Plan Bee Breeding Manual

by Nadine Chapman
and Elizabeth Frost
August 2021

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AgriFutures® Plan Bee

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Glossary

Apiary: A collection of bee hives.

Apiary site: The site that the bee hives are located.

BLUP: Best Linear Unbiased Prediction, the statistical method used to calculate Estimated Breeding Values.

Breeding objective: The goal of the breeding program – the traits to be selected for and the desired direction of selection.

Breeder queen: A queen chosen to produce the next generation, either to produce queens or drones.

Drone mother: A superior queen chosen to produce drones.

EBV: Estimated Breeding Value, an estimate of the genetic potential of an animal relative to the population average.

Genotype: All the genes in an organism.

Heritability: The proportion of variance that is due to genetics rather than environment.

Load: A collection of bee hives that are transported together and managed together in the same apiary.

Measurement error: How accurately you can measure a trait.

Phenotype: The observable or measurable traits of an organism.

Production queen: Queen for general use by beekeepers.

Qualitative measure: A descriptive measure of a trait.

Quantitative measure: The exact quantity of a trait is measured.

Queen breeder: A beekeeper that produces queens that have been selected for traits.

Queen ID and pedigree file: A Google Sheets (free with a Gmail account) or Microsoft Excel file for recording information about queen IDs, pedigrees, and the colonies the queens are in.

Queen mother: A superior queen chosen to produce queens.

Queen producer: A beekeeper who producers queens, but does not perform selection on them through recording of traits.

Repeatability: How closely measures of the trait agree with each other across time.

Reproducibility: How similarly two people would score a trait.

Trait recording file: A Google Sheets (free with a Gmail account) or Microsoft Excel file for recording your breeding objectives and data you have collected during colony evaluations on the traits you are interested in.

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Chapter 1: Introduction

Overview of this document

Chapters 1 to 7 provide an overview of the Plan Bee program, background to the techniques used in the program, how they will improve your business, and the process you will follow to implement the program in your operations. Chapter 8 provides step-by-step instructions for recording your data. Chapter 9 defines all the traits and alternative methods for measuring them.

Plan Bee will support you in your breeding endeavours. You are in control of your breeding objectives: which traits you will record and select for and how often you will collect data.

This manual has been developed in consultation with industry through surveys, interviews, and workshops. It is the culmination of all this feedback. The manual will be a 'living document' in that it will be updated to reflect feedback from those participating in the program and lessons that the project team learns from working with the diversity of queen breeders and beekeepers.

Overview of program management

Plan Bee is governed through both a project management committee and a stakeholder committee. The project management committee (Figure 1) is responsible for project management, governance, communications, and research.

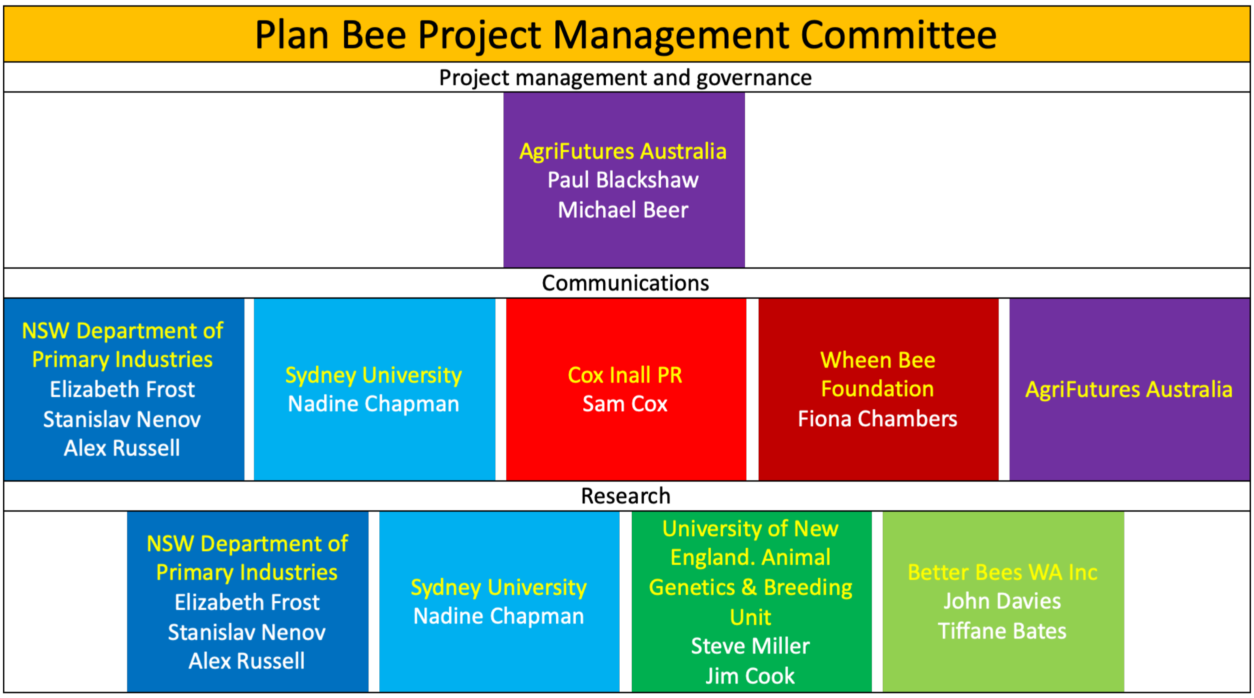


Figure 1. The Plan Bee Project Management Committee.

The Plan Bee Project Management Committee reports to the Stakeholder Committee. The Stakeholder Committee (Figure 2) is responsible for strategic direction and reporting to investors.

Figure 2. The Plan Bee Stakeholder Committee.

Chapter 2: Why the program was implemented

Plan Bee, Australia's national honey bee genetic improvement program, has been initiated to provide the infrastructure necessary to implement modern genetic breeding techniques in the honey bee industry.

The program builds on the considerable expertise in queen breeding in Australia and the progress made in previous and current programs. Plan Bee does not seek to replace these programs, but rather to provide new tools to assist queen breeders in order to speed genetic progress in their own breeding operations.

Without improvements achieved through selective breeding, our agricultural industries would not be where they are today. Humans have been selecting plants and animals for millennia. However, progress has been significantly sped up in the last few decades with new statistical methods and industry-wide programs. Animal industries that do not invest in these new methods typically experience declining profits due to income rising at a slower rate than costs. In contrast, industries that invested in new statistical methods and industry-wide selection programs have experienced booms.

For the first time, we have honey bee pollination-dependent industries involved in an Australian honey bee genetic improvement program. Improvements in honey bee health and productivity will benefit honey bee-dependent horticultural industries by ensuring beekeeping remains a profitable enterprise that can attract and retain beekeepers so our bee population remains strong, and by improving the economic basis of pollination for both the horticultural industries and beekeeping generally. The Australian Government has recognised the importance of the program, and for the first time has provided funding for a honey bee genetic improvement program.

It is time to apply modern breeding techniques to honey bees, as is being done in all major beekeeping countries across the world. Plan Bee seeks to fast-track this in the Australian industry and continue this beyond the life of our current funding from the Australian Government.

Chapter 3: Program goals

Our goal is a sustainable national bee breeding program that meets the needs of industry into the future and that provides a system for evaluating the genetic merit of queens.

We want to assist queen breeders in improving their own stock, if they choose to work with us. Queen breeders will continue to select for the traits that they and their customers are interested in.

In addition, a research population has been established at Tocal College by the NSW Department of Primary Industries (NSW DPI). This population will be used to investigate traits related to pollination, to compare the available methods for measuring traits, and to determine if the expression of traits is genetically linked – that is, if selection on one trait is likely to result in selection for or against another trait. NSW DPI will strategically release limited numbers of artificially inseminated breeder queens to queen multipliers starting from autumn 2022.

Our aim is to build the queen breeding industry by:

- Ensuring beekeepers recognise the importance and value of breeding.
- Driving demand for quality queens.
- Unlocking the potential in the beekeeping industry by producing bees that make more money due to increased production and lower costs of disease management.

Plan Bee aims to provide the following outcomes for our stakeholders:

Queen breeders:

- Pursuit of their own breeding goals; choice over the traits they collect data on and the methods used to collect this data.
- A mechanism to determine the rate of genetic progress.
- A mechanism for breeders to demonstrate the value of their queens through the provision of Estimated Breeding Values and a database of genetic merit.
- The true value of queens is recognised and rewarded.
- Increased demand for genetically superior queens.
- A strong and profitable queen breeding industry.
- An established breeding program that can be adapted to selection for *Varroa* resistance if/when the pest becomes established.

Beekeepers:

- Improved communication of which traits have been selected for and how.
- Evidence of the quality of the queens that they purchase.
- Bees that are more profitable due to increased production and lower costs of disease and pest management.
- An established breeding program that can be adapted to selection for *Varroa* resistance if/when the pest becomes established.
- A strong and profitable beekeeping industry.

Growers:

- Strong beekeeping industry due to improvements in profitability.
- Strong colonies for pollination due to selection for pest and disease resistance.

- Colonies with temperaments that are preferred for pollination i.e. lower risk of aggression.
- An established breeding program that can be adapted to selection for *Varroa* resistance if/when the pest becomes established.

Chapter 4: How breeding works

Phenotype

Bees, like other organisms, vary in observable or measurable traits such as size, weight, colour, aggression, product quantity, and product quality.

These observable or measurable traits form the *phenotype* of the organism. In other words, the phenotype is what you can see or measure to describe the organism. This variation is due to both the *environment* (e.g. feed quality, temperature) and *genetics* (genotype; relatedness established through pedigree or genetic testing).

Genotype

All the genes in an organism form its *genotype*. This information can come through pedigree or genetic testing.

Environment

The environment of a colony includes such things as the beekeeper's management, the load the colony belongs to, the apiary the colony is in, forage opportunities, weather, and the age of the queen.

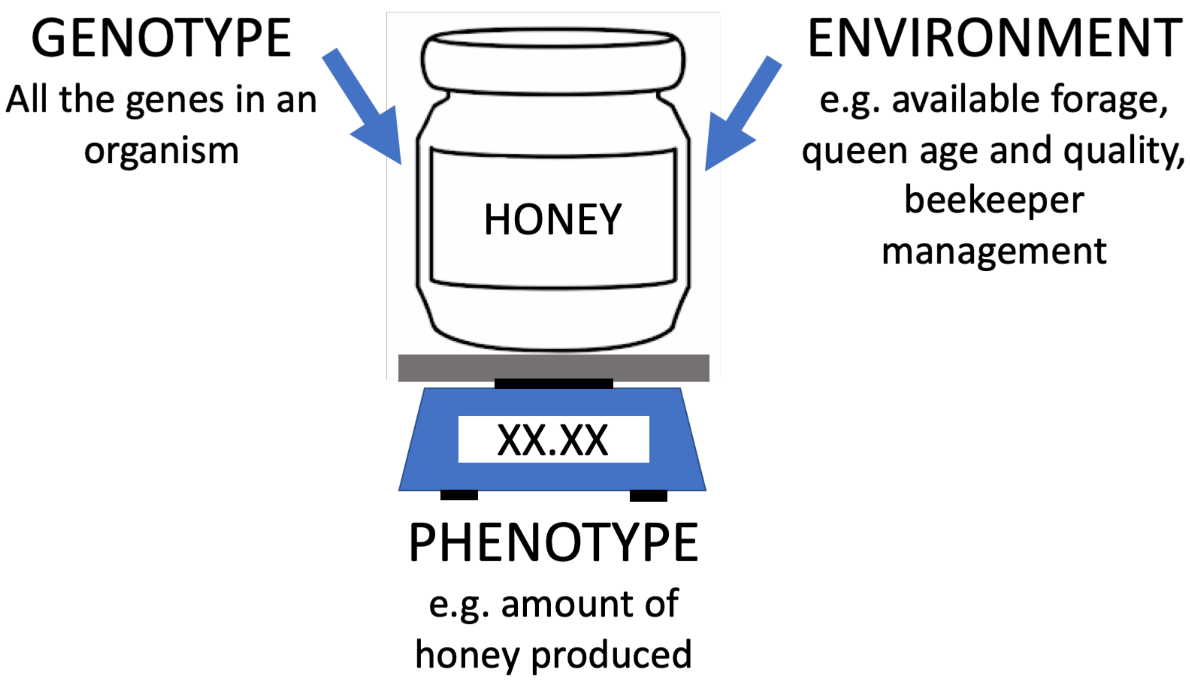


Figure 3. The phenotype of the colony. In this example, the amount of honey produced is influenced by both genetics and the environment.

Heritability

A trait is inherited when a gene from a parent becomes part of the genotype. If there is variation in the genes of the parents, there can be variation in the genotypes of the offspring. However, if all parents have the same gene or genes, there is no variation in the offspring because they will have the same genes, just as the parents have the same genes.

For a trait to be *selectable* there must be *genetic variation* for the trait.

For example, if the queens all have the same genetic makeup but some are bigger than others, the difference must be due to environment. In this case you cannot select for bigger queens because there is no *bigger* gene present. However, if there is a *bigger* gene, then given the same environment, some individuals will be bigger than others. That is, given the same environment, some individuals will perform better because of variations in their DNA (genes or genotype; Figure 4).

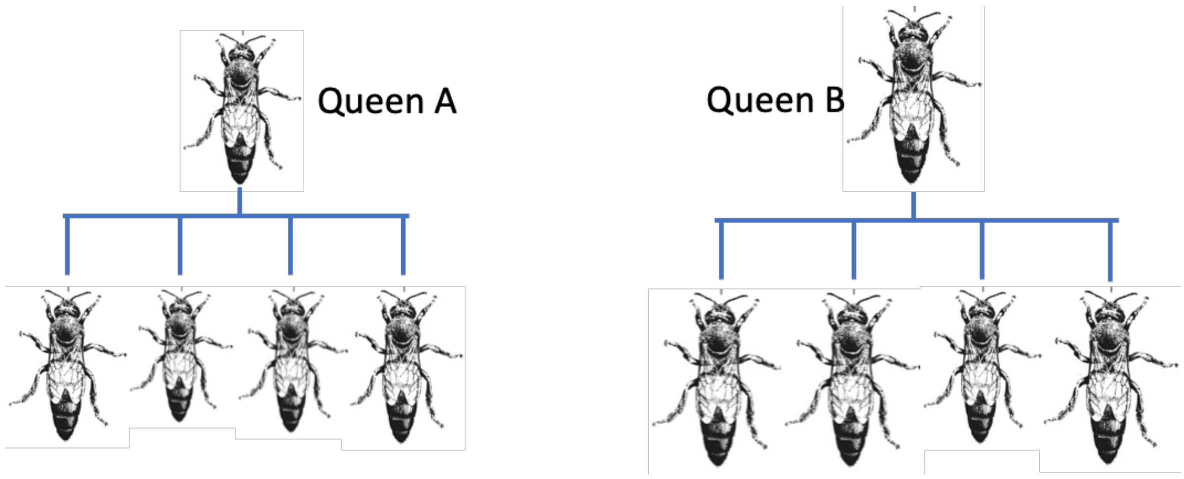


Figure 4. Queen B is bigger than queen A. The offspring of queen B also tend to be bigger than the offspring of queen A. The trait is heritable – some of the variation is due to genetics.

Where this improvement in a trait is due to DNA, it is passed on to offspring (Figure 4). The improved trait is inherited and we say that the trait shows *heritability*.

Heritability is estimated by measuring traits in related and unrelated individuals and determining the association between the values recorded for the trait (the phenotype) and the level of relatedness (the genotype). If heritability is low, it will be difficult to select for the trait because most of the variation is due to the environment.

A heritability of 0.3 indicates that 30 per cent of the phenotypic variation is due to variation in the genes that control the trait. Using the example from Figure 4, 30 per cent of the variation in queen size is due to genetics, with the rest of the variation coming from the environment.

Heritability is not static; it changes with environment and varies between populations. It is also affected by *measurement error*. That is, how accurately you can measure the trait. Therefore, the accuracy of the heritability estimate depends on the ability to measure the trait accurately.

Requirements

The statistical methods used in Plan Bee require evaluation of colonies with approved methods, and information of genetic relationships between the queens that were evaluated either via pedigree records or genetic testing. Breeders already collect some of this information, they just haven't had the tools to make the most of it. Plan Bee and the national database will enable this.

Breeding requires:

- Breeding goals.
- Clearly defined traits and appropriate methods to measure them.
- Accurate records on performance and pedigree.
- Age of the queen.
- Records on a large number of individuals.
- Knowledge of which colonies are kept together.
- Known relatedness between individuals in different apiaries.
- Knowledge of the quality of the colony that the queen inherits, as this will in effect give some queens a 'head start'.
- Management of inbreeding.

Best Linear Unbiased Predictions

Best Linear Unbiased Predictions (BLUPs) are the statistical methods for the production of Estimated Breeding Values (EBVs). These statistics consider the relatedness of the queens. This means that the genetic merit of the queen reflects not just her colony's performance, but also the performance of other queens and colonies that are genetically related. This is because it is expected that related individuals have genes in common and thus should perform similarly.

In addition, the effect of shared environments is considered. There are two types of shared environments:

- Colonies that are kept and managed together in loads or cohorts.
- Apiary sites, which may accommodate multiple loads or cohorts.

The core unit for calculating EBVs is a group of colonies that have been in the *same locations* at the *same time*. These shared environmental effects are accounted for by determining the average for each apiary and load, and adjusting the trait values to account for environmental differences (which are a combination of effects from, for example, weather and food resources).

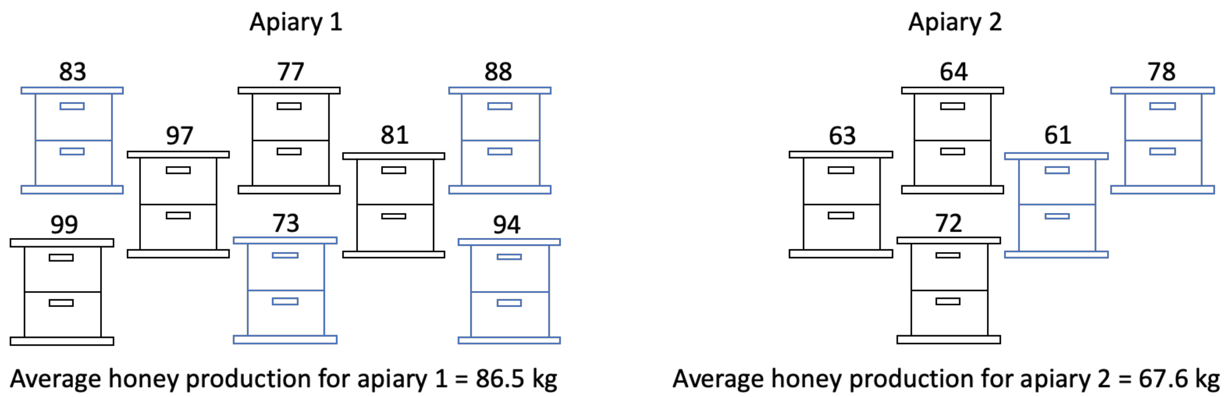


Figure 5. A beekeeper has two apiaries and has two breeder queens. The daughters from the first breeder queen have black colonies. The daughters from the second breeder queen have blue colonies. The beekeeper has placed daughters from each breeder queen at each of their apiaries to control for environmental effects. The average honey production for each apiary is used in calculating Estimated Breeding Values. This is because there is an environmental effect on honey production, with the average Apiary 1 honey production being 18.9 kg higher than that of Apiary 2.

This adjustment requires that the colonies in each load and at each site are related to each other and that there is information on how related they are (Figure 5). The more information that is known about pedigree, the more accurate the EBVs will be. If the colonies are unrelated, then part of the difference in load and apiary averages may be genetic rather than environmental. This too will affect the accuracy of EBVs.

When comparing stock from different queen breeders, it is important to know if these differing stocks are related. The EBV comparisons are more accurate when it is known whether or not the stocks are related. If relationships are unknown, it is difficult to tease out if variation in phenotype is due to genes or the environment (including management styles).

BLUPs account for the accuracy of the information. An apiary average based on a large number of colonies is more accurate than that for an apiary with few colonies. In Figure 5, the average honey production of Apiary 1 (eight colonies) is more accurate than that of Apiary 2 (five colonies). An apiary with 100 colonies would produce a much more accurate estimate than either of these.

In addition, BLUPs and therefore EBVs will be able to account for the age of the queen (particularly important for swarming as one example) and the quality of the colony that a new queen gets introduced to. A queen placed into a colony with a large population of workers will likely perform better than one placed into a small colony. This will not be due to the queen herself, but due to the colony. **Thus, we highly recommend that the number of frames of bees are recorded so that this environmental effect can be taken into consideration when calculating EBVs.**

Estimated Breeding Values

BLUPs produce EBVs for each individual by taking information on phenotype (trait) and genotype (DNA testing or pedigree information) not just for that individual, but for all of its relatives, including relatives from different years (Figure 6).

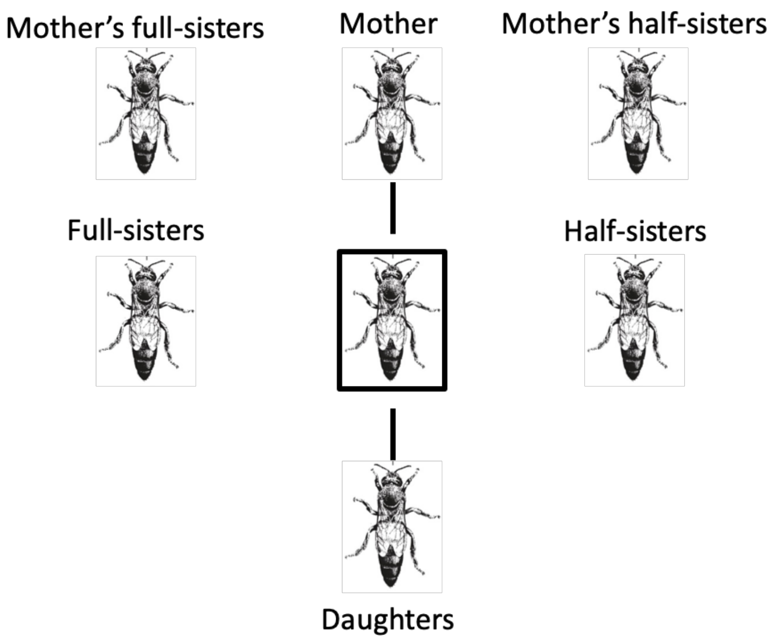


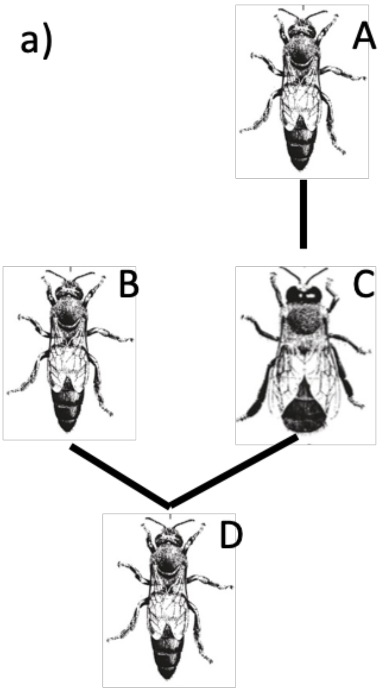
Figure 6. The Estimated Breeding Value (EBV) of the focal individual (in the box) is calculated using information from all of her relatives: mother, aunts, sisters, daughters etc.

An EBV presents how well a colony scored due to the colony's genetics. An EBV of 0.18 for honey means that the colony produced 18 per cent more honey than average due to genetics. An EBV of -0.13 means that the colony produced 13 per cent less honey than average due to genetics. Alternatively, an EBV can be presented as +5 kg, meaning that this colony produced 5 kg more honey than the average.

In Figure 7, queen A's colony produced 14.8 kg more honey than average. If the heritability of honey production in this population is 0.25, then queen A will have an EBV of +3.70 kg. Similarly, queen B's colony produced 20 kg more honey than average, and her EBV is +5.00 kg.

The daughter of queen B (D) inherits half of her genes from her mother, the other half coming from her father. Therefore, the daughter queen is expected to have an EBV that is half that of her mother's, and produce +2.5 kg more honey than average due to the genes that she inherited from her mother (Figure 7).

a)



b)

Individual	Kg honey produced compared to average	EBV
A	+14.8	+3.70
B	+20.0	+5.00

c)

Individual	Calculation	EBV
D (based on A)	+3.70 x 0.5	+1.85
D (based on B)	+5.00 x 0.5	+2.50
D (based on A and B)	$\frac{1.85 + 2.50}{2}$	+2.18

Figure 7. a) Pedigree for individual D. b) The amount of honey colonies headed by queens A and B produce compared to average, and the Estimated Breeding Values (EBVs) for them assuming that honey production has a heritability of 0.25. c) EBVs for queen D calculated using information from different relatives.

The daughter of drone C (D) inherits half of her genes from her father. This half came from her grandmother, queen A. Therefore, queen D's EBV could also be calculated as half of that of queen A: +1.85 kg (Figure 7).

Taking all the available information together, queen D has an EBV that averages the two estimates. That is, her EBV is the average of the EBV calculated based on information from queen A (+1.85 kg) and the EBV calculated based on information from queen B (+2.50 kg) divided by 2. Queen D's colony can be expected to produce 2.18 kg more honey than average due to genetics.

EBVs are calculated using computers due to the large number of related and unrelated individuals included in the databases. The above is a simple example to help you to understand the process. In reality, information about the environment and the number of individuals that have information recorded on them are used by the computer to perform the calculations, which cannot be replicated into a simple example (Figure 8).

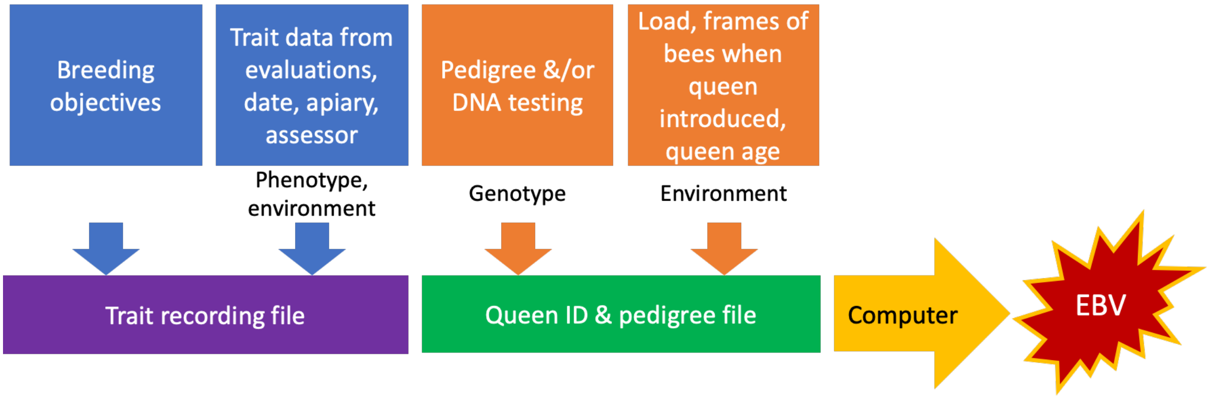


Figure 8. Information about phenotype, genotype, and the environment are put to the Trait recording file and Queen ID and pedigree file, and this information is put into a computer program to calculate Estimated Breeding Values.

Selecting queens to breed from

If only a single trait is selected for, then individuals can simply be ranked by the EBVs for that trait and the best individuals chosen to breed from.

If more than one trait is selected for, then in order to rank candidates for selection it is necessary to combine the values of the traits into a single selection criterion. This selection criterion can be determined by summing the Estimated Breeding Value (EBV) for each trait multiplied by a weighting factor. The weighting factor is the value of that trait to the breeding objective. If all traits are given equal weight, then an average EBV will be generated. For example:

Breeding objective = weight of trait 1 x EBV of trait 1 + weight of trait 2 x EBV of trait 2

It will be up to the queen breeder to determine their breeding objective. Following this, it is their decision which queens they will breed from.

Breeding objectives are discussed further in chapters 6 and 8.

Measuring success

The success of a breeding program is measured as a shift in the average trait values. Conditions vary from year to year, thus there will be variability between years due to environment. However, over longer periods of time there should be measurable change in traits in the direction of selection.

Plan Bee will regularly assess the selection response as part of our monitoring and evaluation plan. These results will be communicated to industry.

Chapter 5: Value proposition

It is estimated that a honey bee genetic improvement program run over a 25-year period will generate gains of more than $41m in honey production using 2021 values, and that this improvement is necessary for the continued economic viability of beekeeping. It is noteworthy that animal industries that do not invest in genetic improvement programs typically experience declining profits due to income rising at a slower rate than costs.

It is essential for the Australian queen bee breeding industry to adopt modern techniques directed toward genetic improvement of honey bees. Australia's honey bee industry supports many other industries through pollination services. Strong, healthy colonies and a strong beekeeping industry are essential to ensuring Australia's food security.

Participation in Plan Bee and using standardised selection will help queen breeders in a number of ways:

- Provide a system for keeping records.
- Provide a means of comparing queens within their own stock, so that they may be sure to select the best queens.
- Over time, provide a means to measure genetic progress: how much impact the efforts are having on the traits selected.
- Provide EBVs, which they may wish to share with customers.
- Knowledge of pedigree to enable both queen breeders and beekeepers to manage genetic diversity within their populations.

BLUP techniques were implemented in Germany in the mid-1990s. From 1970-89, genetic improvement was 0.11% for honey production and 0.01% for gentleness. In comparison, from 2014-2018, genetic improvement was 1.67% for honey production and 1.45% for gentleness, 15 and 145 times the progress, respectively. Traits added between these periods are also experiencing rapid improvement: calmness (1.45%), swarming (1.26%), and a *Varroa*-resistance trait (1.47%). **The same effort went into assessing colonies, but the change in methods used to select queens meant that the work had a greater payoff.** We expect that similar gains can be made in Australia.

Breeding bees is hard and time-consuming work. These efforts must be rewarded if breeders are to continue breeding better bees. The database and EBVs provide a measure of the difference being made, and this will become a valuable tool for bee breeders. EBVs help bee breeders understand and track the value of their stock, and show that their stock is improving year by year.

In addition, Plan Bee will educate beekeepers about the importance of breeding. The costs for implementing the program cannot be borne alone by breeders. Plan Bee will produce a business case to continue the program in perpetuity in a sustainable manner.

Chapter 6: How to implement EBVs into your business

Figure 9 summarises the steps for you to implement the Plan Bee program.

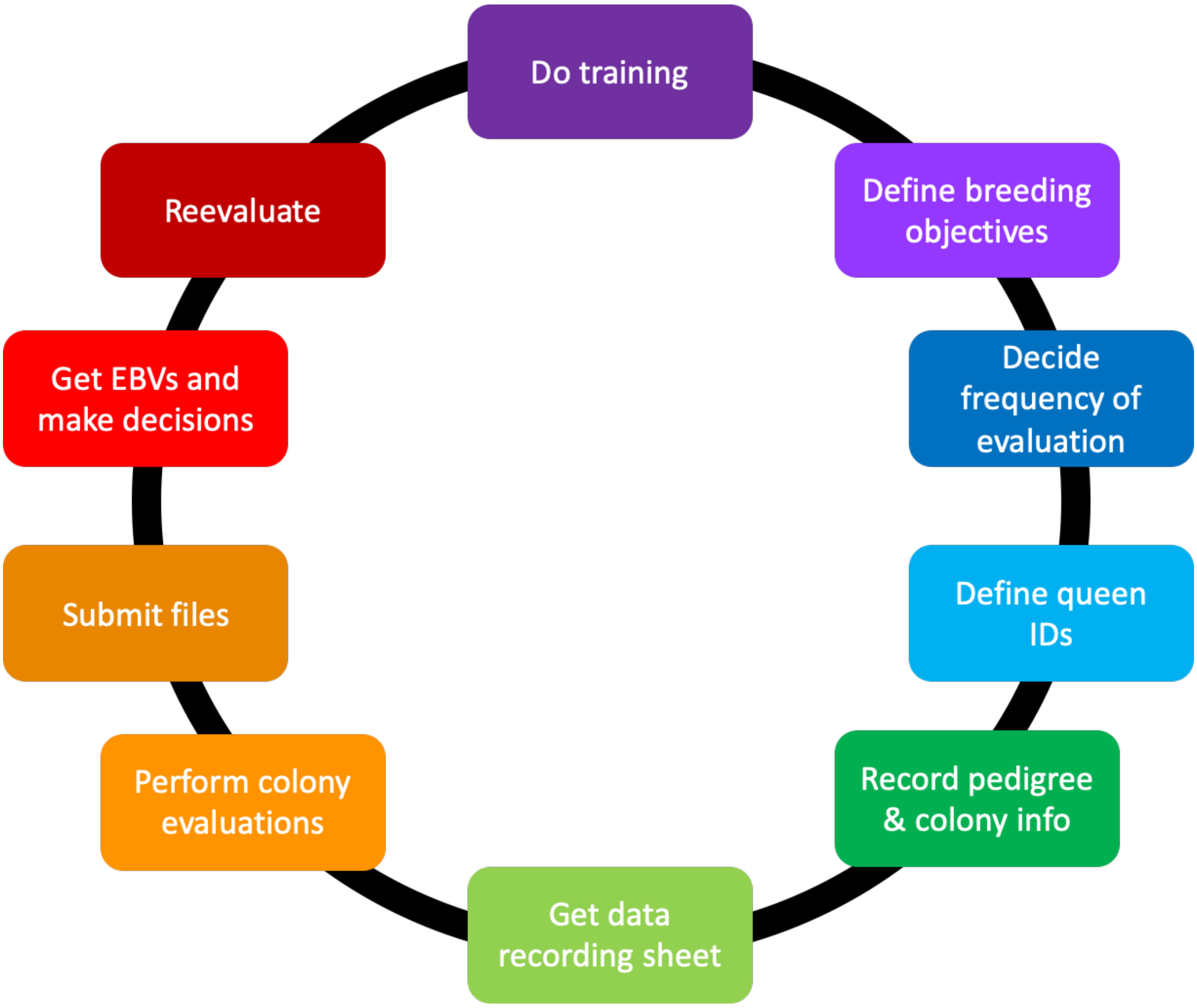


Figure 9. Steps for implementing Estimated Breeding Values into your business through the Plan Bee program.

1. Undertake training

Engage with Plan Bee Breeding Manual training resources (online or face to face) or get in touch with Elizabeth Frost to find out when the next training session will be.

Accurate trait measurements from your colony evaluations are critical to producing accurate EBVs. Preventing measurement error by studying the trait definitions and selection methods in Chapter 9 will increase the accuracy of EBVs and increase the rate of your genetic improvement.

2. Define breeding objectives

Your breeding objective is the traits that you want to select and the direction in which you want to select them. A list of the available traits and the methods available for selecting them can be found in Chapter 9.

Decide what traits you will record data on

Each queen breeder needs to balance the time spent recording traits within the context of their business and the expected outcomes. For example, find the balance between time spent on recording traits and the value added by improved stock to the sale of queens or increase in production.

Computer modelling studies have found that heritability estimates, and therefore EBVs, based on numerous traits are more accurate than those based on single traits. This is likely because some traits are genetically correlated – that is, that a phenotype increase in one trait will increase or decrease the phenotype of another trait. An example of this is colony population and honey production; it is likely that the genes underlying colony population will influence honey production. So, including both traits improves the predictive power of the model.

On the other hand, it is easier to make progress when you select for only a few traits compared to selecting for many traits.

Decide which method you will use to assess each of your chosen traits

Each trait has one or more possible methods for measuring it. Options include:

- Presence/absence
- Scored on a scale of 1 (low, least) to 5 (high, heaps)
- Area to the nearest ¼ frame
- Weight
- Number seen
- Percentage removed

You can use more than one method of assessment for each trait. You may wish to make quick and more frequent assessments with a 1 to 5 score, and occasionally perform a more thorough assessment. For example, you may give the brood area a score of 1 (low) to 5 (high) at most inspections, but record the area to the nearest ¼ frame at certain times of year because you are interested in overwintering.

Decide the direction of selection

Traits can be selected to increase, decrease, or a particular score. For example, if you score the amount of pollen in the brood nest on a scale of 1 (low) to 5 (high) and you consider a score of 3 (average) to be ideal, you can select for colonies to have an average amount of pollen in the brood nest by selecting for a score of 3. Someone else might consider a 4 to be ideal, so they would select for a 4. Another person may want simply to decrease the amount of pollen in the brood nest, and therefore will indicate L (low) as the direction of selection.

Decide what factors or traits will result in a queen being excluded

All colonies with American foulbrood must be euthanised – this removes them from the breeding pool.

There may be other traits that result in you excluding queens as candidates for selection. Decide what these will be. When a queen gets excluded, it is important to record that, so that the information is transferred to the database.

For example, if queens are excluded because their colonies have sacbrood, you should record these colonies as having the disease present and other colonies as having the disease absent. Without this, the information is lost and cannot contribute to EBVs. This will help identify the genetics that contributed to not having the disease in the rest of your population.

As another example, you might wish to exclude any colonies that have a chalkbrood score greater than 3 (i.e. 4 or 5) at any colony evaluation.

Decide if some traits are more important than others

An EBV will be provided for each individual for each trait that you record data on.

Assuming that you want to select for more than one trait, a summary EBV will be calculated that combines all the information into a single score for each individual. If you consider all traits to be of equal importance, then you need not take any action. If you are more interested in some traits than in others you can indicate this by weighting each trait.

As an example, you are selecting for three traits. You choose to spend 50% of your selection budget on the first trait, 30% on the second trait, and 20% on the third trait. The summary EBV will be calculated giving these weights to the respective traits.

Breeding objective

A breeding objective for a beekeeper who is recording data on five traits could be expressed as:

Breeding objective = Trait 1 (H), Trait 2 (L), Trait 3 (3), exclude Trait 4 (P), exclude Trait 5 (>3)

Where (H) indicates that the queen breeder wants to select for an increase in Trait 1, (L) indicates that they want to decrease Trait 2, and (3) indicates that they want to select for colonies that have scored a 3 for Trait 3. (P) indicates that colonies that have Trait 4 present are excluded, and (>3) indicates that colonies that scored a 4 or 5 for Trait 5 are excluded.

If weights are to be used, the breeding objective could be expressed as:

Breeding objective = 50% Trait 1 (H), 30% Trait 2 (L), 20% Trait 3 (3), exclude Trait 4 (P), exclude Trait 5 (>3)

This indicates that 50% of the selection budget is spent on Trait 1, 30% on Trait 2, and 20% on Trait 3.

It is not necessary to use exclusions.

You may have different breeding objectives for different lines.

Your breeding objectives can be reassessed and altered. For example, you may wish to only record honey production in the first year of using the database in order to reduce the recording burden while you assess the Plan Bee system. Later on, you may wish to start recording more traits. Your breeding objectives and the Plan Bee system for assessing them are adaptive.

Your breeding objectives can be communicated by email or in the Trait recording file.

3. Decide frequency of evaluation

Importantly, you do not have to record the same trait or all of them at every evaluation point. Some queen breeders may shortlist colonies for producing breeders based on easily recorded traits, and then add more traits, or different methods for recording those traits, on other potential breeders at a later evaluation point.

The greater the number of times the hive is evaluated and data recorded, the more accurate the EBVs will be. As ever, this must be balanced with the time taken to record traits versus the income doing so will generate.

4. Define queen IDs

Each queen in the database requires an individual identification code. This includes your user ID, an age code, and your studbook ID for the queen. You have control over your studbook code, but it can only be made up of numbers and letters. Further guidance is available in Chapter 8. Queen ID is recorded in the Queen ID and pedigree file.

5. Record pedigree and colony information

There are a number of options regarding pedigree depending on whether your queens are artificially inseminated, mated at isolated mating areas, or open mated, and how much information you have on pedigree. To start with, you may only know which line you queen belongs to, but it is hoped that with time you will start to collect more information on pedigree.

Information records for the colonies include which colony the queen is in, the date the queen was introduced, the date she was superseded or replaced, the load that the hive belongs to, and additional optional information.

Further guidance is available in Chapter 8. This information is recorded in the Queen ID and pedigree file.

6. Get data recording sheet

A data recording sheet is used to record your observations in the field. Each queen breeder will need a personalised data recording sheet, as each individual will have their own set of traits that they record data on.

Guidance on creating a data recording sheet is available from Elizabeth Frost, with further information available in Chapter 8.

7. Perform colony evaluations

Use your data recording sheet to record scores for each of the traits on each of your colonies. Best practice advice for performing evaluations is available in Chapter 7.

8. Submit information

You will need to transfer information from your data recording sheet into the Trait recording file. Your breeding objective will also be recorded in this file.

Submit the Trait recording file and Queen ID and pedigree file (Figure 8) to either Elizabeth Frost or Nadine Chapman via email. They will check the files over before they are uploaded to the database.

9. Get EBVs and make decisions

Plan Bee helps you with your breeding decisions. It produces a report that ranks your queens.

You will get an *individual EBV* for each individual for each trait in your breeding objective. You will also get a *summary EBV* for each individual that combines all the information for that individual into a single EBV. If you choose to weight your traits, then you will get a *weighted summary EBV* for each individual that combines all the information for that individual according to the weight that you gave each trait. The summary EBV and weighted summary EBV are not simple averages of the individual EBVs; they consider the direction of selection for each trait.

You can then select queens to generate the next generation.

10. Re-evaluate

Re-evaluate your breeding objectives. Do you want to change your objective to investigate more or fewer traits, use different assessment methods, or change the weighting of traits?

Chapter 7: Best practice

Spread genotypes across apiaries/loads

Ensure that there is a spread of genetics across your apiaries and loads. Do not keep all the queens from one line together in an apiary and those of another line together in a different apiary, or all the daughters of one queen in a single apiary if you have more than one apiary. This will make it difficult to separate genetic effects from environmental effects.

Pedigrees

EBVs are more accurate the more information is known about pedigree. Accuracy improves the more generations that are included in the pedigree.

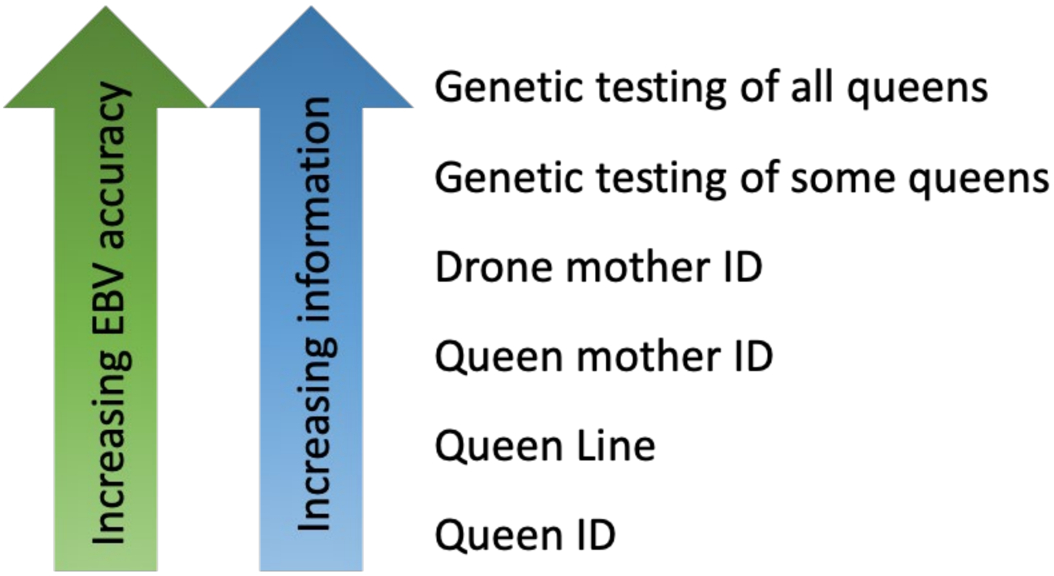


Figure 10. Increased information about pedigree increases the accuracy of Estimated Breeding Values (EBVs).

Controlled mating

Uncontrolled mating can reduce the rate of genetic progress by 47 to 99 per cent.

To completely control mating, artificial insemination should be used. However, this is a time and expertise-intensive method that is used by only some queen breeders, and often only to produce breeder queens.

Mating may also be controlled by providing drone mother colonies at mating sites isolated either by distance (on an island with no resident population of bees) or by time (Horner's use of temperature and light-regulated rooms to alter mating flight times).

Finally, mating can be partly controlled by providing many drone mother colonies at non-isolated mating sites. This should ensure that the queens are most likely to mate with drones coming from the selected colonies rather than surrounding colonies that belong to other beekeepers or from feral colonies in the area.

Number of drone mother colonies

At a minimum, 12 drone mother colonies should be provided per 100 queens being mated. Not having enough mates can be a key reason for queen failure. The number of mates has been associated with colony survival and honey production.

For artificial inseminations, the stand semen dose per queen is eight to 10 microlitres, which represents eight or more drones. Of course, this depends on the average semen load per drone and whether the collected semen is centrifuged. A minimum of 12 drone mother colonies should be managed for inseminations. However, more is advisable considering one Australian study found that queens may naturally mate with an average of 28 drones.

When artificial insemination is used to control mating and the drones are confined to their colony in order to guarantee their pedigree, the queen who produced the drones used in the insemination should be recorded.

Queen labelling

It is important that you record when a queen is superseded. This means that you require some means to differentiate your queens from any new queen that the colony may raise themselves. This may be achieved by clipping wings, applying a paint dot to the thorax, or applying a coloured and numbered tag.

Typically, artificially inseminated queens are labelled with numbered, coloured discs on their thorax. In this case, this queen’s numbered tag on her thorax should be recorded to link to the queen’s unique identification code and hive identification code.

How much progress you can make on a few traits versus many traits

Be aware that the work required to produce a *superbee* is very substantial. A modest increase in specific desirable characteristics, however, is achievable through selective breeding. These improved characteristics must be continually selected for every year in order to maintain desirable traits.

Faster genetic improvement can be achieved for each trait when selecting for a few traits than when selecting for many traits.

Repeatability and reproducibility

Repeatability indicates how closely measures of the trait agree with each other across time. This does not necessarily mean that similar values should be recorded; obviously for many traits there are large seasonal differences. Rather, colonies should *rank similarly* across time.

Reproducibility is, for example, how similarly two people would score a trait. If one person's score average is 4 and the other person scores an average of 2, then the score has low reproducibility. It is difficult to know if the colonies that the second person inspected were truly worse or if they applied different criteria to their scores.

Some suggestions:

- Have the same person evaluate that trait in all colonies.
- If that is not possible, then record who inspects each colony.
- Use the 1 to 5 scoring suggestions in Chapter 9.
- For other traits, work out a definition of scores for your operation.
- Inspect some colonies together so that you can ensure you would score them similarly.
- Perform inspections in a random order to randomise the effect of time of day or how your actions with one colony can affect the behaviour of nearby colonies.
- Perform inspections as feasible, remembering that more inspections will result in more accurate EBVs.

Quantitative versus qualitative methods

Measuring honey on a 1 to 5 scale is a *qualitative* measure. It will give you an idea of how each colony rates compared to others. Measuring the *exact amount* of honey produced by each colony is a *quantitative* measure.

Both types of information are useful, but a quantitative measure is more accurate. However, there is more work involved in determining exactly how much a colony produced than there is in making a visual observation.

Which method you use is up to you as you manage your time, your business and your profit margins.

Management

Be aware that differences in how you manage colonies will contribute to their scores. For example, if you provide supplementary feeding to half your colonies, these colonies could be expected to outperform the other half. Transferring brood from a strong colony to a weak colony affects both.

Queen introduction

In the European breeding program, new queens are introduced to an artificial swarm that has a standardised weight of worker bees. This gives all queens an equal start; thus, differences between colonies are more likely to be due to genetics than environment.

An alternative to the use of artificial swarms is to estimate the population of the colony by counting the number of frames of bees to the nearest ¼. Including this as a trait that you regularly record will help to account for some of the differences in the quality of the colonies that new queens are introduced to.

Colony evaluations should not be conducted until at least six weeks after the colony has been given a new queen, as this is the minimum length of time until the workers are the offspring of the new queen.

Specific traits

The descriptions below detail recommendations for some of the traits in the Trait dictionary (Chapter 9). No traits are required to be recorded, so the presence or absence of the traits in the list below is no indication of their importance. There may be others that are important to you but are not listed here.

Temperament

Temperament can be affected by a number of factors: environmental conditions, weather, time of day, the behaviour of nearby colonies, and how hives are handled by individuals.

Work colonies in a different order each time you score for temperament to determine if colonies are naturally defensive or if they've been set-off before being worked due to disturbance of other colonies nearby.

Recording this trait multiple times across a season will make it more accurate. It is also best if the same person or people record this trait for all the colonies under inspection so that consistency is maintained. If this is not practical, then work a few colonies together to ensure that you would score them similarly (see repeatability and reproducibility above).

Colour

A number of subspecies have been introduced to Australia, and thus a range of colours are possible. Colour can be an indication of subspecies. Different subspecies are believed to express different traits. Colour is a marketable trait when producing queens for sale.

Uniformity in worker colour is a good indicator of queen mating success with a controlled drone pool. Influence of feral or undesirable drones in queen mating is often reflected in workers being a mix of colours.

The desired traits must still be selected, rather than simply using colour as a short-cut for quality or the presence of certain traits. The primary aim should be to produce high-quality queens regardless of their colour.

Size

Size varies between different subspecies and different lines of bees, as well as being influenced by the available resources and the age of the frames the bees are reared in. These factors should be considered if you wish to select for size.

Queen weight

Queen weight is a measurement that is most practical in an artificial insemination program at seven days after queen emergence. Measure queen weight when she has received her first dose of carbon dioxide and is immobile.

Disease

The minimum requirements for evaluation of hive health, according to the Honey Bee Biosecurity Code of Practice, is twice a year, at least four months apart. We therefore suggest that you make the most of these inspections to record the presence, severity or absence of all diseases at least twice a year. You may choose not to include these traits in your breeding objectives, however the option is there, and it would also provide you with data that you can provide to your customers. For example, you may find that different lines vary in the frequency with which they get different diseases.

Hygienic behaviour

We recommend testing hygienic behaviour with liquid nitrogen over the use of a pin-killed brood test.

Hygienic behaviour testing should be done at least twice during the season. Keep in mind that a heavy nectar flow or feeding sugar syrup may stimulate hygienic behaviour and workers to remove chalkbrood mummies from the comb.

A free guide for completing a hygienic behaviour test use liquid nitrogen is available at https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/535604/Testing-for-hygienic-behaviour.pdf.

Frames of bees

Before using too much smoke, quickly count how many frames are fully covered with bees by looking down through the top bars. For a double (colony made of two boxes), bees on frames can be evaluated by looking down at the top bars of the bottom brood box and up through the bottom bars of the frames in the top box. A frame where bees fully cover both sides is recognised as one frame of bees.

Ideally, the population count is made early in the morning before many of the foragers have left the hive. The database will accept the number of frames to the nearest ¼.

Overwintering and spring build-up

Time your inspections so that information on the amount of brood and bees is used for selecting for spring build-up or overwintering ability. This would mean that these traits are selected for, or at least recorded, without any extra work.

The traits for spring build-up and overwintering are marketable traits that have been successfully selected for overseas. Methods to measure these traits are included in Chapter 9. Plan Bee will use the accumulated data to determine which methods are most suitable for Australian conditions and if these measures can offer any early insight about how colonies will perform over the season.

Chapter 8: Data recording

The Queen ID and pedigree file and Trait recording file are not part of this manual. These are files for use in either Microsoft Excel or Google Sheets. Screenshots of these documents are available in the appendix.

The Queen ID and pedigree file is used to record mandatory and optional information about queen identification, the colony they are in, and their pedigrees.

Queen identification

Each queen must be assigned a unique (queen ID) identifier in the database. This is recorded in the Queen ID and pedigree file.

The queen ID is a combination of three codes

- User code
- Age code
- Studbook code

The user code is a four-digit code starting from 1000 and it will be assigned to you (see Figure 11 – XXXX). Please contact either Liz Frost or Nadine Chapman to get your user code.

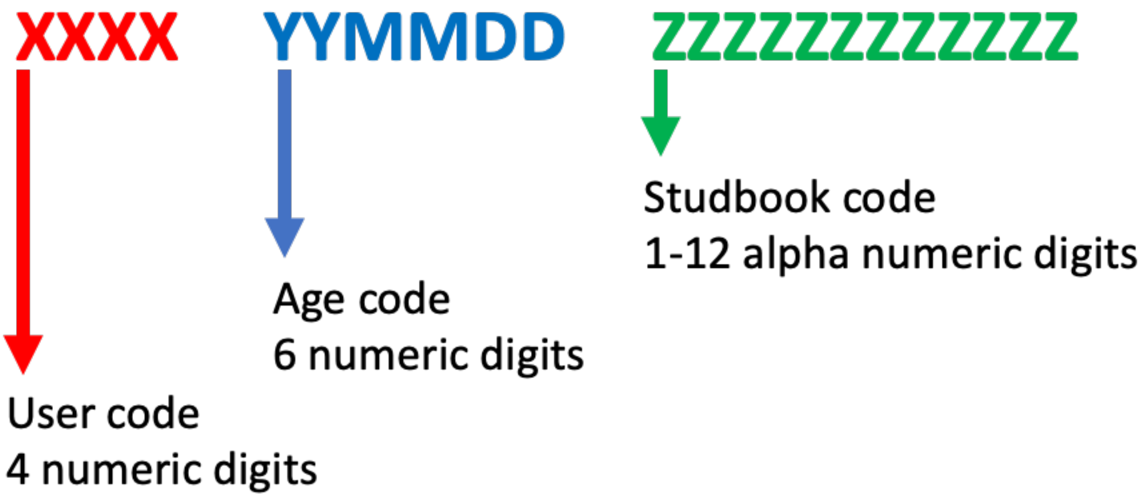


Figure 11. The queen ID is made up of a user code, an age code, and a studbook code.

The age code is a six-digit code. This is the *year*, *month* and *day* the queen was produced, in that order (YYMMDD). Where the day is not known, use 00. Similarly, if the month is not known, use 00 (see Figure 11 – YYMMDD).

For example, 201100 indicates that the queen was produced in November 2020 but the day is not known.

Another example, 200000, indicates that the queen was produced in 2020, but neither the month nor day is known. Placement of 0000 after 20 (noting the year 2020) indicates that there is no information about the month or day.

The system is thus able to cater to different amounts of information.

There is likely to be significant uncertainty about the age of queens as queen breeders begin implementing the program. It is hoped that we will eventually move to knowing both the month and the year the queen is produced.

The studbook code is what you use to identify the queen within your operation. It can be up to 12 digits, combining letters and numbers (see Figure 11 – ZZZZZZZZZZZZ). Note that it does not have to be 12 digits.

It is also possible to record the **previous Queen ID** if you bought her from someone who has records on the queen elsewhere in the database, and they give you her code. This will link data collected by both yourself and the person you purchased the queen from.

Colony information

If you use a **queen tag** you may record this.

The **hive ID** of the colony the queen is in must be recorded.

The **load** the colony belongs to must be recorded. If your colonies are not separated into loads, then use the same number for all colonies or leave blank. It is important to have this information in order to account for environmental variation.

The date (YYMMDD) the queen was introduced to the colony must be recorded if it is known (**date introduced**).

Whether the queen is artificially inseminated (AI), mated at an isolated mating station (I), or naturally or open mated (O) must be recorded under **mating**.

If a colony naturally replaces their queen, then record the **date superseded** (YYMMDD). This may be an approximation. This information is important for ensuring that EBVs are calculated only using the correct time period for the correct queen in the colony.

You may choose to record the date (YYMMDD) that you replace your queen (**date replaced**). The database will also have this date because the replacement queen will have the date that she was introduced into that colony recorded.

If you use a mix of hive types, record that information under **hive depth** and **hive width**.

Queen pedigree

Queen breeders will vary in the level of information they collect on queen pedigree. The more information you provide, the more accurate your EBVs will be.

Record the **line** the queen belongs to. If you do not keep different lines, then record the same code for all queens or leave blank. If you do keep lines but you do not know the line of this queen, then leave blank for that queen.

Record the **queen mother ID**. If this is not known then leave blank.

For queens mated via isolated or open mating, record:

- The **mating yard** if you have more than one if you wish (this does not need to be identifiable to anyone but you).
- An approximate **mating date** (YYMMDD) if you wish.
- The **isolated mating drone mother IDs** if you wish. Each ID should be separated by a comma (,).

For queens mated via artificial insemination, record:

- The date the AI was performed (**date AI**).
- The **drone mother IDs** of the drone mothers used in artificial insemination if known. Each drone mother ID should be separated by a comma (,). Leave blank if necessary or as you choose.
- The **AI drone mother lines**. Each drone mother line should be separated by a comma (,). Leave blank if necessary or as you wish.

You may record the **colour** and **race** of the queen if you wish.

Trait recording sheet

A personalised data recording sheet will be created for you to use in the field when you evaluate your colonies. You must then type this information into the Trait recording file. The Trait recording file also contains your breeding objectives.

Breeding objectives tab

The breeding objectives tab of the Trait recording file lists all the traits and methods against their abbreviation. Place a:

- **Y** in the **record** column against the traits that you have recorded data on.
- **Y** in the **select** column against the traits that you wish to select (as you may record data on additional traits that you do not wish to include in your breeding objectives).
- In the **direction** column, place an **L** if you wish to decrease the trait in the population, a **H** if you wish to increase the trait in the population, or a number (**1-5**) if you wish to select for a particular score.
- If you would like different traits to have different weights, then place a number between 1-100 against each trait under selection in the **weight** column. They must total 100. Alternatively, leave blank.

- If you would like to specify traits that will exclude a queen from consideration as a breeder, specify this in the **exclude** column. This can be a score (e.g. <3) or presence/absence (e.g. P).

Records tab

The records tab of the Trait recording file is where you enter the data you collected during colony evaluations.

Record:

- The **date** (YYMMDD) that the colony evaluation took place.
- The **apiary** that the colony was in (this does not need to be identifiable to anyone but you).
- If more than one **assessor** performed evaluations, then record the initials, name, or number of the person that assessed each colony.
- The **colony ID**.
- The **queen status** using the following codes (you can use other notation in your data recording sheets, but these codes will need to be used in the Trait recording file).
  - QR: queenright, correct queen present
  - V: correct virgin present
  - C: correct caged queen present
  - AINL: artificially inseminated not laying
  - NS: not seen
  - QL: queen-less
  - SRV: self-raised/supersedure virgin
  - SRL: self-raised/superseded laying
  - DL: drone layer
- The number of **boxes** in the colony.
- The number of **supers** on the colony.
- Scores for the traits that you are recording at that inspection under the appropriate column. You may delete any columns for traits that you have not collected data on if you wish.
- You may record **comments** if you wish.
- You may record the time you started (**time start**) and finished (**time finish**) the inspections if you wish.
- You may record the **weather** at the time of inspection if you wish.

Data upload

Email the Queen ID and pedigree file and the Trait recording file to either Liz Frost or Nadine Chapman for checking. The team will check data for outliers or other problems and will contact you if necessary.

Data processing and reporting

The team aim to report EBVs to you via email within two weeks.

Reports will be tailored to your breeding objective. An example of a breeding objective and the report associated with that objective can be seen in Figure 12. The information for each queen is in a separate row; her ID and line is given. An EBV is given for the four traits. There is no EBV given for European foulbrood (EFB) because queens from any colony that had EFB at any time were excluded.

Breeding objective = 50% Honey (H), 20% Aggression (L), 20% Brood viability (H), 10% Chalkbrood (L), exclude EFB (P)

Queen ID	Line	Honey EBV	Aggression EBV	Brood viability EBV	Chalkbrood EBV	Summary EBV	Weighted summary EBV
1001210223W50	1	+0.54	+0.09	+0.12	+0.89	-0.08	+0.19
1001210328B11	8	+0.83	+0.45	+0.55	+0.33	+0.15	+0.40
1001210224N77	3	-0.33	-0.24	-0.11	-0.21	0.00	-0.12
1001210125B6	4	+0.76	-0.13	+0.01	-0.01	+0.23	+0.41

Figure 12. A report for the given breeding objective.

Taking this information, **you** make **your decision** about which queens you will breed from.

Chapter 9: Trait dictionary

Production

Honey

a) Honey production can be scored from 1 (low/little) to 5 (high/heaps) from a visual assessment or by physically picking up a super and classifying the estimated weight.
b) Stored honey can be scored to the nearest ¼ frame.
c) Honey production can be measured by weighing in kilograms the amount of honey extracted or removed from the colony.
d) Colony weight in kilograms can be used as a proxy for honey production.

Pollen

a) Stored pollen can be scored from 1 (low/little) to 5 (high/heaps).
b) Stored pollen can be scored to the nearest ¼ frame.
c) The amount of pollen collected in a pollen trap over a set period of time can be recorded in grams.

Wax

a) The amount of wax can be scored from 1 (low/light) to 5 (high/heavy).
b) The amount of wax produced per colony can be recorded in kilograms.

Burr comb

a) The amount of burr comb can be rated on a scale of 1 (low/little) to 5 (high/heaps) using the descriptions in Table 1.

Propolis

a) The amount of propolis can be scored from 1 (low/little) to 5 (high/heaps).
b) The amount of propolis produced can be recorded in grams.

Royal jelly

a) The amount of royal jelly produced in queen cells can be scored from 1 (low/little) to 5 (high/heaps).
b) The weight of royal jelly collected from 10 queen cups can be recorded in milligrams.
c) The weight of royal jelly collected from a colony can be recorded in grams.

Temperament

Aggression

a) Aggression (amount of smoke needed, attacking) can be rated on a scale of 1 (low/gentle) to 5 (high/aggressive) using the descriptions in Table 1.

Runny

a) Runny (movement and clustering of bees) can be rated on a scale of 1 (low) to 5 (high) using the descriptions in Table 1.

Appearance

Worker colour

a) Rated on a scale of 1 (not the colour I want) to 5 (the colour I want).

Worker size

a) Rated on a scale of 1 (low/little) to 5 (high/huge).

Drone colour

a) Rated on a scale of 1 (not the colour I want) to 5 (the colour I want).

Drone size

a) Rated on a scale of 1 (low/little) to 5 (high/huge).

Queen colour

a) Rated on a scale of 1 (not the colour I want) to 5 (the colour I want).

Queen size

a) Rated on a scale of 1 (low/little) to 5 (high/huge).

Queen weight

a) Queen weight can be recorded in milligrams.

Brood nest

Brood area

a) Rated on a scale of 1 (low/little) to 5 (high/heaps).
b) Scored to the nearest ¼ frame.

Brood viability/pattern

a) Rated on a scale of 1 (low) to 5 (high).
b) Scored as the % viable from a known area.

Pollen in the brood nest

a) The amount of pollen in the brood nest can be scored from 1 (low/little) to 5 (high/heaps).

Honey in the brood nest

a) The amount of honey in the brood nest can be scored from 1 (low/little) to 5 (high/heaps).

Swarming

a) Swarm tendency can be scored on a scale of 1 (low/little) to 5 (high) using the definitions in Table 1.
b) Swarming tendency can be scored as present/absent.

Larval feeding

a) The amount of food fed to workers can be scored from 1 (low/little) to 5 (high/heaps).

Health

Disease

a) Incidence of disease (European foulbrood (EFB), chalkbrood, sacbrood) can be scored on a scale of 1 (low/little) to 5 (high/heaps) using the guides in Table 1.
b) Incidence of disease (EFB, American foulbrood (AFB), Nosema, sacbrood) can be recorded as present/absent. Colonies with AFB must be destroyed.
c) Incidence of chalkbrood and EFB can be recorded as the number of infected cells.

Pests

a) Incidence of pests (small hive beetle, wax moth) can be scored on a scale of 1 (low/little) to 5 (high/heaps) using the guides in Table 1.
b) Incidence of wax moth and small hive beetle can be scored as present/absent.
c) Number of small hive beetles seen when opening the colony can be recorded.

Hygienic behaviour

a) Hygienic behaviour FKB (freeze killed brood) may be recorded as the % removed FKB.
b) Hygienic behaviour PKB (pinned killed brood) may be recorded as the % removed PKB.
c) Hygienic behaviour FKB may be recorded on a scale of 1 (low) to 5 (high) using the guides in Table 1.
d) Hygienic behaviour PKB may be recorded on a scale of 1 (low) to 5 (high) using the guides in Table 1.

Cleanliness

a. Hive cleanliness (debris on bottom) can be scored on a scale of 1 (low/unclean) to 5 (high/clean).

Population

Frames of bees

a. Scored to the nearest ¼ frame.

Adult drones

a. Scored on a scale of 1 (low/little) to 5 (high/heaps).

Drone brood

a. Scored on a scale of 1 (low/little) to 5 (high/heaps).
b. Scored to the nearest ¼ frame.

Overwintering and spring build-up

a. These are calculated (see Table 2) from measures of the number of frames of bees and brood to the nearest ¼ frame at certain times of year, or the amount of honey used over winter. If you are interested in these traits, then you should measure these traits at the relevant times. Some of the options provided have more evidence of heritability than others.

Table 1. Suggested scores for traits on a scale of 1 (low) to 5 (high). Traits that are seasonal do not have suggested scores e.g. brood area, honey production.

Trait	**1 (low/little)**	**2**	**3**	**4**	**5 (high/heaps)**
Aggression	Minimal smoke required, no aggression displayed	Requires more smoke as you progress	Requires more smoke, bees fly around you but do not attack	Stings, posturing, guards recruited	Large amount of smoke needed, multiple stings, persistent attack, chases you, sets off surrounding colonies
Runny	Work as normal	A little movement	Some agitation and clustering	Rush about on combs and box, fairly clustered	Fly off and rush about on combs, bees grouped leaving frames uncovered, no work being done
Swarming	No queen cells, no management required	Queen cells destroyed	Minimal management required – remove brood/honey/add super	Splitting was required	Colony swarmed despite management
European foulbrood	0	1-5 cells	5-20 cells	20-30 cells	>30 cells
Chalkbrood	0	1-5 cells	6-40 cells	41-80 cells	>80 cells
Sacbrood	0	1-5 cells	6-10 cells	10-20 cells	>20 cells
Small hive beetle	0	1-5 cells	6-15 cells	16-30 cells	>30 cells
Wax moth	0	1-5 cells	6-10 cells	11-15 cells	>15 cells
Burr comb	0	30% of space filled	50% of space filled	70% of space filled	100% space filled
Hygienic behaviour (FKB)	0-20% removed	20-40% removed	40-60% removed	60-80% removed	80-100% removed
Hygienic behaviour (PKB)	0-20% removed	20-40% removed	40-60% removed	60-80% removed	80-100% removed
Worker size (length)	12 mm	13 mm	14 mm	15 mm	>15 mm
Drone size (length)	<15 mm	15 mm	16 mm	17 mm	>17 mm
Queen size (length)	18 mm	19 mm	20 mm	21 mm	22 mm

Table 2. Summary of traits and the options (a-d) for recording them, and the abbreviation used in the Trait recording file.

Trait	a: Trait abbreviation	b: Trait abbreviation	c: Trait abbreviation	d: Trait abbreviation
Honey	1 (low) - 5 (high): HonS	Weight of honey: HonW	Area to ¼ frame: HonF	Colony weight: HonC
Pollen	1 (low) - 5 (high): PolS	Area to ¼ frame: PolF	Weight in pollen trap: PolW	
Wax	1 (low) - 5 (high): WaxS	Weight: WaxW		
Burr comb	1 (low) - 5 (high): Burr			
Propolis	1 (low) - 5 (high): PropS	Weight: PropW		
Royal jelly	1 (low) - 5 (high): RJS	Weight from 10 cups: RJCp	Weight per colony: RJCy	
Aggression	1 (low) - 5 (high): Agg			
Runny	1 (low) - 5 (high): Runny			
Worker colour	1 (poor) - 5 (good): WC			
Worker size	1 (low) - 5 (high): WS			
Drone colour	1 (poor) - 5 (good): DC			
Drone size	1 (low) - 5 (high): DS			
Queen colour	1 (poor) - 5 (good): QC			
Queen size	1 (low) - 5 (high): QS			
Queen weight	Weight: QW			
Brood area	1 (low) - 5 (high): Brood	Area to ¼ frame: BroodF		
Brood pattern/ viability	1 (low) - 5 (high): BP	% empty cells: BV		
Pollen in brood nest	1 (low) - 5 (high): BNP			
Honey in brood nest	1 (low) - 5 (high): BNH			
Swarming	1 (low) - 5 (high): SwaS	Present/absent: SwaPA		
Larval feeding	1 (low) - 5 (high): LF			
European foulbrood	1 (low) - 5 (high): EFB	Present/absent: EFBPA	# of infected cells: EFBN	
American foulbrood	Present/absent: AFB			
Nosema	1 (low) - 5 (high): Nos	Present/absent: NosPA		
Chalkbrood	1 (low) - 5 (high): CB	Present/absent: CBPA	# of infected cells: CBN	

Sacbrood	1 (low) - 5 (high): Sac	Present/absent: SacPA		
Small hive beetle	1 (low) - 5 (high): SHB	Present/absent: SHBPA	# beetles seen when open hive: SHBN	
Wax moth	1 (low) - 5 (high): WM	Present/absent: WMPA		
Hygienic behaviour	% removed FKB: FKB	% removed PKB: PKB	1 (low) - 5 (high) FKBS	1 (low) - 5 (high) PKBS
Hive cleanliness	1 (low) - 5 (high): Clean			
Frames of bees	Area to ¼ frame: Bees			
Adult drones	1 (low) - 5 (high): Dro			
Drone brood	1 (low) - 5 (high): DroBS	Area to ¼ frame: DroBF		
Over-wintering*	Frames of bees in spring ÷ frames of bees in autumn: WinBees	Frames of brood in autumn ÷ frames of bees in autumn: WinR	Colony weight before winter - colony weight after winter: WinW	Weight honey before winter - weight honey after winter: WinH
Spring build-up*	Frames of bees in spring ÷ frames of bees in winter: SprBees	Frames of brood in spring ÷ frames of bees in autumn: SprR	Frames of brood in spring: SprB	

* These are calculated from information on other traits if performed at the applicable time. Therefore, they are not recorded, the traits used in the equations are recorded instead.

Appendix

Appendix 1: Screenshot of the Queen ID and pedigree file

Age code	Studbook ID	Queen ID	Queen tag	Hive ID	Load	Date introduced	Mating	Date superseded	Date replaced	Hive depth	Hive width (frames)	Line	Queen mother ID	Mating yard	Mating date	Isolated mating drone mother IDs	Date AI	AI drone mothers IDs	AI drone mother lines	Previous Queen ID	Colour	Race

Appendix 2: Screenshot of the records tab of the Trait recording file

Date	Apiary	Assessor	Colony ID	Queen status	Boxes	Supers	HonS	HonF	HonW	HonC	PolS	PolF	PolW	WaxS	WaxW	Burr	PropS	PropW	RJS	TJCp	RjCy	Agg	Run	WC	WS	DC	DS	QC	QS	QW	Brood	BroodF	BP	BV	BNP	BNH	SwaS	SwaPA	LF	EFB	EFBPA	EFBN	AFB	Nos	CB	CBPA	CBN	Sac	SacPA	SHB	SHBPA	SHBN	WM	WMPA	FKB	PKB	FKBS	PKBS	Clean	Bees	Dro	DroBS	DroBF	Comments	Time start	Time finish	Weather

Appendix 3: Screenshot of the breeding objective tab of the Trait recording file

Record: place a Y against the traits that you have recorded data for

Select: place a Y against the traits that you wish to select for

Direction: direction of selection; L for low, H for high, or a number if you want to select for a particular score

Weight: If you would like different traits to have different weights then place a number between 1-100 against each trait under selection. They must total 100. Alternatively, leave blank and an overall average EBV will be generated. Each trait will have an EBV produced for each individual regardless of whether you apply a weight

Exclude: Traits that will exclude an individual from consideration. These can be a score (e.g. <3 for "clean") or presence/absence (e.g. P for "SacPA")

Score: 1(low) to 5 (high)

Trait	Abbreviation	Record	Select	Direction	Weight	Exclude
Honey score	HonS					
Honey to 1/4 frame	HonF					
Honey weight (kg)	HonW					
Colony weight (kg)	HonC					
Pollen score	PolS					
Pollen to 1/4 frame	PolF					
Pollen in trap (g)	PolW					
Wax score	WaxS					
Wax weight (kg)	WaxW					
Burr comb score	Burr					
Propolis score	PropS					
Propolis weight (g)	PropW					
Royal jelly score	RJS					
Royal jelly weight 10 cups (mg)	TJCp					
Royal jelly weight per colony (g)	RjCy					
Aggression score	Agg					
Runny score	Run					
Worker colour score	WC					
Worker size score	WS					
Drone colour score	DC					
Drone size score	DS					
Queen colour score	QC					
Queen size score	QS					
Queen weight (mg)	QW					
Brood area score	Brood					
Brood area to 1/4 frame	BroodF					
Brood pattern/viability score	BP					
Brood pattern/viability %	BV					
Pollen in brood nest score	BNP					
Honey in brood nest score	BNH					
Swarming score	SwaS					
Swarming presence/absence	SwaPA					
Larval feeding score	LF					
European foulbrood score	EFB					
European foulbrood presence/absence	EFBPA					
European foulbrood cell count	EFBN					
American foulbrood presence/absence	AFB					
Nosema presence/absence	Nos					
Chalkbrood score	CB					
Chalkbrood presence/absence	CBPA					
Chalkbrood cell count	CBN					
Sacbrood score	Sac					
Sacbrood presence/absence	SacPA					
Small hive beetle score	SHB					
Small hive beetle presence/absence	SHBPA					
Small hive beetle count	SHBN					
Wax moth score	WM					
Wax moth presence/absence	WMPA					
Hygiene freeze killed brood % removed	FKB					
Hygiene pin killed brood % removed	PKB					
Hygiene freeze killed brood score	FKBS					
Hygiene pin killed brood score	PKBS					
Hive cleanliness	Clean					
Frames of bees to 1/4 frame	Bees					
Adult drones score	Dro					
Drone brood score	DroBS					
Drone brood to 1/4 frame	DroBF					
Over-wintering frames bees	WinBees					
Over-wintering brood ratio	WinR					
Over-wintering colony weight	WinW					
Over-wintering honey weight	WinH					
Spring build-up frames bees	SprBees					
Spring build-up brood ratio	SprR					
Spring build-up frames brood	SprB					
SUM OF WEIGHT					0	

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