Proposed Project: Breeding for *Varroa* Resistance in Ireland

**Proposed Goal**

To enable the Native Irish *Apis mellifera mellifera* to re-colonise back into the wild. This is done by improving the health and survivability of the Amm in Ireland though selective breeding for *Varroa* mite resistant traits. Other benefits include better quality bees for beekeepers with minimal, if any, *Varroa* treatment and improved genetic diversity and mating capabilities.

**Introduction**

*Varroa* Mite

The *Varroa* mite is currently the most serious problem affecting honey bee health in Ireland. It is an external parasitic mite that attaches to the bee feeding off larvae and adults. Not only does their action of feeding on bees severely damage colonies they are also a vector for other diseases, in particular, viruses. Once infected, colonies not treated will die out in a few years. The mite transferred from the Asian to the European honey bee over forty years ago and reached Europe in the 1970s and then spread to Ireland in the 1990s. As a result of *Varroa* it has been said that there are no wild bees remaining in Ireland. Any bees found in the wild are presumed to be from recent swarms escaped from beekeeper colonies.

*Apis mellifera mellifera*

*Apis mellifera mellifera* (Amm) is the northern dark European honey bee. At one stage this bee covered the bulk of Northern Europe, however now due to movement of other subspecies by beekeepers there are very few areas of pure Amm. One of the areas where there is a high percentage of pure Amm is Ireland. This is in no small part due to the impressive breeding work by groups such as the Galtee Bee Breeders Society. The very wet weather and short season that the Irish climate brings has quite likely led to a unique local ecotype or Irish variety of Amm.

*Varroa* mite resistance

Bees have been selectively bred for desired traits for probably as long as there has been beekeeping. Since 1995 there has been a significant body of work from a number of countries that demonstrate that it is possible to selectively breed the honey bee for resistance to the *Varroa* mite. Bees that have the ability to minimise the amount of *Varroa* in the hive are said to be mite resistant. The US
Dara Scott has been particularly proactive in organising breeding programs all over the country that promotes with this, see Fig. 1.

The key potential advantages of having mite resistant bees are as follows.

- No treatment for Varroa needed
- Fewer colony losses
- Greater production of honey and bees
- Re-introduction of bees into the wild
- Enhanced biodiversity
- Reduced inbreeding due to large feral gene pool
- Improved queen mating due to large feral gene pool

If a project such as this is not developed, it will mean that beekeepers will continue to treat their bees indefinitely and suffer the negatives that the mite and continued treatments bring. As breeding projects become more successful outside of Ireland there will be a higher probability that Irish beekeepers will begin to import Varroa resistant bees into Ireland. Not only will this quickly erode our native population of Amm as the imported strains begin to survive in the wild, but it will also pose the additional threat of the introduction of extremely serious diseases through importation such as the small hive beetle, the mite Tropilaelaps clareae, the Asian hornet, or other new viruses.

Previously, selection for mite resistant bees was dramatic and led to very heavy colony losses. Methods such as the “Bond method” were used. This, typically, is where a large number of colonies, 100+, were left untreated for Varroa. Colonies that survived, usually a small number in the single digits, were then used as breeding stock. This gave successful results but with a high cost. More recently and in particular in the US bee breeders are using a more gentle method of selecting for mite resistance. The method is relatively simple. I have spoken the well-respected beekeeper Randy Oliver in the USA (www.scientificbeekeeping.com) and Dr Robert Paxton and Dr Tomás Murray of Queens University and Halle University and all three support this project.

The proposed project can be simplified to two key steps for beekeepers:

- Before treatment of the bees for Varroa, the bees are assessed for the percentage of Varroa present in the colony. This can be done via a simple powdered sugar shake method which will give an accurate percentage of mite infestation in the colony.
- The colonies with the most desired traits and the lowest Varroa count then form the basis of future breeding.

While this method will take somewhat longer to show efficacy, it is a simple, proven method that does not add cost to the beekeeper and does not involve colonies losses. It can be expected that with a 5 year time frame that the benefits of the work will be evidenced. I have been in contact with Randy Oliver and Adam Finkelstein who both had great success with this method and started seeing results within 3 years. Depending on success and the size of the apiary, it is anticipated that within a 5-10 year period the beekeeper can expect minimal to no treatment for Varroa. Sometime thereafter it would be expected that escaped swarms from these colonies would begin to be able to survive in the wild. These colonies would be able to continue breeding and, over time, would develop better resistance through natural selection.
This selective breeding is likely to be the only long-term solution to the devastating problem of the *Varroa* mite. With minimal cost and effort and a small amount of patience an all-island policy of selective breeding for mite resistant across the island of Ireland can ensure there is a future for bees, beekeeping and pollinated biodiversity in this country.

**Proposed Methodology**

This project is to be an all-island project combining beekeepers north and south of the border. The project will focus in particular on beekeepers that produce queens and bees for supply, especially at the early stages, but it would be desirable to have involvement from all beekeepers.

There are four key parts to the project that are needed to enable its success:

1. Selection of the stock for mite resistant traits
2. Breeding from stock with mite resistant traits
3. Database management
4. Outreach

**Selection of the stock for mite resistant traits**

There are a number of methods to assess whether colonies have mite resistant traits. Something that is simple and quick and inexpensive for the beekeeper to use is needed. Currently, the best practice in the US is the “shake method” which reliably evaluates the percentage of mite infestation in a hive. This will provide an accurate snapshot of the percentage of mite infestation in a colony. Other methods such as assessing the mite drop or damaged mites have been found to be subjective.
to colony numbers, animals attacking fallen mites and other variables, but most importantly they do not give an accurate measurement of the mite infestation in the hive which is the fundamental objective.

The other very significant advantage of the shake method is that it allows the beekeeper to know what his Varroa levels are in a colony and whether treatment is needed or not.

There are a number of shake method can be performed using either:

- Powdered (icing) sugar
- Alcohol (a bit easier but more expensive and harder to get alcohol)
- Dishwasher powder

Upon further research the powdered sugar method should be the standard method for the following reasons.

- Ingredient easily accessible and cheap
- Have confirmed it does not kill bees
- Lessens the chance of killing queen

For further information on sugar shake method see:

- Appendix 1
- [http://www.youtube.com/watch?v=ZvWfGMvy_zs](http://www.youtube.com/watch?v=ZvWfGMvy_zs)
- [http://www.youtube.com/watch?v=URuhrEJOIM](http://www.youtube.com/watch?v=URuhrEJOIM)
- [http://www.youtube.com/watch?v=XE7K2n8St_g](http://www.youtube.com/watch?v=XE7K2n8St_g)
- Appendix 2 talks about using alcohol but has other good information

Advantages of the shake method are:

- Simple and easy to learn
- Inexpensive
- Quick
- Provides a consistent method for all beekeepers
- Accurate
- Does not need to be done on site (bees can be put in bag and tested later)

It is important to note once the percentage of Varroa has been established in the colony the beekeeper can then treat for Varroa as normal. If treating for Varroa it is imperative that the beekeeper treats all hives equally at the same time. This allows for a fair comparison of mite levels when it comes round to selection time again, which should be at least 4 brood cycles after the most recent treatment.
Proposed method:

- Collect 100 ml of bees, which is equal to 300 bees, from colony brood frames and put into jar with mesh lid. Jar should have 100 ml level marked on it for easy measurement.
- To collect the bees one can roll the jar down along the frame or alternatively shake bees off frames into a bucket and pour into jar.
- Do not collect queen.
- Add a few tablespoons of icing sugar and gently shake around. You want enough sugar to thoroughly coat all the bees.
- Leave for 1-5 min for the bees to heat up the jar and make mites fall off, you can be closing the hive up at this stage.
- Shake onto white tray with water added. This will dissolve the sugar, make mites easy to see and prevent them from blowing away. Alternatively a white sheet can be used spraying water as you go to prevent the wind blowing mites away.
- Continue shaking until no more mites fall.
- Count mites and calculate percentage of mite infestation. This is done by dividing the number of mites by three i.e 15 mites fallen equals a 5% mite infestation.
- The beekeeper will want to treat if mite infestation is over 10%. Some treat anything over 2% infestation.
- Fill out the mite infestation checklist and post back to X

Actions Required:

- This method may need to be tweaked for Irish beekeeping.
- Need to create a checklist for beekeeper to fill to include at a minimum:
  - Date of inspection
  - When treated last and how
  - Did hive swarm
  - Was there a broodless period at any stage
  - Period of inspection
- NIHBS could provide beekeepers with a shake kit with shakers as we could buy in bulk to make it cheaper for beekeepers to purchase (I am currently looking into a cheap source in Germany)

Breeding for mite resistance

Once beekeepers know which of their stock has the least amount of Varroa they can then make an informed decision about which stock they wish to breed from. It is important to note that beekeepers should ensure that they are breeding from workable, productive bees in the first instance. The beekeeper can choose whether to prioritise breeding from his stock with low mite counts and/or to re-queen his stock that has the highest mite counts.

Actions Required:

- Developing regional breeding apiaries
• Explore incorporating a certificate or something similar that shows if the beekeeper is involved in the project to inform purchasers of Nucs/Queens thus incentivising selective breeding for mite resistance within the breeding community.

Database

To further aid this project the creation and maintenance of an island-wide database would be advantageous. This database would include all the key breeders in the project and any other beekeepers that wanted to participate subsequently. It could be maintained by an association or possibly some universities may also be interested in this project.

I am currently speaking with the Zoology Department of NUI Galway as they are very interested in the project. This department would also be able to help identify makers that are found in bees with lower mite levels. They would also be able to quantify the genetic diversity in order to prevent inbreeding and may be in a position to monitor diseases. With the creation of such a database progress could be easily monitored and it would be clear to see if some groups are having particular success and which groups need additional help. If successful groups were willing their stock could be distributed to other groups to help the project progress further.

Advantages of the database

• Track overall progress of the project
• Identify groups having exceptional success and use this to aid other groups
• Establish if different methods are providing different results
• Prevent inbreeding
• Aid in establishing bees with genetic markers for mite resistance

Actions required:

• Set up database
• Partner with University/ies

Outreach / Civic Engagement

This is the last but possibly most important point. The project will need consistent outreach to ensure that beekeepers are aware of it and that they see the merits and benefits of the project for themselves, the bees and the environment.

Outreach should compose of but not be limited to:

• Emailing, flyers etc.
• Articles in beekeeping magazines
• National Media (A project to bring the bees back to Ireland would surely garner interest)
• Workshops and Public Lectures

Outreach will need to be consistent in particular for the first 3-4 years or until some obvious success has been obtained. If not beekeepers will quickly become frustrated when they do not see success in the initial years of the project. Corporate sponsorship may also come available as it would be great PR for any company especially if we are getting national media involved.
Actions required:

- Establish an outreach plan spanning the period of the project, with a particular emphasis on the first 3-4 years

Conclusion

With support from beekeepers throughout the island of Ireland this project has a very real possibility of improving our beekeeping stock, improving queen mating, improving biodiversity and getting bees back in the wild once again. If successful, Ireland would be the first country to take on a national and cross-border project such as this and be a world leader for other countries to follow in the future.

Important point to note

For this to be successful it will need the participation of as many groups and individuals as possible and careful management. The main reason for this is that if only one or two groups are successful their stock will be used across the country; this will lead to a limited number of sexual alleles and the possibility of inbreeding or poor stock.

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1 http://www.sicamm.org/WhatApis.html
2 http://en.wikipedia.org/wiki/European_dark_bee
3 Breeding for resistance to Varroa destructor in North America* Thomas E. Rinderer1, Jeffrey W. Harris1, Gregory J. Hunt2, Lilia I. de Guzman1
4 Breeding for resistance to Varroa destructor in Europe* Ralph Buechler1, Stefan Berg2, Yves Le Conte3
5 http://www.vpqueenbees.com/awa/
6 http://www.beelab.umn.edu/prod/groups/cfans/@pub/@cfans/@bees/documents/asset/cfans_asset_317463.pdf
7 http://www.extension.org/mediawiki/files/6/6d/Standardized_Varroa_Sampling_Bees.pdf
9 http://scientificbeekeeping.com/fighting-varroa-reconnaissance-mite-sampling/
10 As per conversation with Flemming Gejsnaes at Gormanston 2013
11 As per conversation with Flemming Gejsnaes at Gormanston 2013
12 Fine-Scale Linkage Mapping Reveals a Small Set of Candidate Genes Influencing Honey Bee Grooming Behavior in Response to Varroa Mites Miguel E. Arechavaleta-Velasco1, Karla Alcala-Escamilla2, Carlos Robles-Rios1, Jennifer M. Tsuruda3, Greg J. Hunt3*
13 High-Resolution Linkage Analyses to Identify Genes That Influence Varroa Sensitive Hygiene Behavior in Honey Bees Jennifer M. Tsuruda1*, Jeffrey W. Harris2, Lanie Bourgeois2, Robert G. Danka2, Greg J. Hunt1
Appendix I

Possible shake methods:

Method 1 *(Note the “Gizmo” is not needed. We could just adapt a jar or plastic container.)*

*Image on next page...*

The following is the official YouTube video for the project:

http://youtu.be/PYjGDF-jZIs
Gizmo Operating Instructions

University of Minnesota Instructional Poster #171, Gary S. Reuter, Katie Lee, Marla Spivak Department of Entomology

Method to accurately measure 300 bees and determine the infestation of varroa mites.

www.extension.umn.edu/honeybees

This device is an important tool to help sample Varroa mites on adult bees. It can be used to provide an estimate of the level of mites in a single colony or an entire apiary. It is designed to provide a standard, quick, and non-destructive way to back the levels of mites in colonies.

WHY SAMPLE is a standard way?
- Be informed: know thy enemy
- Decrease use of miticides
- Reduce chemical residues in hive
- Save time and money
- Develop regional treatment thresholds
- Breed queens from colonies with low mites

1. Assemble unit as shown above. Initially, the holding jar on the Gizmo should be assembled without the screen under its lid. The beam insert at the bottom of the measuring tube should not be removed; it determines the number of bees per sample.

2. Turn the rotating cylinder COUNTERCLOCKWISE to open the measuring tube into the funnel. The measuring tube holds approximately 300 adult bees.

3. The number of mites in a sample reflects only the mites on adult bees. To find the percent infestation of a whole colony (mites on adults plus those hidden in pupae) use the conversion chart or formula: (mites per 300 bee sample / 3) * 2. The chart can be used to determine the mite infestation of a colony or an apiary (8, 300 bee samples).

4. Sampling a Colony. Shake bees from a brood (eggs, larvae, pupae) frame onto a honey bee sample “thing” (a 12” x 18” piece metal flashing bent into a V-shape). Check for the queen before shaking the frame! DO NOT put the queen in the sample!

5. Dump the bees into the funnel, then raise the bottom of the jar on something solid 3 times to compact bees into the measuring tube and then quickly turn the rotating cylinder COUNTERCLOCKWISE ¼ turn to trap the bees inside the measuring tube.

6. Dump any excess bees back into the colony. If there were no excess bees in the funnel there were not enough bees sampled. In this case, a new sample must be collected for the measurement to be accurate.

7. Turn the rotating cylinder COUNTERCLOCKWISE until it stops and rap the jar to force the bees from the measuring tube into the jar. Samples of bees from up to 2 colonies can be added to the jar before measuring mite levels.

8. Testing for Varroa. Rap the jar to get the bees to settle to the bottom. Remove the jar from the Gizmo and quickly secure the lid with the screen on the jar.

9. Add about 2 tbsp of powdered sugar. Shake the jar to coat all bees with powdered sugar. Let the jar set in a shady place for one minute.

10. Shake the jar vigorously with the screen side down over a white dish for one minute. Mites and sugar will fall into the dish. Add a small amount of water in the pan to dissolve the sugar, making the mites more visible. Count and record the number of mites.

11. Open the lid and pour the bees back into a colony. It is not important to separate the bees back into the colonies from which they originally came. Clean the Gizmo and continue testing the next colony or colonies.

12. Sampling an Apiary. Sample a total of 8 colonies. Select every fifth colony in the apiary to sample until 8 colonies are sampled. You can combine the samples from two colonies into one sample.

For more information on how this sampling procedure was derived and treatment thresholds, please read the article in American Bee Journal, December 2010, or in J. Economic Entomology, 2010; vol 103(4): pp. 1039-1050.
Method 2


With the right equipment and a bit of practice, the alcohol wash takes only about two minutes per hive. There are a number of tips and tricks involved, and in this case a picture is worth a thousand words. So I’ve made a photo essay of the technique.

Step 1 – Pull out a brood frame from middle of the cluster in the upper brood box. It does not appear to be all that critical that the frame actually contains brood.

Step 2 – Make sure that the queen is not on the frame! The one drawback to this method is that you must sacrifice 300 bees (and you don’t want one of them to be the queen). I don’t like killing bees any more than anyone else, but to me the sacrifice of a few bees in order to prevent the death of a million bees in a yard is akin to taking a blood sample (or mowing your lawn). Disclaimer: to avoid eye injury, you should always wear a veil when working bees!

Step 3 – If you see the queen, either remove her, or sample another frame. I like it when I see the queen (which is quite often when I take the frame from the center of the cluster), since I am then home free!

Step 4

Step 4 – Shake the frame to dislodge the bees into a tub. Tip: the technique for shaking is to make the frame bang down-up-down sharply and hard between your loosely-held thumb and middle finger, making sure that your thumb never clamps down (which would cause the bottom of the frame to swing wildly). Alternately, you can hold the frame vertically, and bang the ear against the bottom of the tub (the tub would need to be on a hard surface so that you don’t punch a hole through it!).

Step 5 – Allow the older bees (which carry fewer mites) a few seconds to fly off. If you hadn’t already confirmed that the queen is not in the sample, she is easy to spot at this moment.
Step 6

Step 6 – Shake the bees into one side of the tub and use a ½ cup (120 ml) measuring cup to scoop up the bees, then shake them off dead level (equal to 300 bees). If nectar is shaking from the frame, making the bees sticky, then hold the cup below the bees, and jiggle the tub to fill the cup. Tips: use a stainless steel cup that measures ½ cup to the rim, and a white Rubbermaid® dishwashing tub. The curve of the Rubbermaid brand tub corners exactly matches that of the cup, making for easier scooping. Dump the bees into a wide mouth pint jar of alcohol.

Step 7 (Option A)

Step 7 (Option A) – Use a round bottom white bowl with a kitchen sieve that fits it closely. Fill the bowl with rubbing alcohol so that it is at least two inches deep in the sieve. Dump in the jar of bees and stir and shake them vigorously to dislodge the mites. Keep washing until no more mites fall off.
Step 8

Step 8 – Lift the sieve and count the number of mites. It helps to pour off the excess alcohol for better visibility.

The sieve and bowl work fine, but for taking multiple samples in the field, a “mite shaker” jar is a big improvement. There’s a photo of my prototype at “Reconnaissance,” but Dr. Medhat Nasr designed one that is a great improvement. It is available for purchase in Canada, but I have not yet seen it offered in the U.S. So I’ll show you how to fabricate one yourself!

Take two clear plastic jars (plastic jars are much lighter weight for shaking). Make sure that the bottoms are molded smoothly so that it will be easy to view mites. Empty the jars.

Use a tomato paste can with both ends removed, heated in a flame until red hot at the edge, to melt a hole through the center of each lid. Use a clamp to hold the can, and have a responsible adult help you, since there is an element of danger involved.

Cut out a circle of $\frac{1}{8}''$ hardware cloth to fit inside the rim.
The lids are made from polypropylene, to which virtually no glue will stick (I tried several, including polyolefin hot glue—all failed when soaked in alcohol). Luckily, polypropylene can be easily heat welded with an ordinary soldering gun. Make sure that you get a good, deep weld, as the weld may crack with use (but is easily repaired by rewelding). You probably shouldn’t hold it against your belly as I am doing!

Here is the final mite monitoring kit – compact and field ready. It includes a fine kitchen sieve, so that the penny-pinching beekeeper (me) can reuse the alcohol. For additional savings, I cut the 70% alcohol in half with water (be sure to relabel the bottle). Some suggest using windshield washer fluid, but I find that it foams too much, and lacks the clarity of plain rubbing alcohol. In a pinch, you can also use water with granulated dishwashing detergent.
Step 7 (Option B)

Step 7 (Option B) – Fill one jar about 2/3rds full of alcohol, and pour the bees into it. (OK. I’m really pinching pennies here with reused alcohol).

Step 8

Step 8 – Screw the lid and upper jar on top.
Step 9

Step 9 - Invert the shaker so that the bees are in the upper jar. Shake vigorously for twenty seconds, then after the last up shake jiggle the jar as the alcohol drains through the bees. If you don’t jiggle, some of the mites get stuck in the bees from time to time (I’ve tested).

Step 10

Step 10 – Look up through the bottom of the jar. It takes about 30 seconds for all the mites to sink to the bottom, depending upon the swirl. Adjust the view so that the sun illuminates the mites clearly. If you’ve shaken vigorously enough, you will generally see a dislodged bee stinger or two. Test the efficiency of your initial shakes by repeating with fresh alcohol to see whether you recover any additional mites—you shouldn’t!
Step 11

Step 11 – Count the mites. There are 16 in this sample of 300 bees, which you divide by 3 to get percent infestation (mites per 100 bees)—in this case, slightly over 5%. I treat at anything over 6 mites during summer (2%), so no need to count any higher. Surprisingly, I often see light-colored mites.

Holy cow! Too many mites—approximately 72, or a 24% infestation! This colony (not one of mine, thank God) is in the severe danger zone. (Note the stinger at the far upper left).
Step 12

Step 12 - Discard the bees and filter the alcohol for reuse. Make sure that you check the emptied jar for residual mites (I just pour a bit of alcohol back in, swirl, and dump). Tip for commercial beekeepers: Make two shaker jars, so that one can be settling on top of a hive while you start taking a sample in the next one!