow and bloom. The long grass area had about fifty times as many insects as the short grass area. One of the commonest wild flowers that benefitted from reduced cutting was black knapweed, which is extremely attractive to honey bees.

Videos from the LASI YouTube Channel 'LASI Bee Research and Outreach'

- Quantifying variation among garden plants in attractiveness to bees and other insects. <https://www.youtube.com/watch?v=4u2LeTPGo9w>
- LASI Research on the attractiveness of ornamental garden flowers to bees and other insects. https://www.youtube.com/watch?v=stMgzQ1_kVA
- How to determine good plants for your garden by counting insects. <https://www.youtube.com/watch?v=I8BgUjI0ayU>

Waggle Dance Decoding: Investigating Honey Bee Foraging

by Francis L W Ratnieks

The honey bee waggle dance is a unique communication behaviour made only by honey bees. So remarkable is the dance that the Austrian scientist Karl von Frisch was awarded a Nobel Prize in 1973 for its discovery. The decoding of waggle dances has given LASI researchers a special opportunity for studying honey bee foraging – no other animal ‘tells’ researchers where it has been foraging. By videoing and decoding the dances made by bees living in observation hives, we can study their foraging over an area of almost one hundred square kilometres and at any distance. The results tell us how the bees are using the landscape for foraging. This tells us what can be done to make the landscape more bee-friendly.

The main reason why the number of hives in Britain has declined over the past century is almost certainly because bee food supply, flowers providing nectar and pollen, has declined. The intensification of agricultural land, which covers 80% of Britain, is the major factor and has reduced the value of the countryside to bees and other wildlife. Flower-rich hay meadows have almost all been ‘improved’, arable fields have fewer weeds, and clover is less used in pastures. If we want to make Britain a more bee-friendly country we need to increase the numbers of flowers. But to do this in the most effective way, and to maintain food production, we need better information on how foraging honey bees use the landscape.

One main way that LASI has been researching honey bee foraging is by ‘listening’ to the bees. That is, by decoding their waggle dances. Waggle dances are made by workers foraging at high quality flower patches. The dance communicates the direction and distance of the flower patch to the dancer’s nestmates. We first video the dances using observation hives. We then decode the dances by playing them back frame by frame on a computer to measure the waggle run angle, which gives the direction of the flower patch from the hive, and duration, which gives distance. We can then map the dances to determine where the bees are foraging and the distances they travel.



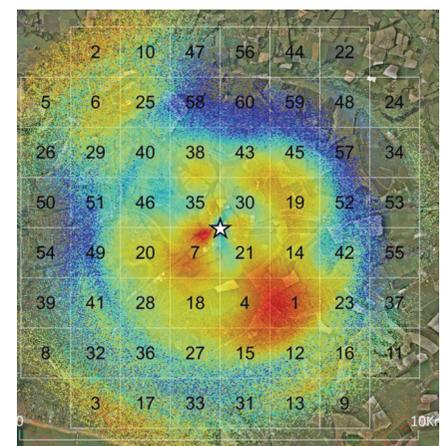
A honey bee pollen forager making a waggle dance back in the hive. Photo by C. Grüter.

We began by studying the dances made over a whole foraging season (two seasons actually), which runs from March to October. This had never been done before. The results showed a clear seasonal trend, in which average foraging distance was low in early spring, March and April, at less than one kilometre. Average distance increased in May and June until by July and August it averaged two to three kilometres, before reducing again in September and October. Honey bees do not travel long distances to forage for the fun of it, as this wastes time and energy. The longer summer foraging distances tell us that summer is a more challenging season in which to find high quality patches of flowers. It also tells us that this is the season to focus on if we want to help the bees with more flowers.

So far, we have also used dance decoding in five other projects. We have compared the foraging in different land use types, and also in urban versus rural areas. It seems that foraging is not significantly different in urban versus rural areas, indicating that towns are neither better nor worse than the countryside, but there is more foraging in countryside areas that benefit from ‘high-level stewardship’. We have also investigated foraging on two crops, apple and oil seed rape. The results from the study of oil seed rape show that bees will not travel more than 1.5–2km to spring-flowering oil seed rape, but travel further to summer-flowering rape. This fits well with our results showing that bees forage at greater distances in summer than spring.

Videos from the LASI YouTube Channel ‘LASI Bee Research and Outreach’

- Dancing bees cast their votes on the best land-types and areas for their food collection. https://www.youtube.com/watch?v=AMUIKOF_Tck
- Using the honey bee waggle dance to understand seasonal foraging challenges. <https://www.youtube.com/watch?v=yELA7pvNUQI>
- Apple pollination and the honey bee waggle dance. <https://www.youtube.com/watch?v=hbGRWoqJVQs>



Honey bee foraging intensity in 94 square kilometres of land around LASI over two foraging seasons by decoding more than 5,000 waggle dances over 2 years. LASI is the star in the centre. A foraging hot spot can be seen 2–3 km to the south east of LASI. This is an area of down land and is especially visited by honey bees in the summer. LASI is surveying this area to determine the most attractive sub-habitats and flower species for honey bees and other flower-visiting insects. From Couvillon et al. 2014b.

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Hygienic Behaviour: Natural Disease Resistance in the Honey Bee

by Francis LW Ratnieks

The easiest way to maintain healthy hives would be if the bees themselves controlled their pests and diseases. This is not an impossible goal as honey bees have many defence mechanisms. One of these is hygienic behaviour, in which worker bees remove dead and diseased brood from capped cells, thereby slowing down or halting the spread of the infection. Many serious honey bee diseases are brood diseases, or have a crucial stage in their life cycle in brood cells. Varroa mites breed in brood cells and also transmit viral infections when they feed on brood. LASI has been breeding hygienic bees, carrying out research on hygienic behaviour, and running workshops to train British beekeepers on how to test for hygienic behaviour in their hives. LASI hopes to be able to supply beekeepers and queen rearers with hygienic queens, which commenced in 2014.



Killing a patch of capped worker cells using liquid nitrogen to determine the level of hygienic behaviour in a colony. The frame is now photographed, placed back into its hive, then checked two days later to determine the proportion of freeze-killed brood cells that have been cleaned out. Each hive is tested three or four times to determine a meaningful average hygiene level.

Hygienic behaviour is a natural defence against brood diseases. Hygienic workers clean out cells containing dead or infected brood. Hygienic behaviour is not learned. Rather, it is an inherited trait. Previous research has shown that chalk brood and American foul brood can be controlled by hygienic behaviour.

LASI research has investigated both basic and practical questions about hygienic behaviour, including whether it is effective in controlling varroa and viral diseases, how to test for hygienic behaviour, and how to supply beekeepers with hygienic queens.

Hygienic behaviour is widespread but uncommon. Surveys typically show that only about 10% of hives are hygienic. To determine how hygienic a colony is, patches of capped brood are freeze-killed with liquid nitrogen, photographed, returned to the hive and then checked two days later. The proportion of brood cells that have been cleaned out gives the hygiene level. Colonies that clean 95% or more are considered fully hygienic. At LASI, we have been able to breed fully hygienic bees, some even 100%.

One basic research project investigated one possible reason why hygienic behaviour is rare. We determined whether colonies with higher hygiene levels also removed more healthy brood. We found that this was not the case. As far as we know, from this project and from research in the USA, hygienic behaviour is not costly to the colony.

A colony's hygiene level is not constant and we normally test each colony three or four times to get a meaningful average. We investigated factors that may cause the hygiene level to vary. We manipulated the amount of brood, by adding or removing brood frames, and also 'nectar flow' by feeding some colonies with syrup. Neither

factor influenced the level of hygienic behaviour, nor did colony size. This is encouraging as it shows that a beekeeper need not worry about these factors when quantifying hygiene levels, for example in choosing a hygienic breeder colony for queen rearing.

“ We determined whether colonies with higher hygiene levels also removed more healthy brood. We found that this was not the case. As far as we know, from this project and from research in the USA, hygienic behaviour is not costly to the colony. ”

How can queen rearers produce hygienic queens? We compared the hygiene levels of colonies headed by queens reared from fully hygienic breeder colonies. Half were allowed to mate naturally. The others were instrumentally inseminated with drones from hygienic colonies. Several months later when each colony was full of the new queen's workers we quantified hygiene levels. Colonies headed by instrumentally inseminated queens had higher hygiene levels, but the colonies headed by open-mated queens were also highly hygienic. This shows that queen rearers could supply open-mated queens to beekeepers wanting hygienic hives. Queen rearers could supply beekeepers with young, virgin hygienic queens to mate locally. LASI research has shown that it is simple to keep virgin queens alive in cages for one week prior to mating.

LASI research has also shown that hygiene can control varroa and the viral diseases it transmits. We tested varroa population build up in 42 colonies with different hygiene levels and found that fully hygienic colonies have only 40% of the varroa build-up. We also found that hygienic colonies had lower levels of deformed wing virus (DWV). This is important as DWV can kill colonies. ■

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