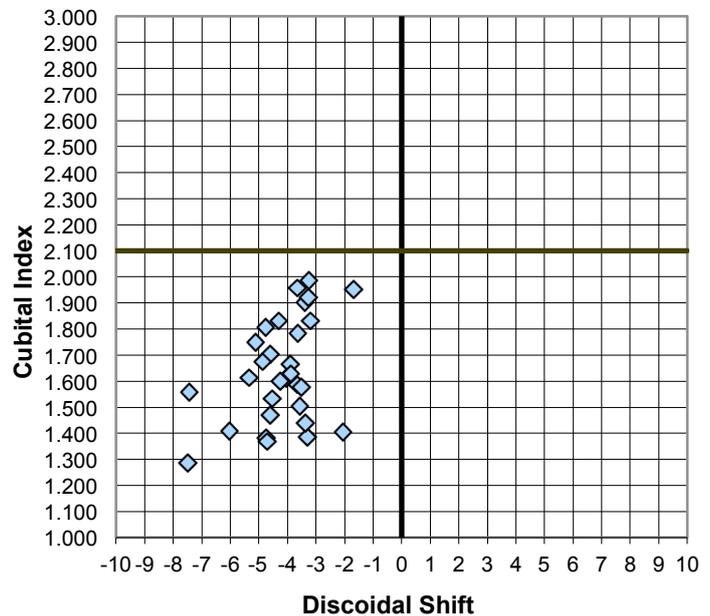


## Scottish Mainland A.m.m. - A Dark Bee Breeding Project – Margie Ramsay

Fig 1  
Queen D2 1/6/2010

In 1905, just before the First World War there was a 20 year long bee plague called Isle of Wight disease which was considered by many, including bee breeder Brother Adam, to have eradicated the native subspecies of dark European honeybee *Apis mellifera mellifera* from our Isles. To make up the losses imports of foreign subspecies, which had started in 1859, were increased. Since then regular imports of *Apis m. carnica*, *A.m. ligustica* and other races have become the norm. Crosses between these bees have given a range of hybrids in the British Isles. However, pure populations of A.m.m still exist in several areas of Scotland as identified by the Scottish Bee Survey carried out by John and Morna Stoakley in 1992. A few of the Scottish mainland A.m.m. apiaries identified by Morna Stoakley using manual wing morphometry analysis were still found to have maintained their purity (Fig 1) when re-examined in 2010.



Source bees from North coast on the Scottish Mainland.

Through negotiation with SBA members John Durkacz and Irene and Alasdair Joyce brood was annually obtained from some of these Scottish mainland A.m.m. colonies and used to graft queens yearly from 2010 to 2012. These queens were ultimately used to establish colonies of A.m.m. at the Scottish Natural Heritage Reserve near the inland mountain range of Beinn Eighe, Kinlochewe, where it was possible to establish a pure A.m.m. apiary without the purchase or removal of any colonies or live bees from the source stocks on the North coast of mainland Scotland. This allowed an increase in A.m.m. population and range while preventing any depletion of the limited number of source hives situated 200 miles away. I would like to thank the beekeepers who provided the brood and comb for grafting; this work would not have succeeded without their generosity.

The project started in 2010 at a coastal apiary in Wester Ross with grafting of known A.m.m. brood from remote stocks of dark bees kept at apiaries 200 miles to the North on the Northern mainland coast of Scotland. The successful raising of virgin queens from these stocks was reported in *Bee Improvement and Conservation* 38 Late Spring 2012 pp15 -16 (see SBA website [www.scottishbeekeepers.org.uk/PracticalBeekeeping/Research.aspx](http://www.scottishbeekeepers.org.uk/PracticalBeekeeping/Research.aspx) ).

Year 1. July 2010 – The timing of harvesting and hive selection was donor dependant. Where available, one piece of comb containing eggs and young larvae was cut from a brood frame using a fresh sterile scalpel for each colony. Comb blocks of roughly 2x3 inches were cut from a central brood frame from between 2-4 populous hives at the source apiaries. Sections contained eggs and young larvae but often brood of other ages too.

Only old comb was used due to the destructive effect of harvesting on the donors frames. No serious brood diseases were identified. The harvested brood was freed of any adult bees and boxed in plastic cut comb honey boxes, sealed with sticky tape and labelled with the date, source hive and any other necessary identification using a marker pen and then packaged in a plastic bag for transport. In 2010 the temperature of the brood during transport was carefully controlled at near body temperature (32oC), but in subsequent years average internal car temperatures were found to be adequate over the 4 hour drive back to the home apiary. However temperature/humidity may have to

be more carefully controlled under different transportation conditions as attempts to use the Royal Mail to post boxes of brood were unsuccessful. On arrival at the home apiary brood sections were taken from their boxes and pinned between the brood frames of queenless starter nucs. The next day (20<sup>th</sup> July) a Chinese grafting tool and scalpel were used to graft the smallest larvae into frames holding plastic cups each containing a tiny droplet of water; this was done either in the apiary or car. Frames of grafts were labelled and transferred into reception nuclei of queenless bees which were fed pollen substitute and syrup. Six queens were produced to point of lay and became the next years drone mothers, as even if mated to local hybrids pure A.m.m mothers will still produce pure sons. Because drones are haploid and only contain the DNA of their mother they can be mated to subsequent batches of virgin A.m.m. queens grafted from the source apiary to produce a pure line.



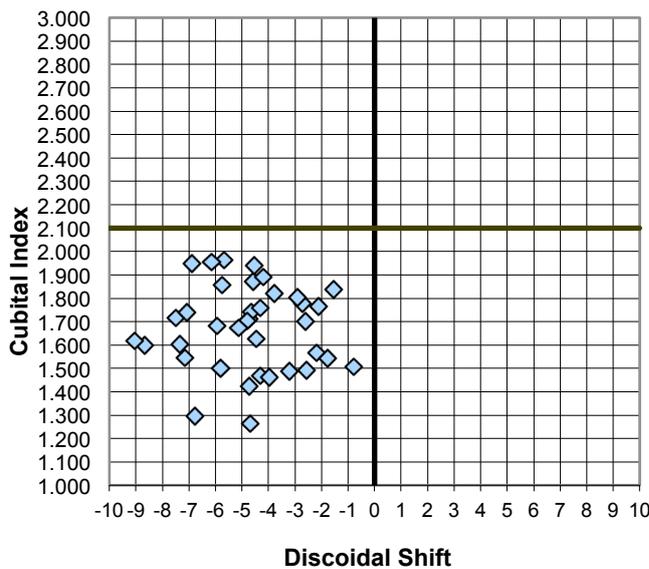
A.m.m. queen produced by grafting.

Year 2. In 2011 another trip was made to source more brood to graft queens for 2011. A batch of virgins was again produced using the same process as before and naturally mated with the drones from the previous years A.m.m. mothers. (It would NOT have been possible to breed pure queens from the previous year's out-mated mothers.) Unfortunately the weather that summer was very poor and all virgins were lost or became drone layers. It was also decided that the coastal apiary was no longer sufficiently remote from other apiaries to give satisfactory mating success by drone flooding using drone comb in selected hives.



Year 3. 2012. A suitably remote site for mating of grafted A.m.m. virgins was needed, and the Director of the Scottish Natural Heritage Reserve at Kinlochewe gave permission to site a mating apiary at the field station of the mountain nature reserve at Beinn Eighe. No other beekeepers/feral colonies were known to be in the area at that time and drone-free mating hives housing virgins were set up as a test, as no successful mating was achieved a number of hives were established on the reserve. New queens were successfully grafted, mated and reared from more brood retrieved from the source apiary and these virgins were successfully crossed with the drone offspring from the 2010 mothers.

**Fig 2**  
**Queen D24a 10/04/13**



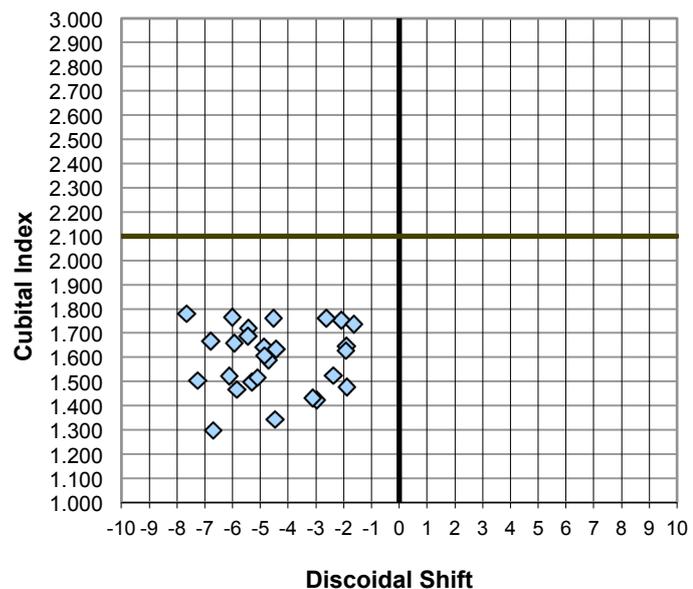
Queen mated at Scottish mainland mountain apiary Beinn Eighe, Kinlochewe.

After allowing new queens time to replace all the workers in the colony with their own offspring a test of their progeny purity was done. As different subspecies of honeybee have slightly different wing venation and their hybrids show a scatter distribution this test can give an objective assessment of breeding success (Ruttner, 2004). For a test about 30 young bees were collected from the brood box and frozen to kill them, their right wings were removed and the veins measured (Dews and Milner, 2004). This was done using a USB microscope and the computer programme Beemorph (Talbot, 2002) which can be downloaded

via the BIBBA website [www.bibba.com](http://www.bibba.com)

Years 4-5 2013 and 2014. Four mother queens were used to establish breeding lines, with seasonal rotation of drone production between these lines. Morphometry plots of two colonies sampled in 2013 (Fig 2) and 2014 (Fig 3) are shown. The wing morphometry of bees was tested via Ewan Campbell by Jim McCulloch in July 2013 and the CI/DsA% was 100% (pers. comm.) On-going culling of queens and drone brood has been carried out based on morphometry plot results and

**Fig 3**  
**F<sub>2</sub> Queen D22a 19/4/2014**



other dark bee characteristics such as disease prevalence –

**Daughter queen naturally reared and mated at Kinlochewe**

particularly selection against chalkbrood. Hives had been tested for acarine at Aberdeen University in 2013 and showed 1 weakly positive bee. Kinlochewe and the source apiary are both varroa-free areas and as such braula is present. Some selection was also made for white cappings and brood pattern (compact brood nest with pollen stored below brood). Colonies did not exhibit following and nor overly defensive but did rarely produce a ‘Pet Hive’: such a hive has arisen once at the source apiary and once at Beinn Eighe, the non-response of such bees to the beekeeper, wasps and robbing makes them difficult to maintain for long. Other dark bee attributes were also encouraged including supersedure and longevity (line mothers being allowed to fulfil their maximum natural life cycle of 3-4 years if possible). There have been no over wintering losses as yet.

Morphometric testing showed that *Apis mellifera mellifera* colonies were successfully produced using the grafting and mating methods described. A.m.m was confirmed by mitochondrial DNA testing (mtDNA) of 5 worker bee samples from each of 6 hives by Dr Ewan Campbell at Aberdeen University (Campbell, E., & Ramsay, G. 2015). Mitochondrial DNA is an extra-nuclear genome passed on by the female parent to all of her offspring. The circular mtDNA was cut up using an enzyme called *Dral* to give pieces that are highly variable between the different honeybee lineages so that the degree of hybridization can be analysed. In addition a short section of nuclear DNA (inherited from both male and female ancestry) was also tested and compared to known genotype libraries; from these results it was shown that, “For the Kinlochewe bees, the *Dral* markers were exactly as expected for *Apis mellifera mellifera* for each hive tested”, (Campbell, E., & Ramsay, G. 2015).

To help prevent hybridization there was selective culling and swamping of the surrounding 12km diameter area around the breeding site. Also neighbouring new beekeepers were encouraged to buy bees from the project. Instrumental

insemination was not employed. This work shows that in some suitable areas it is possible to reintroduce and produce the native European dark honeybee, and to achieve them without depletion of currently limited A.m.m stocks. Hopefully this could help to conserve *Apis mellifera mellifera* as a distinct race and increase colonies of our native dark honey bee.

As a postscript, after the completion of this work I was interested to read historical research by Will Messenger which found that a similar backcrossing strategy had previously been employed in the 1940's by Evan Evans of Mountain Grey Apiaries, Yorkshire to breed Caucasian queens (Messenger, W., 2014).

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