Translation of the contribution to the course Specialist/Teacher Queen Rearing 2014-2015

I Estimation of breeding values and selection

II Selection for Varroatolerance

III Beebreed

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13 December 2014. English version 15 November 2015

Part I: Estimation of breeding values and selection

a. Introduction: The notion 'breeding programme'

Estimation of breeding values and selection are both elements of a breeding programme. A breeding programme is a set of activities aimed to improve the quality of a population of honey bees every generation. This can be achieved by the selection, every generation, of the better colonies to produce offspring. In principle this selection process takes place within a closed population, i.e. without the introduction of breeding stock from outside.

Before discussing elements of a breeding programmes it is useful to talk about the position of such a programme in the context of genetic improvement of honey bees. The point is that colonies that take part in the breeding programme in terms of systematic selection represent only a small part of all colonies kept by beekeepers.
My first observation concerns the multiplication of improved breeding stock (colonies, queens); my second observation concerns the use of cross breeding in relation to genetic improvement of honey bees. I use the concept breeding programme in a narrow sense: improved stock from such a programme can be used for multiplication or can be used for cross breeding. I do not include multiplication or cross breeding itself in the term breeding programme.
After having discussed multiplication and cross breeding the body of this course deals with elements of breeding programmes in this narrow sense, the estimation of breeding values and selection in particular. In the improvement of livestock the population of animals taking part in the breeding programme in the narrow sense is called the nucleus.

Breeding programme and the multiplication of improved stock

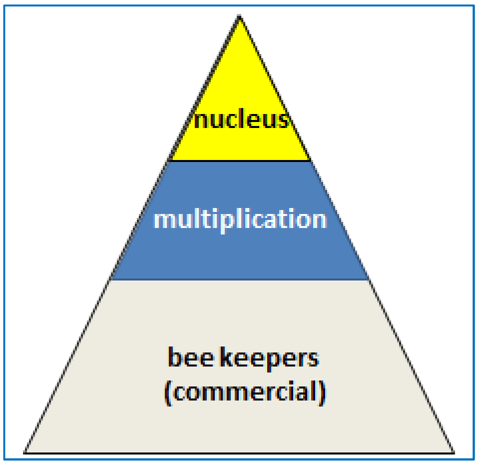


Figure 1. The production pyramid

In the case of farm animals breeding programmes often are positioned in a so called production pyramid (Figure 1). In this figure the breeding programme is called nucleus. Within the nucleus the systematic genetic improvement takes place of, for example, laying poultry. In the multiplication phase large numbers of hens are produced from improved parents and these hens are used in the commercial phase for egg production (Figure 1). Generally the number of animals in the nucleus is a lot smaller than

in the multiplication phase and in the commercial phase. In the situation of honey bees commercial seems not an appropriate term because most bee keeping is not for commercial purposes at all. This phase may be indicated as 'beekeepers', the collection of ordinary beekeepers who do not select systematically nor produce improved stock for other beekeepers. Moreover, in the situation of honey bees, nucleus and multiplication usually are not separated. Beekeepers who work on the systematic genetic improvement of their stock generally also operate as multipliers in the sense that they rear many queens from there improved stock which will be made available –be it virgin or mated- for other beekeepers.
When a beekeeper (or more often a group of beekeepers) intends to set up a breeding programme it is usefully to consider from the beginning how multiplication will take place: How can other beekeepers take profit from this initiative by receiving improved stock.

Breeding programme and cross breeding

In farm animals (pigs and poultry in particular) the nucleus is formed by so called pure lines or breeds. In the multiplication phase (in multiplier herds) these lines or breeds are crossed to produce crossbred stock for the commercial phase (production of meat, eggs). In that way the systematic genetic improvement of these lines or breeds is combined with effects of cross breeding (of which heterosis is one example).
In the situation of honey bees I don't think such a system of systematic cross breeding is feasible because it requires a well-developed infrastructure which doesn't fit the beekeeper. For that reason I will discuss selection and cross breeding from a different angle, as illustrated in Figure 2.

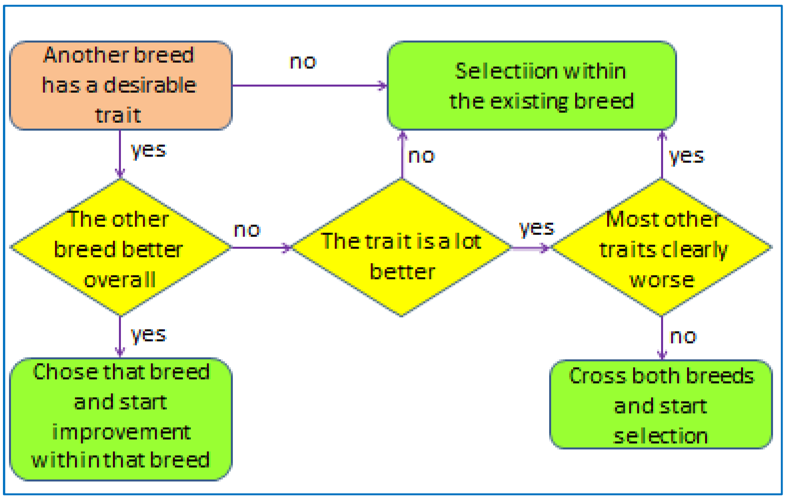


Figure 2. Selection with a breed or cross with another breed?

This figure is a decision tree before starting a breeding programme, given the intention of selection within a particular breed, **plus** the question whether the contribution of another breed (or other breeds) would be useful.
The figure deals with a number of questions which subsequently need to be answered. The first question, upper left in the figure, is: Is there a breed that for a desirable trait is superior to the own, existing, breed. As an example may serve the resistance to Varroa. If there is a breed that is better for that trait than your own breed the answer to the question is "yes". Subsequently a logical question is whether that other breed is better overall, for essentially all relevant traits. If that is the case (the answer is "yes") it seems useful to start the breeding programme with that other breed. If the answer is "no" (not overall better) than the question is whether the other breed is a lot better for the desired trait or a little bit. If it is only slightly better (the answer is "no") it is wise to forget about this other breed and select within your own breed. There too desirable genes will be present be it at a lower frequency. If the answer is "yes" the next question is whether the other breed is clearly worse for other relevant traits or more or less an equivalent. If it is clearly worse then again: forget about it. Only in the case that for other relevant traits the other breed is only slightly worse (or

similar) it can be considered to produce a cross between both breeds (produce a so-called synthetic) and start the breeding programme with this. Within this synthetic the breeding programme goes as usual: estimate breeding values and select. Cross breeding here is a one-time exercise and continued selection is done within this new population.
My conclusion from Figure 2 is that cross breeding with another breed to introduce a desirable trait generally is not useful. The drawbacks usually are larger than the merits.
Another issue with regard to cross breeding is connected to the local adaptation of bee breeds. In case bees are kept that are very well adapted to a local environment, cross breeding with a breed or breeds from elsewhere doesn't seem a good option. In the context of Figure it may be argued that the other breed is not better overall and may have an interesting trait, but most traits are clearly worse.

It is of interest to note that Buckfast (currently to be seen as a synthetic breed or a line) finds its origin in cross breeding of various breeds with the indigenous British bee, A. m. mellifera. Whether initially this population really was crossbred with A. m. ligustica or that it in essence A.m. mellifera was replaced by A. m. ligustica is to be seen, but in any case, over the years many other breeds were introduced. I wonder if Brother Adam would have chosen his strategy with less time at his disposal as was used in retrospect.

b. The theory of breeding programmes within one breed or line

Selection and rearing are elements of a cycle which is repeated each generation. In principle these activities are similar each generation and the purpose of the activities is that with each generation the population of honey bees improves a little bit compared to the previous one. Figure 3 depicts such a cycle. First of all I will discuss the figure from the viewpoint of an individual queen breeder and subsequently from the perspective of a programme of collaborating breeders.

The breeding cycle for an individual breeder

The discussion of this figure starts at the top, with the selection of a queen or in fact a colony. On the basis of the quality or the performance of the colony the breeder can choose the colony (or colonies) of his preference. That's the **first selection step**: the selection of the favourite queen. From this queen a number of young queens can be reared and mated to drones with desired pedigree. This is the **second selection step**: the selection of drones mated to the young queens. In fact not drones are selected but colonies with queens producing the drones (drone-producing colonies, each with a drone-producing queen). Mating needs special attention because in nature queens are fertilized by many drones, up to twenty, during the nuptial flight(s) of the queen. Without special measures the ancestry of the drones will not be known

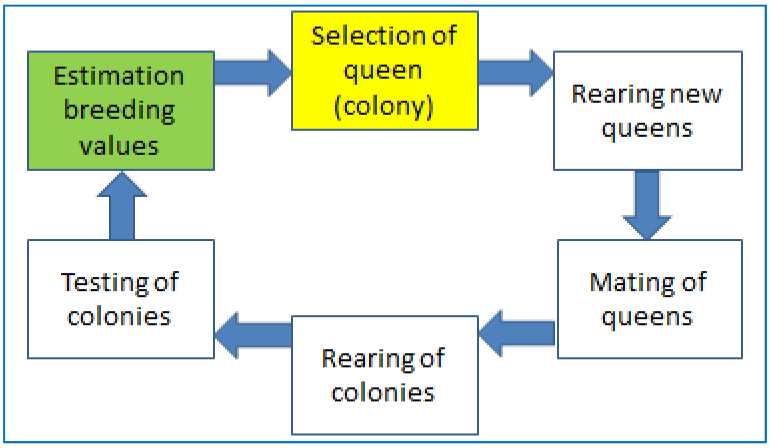


Figure 3. The breeding cycle, with rearing, two selection steps (the queen and the mating) and the estimation of breeding values.

such that the second selection step doesn't have the effect it can have. To put it a bit extreme: A breeding programme without control of the drones doesn't make sense. The quality of colonies continuously falls back to the population mean by the effect of non-improved drones.
To control the drone-side there are two alternatives. 1st The breeder travels with unfertilized queens to a mating station on an island or an isolated location elsewhere, with colonies with drone-producing queens of known genetic merit. 2nd The young queens are fertilized by instrumental insemination with semen collected from drones from queens with known genetic merit. After successful mating queens produce their colonies. Observations usually are done on the colonies in the following year after the winter, and the purpose of observing is to judge the quality of the queens and their colonies. Often selection decisions are made on the basis of these observations as such. Better, however, is to analyse the observations and to convert them into estimated breeding values because that provides a better insight in the *heritable* quality of the colonies and queens. This is important because the observations as such are not only affected by the genetics of queen and colony, the breeding value, but also by environmental factors.
In a separate paragraph the estimation of breeding values is discussed. .

The breeding cycle as part of a breeding programme

The purpose of breeding programme is to genetically improve the bee population as a whole, that is, the colonies of the participating breeders. The simplest way to look at it is to consider it as a collaboration of a number of breeders each working at the genetic improvement of their colonies, but collectively working for the improvement of the population at large.
The breeding cycle then doesn't differ from that of an individual breeder. Figure 3 therefore also holds for a breeding programme. However, there are differences. The most important one concerns the queens from which drone-producing queens are reared for mating stations or for the production of drones for AI. From this point-of-view the second selection step, i.e. selection for mating, is a decision on the level of the programme. Although the individual breeder decides to which station to travel with his young queens, or with what semen to inseminate his queens, the drone-producing queens available is a matter he has to live with: that's decided on the level of the programme.
Another difference is that an individual breeder can decide for himself about the relative importance of traits. On the level of the programme, however, some agreement is required about this issue for systematic genetic improvement in a common direction to be achieved. This direction usually is called the breeding goal and is an important element of the breeding programme.
Generally speaking the genetic improvement per generation can be larger in a breeding programme than it can be for an individual breeder working on his own. This is purely a matter of scale. When various breeders collaborate every year a wealth of information becomes available on a large number of queens and their colonies, from which the best can selected to populate the mating stations. So strong a selection is not possible for an individual breeder because he only can select from a smaller number of candidates (his own colonies and queens). Also, in the situation of a programme, breeding values can be estimated more accurately when all observations of the collaborating breeders are analysed simultaneously. As a consequence of the larger number of colonies stronger selection is possible on the basis of more accurately estimated breeding values.

c. Elements of a breeding programme

In this paragraph I discuss two elements of a breeding programme I touched upon before: the breeding goal and breeding value estimation. Subsequently I discuss the factors affecting the size of genetic improvement.

Breeding goal = direction

The breeding goal is the aggregate of traits for which one wishes to improve the population of honey bees. The breeding goal provides/represents a direction. For example a higher yield of honey, a stronger degree of calmness, a better ability to deal with the Varroa mite. The idea is not really to arrive at the “perfect bee” (because she doesn’t exist) but to gradually, step by step, improve a bee population for a variety of traits. In general there are three groups of traits:

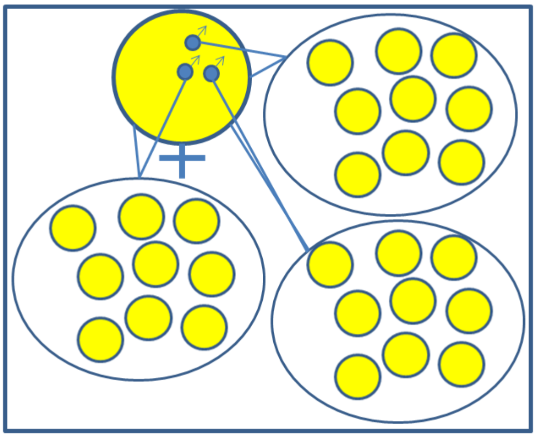
- Traits related to honey yield
- Traits related to behaviour, relevant for the ease with which to handle bees
- Traits related to the resistance to disease

Honey yield combines many underlying traits, like the ability of workers to find and collect nectar, the health of workers, the size of the colony and with that the egg-laying capacity of the queen. How to incorporate honey yield in the breeding goal merits some discussion. Perhaps life-time yield is the trait to be preferred. This choice clarifies that the trait one measures not necessarily is the trait in the breeding goal. In this case one measures honey yield in the first season and perhaps in the second as well. But certainly not life-time because then the queen by definition is dead after the trait (life-time yield) is measured and from her no young queens can be reared. When looking at the size of genetic improvement this issue will be addressed once more.
Behavioural traits have to do with defensive behaviour but also with swarming behaviour. As for disease resistance one can think of susceptibility for chalk brood or the capacity to deal with the Varroa mite. Just as with honey yield, for Varroa resistance it is not obvious which trait to include in the breeding objective. We think of it as a combination of the quality of workers to identify and remove infested brood, plus a property of the brood slowing down mite reproduction, plus polishing behaviour of workers diminishing the entry of mites into a brood cell, etcetera. In practice it will not be possible to measure all these traits and instead of that one may measure numbers of falling mites over certain periods or observe hygienic behaviour. I will come back to this in part II.

Estimation of breeding values

To discuss the way breeding values can be estimated it is useful first to discuss what is meant by the term “breeding value”. The breeding value of a queen is defined as the expected quality for a particular trait of her progeny in an average environment and when mated to average-quality drones. When progeny is kept in a better environment better results can be expected, not caused by the genetics of the queen. 2nd When she was mated to above-average drones results can be expected to be better than predicted on the basis of the queen’s breeding value.

It is important to realize which queen we are talking about and which progeny. Figure 4 represents a colony with (upper left) a queen fertilized by three drones. From a genetic point of view in the colony there are three groups of workers, all descending from the same queen, but each group from a different



spheres) and three groups of workers, all descending from the same queen but each group descending from a different drone.

drone. In reality in a colony there may be 10 to 20 groups of workers.
When talking about the expected quality of progeny in fact we talk about the expected quality of all workers in the colony. Honey yield, behaviour and disease resistance are expressed on the level of the colony, of the set of workers in it, not on the level of an individual worker or on the level of the queen. When talking about the breeding value of a queen, we talk about the expected quality of workers descending from that queen, in fact the quality of the colony of which she herself is a part. The quality therefore is not a one-animal issue but emerges from the collaboration of then thousands of workers plus their queen.

Figure 5 illustrates the factors contributing to the quality of a colony. There are genetic factors (yellow) and environmental factors (green). There is a variety of environmental factors, like weather, the quality of flowering, the way the beekeeper treats his colonies, presence of diseases, etc. Genetically on the one hand there is a contribution of the queen for example by regulatory effects through pheromones or certain aspects of her egg-laying capacity. This contribution to the quality of the colony depends upon the breeding value of the queen herself, through which she herself influences colony's quality. We call this contribution the *queen effect* and relevant is the breeding value of the queen for queen effect. On the other hand the quality of the colony is affected by the workers, how they behave, how well they collect nectar, the degree to which they are able to detect brood cells with Varroa mites, open those cells and remove the brood, etc. We call these contributions *worker effect*, which genetically is determined on the one hand by the breeding value for worker effect of the queen (contributes half) and on the other hand the breeding value for worker effect of drones (contributing the other half). Or, more precisely, the average of breeding value for worker effect of the drone-producing queens contributing the drones for mating. One finds that the genetic worker effect on traits like honey yield, calmness or Varroa tolerance is somewhat larger than queen effect, but both are relevant. It is a rather complex situation which has to be accommodated by the way breeding values are estimated. However, there are appropriate methods to estimate the breeding values for honey bees. These methods in essence compare colonies with other colonies tested on the same location (in the 'same' environment). The genetic level of the different locations can be derived making use of the pedigrees of the colonies on the different locations and to be effective in principle colonies on different locations need to be relatives. A very effective way is to test groups of sister colonies on more than one location but that's not a prerequisite to be able to determine the relative genetic level of locations. It is sufficient when colonies are related. Breeding values of individual colonies are estimated by the comparison of colonies within test locations, furthermore taking into account the difference in genetic level

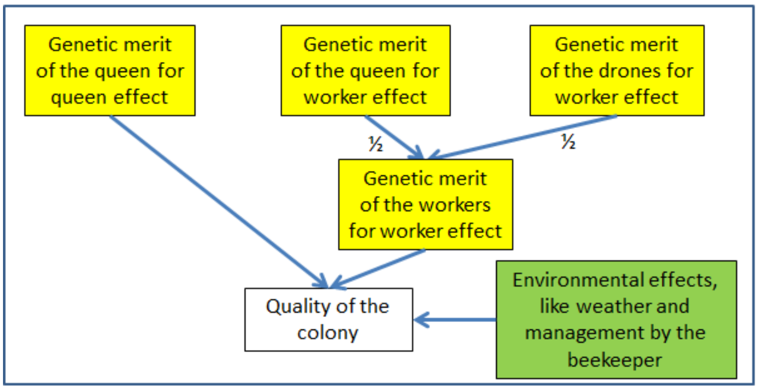


Figure 5. Effects of genetics and environment on the quality of a colony

between test locations[1]. Currently there is only one programme where the required statistical analyses are done: The Beebreed programme. This is discussed in part III.

d. Factors affecting the size of genetic improvement

In this paragraph I broadly discuss the factors affecting the size of genetic improvement, i.e. the speed with which the population mean changes genetically in the course of time of the breeding program. Figure 6 summarizes these factors. The triangle is called "delta" and is the symbol for change. The top of the figure gives two formulae both useful to predict the annual genetic improvement, ΔG. The first formula relates to the situation where the average estimated breeding value of selected parents (queens and drone-producing queens) is known. The expected annual genetic improvement is proportional to this average estimated breeding value (as a deviation of the mean estimated breeding value of all animals in a particular year). Furthermore the predicted annual genetic improvement is larger when the average generation interval of parents is lower, i.e. when a new generation replaces the previous one faster. The formula can also be expressed in another way and is given to the right of the first formula. The average breeding value of parents equals the product of the accuracy with which the breeding values are estimated, the selection pressure (expressed as selection intensity[2]) and the size of the genetic variation (the standard deviation of true breeding values). The accuracy is higher when the heritability of a trait is higher and also when a trait is measured repeatedly. When, for example, the

$$\Delta G = \frac{\text{breeding value parents}}{\text{generation interval}} = \frac{\text{accuracy} \cdot \text{intensity} \cdot \text{variation}}{\text{generation interval}}$$

ΔG	Annual genetic improvement
Accuracy	Heritabilities Repeated measurements Observations on full sister colonies (and other relatives) Relation with traits in breeding goal
Intensity	Selection of the top x%
Variation	Genetic variation (standard deviation) traits in breeding goal
Generation Interval	Age of parents at birth of next generation

Figure 6. Factors affecting annual genetic improvement

[1] A bit more detail which goes beyond the purpose of this course. When estimating breeding values in honey bees the breeding value of interest is that of a future unfertilized queen. This estimate equals the estimated breeding value of the colony she was reared from. The true breeding value of such a queen will differ from the estimate of the colony for a variety of reasons, for example because she descends from a particular drone and not from an average one. But the best estimate is that of the colony's. Furthermore, the breeding value of interest is the sum of the estimated breeding value for worker effect of the colony plus that for queen effect of the colony. Currently I'm analysing Austrian data of Biene Österreich (Austrian Bee) to estimate genetic parameters (heritabilities and genetic correlations) but also to estimate breeding values. In the statistical model used, apart from the mean, there is only one so called fixed effect. This is the effect of the test location where a set of colonies is tested. The test location is a combination of year, breeder and location within breeder and therefore includes effects of region (mountains or valleys for example), climatic effects and managerial differences between breeders. Furthermore the model includes the effect of workers and the effect of queens. The pedigree needed to connect all individuals and to disentangle genetic and environmental differences between test locations includes all colonies (groups of workers), all queens (called dams) and all sires (groups of drone-producing queens), and –if present- their parents.

[2] Technically: The selection intensity is a function of the proportion selected. If selection concerns the individuals above a certain truncation point in a normal distribution the selection intensity is the mean value of selected individuals in a normal distribution with a standard deviation of one.

mite fall in spring is counted three times instead of once, the accuracy of estimation will be higher. Furthermore the accuracy with which breeding values are estimated increases when not only information on the colony itself is considered, but also observations on colonies which are genetically related (queens and/or drone-producing queens share common ancestors). In other words, when not only the colony itself is considered, but also sister colonies, the parental colonies and colonies more distantly removed. Also, but that is often ignored, the accuracy is influenced by the degree to which the trait which is measured is informative on the trait in the breeding goal. As an example, when the breeding goal trait is life-time honey yield, the accuracy is higher when not only honey yield in the first production year is taken into account but also that in the second.

The formula for ΔG is a very powerful tool to think about the optimal design of a breeding programme. A certain measure often works out positively for one factor, but negatively for another. Again the example above, when honey yield is measured in two production years, but as a consequence of that queens are not selected after the first but after the second production year, the generation interval will increase. Whether it is advantageous for ΔG to measure in two instead of one production years depends on the balance between the increase in accuracy and the increase in generation interval. Also, when measuring two years, a number of queens no longer will be a candidate for selection (because they are dead) such that also the selection intensity is affected (that is: affected downward).

In order to make optimal choices one has to take into account all plusses and minuses, not only for one trait, but also for all traits in the breeding goal simultaneously.

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Part II: Selection for Varroa tolerance

Introduction

Since the eighties of the previous century honey bees worldwide are threatened by the Varroa mite. The mite lives in a rather peaceful host-parasite-relationship with A. cerana where the mite predominantly occurs in drone brood. A number of years ago the parasite migrated to A. mellifera. This species (until now) is not equipped to live together with the mite. The mite parasites the brood and weakens it, leading to weaker bees. Additionally Varroa infestation increases the risk of infection of the bees by viruses, both because mites transmit various viruses but also because the mite damages the skin of the pupae which facilitates infection.

The most frequently used instrument to promote co-existence of the honey bee with the mite is the use of chemicals like oxalic and formic accids. It is tempting, however, to promote resistance (or tolerance) by genetic selection. The term tolerance implies that the infestation (in this case with the Varroa mite) isn't decreased as such, but that the bee can live with it. Resistance implies that the infestation is actively decreased. In practice the difference is not very clear realizing that many ways to work genetically on Varroa lead to a lower infestation (therefore resistance?) while on the other hand the level of infestation may not become zero (therefore better to speak of tolerance?). I'm using the term tolerance not bothering about the difference between tolerance and resistance.

In part I I introduced as trait in the breeding goal the quality of workers to identify and remove infested brood, plus a property of the brood slowing down mite reproduction, plus grooming behaviour of workers reducing the entry of mites into a brood cell, etcetera. One might even consider a broader definition by choosing as breeding goal trait the ability of colonies to keep the infestation on a level low enough to survive the winter without (chemical) treatment. Related to the definition one has to decide what to measure, not too difficult to implement, but informative.

Measuring Varroa infestation

In essence there are two ways to measure the level of infestation with Varroa. Both methods involve counting: counting the fall of dead Varroa mites on the drawer and counting the mites present on bees, respectively. Counting of mites present on bees can be done by freezing a sample of bees (or after killing in alcohol), then after washing with soapy water rinsed over a double honey sieve. The bees are checked by the upper sieve and the mites on the lower one. Another method involves shaking of a sample of bees with dry icing sugar. The bees start to groom which removes mites from the bees. Shaking bees and sugar over a sieve will separate bees and mites. The advantage of this latter method is that the bees can be returned to their colony.

Selection for Varroa tolerance

Growth of the mite population

One method to select for Varroa tolerance is the measurement of the growth of the mite population. The idea is that a low growth is preferred. Apparently the colony controls the mite population. A simple way is counting falling mites on the drawer down in the hive. I discuss two ways to use this information. The first way is used by DDB (a group of collaborating beekeepers in The Netherlands called De Duurzame Bij –the sustainable bee). In this situation the mite fall is counted frequently and the growth of the mite population is related directly to the number of falling mites. The development of mite fall can be studied graphically but also by treating the data algebraically. Mr Henk Kok achieves this studying the cumulative number of fallen mites (c). This parameter c exhibits a so called exponential development. By a mathematical trick (taking the natural log) the following equation emerges:

$$ln(c) = \mathrm{a} + b \cdot t$$

The advantage of this equation (a function of t, time) is that it is a straight line. In this equation $ln(c)$ is the natural log of the cumulative number of fallen mites, a is a constant (which is different for each colony) and t is time (week number, for example). Henk Kok especially is interested in the parameter b, describing the speed with which $ln(c)$ increases per unit of time. The smaller b the better the colony.

Also in the framework of Beebreed a parameter for the growth of the mite population is used. To estimate the growth of the mite population measurements are done in two different periods approximately 15 weeks apart. One measurement is done around the first flowering of willows in spring and the second early July. In the first period during three weeks the mite fall on the drawer is counted weekly and in the second period the number of mites on bees is counted in about 30 grams of bees. These counts are included in a parameter for the growth of the mite population which looks like

$$\frac{the\ number\ of\ mites\ in\ summer\ per\ 10\ grams\ of\ bees}{the\ number\ of\ mites\ fallen\ per\ day\ in\ spring}$$

In reality the parameter[3] looks a bit more complicated to avoid that a division by zero occurs and that the log has to be taken of zero (both being impossible). The log is taken to promote that the parameter is distributed more symmetrically. In fact the parameter equals:

$$\log(1 + 10.\frac{1 + the\ number\ of\ mites\ in\ summer\ per\ 10\ grams\ of\ bees}{1 + the\ number\ of\ mites\ fallen\ per\ day\ in\ spring})$$

The bee institute of the US Ministry of Agriculture in Baton Rouge for many years has been running a research programme to select for Varroa tolerance. In this programme growth of the mite population was measured during about 10 weeks. This was done in artificially created colonies without brood, with a queen and about 8000 workers from a large cage with workers infested with Varroa (Harbo

[3] Abschlussbericht über die wissenschaftliche Betreuung bei der Durchführung des Forschungs- und Entwicklungsvorhabens. Einsatz und Erprobung von Prüfparametern zur Selektion einer varroatoleranten Honigbiene in der Praxis – 03UM008 -2007.

and Harris, 1999)[4]. In this situation after a short period the colonies only differ with respect to queen and brood while later on also the workers differ. Selection was based on the number of mites after 10 weeks divided by the initial number of mites. During these 10 weeks the mites have about 3.7 cycles of reproduction. In one period during the cycle the mites are in the brood (about 12.5 days) and in the other period on the workers (about 6.7 days). The latter period turns out to be far more variable than the first. Harbo and Harris describe how the growth of the mite population can be subdivided/described by the increase when in the brood, the decrease of the number of mites when on workers and the number of cycles of reproduction. The authors suggest that the variation in the growth of the mite population in the beginning of the 10-week period primarily is caused by the brood (which must be so because workers and mites are similar across colonies), while later on it is caused by the mites, influenced by the workers. Additionally they studied in different parts of the 10 weeks the suppressed reproduction of the mites.

Suppressed reproduction of mites

Another way to approach Varroa tolerance is to look at suppressed mite reproduction. Suppressed mite reproduction is expressed by a high frequency of infested cells that contain an adult female mite (mother) without young. In the research of Harbo and Harris (who initially selected for slow growth of the mite population) it appeared after a number of years that in fact selection had been for suppressed mite reproduction. They concluded from their research that the suppressed reproduction in the later phases of the 10-week period offered the better perspectives for selection. In retrospect the consequence of this choice for selection work is that the trait is primarily **a property of workers** and not of brood. Suppressed mite reproduction also appears in the Gotland-experiment[5]. In that experiment colonies were moved to the Swedish island of Gotland. On the island no treatment against Varroa was applied. After a number of years a limited number of colonies had survived that, apparently, were able to deal with Varroa. Research of Behrens et al.[6] suggests that this points at **a property of the brood**. These authors reared F1-queens from Gotland queens (through the mating of the Gotland queens with non-Gotland drones). These F1-queens on the one hand produce drones with predominantly Gotland-genes and on the other hand drones with predominantly other genes (and mixtures of course). As a matter of fact, looking at only one gene in the F1 with a Gotland-allele and a non-Gotland allele there are only two types of drones: 100% Gotland and 100% non-Gotland. Behrens' suggests that drone brood predominantly appears in two types with in the one type many mites with suppressed reproduction. Because both types of drone brood appear in the same colonies this difference must be due to the brood and cannot be due to the workers.

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[4] Harbo J.R., Harris J.W. (1999) Selecting honey bees for resistance to *Varroa jacobsoni*, Apidologie 30: 183–196.

[5] Fries, I., A. Imdorf, and P. Rosenkranz. 2006. Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. Apidologie 37:564–570.

[6] Behrens D, Qiang Huang, Geßner C, Rosenkranz P, Frey E, Locke B, Moritz RFA, Kraus FB. Three QTL in the honey bee *Apis mellifera L.* suppress reproduction of the parasitic mite *Varroa destructor.* Ecology and Evolution 1, 2011: 451-458.

Hygienic behaviour: general hygienic behaviour and Varroa Sensitive Hygiene (VSH)

To test general hygienic behaviour pupae are killed in their cells and afterwards it is observed whether workers have opened the cells and removed the brood. Killing can be done by a needle (pin test) but also by liquid nitrogen. In the context of Beebreed the pin test is used.

In the research earlier referred to on selection on slow growth of the mite population, selection appeared to have been for Suppressed Mite Reproduction (SMR) and after still a number of generations it in fact turned out to be Varroa Sensitive Hygiene (VSH). It turned out that colonies with VSH-workers are particularly equipped to detect cells with reproducing mites and their removal. Cells with non-reproducing mites are also detected and removed but in a far lower degree. This phenomenon is illustrated by Table 1 (Harris, Danka and Villa, 2012[7]). The table gives the results of an experiment in which during 10 weeks Varro-infested brood is placed in colonies either selected for VSH or not selected for VSH (non-VSH). In the case of non-VSH after 10 weeks 6.7% of cells was infested with reproducing mites, while in VSH-colonies this was 0.7%. Because in both types of colonies similar infested brood was supplied it can be concluded that VSH-workers detect and remove infested cells to a high degree. In case of cells with non-reproducing mites the difference is a lot smaller. As a consequence in VSH colonies far more SMR is observed than in non-VSH colonies. In case of non-VSH the ratio reproducing: non-reproducing is 6.7:1.4≈5:1, while in case of VSH this is 0.7:0.5≈1:1.

	VSH	non-VSH
reproducing mites (%)	0.7	6.7
non-reproducing mites (%)	0.5	1.4

Table 1. Effect of VSH on detection and removal of cells with mites with and without progeny

The American researchers assume that the difference between VSH and non-VSH finds its basis in only a few genes. In fact they assume the presence of two genes in essence doing the job. Currently also in Europe at a number of locations research is going onto verify this finding.

Selection

Selection for Varroa tolerance can be done in a number of ways. On the basis of the discussion above I mention three ways:

1. Select for slow growth of the mite population using measurements as in DDB.
2. Select for a combination of slow growth of the mite population and pin test (the Varroa-index). This is currently used by Beebreed.
3. Select for VSH.

In the case of Beebreed it should be noted that there is a specific programme which functions within Beebreed with a focus on Varroa. This is the AGT-programme (Arbeitsgemeinschaft Toleranzzucht [Working Group Tolerance Breeding], http://www.toleranzzucht.de/). The AGT-programme puts special emphasis on Varroa tolerance. This is not only done by selection on the Varroa-index, but additionally by using mating stations specific for AGT where drone-producing colonies should survive the winter without treatment before being used for reproduction. On the mating island Norderney

[7] Harris HW, Danka RG en Villa JD. Changes in Infestation, cell cap condition, and reproductive status of *Varroa destructor* (Mesostigmata: Varroidae) in brood exposed to honey Bees with *Varroa* Sensitive Hygiene (2012), Annals Enomol Soc America105: 512-518

annually drone-producing colonies are available from the AGT-programme for use by any breeder who is interested in the material.

With respect to VSH currently at the research institute in Kirchhain queens are reared from AGT-colonies and tested for VSH. If this effort is successful and selection will have been carried out for a couple of generations it may be that VSH-drone-producing-queens will become available on Norderney.

During this course Bart-Jan Fernhout talks about a selection programme in the context of Arista Bee Research (http://aristabeeresearch.org/). This programme focuses on VSH, in first instance with Buckfast[8]. I don't discuss this here.

[8] Two participants in the Dutch branch of Beebreed, Tieme Wanders and Bart Barten, this year similarly work on VSH using Beebreed stock

Translation of the contribution to the course Specialist/Teacher Queen Rearing 2014-2015

E.W. Brascamp

Part III: Beebreed

Structure of the bee population

Strictly speaking Beebreed is a programme engaged in the estimation of breeding values of queens. Annually in February breeding values are published on the internet (www.beebreed.eu). These breeding values are used on a large scale in a number of breeding programmes with Carnica, in particular in various German states, and can be seen as a combination of nucleus breeding and multiplication, with annually some 6000 new queens and their colonies. The drone side on the one hand is covered by AI and on the other hand by mating stations on islands but also on the main land. The mating stations are assumed to be isolated such that fertilization by drones of desired pedigree

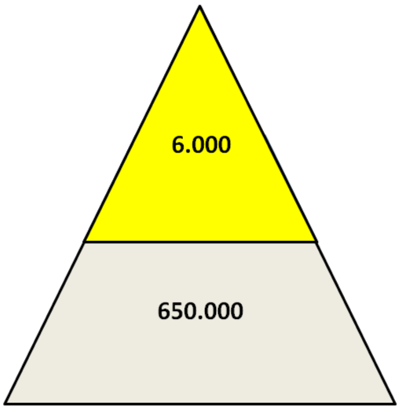

Figure 7. The number of colonies in the German production pyramid.

colonies	5689			
stations	124			mean no of drones
sires	116	colonies	mean	
AI	62	2023	33	2.8
island	12	2032	169	13.5
land	39	1564	40	16.3
AGT+	3	70	23	3.0

Table 2. Characteristics of the German nucleus and multiplication (Carnica) on the basis of the database of Beebreed (sires of colonies born in 2012). For details see text

is guaranteed. In particular for mating stations on the main land this issue deserves permanent attention. Figure 7 gives the production pyramid in Germany. Table 2 provides details about nucleus-mutiplication. The numbers are based upon counts in the Beebreed data base (www.beebreed.eu), in which also an overview is available of drone-producing queens used. The numbers refer to matings which took place in 2012. It concerns 5.689 colonies resulting from matings on 124 mating stations including AI. The last line (AGT+) refers to AGT-mating stations (not Norderney) where, in contrast to the common situation, sometimes drone-producing queens are no full sisters. At the 124 mating stations there were 116 different sets of ancestors (or if you like 116 different dams of drone-producing queens). In a limited number of cases the same dam occurs at different mating stations in particular at different stations with AI or on an island and AI. The heading "colonies" refers to the number of colonies and the heading "mean" refers to the average number of colonies. When fertilization is by AI, the number of resulting colonies in average was 33, while at island the number was 192. The heading "mean no drones" refers to the average number of drone-producing queens at a mating station. For AI this usually is 3. At islands the variation is very large. The average is 13.5, but at Norderney there were 35 drone-producing colonies and at Helgoland only 3.

The ratio between nucleus+multiplication and the number of colonies of common beekeepers is about 1:100. You may therefore say that the activities of the breeders in nucleus+multiplication have a hundred-fold effect on the bee population as a whole. Sometimes very directly (due to material obtained by beekeepers from nucleus-multiplication) but also indirectly because gradually the drones used for non-controlled mating will genetically follow the trend in nucleus+multiplication.

Apart from Germany, Beebreed is also used in Switzerland and Austria (the Austrian Carnica Association, ACA), respectively with 10 mating stations and 758 colonies and 26 mating stations and 1238 colonies. Also at a far more limited extend in Belgium and The Netherlands.

Selection decisions

Which queen to be selected for the rearing of drone-producing queens on islands generally is decided by the leadership of breeding programmes in the different German states. For AI decision making is more diverse. In the German programmes drone-producing queens at a particular mating station descend from one queen, so are full sisters. In that way the pedigree of drones used for fertilization is more uniform and breeding value estimation is easier[9]. The fact that the drone-producing queens are full sisters can be used to estimate selection pressure. Annually about 115 queens with colonies are selected for the rearing of drone-producing queens. These 115 queens are selected from about 6000 colonies which would make the selection pressure about 1:50, potentially a selection of the best 2%. Looking at it this way is overstating the case because these 6000 colonies in fact are for example 600 groups of 10 full-sister colonies, which means a selection of 115:600=1:5, but this in turn is too pessimistic. The truth is in the middle.

Selection decisions on the dam side are completely in the hands of individual bee breeders. The bee breeder decides from which colonies young queens are reared. In principle this selection can take place within the colonies owned by the breeder himself, but obviously also colonies of other breeders are at disposal.

Apart from who decides it is of course of utmost importance for what criterion selection takes place. In part I I discussed that in principle selection should be aimed at the breeding goal and the purpose of breeding-value estimation is to identify colonies (young queens from that colony) with suitable breeding values in relation to the breeding goal.

Breeding goal

Within Beebreed the breeding goal contains 5 traits which are given in Table 3.

Table 3. Traits in the breeding goal of Beebreed and their relative weight

	honey yield	defensive behaviour	calmness during inspection	swarming drive	varroa-index
weight (%)	15	15	15	15	40

The breeding goal includes honey yield, three behavioural traits and the Varroa-index. The Varroa-index combines the growth of the mite population (according to the formula given in part II) and results of the pin test. To understand the weighing of the traits it is important to realize that

[9] In fact, for the current Beebreed algorithm common ancestry of drone-producing queens is a prerequisite. If that is not the case (as sometimes in AGT) the male-side of the pedigree is ignored.

Beebreed standardizes the standard deviation of the breeding values. All five traits get the same standard deviation. In units of standard deviation the first four traits receive the same weight (15%) while the weight of the fifth trait (the Varroa-index) is about 2½ times as large. Individual breeders, however, can choose other weights, when desired.
It was discussed in part I that traits in the breeding goal need not to be the same as those observed. From the examples given there that seems quite logical. Nevertheless, in Beebreed the traits observed and the traits in the breeding goal are the same.

Estimation of breeding values
The estimation of breeding values in Beebreed follows Figure 5 in part I. That is to say that the observation on a colony is taken to be affected by the breeding value of the queen for a trait (as a consequence of egg-laying capacity etc) and also by the breeding value of workers (as a consequence of behaviour, flying capacity, etc). Both breeding values are estimated separately for each queen. The published breeding value is the sum of both[10].

Table 4. Estimated breeding value of queen 17 27 28 2011, as published February 2015

	weight	breeding value	accuracy
honey yield	15	125	0.44
defensive	15	125	0.66
calmness	15	124	0.68
swarming	15	119	0.44
Varroa-index	40	129	0.55
Total index		133	
chalk brood		104	

Table 4 gives the estimated breeding value of a particular queen (17 27 28 2011[11]) as can be found on website of Beebreed. These breeding values have a standard deviation of 10. Furthermore the breeding values have a standardized mean of 100, the average of all breeding values of queens born during the last five years.
The queen in the table certainly has high breeding values. For all five traits the breeding value is far above average, often more than 2 standard deviations. The table also gives the total index, which results from weighing the individual traits with the weights given in the table 3[12]. The last column provides the accuracies with which the breeding values are estimated. Theoretically these accuracies[13] are numbers between 0 (the estimated breeding value has no predictive value at all with respect to the quality of progeny), and 1 (perfect predictive value as to breeding value, not with respect to environment of course). The differences in accuracy between traits is caused by differences in the level of heritability. Recently also a breeding value for chalk brood is published, but only a limited number of breeders scores this trait.

[10] See note 1.
[11] To be sure, this refers to the breeding value of a young queen reared from queen 17 27 28 2011.
[12] It is often asked how the total index can be higher than one of the components. This is caused by the fat that multiplication of the breeding values of all queens in the data base with the weighing facts gives breeding values with an average of 100 (for the last 5 years) but a standard deviation lower than 10. Beebreed upscales the standard deviation to ten such that values above 100 become bigger and below 100 smaller.
[13] I think that these accuracies refer to those for the estimated breeding value of the colony, not of a future queen reared from the colony. That accuracy is about 40% lower.

The estimation of breeding values takes place using a so called multi-trait animal model[14]. These words imply that the breeding values for all traits are estimated taking into account their mutual genetic relationships. Furthermore that all animals (queens in our case, their colonies and the sets of drone-producing queens) are connected by a pedigree. For a reliable analysis it is important that there are various colonies on one test location because one of the basic elements of the analysis is the comparison of colonies *within* test location. Within a test location the environmental effects influencing the performance of the colonies are assumed to be the same. To compare colonies *between* test locations the pedigree is used. As an example: In the most simple case there is a second test location with colonies with a pedigree as those on the first. In that case the differences between both test locations are taken to be completely non-genetic (because the pedigrees of their colonies are the same) but purely environmental. In reality the colonies at the two locations will have different pedigrees but they nevertheless will be related because they share dams or sires in earlier parental generations. The analysis then estimates the degree to which the difference between the two test locations is genetic and environmental. Clearly the analysis becomes easier (and more reliable) if there are colonies with different pedigrees on a test location (for example 3 of one and 3 of another) while on the other hand each pedigree also is represented with a number of colonies on another test location. Such "rings" are advocated by Beebreed. In other words, the pedigree is used to disentangle genetic and environmental effects on the test locations. The pedigree is also used to base the estimation of breeding values not only on the observation of a colony itself, but on the observations on family colonies. Obviously closely related colonies have more to offer than distant relatives which in the analysis translates to the genetic relationships between colonies, following from the pedigree.

Traits measured

The five traits in Beebreed are measured or judged by the breeders as follows. Honey yield is measured in kg. Defensive behaviour, calmness and swarming drive is scored a number of times subjectively on a scale of 1-4. For the first two traits the average score is taken while for swarming drive the lowest score is taken. Details about the measurement of Varroa-traits were discussed in part II. Further details about this and how to measure other traits can be found in the Methodenhandbuch[15] van AGT.

Genetic improvement

In 2013 Professor Kaspar Bienefeld (director of the bee institute in Hohen-Neuendorf and responsible for the estimation of breeding values in Beebreed) published estimates[16] of the genetic improvement for honey yield, calmness and Varroa-index. The results for Varroa-index are given in Figure 8

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[14] Noticeable Success in Honey Bee Selection After the Introduction of Genetic Evaluation Using BLUP. Kaspar Bienefeld, Klaus Ehrhardt, Fritz Reinhardt, translated by Kirsten S. Traynor. American Bee Journal, August 2008, 739-741

[15] http://www.toleranzzucht.de/fileadmin/websitedateien/pdf/Methodenhandbuch_2._Auflage_2013-03-13.pdf. Een Nederlandse bewerking staat op www.beebreed.nl, Marjan Brouwer, Rein Kakes en Tieme Wanders, 2008, en aangevuld 2013.

[16] Bienefeld K, 2013. Von der Theorie in die Praxis. Deutsches Bienen-Journal, 8: 20-21

(Bienefeld, 2013). The figure shows that between 1990 and 2004 the annual genetic improvement was 0.18 units per year. In the 10 years after 2004 this was 0.72 units per year. The units relate to the standard deviation of 10 units. With an improvement of 1 unit per year in 10 years time the population mean would increase with one stand deviation unit. The reason to split the series of points in two periods (until 2004 and thereafter) is that about in 2004 breeding value estimation shifted to the method currently in use. In table 5 I summarize the results of Bienefeld for all three traits concerned.

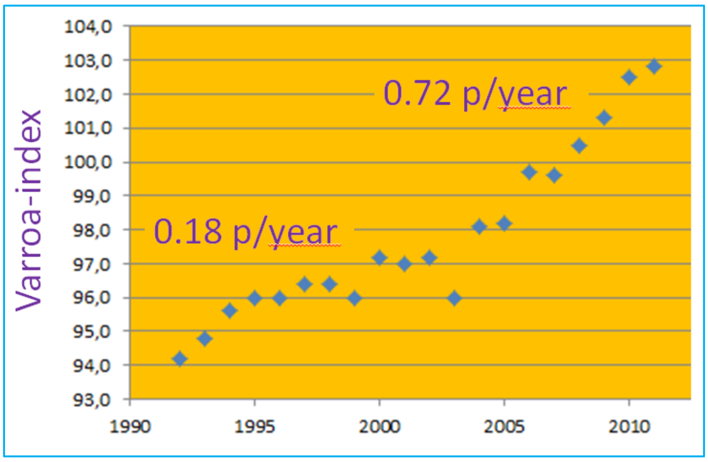

Figure 8. The genetic improvement for Varoa-index between 1991 and 2011 for the Carnica population in Beebreed (re Bienefeld, 2013, see footnote 16)

Table 5. Genetic improvement in points breeding value per year for honey yield, calmness and Varroa-index (Bienefeld, 2013, see footnote 16)

Trait	1972-1990	1993[1]-2012
Honey yield	0.05	0.65
Calmness	0.01	0.44
	1993-2004	2005[1]-2012
Varroa-index	0.18	0.72

[1]Start of the estimation of breeding values. The start differs between Varro-index on the one hand and the other traits on the other.

Summary

1. Genetic improvement in farm animals has a firm theoretical basis which utilizes principles of genetics. These principles equally hold for honey bees. Some adjustments need to be made, however, due to the reproductive system of honey bees. In particular the fact that groups of workers (and queens) derive from the same drone causes differences.
2. Important elements in a breeding programme are the breeding goal (where to go for), the traits observed (observations to be used to estimated breeding values) and estimated breeding values (upon which selection can be based).
3. The annual genetic improvement is a function of the selection pressure, the accuracy with which breeding values are estimated, the variation of breeding-goal traits and the generation interval.

4. In honey bees traits (measured on the level of a colony) are not only affected by the breeding value of workers, but also by that of the queen. And of course by the environment.
5. To measure the development of Varroa infestation it is useful to count the fall of mites on the drawer on certain moments.
6. There is a discussion about the best way to measure Varroa tolerance from the viewpoint of selection. Of interest are the developments with respect to Varroa Sensitive Hygiene (VSH). If expectations come out this trait should be included in breeding programmes.
7. Beebreed is a system of estimation of breeding values. The estimated values are used by associations in various states of Germany to select the colonies from which drone-producing queens are selected to be used for AI, or to be placed on mating stations. The estimates of breeding values are based upon observations on annually about 6000 colonies in Germany and considerable numbers in Switzerland and Austria. Also in Belgium and The Netherlands there is growing interest in Beebreed. Breeding values of course are not only useful to get high quality drone-producing queens but also for individual breeders to select their colonies for further breeding.