

Introductory study for breeding Varroa resistant bees

Final report

2004

by

Tore Forsman, Per Ideström and Erik Österlund

of

SWEDISH BEEKEEPING ASSOCIATION

SWEDISH BEEKEEPING ASSOCIATION
TRUMPETAREVÄGEN 5
S-59019 MANTORP
SWEDEN

www.biodlarna.se

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Introduction to the preliminary report

The aim of this study is to present a concept to achieve bees that can be kept without any use of chemicals to control the varroa mite. This concept is not presented as truth. It is presented as a possible concept that can be tested by scientists and beekeepers to confirm or falsify.

As a base for this concept we have used documentation that present claims of success in this area. We deal with this documentation as if it presents the truth. We can't sort out *if* the documentation is presenting the truth. Those that find this concept we present useful, will hopefully help to confirm or falsify parts of or the whole of this documentation.

Much of the documentation is not of a strict scientific kind, as such a documentation is usually presented today. It is more of an anecdotal type of documentation. But as so much of scientific tests in a strict way of looking at such work lack in this area, to be able to cover the area as much as possible we have to take in account also this sort of documentation.

Now, also anecdotal type of documentation is though not useless in qualifying as a base for conclusions. Conclusions are drawn in response to how this documentation is confirming or falsifying a hypothesis or a theory. These conclusions are drawn in the purpose of making further investigation in this matter. The documentation may also give ideas for further tests or modification of the hypothesis or theory. Also the border between anecdotes and tests that qualify to be called scientific is floating, isn't it? Of course a strict scientific test may give a better base for a better conclusion.

That anecdotes of an enough covering type can give a good base for workable conclusions is shown by the fact that no strict scientific test exists that come up with the conclusion that Africanized bees are resistant to the varroa mite (or the varroa mites). Anyhow all involved agree that this is the case. That is due to the overwhelming presentations of *surviving* and thriving bee colonies over many years. It seems though that this conclusion don't apply to every single bee colony in that group that many would classify as Africanized. This is not surprising, as a variation exists in every bee stock. This is of course also the case when it comes to other

bee types that are described to have a higher varroa resistance than the average bee of today of *Apis mellifera*. If these descriptions are true, which we take for granted in this study, others in other types of circumstances will have similar experiences. Reality will hopefully confirm or falsify this.

If all the documents reflects the truth they must harmonize. That is, if there are parts of them that seem to contradict each other, there must be a way to interpret or understand them so that they harmonize. It may mean that we have missed some important part in our understanding of this subject. On the other hand if it seems impossible to get all documents in harmony, some parts of one or more, or entire documents may not be true. But if this is the case someone must be describing smaller or bigger lies. This we though find improbable, due to among other things that these different documents in many parts do harmonize in an appearant way. Also we have got to know many of these people that are behind these documents and find it improbable that they have presented lies.

We are convinced that the documents presented as a base do harmonize in all important details. That gives interesting implications of the possibilities to explain why some have more difficulties than others with a certain concept. We are also aware of that there may exist parameters that we haven't stressed enough, or that we may have overlooked. We hope that others can fill in the gaps we may have missed.

At last though reality will tell if the concept we present here will bring any positive results for beekeepers and the bees themselves. This is of course the purpose of this study.

Tore Forsman, Per Idestrom and Erik Osterlund

Introduction to the final report

The aim of this final report is to add comments to the preliminary report and due to these make revisions of our summary of the used methods and our suggestions for outlining tests. We also discuss the given comments shortly.

The preliminary report of 'Introductory study for breeding varroaresistant bees' was sent to a selected number of scientists and experienced beekeepers, who were asked to give their comments to the study, short or detailed. It was also sent to those whose experiences are described and to those that in different respects have contributed. We have received a number of comments. All that have been asked to give comments have not been able to answer, but we are very grateful for the comments we've got. All of the comments are valuable, short as well as more detailed. John Kefuss has given us more valuable information about his work. Dee Lusby wanted to give some response, which we have included in the section of comments

It has been discussed many times which term(s) to use when describing the bees ability to cope with, survive and live normally with a low or high population of mites or with no mites at all. We have made it simple for us and used the term resistance for all the varieties of the bees ability in this respect. Among many this is how the term is used, right or wrong as it may be. Right is at the end how the majority use a term, and that's the way we should use it if we would like to have the most understanding of what we are communicating.

We have collected the comments and include them here in the final report, as well as our response to them. The comments have also resulted in some changes in our summaries of the documents given in the preliminary report and our suggestions for making tests based on the described methods. This is the chapter in the preliminary report named 'Concept for obtaining honeybees of *Apis mellifera* that don't need any treatment activity against the mite *Varroa destructor*', and it's of course included in its revised form in this final report.

In the preliminary report, to make it easier

for those that were asked to make comments, we had copied documents that described the experiences of those that claimed total or close to total success in obtaining varroaresistant bees. The economy didn't allow us to do this again, but we include here in the final report a list of the documents in the preliminary report, which hopefully will help those that don't have access to the preliminary report and want to look further in the documents we have used.

Once again we want to stress that in this work we don't make any conclusions of which selection or management methods we think are successful. We give a summary of them and on the basis of the descriptions of the methods used we make suggestion that might be used to try to repeat what have been done by others. So many methods are suggested that we understand that those who want to use this study as inspiration for obtaining bees that don't need any treatment activity against the *Varroa* mite probably will make a selection among these methods. Even if we don't describe what we think are the best choices, we of course have opinions of our own. Also, we who have done this work don't agree in all of our opinions. We can work together anyhow, which should be the characteristic of us all involved. You can guess from this report some of our opinions and some of you who read this know some or all of us enough to know. What is important, whatever our opinions may be or may be not, is that you take the ambition seriously, and hopefully make it your own, the devotion to get *Varroa* resistant bees. We also want you to excuse us for our limited knowledge in the English language and hope you can find the descriptions presented useful.

Tore Forsman, Per Ideström and Erik Österlund

Concept for obtaining honeybees of *Apis mellifera* that don't need any treatment activity against the mite *Varroa destructor*

To have bees that don't need any special treatment activity is a dream for every beekeeper, we hope, also for us that are behind this study. We are "brave" enough to form a hypothesis that this is possible for every beekeeper that wants it and is able to do the work needed. We present this hypothesis because of the documents we have found on the subject.

THE GOAL

The goal for this study is to present ways to confirm or falsify the hypothesis. It's only those who try who will know.

METHODS

We have collected documents that describe experiences and tests concerning claims of success in breeding or keeping bees that don't need any treatment activity, or almost any treatment, against the mite *Varroa destructor*. These reports then support our hypothesis. We want these successful concepts to be tried also in other areas of the world. Is it possible to use them also in our part of the world? Can this hypothesis be further supported with positive experiences from other climatic and environmental conditions?

We have tried to present the essentials of the different methods and possibilities described and tried to make a "manual". This manual is made to fit every type of circumstance that forms the start for a project with the goal described in the hypothesis above. You may thus start with the bees you have or with whatever suits you concerning the type of bee. With an unselected bee you start from scratch. With a bee that already is selected somewhat you may start a little later in the manual. You may choose to use many selection tools that require quite some work, or you may choose to rely more on the results concerning survival.

SOME BASICS

The first two parts of the list of documents deals with the fact that honey bee colonies have an defense system which consists of many different parts, from the micro organism level (Immune system) to the colony level (and even apiary level). And bee colonies differ in their ability to fight different types of enemies. Also we as beekeepers influence this fighting ability by our management system, many times in a negative way also when we help the bee colony with for example treatment activities. This is important to remember so that

in a breeding type of set up, or search for management systems we have to take this in account. For example, bee colonies that are not treated for varroa with chemicals (pesticides, acids and oils) of any kind can stand a higher mite/virus pressure before going downhill in such to such an extent that it can't recover and survive. Also in such a task as this, we have to allow for bee colonies to die, or rather identify those early enough and eliminate their influence on the neighbouring bee colonies. The goal is not to save susceptible colonies, but to have them identified and thus also to help to recognize those others more resistant which are to be bred from for the next generation.

Cell size

Many reports today say a smaller cell size than what is usual on most wax foundations sold today contributes to a better survivability of the bee colony. Smaller cell size was used as standard in the early days of wax foundation, most common was just below 5.1 mm cell size (5 cells in a row making one inch).

Eric H. Erickson

Dr Eric H. Erickson, Tucson, Az, USA says bee colonies survived much better on a cell size about 5.1 mm in width, compared to the usual 5.45 mm. He speculated among other things that the fertility of the mite might be influenced.

Dee and Ed Lusby

Dee and Ed Lusby in Tucson Az, USA, has rescued their organic bee business by using 4.9 mm cell size and no chemical whatsoever. They stress today the occurrence of twice a year or so premature uncapping of sealed brood infested with varroa mites as a probable contributor to better survivability. In some colonies the mite population seems to be quite high once or twice a year.

Dennis Murrell

Dennis Murrell in Wyoming even says that after he's had all his colonies "stabilized" on 4.9 cell size (after which event about half of the brood combs of this cell size is enough) he can shift queens to whichever source of queens. And these colonies "forced" to live on 4.9 mm cell size have no problems living well with a very small mite population all through the year, even if the bees born in this cell size have big problems drawing 4.9 foundation correct. When his colonies were in the state of sta-

bilisation as he calls it (first year(s) of downsizing of cell size) his colonies had high mite populations (though varying), bees with deformed wings (in varying numbers) and premature uncapping of brood, so called bald brood (and sometimes chewing out of the uncapped brood). The mite population in some of the colonies then was very high. All the bees were in the same apiary.

Roger White

Roger White has unselected (for survivability of varroainfestation) bee colonies surviving, and now producing honey, for several years without any treatment. Though once a year they show bees with deformed wings, which they have recovered from when next season starts. If though he uses dronecomb removal to lower the mite pressure he has not seen any wing damaged bees at the end of season. He has tried that in one colony. He has also seen the premature uncapping of brood in these colonies. These colonies with small cell size are kept in an apiary of their own.

Thomas Kober

Thomas Kober in Germany started to downsize his cellsize in 2002. He wintered his 200 colonies without treatment, except the 20 still on entirely big cell size. In spring 2003 he had 51 colonies left mainly early splits on 4.9 mm cell size made the previous year. Some of the treated big cell size colonies survived too. This was the winter with the high winter mortality all over Europe. The small cell colonies developed rapidly and he made enough splits to again winter 200 colonies, again without any treatment. In april 2004 he has experienced a 30% loss, mostly splits he made late in season 2003. The colonies that survived are the spring of 2004 much stronger than the spring before. He is very confident he is going in the right direction. Of course he bred from the best survivors from the year before.

Hans-Otto Johnsen

Hans-Otto Johnsen in Norway has used very little chemicals in his hives since the very arrival of the mite in his apiaries about 1997, in practise nothing. He has experienced a varied number of mites in his hives. He has downsized his cell size during a number of years now and the biggest problems concerning varroa resistance he has had in colonies with bigger cellsizes and unselected bee stock. The main bee stock in his operation is the Elgon bee, which according to several experiences has a bigger survivability than the average bee. A few colonies he has actually killed due to close to crashing. The biggest problems have also showed up in "old" colonies. In splits and new colonies there are normally no problems. At least when he is able to keep them

in apiaries of their own. In spring 2004 he had 600 healthy and thriving colonies out of 700 in autumn. 26 died of cattle (!) and 7 of a fallen pine tree.

Debate

Mia Davidsson in Sweden made a test in 1992 concerning varroa reproduction in different cell sizes. Michelle Taylor in New Zealand made a test in 2002 with the same purpose. Both studies found no difference in mite reproduction ability in different cell sizes. The tests were done with bees born in big cells and with cell sizes of different sizes in the same colonies. There were no colonies "stabilized" on different cell sizes and kept apart. Such studies requires more time of course.

The results don't contradict the experiences of the beekeepers mentioned above, as all of them have experienced high mite populations, at least once, in spite of small cellsize. At first it was suggested that the Africanized bee (AHB) in South America/Mexico was resistant due to low mite fertility. Today the mite fertility in Mexico is as high as in European bees. Still the AHB is resistant. There seems to be other factors that are more important.

Prof. Ingemar Fries made a test 2001-2002 in Sweden concerning the mite popluation increase. 7 colonies each in two groups, one on 5.0 mm cell size, the other on 5.45. The small cell group was downsized during the test period, thus not stabilized before it started. The groups were not kept apart in different apiaries. As far as we know all the queens were not sisters mated the same way. Year two the natural downfall of mites in the small cell group was half of the other in the beginning and middle of season. At the end there was no difference. It seems from the experiences from successful reports of "varroa resistant bees" that keeping other types of cellsize colonies "isolated" from small cell colonies is extremely important to avoid evening out of mite populations and "infestation" by big cell bees through drifting into small cell colonies. Not only that they might bring mites, but those bees may be different enough pheanotypically to influence the total behaviour of a bee colony.

In a survivability test (for selection purpose), which is what resistance is all about (survival), a test group has to be kept so that colonies are allowed to come close to death or die, while they are not "infecting" the other colonies with extra mites then what they produce themselves and with pheanotypically less good bees. Here's the difficult part. To be able to let survivors survive on their own credit, without the negative influence of neighbouring dying or close to dying colonies. If a colony survive in such an environment it's already at the goal, from a selection point of view, being able to stand a high reinfestation and drifting pressure.

The test of Prof. Fries didn't answer the question, if small cell bee colonies have a better survivability against the varroa mite than big cell colonies, also because the test was inhibited before the colonies began to show crashing symptoms..

Downsizing problems

The only problem in connection with small cell-size that is reported is the difficulty to get bees to draw small cell size correctly. This may be the most important reason for a commercial beekeeper not to try to use small cell size. But in a project like we are describing here it's no objection at all. We find that there exist no reason in a project like this to reject to work the bees on small cell size. For scientists, who wants to find out the value, or disvalue, of different parameters, there may exist reasons not to use it. But here we want to make use of all the described experiences to make the best possible set up of steps for obtaining the goal described, for breeders and interested beekeepers. Therefore it's quite evident that we suggest the use of small cell size.

Micro fauna and miticide residues in wax

In Germany they have not had as evident positive experiences from small cell size concerning survivability, as from other places. This is interesting and if this experience is confirmed during the coming years (still those in Germany working with small cell size state that it has evident advantages also concerning survivability). There may be something special in the German environment in comparison with Arizona, Wyoming, Cyprus and Norway.

Remember we are dealing with all these reports we have as if they are true, and no fakes (we can't find any reason why they should be fakes). We have found a couple of possible relevant differences that may help to explain. For 25 years there has been a very efficient and intense use of different chemicals (including more and more acids) in all or almost all the bee colonies in Germany. This has most probably wiped out the micro fauna, that is otherwise present in a normal bee colony. It also has caused accumulation of miticide residues in the wax. The micro fauna in the bee colonies consists of many different insect like organisms. More than 40 it is said. One is recognized to eat fungi mycellium. And who knows what role they all play in the immune system of the bee colony.

Many miticide residues in the wax has negative influences on the nerve system of the bee as many miticides used are nerve posions. We don't know enough of the effects of such residues and eventual synergetic effects with residues from drugs sprayed on crops.

BREEDING METHODS USED

Eric H. Erickson

Eric H Erickson's successful breeding method has been described in for example American and Swedish bee press. He began by searching for bee colonies that survived the varroa mite without treatment better than other bees. One such tests of his own ended with four surviving colonies, which by the way were kept on cellsize 5.1 mm. He were given other colonies. And all those formed the population he began with. An enough isolated area in which these colonies were brought was also an important part. He also kept track of the mite population by the alcohol wash method and took away those that had a higher mite population than what he had set as the upper limit (which was lowered later on). Or he treated those and shifted it's queen. The aim was to let these not so good colonies have no negative influence on the other colonies. But also to let them survive to give a crop as the cooperating beekeeper was a commercial beekeeper, Lenard Hines. In October 2003 Erik Österlund and Hans-Otto Johnsen visited Eric Erickson and Lenard Hines. Hines today no longer use any chemicals against the mite in his 500 colonies. Except in two test apiaries. These apiaries are kept for the Tucson lab to use as they want. In return they breed all the queens he needs. Hines today select the queens from which the lab breed for him. About 30% of the combs in his colonies have cellsize 5.1 mm. It is difficult to know how much they influence the result they have obtained. Today Hines seldom monitor the mite population in his bee colonies.

John Kefuss

John Kefuss in France and Chile uses a similar system as Erickson and Hines. You can say that Kefuss in a way has at least partly confirmed the approach of Erickson and Hines. (Or they have confirmed each other.) Kefuss started out using queens that he thought had a higher resistance than average bees. He used Intermissa queens from North Africa and also one control Carnica queen that showed better resistance (as the Intermissa queens had). He then tested his queens for hygienic behaviour, by freezing a small piece of broodcomb, putting it back and checking the time the bees used to remove the dead brood. In the beginning of his test he also bought mites (!) to quicken up his first tests. He bought brood frames from heavily infested bee colonies and distributed evenly in his test apiary.

He breeds many daughters from the best queens and distribute in his apiaries. Those that survive and perform the best he uses as breeders in coming generations. He doesn't regularly monitor the mite populations in his bees. Like Hines in Arizona he hasn't used any chemicals in his colonies for many

years. The only colony that survived the "live and let die"-test on the island Unije in the Adriatic Sea had a queen from Kefuss. So it survived in spite of a very heavy reinfestation of mites, or a possible reinfestation (It may have kept foreign bees from entering the colony). He doesn't use small cell size.

Kefuss uses the following methods for monitoring mites. A soapy water wash (1 drop liquid detergent in about 500 ml water) is made on a sample of 250 bees or more after counting. The bees are shook in the soapy water, then washed over a double screened honey strainer. Varroa mites are recovered for counting from the bottom strainer. Varroa adults, daughters and immatures are counted in one hundred cells of capped brood (brood has purple eyes and tan colored bodies).

Kirk Webster

Kirk Webster in Vermont saw that the same type of bees lived longer if the brood area was restricted compared to when they had unrestricted brood area. Therefore it was possible to easier observe differences in resistance in such colonies with restricted brood area. He achieved this by a nuc system, in which he is making new nucs with the help of overwintering nucs. And these nucs are not treated with any chemicals (pesticide, acid or oil). The nucs are also kept in apiaries of their own to avoid reinfestation of mites from the big honeyproducing colonies. He also uses an isolated mating station in the mountains. There he places colonies with daughter queens of the best survivor colonies, somewhat more than 20 of different lines/origin. Today his stock consists of about 70% Primorski-heritage. At present he hasn't treated his stock for 2 to 5 years (different groups). He has increased the survival rate in the honeyproduction colonies over winter from 0 (zero) to 30%, in the nucs from a few % to more than 90% in the early made nucs and 60% in the nucs made late in season. The baby nucs have wintered well almost from the beginning without treatment (he treated them just a few years after the arrival of the varroa mite). His cell size has been mostly between 5.2 and 5.3. Now he has invested in a mill producing cell size 5.1 mm. One of the reasons is to avoid pesticide contaminated wax. He doesn't monitor the mite population in any way. And he doesn't prevent any colony from dying (and reinfestate other colonies/nucs with mites).

Alois Wallner

Alois Wallner in Austria uses a quite differnt method than the others. In contrast he uses just one source of bees, the Carnica bee. As he has about 700 colonies it is possible for him to make progress inside his bee population without to much inbreeding effects. As he has so many colonies he

dominates his area and thus has quite some control of the matings for his queens, only due to this. He though also brings his breeder queens to his breeding apiary. And these colonies in this apiary he doesn't treat with anything. The honey producing colonies he treats twice a year against varroa with small amounts of formic acid in late summer (too small amount for susceptible bee colonies). He though plans to stop treating these too. His winter losses are low.

He does monitor the mite population you can say, but in a simple way. He takes random samples of worker pupae, less than 100. If 50% or more of them have mites he treats the colony with formic acid right away (this never happens anymore). A low infestation rate is a selection criteria. The colonies with the lowest percentage of worker pupae infested are probable breeders.

Also he checks natural fallen mites in the bottom debris, less than 100. He checks for damages. Those colonies that have the highest damage % of the mites are most likely to be selected for breeding.

SMR (surpressed mite reproduction) -bees

On pure SMR-bees varroa mites reproduce very little or not at all. Pure SMR-bees are not claimed to be good production bees. The aim with them is to provide beekeepers with a bee to start with in selection for increasing varroa resistant in their bee stock. It's a source better to start with than the average bee. It places you a step on the way. The inbred queens produce colonies that do survive without treatment, but as they are inbred they are unreliable as production queens. And as soon as they have shifted their queen the fertlilty of the mite raises considerably, but is still much lower than in the average bee colonies.

Now this phenomen, low fertility of the mite, is a trait among the "natural" resistant bees mentioned earlier. But it's not the trait that seems to be the most important one (though probably important), among the AHB (Africanized bees). But the SMR-bees do provide you with good traits and can thus be valuable for those doing breeding work, maybe expecially in combination with other bees with other resistant traits.

Also, the selection criteria used by the scientists that have produced this bee, can also be used by other beekeepers/breeders.

1. Find 20 pupae with dark brown/reddish mites.
2. Count how many of these cells with these pupae also contain more mites with lighter colors. These lighter colored mites are offspring of the dark ones. Those colonies with the fewest % of mites with offspring are selected for breeding.

You can also make a still more monitoring by taking a alcohol wash of a little more than 100

bees and count bees and mites found. Also uncap at least 100 worker pupae. Count the mites found. Then you have a ratio for % mites on the bees and % mites in the brood. Those with highest quota for % mites on the bees compared to % mites in brood are selected as breeders (more mites present on the bees, where they don't reproduce, instead of in the brood).

BEE STOCKS DESCRIBED TO HAVE HIGHER THAN AVERAGE RESISTANCE

Besides breeding methods it exists a couple of commercially available bee stocks that are described as having a higher varroa resistance than average bees. As always there are different opinions about bees, also these bees. If they don't fulfil the expectations of the buyers these will not continue to use them. That's a quite obvious "law".

Primorski bees have been imported first to USA and Denmark and there selection and breeding have been done. Primorski bees are now becoming one of the most important bee strains in USA. Many reports of better resistance are seen. Also there are reports of a variation in production ability and management traits of the bee. Of course the longer time is elapsing the more selected the stock will be and the use of the bee is increasing in USA. There are Primorski bees also in Europe, but not in big numbers. In some environments it seems no treatment at all against the varroa mite is needed.

Elgonbees is a combination type of strain, bred according to Buckfast principles since 15 years. The bee is mostly available in Scandinavia, as it is here the breeding work mainly is done. There are a number of beekeepers with this bee that are not using any chemicals against the varroa. It is evident this type of bee is not evenly resistant in the whole strain. Selection is important. Also you make best use of it when no other bees are kept in the same apiary. Even if some individual colonies seem to resist reinfestation, the stock as a whole is susceptible to reinfestation from colonies with high number of mites, especially if placed in the same apiary. Reinfestation of course should be avoided whichever type of bees is used.

BASIC ENVIRONMENTAL CONSIDERATIONS

No chemicals of any kind

To start with it's evident that to help the bees to have as high health status as possible is of utmost importance when selecting bees for anything, especially for resistance against diseases and pests. Therefore to use chemicals of any kind in the bee colony can't help them achieve that, especially

chemicals we know kill them if given too much. A lower amount which don't kill them but the target bug, will most probably more or less though harm the bees and/or their way of functioning. This is the case for every kind of chemical (pesticide, acid or oil) treatment against the varroa mite.

All colonies in the apiary managed the same

Here it's also evident that as all bee colonies in an apiary more or less share their bees, all the bee colonies in an apiary must be managed the same way. If you want control colonies you have to have them somewhere else, the best then of course whole control apiaries. But this is a breeding project with the goal of achieving progress in resistance in a population. This is not a project to find out if any kind of selection method is better than the other or if any kind of bee is better than the other in achieving resistance. These are also good goals, but not for this study. Bees from unselected colonies mingling with our bees will interfere with what we are trying to achieve. Time will tell if this type of work suggested here will result in progress, namely in a longer period without special help from the beekeeper to fight the mite. And finally a bee and management that need no treatment activity against the varroa mite.

Healthy micro fauna

There are many things we know little about in the bee colony. For example the micro fauna. All these, at least 40 is mentioned, small organisms, mites and insects of different kinds that also live in the bee colony beside the honeybees. At least one of them eat fungi mycellium and is said to help keep chalk brood down. How much this microfauna contribute to the varroa and virus resistance we don't know. But it's not unlikely it does in at least some respect. Therefore it's positive if this micro fauna is present in as normal degree as possible. And we know that at least acids and pesticides wipe out the microfauna as well as the target mites, so that's an additional reason not to use these chemicals. But if they have been used in an efficient way for many years one can wonder if there is any microfauna left at all. This may pose a problem in getting as normal an environment as possible. It is probably wise in a breeding project to try to get as many bee colonies to start with which has been treated as little as possible. Maybe get a feral colony or a few from someone that we know haven't used chemicals, at least some colonies, from which bees and combs can be distributed among the bee colonies that will be used to select among. In coming years hopefully the microfauna will get back to normal status.

Pesticide residues in wax

There are few places on earth today that have wax

free of residues of different kinds. Chemical residues influence life in different negative ways. In very small amounts probably in such a small way the bees can handle it if they are strong enough in other traits. Especially difficult to handle and to have knowledge about are the synergetic effects that can take place between different chemicals. The best think we can do is to make efforts to have as clean wax as possible. It may be difficult, but the obvious method is to use but cappings wax for making new wax foundation and renew combs in bee colonies we strongly suspect are contaminated. Another source is wax from areas where miticides are not used. Watch though out for other types of contamination.

Honey as winter food

Some stress the use of honey as winter food instead of sugar. There are also experiences in Varroa infested areas (also in colder climate) of colonies left on their own (no honey taken and no sugar given) that seem to survive much better than otherwise similar colonies. Why not leave as much honey as you find possible for winter food. (There are though examples of certain types of honey that seem to stress the bees quite a bit during winter, some with high ash content, others that form hard crystals.)

Summary of basics

Thus some general things seem to be important to achieve succes:

1. All the colonies in the same apiary are managed the same way.
2. Have the colonies spread out in the apiary as far away from each other as possible to avoid drifting.
3. Don't keep many colonies in an apiary. How many? Maybe 6-12, it's hard to say, but at least few in the beginning of the project when the material is uneven. It's better to use more apiaries with fewer colonies to avoid reinfestation. The probability of many high mite population colonies in an apiary will decrease that way.
4. Also try to make sure that apiaries that may spread mites heavily isn't placed close to your project apiaries.
5. Use no chemical whatsoever in the project. Or at least very little. When used, the goal is not to save a colony as colonies has to be allowed to die, the least good ones, but to hinder reinfestation of the other colonies.
6. If mite populations have to be kept low to hinder reinfestation of other colonies this is possible by other means, for example by removing all capped brood in two stages with for example 9 days in between. Remember this is done not to keep as good a producing colony as possible, but to hinder reinfestation of the other colonies.

7. Make sure that the microfauna is as little disturbed (and maybe helped back if possible) as possible in the bee colony (compare with no 5 above).

8. Use combs which are drawn on wax foundation made from as chemical free wax as possible.

9. Try to leave as much honey as you find possible for winter food. After all that's what the bees "expect" for winter food.

SUMMARY OF THE METHODS USED

The bees

Before starting a breeding project we must decide which bees to use for the project. And this of course involves which types of bees are possible to use. And we can have different idéas that influence the choice.

1. Most of the examples in the documentation involve a mix of different origin of bees. The beekeepers/scientists have in different ways looked for what they think or hope are the most resistant they can get hold of, regardless of their origin. One sort of bee though is evidently avoided, or at least not sought for, the Africanized bee.

2. Wallner in Austria though is working with only the stock present close to him, which is a Carnica (Carniolan) type of bee. Harbo/Harris also choose to work with a local type of bees (but these bees of course are more or less a mix already, but not selected for varroa resistance previously).

The mix-people (group 1) work more with survival of their colonies more than monitoring mite population in them. The non-mix-people (group 2) work more with certain monitoring tests. Both groups have achieved results. We would say that working most with survival of the colonies is what we all want to end up with. It seems it's easier to begin with a hunt for colonies that can be named some sort of survivors to get as good a start as possible of the project. But it seems possible to start with any kind of bee, for example your own local bee (which probably has the advantage of in other respects being adapted to your area). Here a geneticist would tell us though not to start with a bee with too small genetic variation. The genepool need to have enough variation to allow for selection without getting a bee that will be at risk ending up with low vitality and disease problems due to inbreeding.

Non mixed bees

Especially when you start with a pure race or just the stock you have locally without first going for a search of as resistant bees as possible to use in your project, these monitoring techniques used in

the described projects may be important:

Wallner:

1. Check up to 50 worker pupae. Look for mites. Count how many pupae are infested with mites. Divide with the number of pupae checked and get the percentage of the pupae infested. The lower the better for selection. Do it more than once during the season.

2. Check up to 50 (it may be hard to find that many sometimes, and at other times easy) mites and look at them with a magnifying glass (up to 20 x). Count those mites with injuries and divide with the number of mites checked and you get the percentage injured, the Varroa Killer Factor (VKF). Do it more than once during the season.

3. The colonies selected for breeding purposes are enough many to fill an apiary. For many years now this apiary isn't treated with anything. In this apiary the matings of the new queens are achieved.

SMR:

1. Find 20 pupae with dark brown/reddish mites. Count how many of the cells with these pupae also contain more mites, now with lighter colors. These lighter colored mites are offspring of the dark ones. Divide the number of cells with mite offspring with the total number of cells with mites and you get the fertility percentage. Those colonies with the relatively fewest mites (lowest fertility%) with offspring are selected for breeding.

2. Make an alcohol wash of a little more than 100 bees and count bees and mites found. Divide the number of found mites with the number of bees and you get the percentage mites on the bees.

Uncap at least 100 worker pupae. Count the mites found. Divide the number of mites with the number of pupae checked and you get the percentage of mites in brood.

Divide the first % (mites on the bees) with the second % (mites in brood) and you get a ratio for mites on bees and in brood. The colonies with highest figure achieved this way are selected. The longer the mites are on the bees compared to in the brood the slower the reproduction speed.

The alcohol wash can be made in a little bit different ways. One quite easy method is to take a plastic jar (for 500g honey) with wide opening, fill it with 1/3 alcohol, take a comb with bees (the third with bees from one side or the back to avoid the queen and the outermost bees with fewer mites. For the same reason don't take bees close to the entrance.) Scrape bees into the jar to almost fill the alcohol volume. It will be 100-200 bees. Put on the lid and save for later measuring. Shake the jar for a minute before pouring them out on the double sieve. Pour on water heavily from the tap.

The mites stay in the fine mesh part and the bees in the upper big mesh sieve part.

The progress in selection and breeding is secured by instrumental insemination, not in an isolated apiary.

Mixed bees

Erickson, Kefuss and Webster first tried to get hold of better than average bees concerning resistance to start with.

Eric H. Erickson

Erickson received colonies from people that in different ways had found survivors. Also he collected a number of colonies and placed them in an isolated apiary, didn't treat them and followed their progress. The best survivors of those he used further. The origin of the queens was quite local though even if it was varying types of bees.

1. Colonies that probably or hopefully filled their expectations were placed in an isolated area in Arizona. This was well before the arrival of the AHB.

2. The best were bred from and the queens mated in that apiary.

3. Mites were monitored by alcohol wash a number of times throughout the season. A limit for % mites on the bees were decided, first 15% later on 10%.

4. As soon as a mite population came above that limit the colony was taken out of the apiary or treated to lower the mite population. The queen was shifted to a queen bred from a colony with better figures and performance.

5. The effect was that reinfestation of mites was kept to a minimum and matings were achieved with drones from untreated selected colonies.

6. The area with apiaries with selected queens were spread by introducing selected queens in the closest apiaries further and further away around the first apiary.

John Kefuss

Kefuss collected queens from North Africa and from various other sources that seemed probable to have higher resistance. The colonies with these queens were placed in the same apiary which were as isolated as possible.

1. Most control colonies and some test colonies died. The surviving colonies were allowed to supersede their queens.

2. He then performed the hygienic test by freezing a small piece of sealed brood comb, put it back and measured the time until it was cleaned out by the bees. The quickest colonies were selected.

3. Daughter queens were bred and mated in that apiary. They were then spread evenly in apiaries in

which the colonies were not treated.

4. No special monitoring of mites were done, other than by the eye. The health status of the colony was of course judged. The best survivors were brought back to the central apiary.

5. Pollen collection is measured. The best hygienic pollencollectors of the best survivors are selected as breeders.

6. Mite populations are measured and queens are bred from those with lowest mite infestations.

Kirk Webster

Webster uses a three part program:

1. Honeyproducing *colonies* with unrestricted brood area, kept in apiaries of their own.

2. *Nucs* on 4 or 8 frames, overwintered as such and producing brood frames for additional nucs. (part of the nucs replace winterlosses of the honeyproducing colonies), kept in apiaries of their own.

3. *Babynucs* for mating queens, consisting of 4 or 8 babyframes, 232 mm high x 195 mm wide (half Langstroth), kept in apiaries of their own over winter, but at mating station during season though not *very* close to the drone colonies.

He checks survival and overall health status and other normal traits like swarming, temper, honeyproduction and overwintering ability. He breeds from the survivors and the best queens in the best sistergroups. He uses no chemical today. There is no pesticide that has any relevant effect on the mite anymore where he lives. And other types of chemicals are too unreliable he says. He does no monitoring at all of the mite population.

Mixed bees to start with

Primorski bees and Elgon bees do not belong to any special type of selection system. They are mentioned for those that may want to start with bees that are described to have a better varroa resistance than average.

Other bees to start with, are survivors (that may be real survivors concerning varroa, if not new swarms) found in abandoned hives. It may be colonies that have survived in a beekeepers apiary that hasn't been treated against varroa for many years. It may be SMR-queens that you buy, if it's possible where you live, and it may be queens (or breeding material) from John Kefuss in France. It may also be from some other beekeeper that have been working with varroa resistance breeding for some years.

Or you may want to start a project with bees from a specific source due to special reasons, for example to keep a local strain/race of bees. This we can call starting from scratch.

Find out the possibility of making progress

As this project is not describing a commercial outfit, but a varroa resistance breeding project, we will make certain choices in our proposals for the possible tests we describe. As we have described the different ways of how the beekeepers and scientists described work, it's not difficult for anyone to modify according to their own preferences. This project is neither a test for comparing different approaches or finding out if certain parts are true or better than others. We have taken these descriptions for true and use them to form a way to work in achieving varroa resistant bees. Then you can try this out (with eventual modifications of your own) in your own area to see if it will take your bees in the right direction concerning varroa resistance. Our hopes are that it will.

SUGGESTED METHODS

Basics

1. As a small cell size is of no disadvantage for the bees, but maybe though so for the beekeeper who downsizes, it is a basic in a project like this, due to the reports.

2. Make sure, or as sure as possible, that you have a microfauna in your colonies, or try to improve it, by for example get a nontreated colony from somewhere to mix in bees and combs with your bees.

3. Make efforts to use as residue free wax as possible in your combs.

4. All the colonies in an apiary are managed the same way.

5. Place only 6-12 colonies in an apiary to begin with. If you loose all colonies in an apiary due to domino effects you don't loose all and not so many. A project like this can have as many colonies you are able to supply it with, but it is said by Brother Adam once that 100 colonies is a minimum for being able to make progress. But start with as few as you can set aside for this and cooperate and exchange breeding material with others maybe (you have to cooperate if you are small to avoid inbreeding and a lowered immune system just because of the small number). Try to work at least 3 km from other beekeepers, not for the safety of their bees, because you never get any hive out of control, but for the safety of *your* bees.

5. Plan for more than one small apiary (placed "together"), as isolated as possible from other bees.

6. The new queens bred are mated in the center of these project apiaries, or in the center apiary. Instrumental insemination and mating islands can be used sporadically to try to make quick improvements. But there is an important point in using matings like described, to keep the genetic

variation high and thus avoid inbreeding problems and health problems related to that. Also drones flying freely from untreated colonies may well be an advantage to get the best drones to mate with the queens concerning varroa resistance. If you get occasional wrong matings it will delay the progress but little, if progress is achieved.

The start

You can start from *scratch* (1) or you can start somewhat *on the way* (2), two different choices.

1. You may start with a pure race or local unselected bees.
2. You may start with a collection and mix of bees that hopefully are more resistant than average bees.

Scratch bees

You can *choose* between all the methods used by Alois Wallner (two methods), the SMR-team (two other methods), the hygienic test (which Kefuss uses and a premature uncapping test, or use them all. The easiest are the two Wallner, the first SMR-method and the premature uncapping test.

1. Check up to 50 worker pupae. Look for mites. Count how many pupae are infested with mites. Divide with the number of pupae checked and get the percentage of the pupae infested. the lower the better for selection. Do it more than once during the season.

2. Check up to 50 (it may be hard to find that many sometimes, and at other times easy) mites and look at them with a magnifying glass (up to 20 x). Count those mites with injuries and divide the number checked and you get the percentage injured, the Varroa Killer Factor (VKF). Do it more than once during the season.

3. Find 20 worker pupae with dark brown/reddish mites. Count how many of the cells with these pupae also contain more mites, now with lighter colors. These lighter colored mites are offspring of the dark ones. Divide the number of cells with mite offspring with the total number of cells with mites and you get the fertility percentage. Those colonies with the relatively fewest mites (lowest fertility%) with offspring are selected for breeding.

4. Pinkill 100 capped brood in a rhomb with each side consisting of 10 cells (easier than freezing a piece of comb and put it back for removal of dead brood). Use a very thin needle to get just a small puncture hole. Check every 12 hours for removal of dead brood. The quickest colonies are selected as breeders.

5. (Maybe an alternative hygienic test.) At the beginning and end of brood periods, often spring and late summer (not common in peak of brood or honeyflow) check debris for "natural" fallen

mites. Count a number of mites, the more the better. Count not only dark, but lighter brownish and whitish (including smaller types). These can be sign of cleaning out mites from brood, they are immature. (premature uncapping). Divide the number of lighter and whitish with the darker. The highest figures are selected for breeders.

The second SMR-method, checking for the ratio of mites on bees and in brood is a bit more complicated, but a good method. Description is given earlier. Pinkilling hygienic test is described more in depth elsewhere.

"On the way" and scratch bees

In the beginning of the project always keep the brood area restricted to 4 or 8, or 5 or 10 frames, depending on the box used. Divide a normal brood box in two halves and let the nucs reside in there. When the nuc gets too strong, make more nucs. Place new nucs in apiaries with only new nucs. If you get too many nucs, sell some. *If you are quite sure your bees show good resistance use production apiaries as described below already from the start.*

Winter the nucs as nucs/small colonies. You can stack them on each other two or three high and pack them two stacks (or more) together (in northern climates). Let them keep as much honey as you find possible for winter. Be quick in spring just before cleansing flight and move them apart to avoid drifting during cleansing flight.

The nucs will live longer and give you better chance to see difference in varroa resistance between the colonies, than big production colonies with unrestricted brood area. Breed of course from the best.

If or when nucs show bad unhealthy varroasis symptoms, take away all capped brood and destroy in two stages with for example 9 days in between. Shift the queen as soon as possible. If a nuc is too small to winter, combine it with another with a sister queen. There's no need to find the queen then.

"On the way" bees (the scratch bees may well be on the way too now)

When the survival rate of the nucs over winter are at least 70% two year in a row, divide your bees in nuc apiaries and honey production apiaries. One production apiary to begin with. Make the center apiary a production apiary.

Keep the nuc apiaries as above.

Keep production apiaries as normal apiaries. Do the Wallner, SMR and hygienic-checks if you find that suitable. Though pure survival tests become more important now when you are running production apiaries.

You can choose to monitor mite levels by alcohol wash method in the production apiaries, at

least the first year(s) three times a season with at least three weeks in between. Find out a limit for mite population, maybe 15%, maybe 20% to begin with, 10% later on. When mite level is above your limit level, remove all capped brood in two stages with 9 days in between. Shift queen as soon as possible. This is done mainly to hinder reinfestation of other colonies, not to keep the colony alive, which it though may well do. Don't use drone brood removal as a regular method as this is blurring the colony's own resistant ability (and may select for mites preferring worker brood). When needed remove *all* capped brood twice as described. On the contrary you maybe can allow up to 10% of drone wax on a few combs to give the mites "playroom" thus damaging worker pupae less, for example when many winterbees are born, and to supply with drones for matings.

Most important, keep track of the overall health status of the colony. A few wingless bees in times with little brood is not an enough bad sign. Also then check the colony strenght, the harmony of the colony, other eventual virus symptoms. And if premature uncapping of capped brood with mites occur (this is mostly seen as a positive sign). Occasionally many mites in the debris *may* be a positive sign of cleaning out mites, if there are high % injured mites and lighter colored mites.

If a colony, regardless of the mite level, show an unhealthy status with declining bee strength, wingless bees (more than just a few), the colony sound when opening the hive is not an even low buzz, but staccato sounds, cloudy wings maybe on the bees, maybe many crawling bees outside the entrance. Consider killing such a colony at once to avoid reinfestation of the others. If symptoms are not very bad but bad enough take away all capped brood in two stages and destroy (put in a freezer overnight then in a big colony somewhere to remove the dead brood, you may need to save the small cell sized combs if they are well drawn).

- Again, remember that in a project like this the goal is not to keep all colonies alive, it is to eliminate the bad ones, in one way or the other.

- The best production colonies concerning resistance and production are moved to the center apiary. The new queens are mainly mated here.

A SIMPLE RECIPE

The experiences looked into by us are of such a character that some may want to try a simple test like the following.

1. Get your bees down on a smaller cell size 5.1 mm (5 cells to the inch) and/or 4.9 mm on as clean wax as possible.
2. Use whatever bees you choose.
3. Have no other colonies than the above in the apiary.

4. Place the apiary fairly isolated. Maybe at least 3-5 km (2-3 miles) from other bees are enough?
5. Monitor mite amount in the colonies three times per season. When mite amount is above 15% on the bees make divides of the colony- and requeen with offspring of more resistant colonies. Make divides anyhow from the least good ones and requeen. If some colonies are looking bad due to varroaosis take away all capped brood twice with about a week in between. If really bad remove the colony.
6. Let the young queens mate in this fairly isolated apiary, preferably in small baby nucs as experience seems to indicate that matings take place closer to the home apiary then.
6. Combine too small colonies in autumn
7. Let the bees keep as much honey as you find possible for winter food.
8. Exchange breeding material with others working the same way.
9. As progress takes place widen the area with other apiaries kept the same way.

AT HOME

When the survival rate of the production colonies is 70% or more two years in a row, you're almost "at home". Now you can make the nuc division as big as is suitable for you by other reasons than keep the bees alive long enough to be able to make selection for the best survivors. Also you can place your new splits wherever you want. Life is (almost) back to normal. Now the goal is to make the area wider with survivor bees.

We find it urgent that these methods and strategies described here are evaluated as soon as possible, for the benefit of all beekeepers and the bees themselves.

CONCLUSION

It exists claims, and documents with experiences and descriptions of these claims, of total and close to total success in obtaining bees that don't need any form of treatment activity against the mite Varroa destructor. Due to this we formed the hypothesis that it is possible to obtain such bees for every beekeeper that wants it and is able to do the work needed.

The goal for this study is to present ways to confirm or falsify the hypothesis.

With the help of the tests that have been suggested, inspired by these experiences and descriptions, we find that it would be possible to confirm or falsify the hypothesis to be valid also in other areas of the world than where the experiences are made. •

Comments to the preliminary report

ERIC ERICKSON:

First, the authors are to be congratulated and commended for having compiled a comprehensive document detailing all aspects of the problems of producing and maintaining Varroa resistant honey bees. It was a monumental undertaking since it required a survey of all pertinent scientific literature worldwide.

My comments below reflect my attempt to put myself in the shoes of the average beekeeper wanting to acquire and maintain Varroa resistant stock..

- A. I think that something like the following should be clearly spelled out for beekeepers.

Basically there are two ways to acquire Varroa resistant stock.

1. Import resistant stock developed elsewhere. The principle limitation here is that this stock may not be well adapted to the local environment.
2. Breed for Varroa resistance out of locally adapted stock. The principal limitation here is the level of beekeeping skill possessed by the beekeeper(s).

Both ways are susceptible to dilution over time (~1+ years) depending upon the genetics of susceptibility of feral and managed colonies within the mating range of supercedure queens from the resistant colonies. This dilution will lead to failures.

Managed susceptible colonies maintained via chemical treatment will produce drones that will quickly compromise efforts to maintain resistant stock via matings between 'resistant' queens and 'susceptible' drones.

Feral colonies in the area can provide similar compromise, but to a much lesser extent since they are

already somewhat Varroa resistant. Our research has shown that feral colonies are not as resistant as our colonies specifically bred for resistance. (I do not see that you have cited this work - I think that it would be helpful and should be included in the section on reinvasion)

- B. I am assuming that the intent is to put the final report in the hands of beekeepers for their use. Based on this assumption I believe that the structure of the report will likely intimidate the average beekeeper. I think that the last section "Concept for obtaining..." should be placed at the beginning of the document. This, the "how to" part, will be of primary interest and should be simplified in a 'recipe' format. The balance will serve as references for further reading for those who are inclined to do so. Thus, I would divide the references up into two major categories as in A – 1 and 2 above using the other headings as you have them. This will allow beekeepers to move directly to their areas of major interest. Of course the section on the immune system will have to stand alone. Similarly, discussion of viruses needs to stand alone. While we know little about the subject of viruses, we do know that they exist and may be transmitted by Varroa. However, when breeding for resistance it needs to be pointed out that we do not know whether genetic resistance is to Varroa, viruses or both. Which ever, breeding does work.

Finally, I would add that I presume that the final draft will be structurally uniform.

I hope that I have been of service.

Dr. Eric Erickson, Tucson, AZ, USA

JAMES FRAZIER:

I am happy to offer an ad hoc review of your Introductory study for breeding varroa resistant bees. I am Professor of Entomology at Penn State, the husband of Maryann Frazier, and an insect physiologist with specialization in chemical ecology, behavior, and neurophysiology of insects. My wife and I have done some studies of solitary bees together, but she has the majority of direct experiences with honeybees and mites.

As a complex problem with worldwide scope and emerging stories of successes, your collection of literature both scientific and popular into one easily accessible document is a real contribution to the field. Having such a wide spectrum of information in one cover certainly promotes a systematic evaluation of the many suggested successes and helps to promote a standardized source of information for people to reference when dealing with tests or quoting information found in these accounts. The publication of this booklet will be of significant benefit in promoting testing of the suggested hypotheses and save much time for those interested in solving this problem.

Your efforts to extract the major points of each method to show success is also helpful to those wanting to test these ideas themselves. The summaries are quite accurate and will save additional time and promote the testing of ideas, which as you state is the main goal of the project. Placing this at the end makes it easily referenced as well.

You begin the book by offering some scientific articles on honeybee immunity. These are of course not the only scientific articles, and a helpful addition might be the names or organizations where people can look for new information coming out in the future. A list of scientific journals where such new information is likely to be found would also be helpful. While the understanding of insect immunity is an expanding area of current research, I must offer an objection to the use of the term “immunity” for all levels of honeybee defenses from the innate hemolymph based immunity for which the term is correctly used, to the behavioral resistance of hygienic behavior and on to include the microfauna and other genetic based

differences among colonies. While collectively these factors all may contribute to a reduced level of mites, I would not favor referring to these as colony immunity.

The genetic differences that occur among bee populations worldwide and the selection of more resistant individuals, regardless of the mode of resistance are an important part of the different studies and methodologies presented in your collection of documents. What is missing from many of these reports are detailed records of what has actually been done. In your manual section at the end, you do a nice job of presenting summaries of the various methods and ideas, but do not actually encourage the experimenting beekeepers to keep very detailed and thorough notes of their studies. You suggest some different fairly standardized methods for evaluating hygienic behavior in hives, but don't actually say how the data should be kept or what other variables should be accounted for in the record keeping. Any additional directions you can offer the readers for standardizing their methods and keeping accurate records so that others may question what they have done and perhaps eliminate some confounding variables in the interpretations may make each persons efforts more valuable overall.

I commend you and your collaborators on this effort, and hope that you will find these comments useful for the final product.

James L. Frazier
Professor of Entomology, PA, USA

MARYANN FRAZIER:

As a senior extension associate with primary responsibilities in the area of apiculture I found this work very useful. There are three main aspects of this publication that make it particularly valuable: the fact that it consolidates two areas (small cell size and breeding for resistance) of the non-chemical effort to combat Varroa destructor in one location, it does so on a world-wide basis, and while the summary is the cornerstone of the publication, including the original articles allows the reader to have access to the details of the original work. It is also produced in such a way that it gives beekeepers and/or breeders first, hope that it is possible to control varroa without the use of chemical pesticides and second, some tools and concepts that should allow them to effectively reduce mite levels and even successfully control varroa without the use of chemicals. It does seem that the title of the document is a bit narrow considering the scope of its content. The use of smaller cell-size is not really a breeding technique, but is obviously a very worthwhile technique for reducing varroa mite populations. Perhaps the title of the publication could be changed to reflect its broader nature. Would it be possible to include abstracts of the non-English articles? While I think the information from most of these must be included in the summary, I found myself searching for more details concerning individual articles mentioned or going through the document wishing I had a summary of a particularly interesting-looking article. Some specific comments

The possible lack of micro fauna is given as a possible explanation for the lack of positive experience concerning survival of colonies with small cell size in Germany. It is suggested that the lack of micro fauna might be explained by the use of chemicals for the past 25 years. But is it not reasonable that the use of chemicals over such a long period of time could have other negative impacts that could result in the reduced survival (in colonies with smaller cell-size compared to "normal" cell-size colonies)? For instance, couldn't the accumulation of these chemicals in the wax be having a negative impact on the physiology and even the behavior of the developing, and perhaps even adult bees

leading to a lack of survival? The use of the term nymph (English) typically refers to the immature stage of insects with incomplete metamorphosis. Rather than nymph, larva is used to describe the immature (feeding) stage of insects that have complete metamorphosis. In some places (summary), the sentence structure made the document a bit difficult to understand. Thank you for giving me the opportunity to review this important document and I look forward to the final work. –

Maryann Frazier
Senior Extension Associate
University Park, PA 16802, USA

SEPPO KORPELA:

For some time ago I received from you a booklet "Introductory study for breeding varroa-resistant bees". I have browsed through it but not read every page. Most of the articles included I have read before. I, however, read the introduction and the last chapter "Concept for obtaining...". My comments are quite brief. I think that the methods and principles in developing the varroa tolerance become clear by reading the articles included and especially the technical "guidelines" should be helpful for every beekeeper interested in seeking bee strains more tolerant to varroa than the present stock. For instance, the requirements for the testing are well written. I could add to this point that keeping control colonies and the colonies to be tested in the same apiary could really produce wrong results, as the mite numbers can equalize very rapidly. In our nordic project paper on varroa population dynamics (Korpela, Aarhus, Fries & Hansen 1992, *Journal of Apicultural Research* 31: 157-164) we write, in results: "After treating five colonies of group 1 in autumn 1990, Apistan treatment killed equal numbers of mites in the remaining colonies in autumn 1991, both in treated colonies (n=5) and in untreated colonies (n=8): 6401+-1178 (mean +- s.e.) and 5733 +- 820 mites/colony, respectively. In discussion we write: "After treating five colonies of group 1 in autumn

1990, the mite populations in treated and untreated colonies equalize during late summer and autumn 1991 probably because of drifting and robbing as suggested by Sakofski et al. (1990), Buchler and Hoffmann (1991) and Greatti et al. (1992). This result stresses the importance of treating all colonies of an apiary simultaneously.”

I only have one note on the english usage: the plural of “pupa” is “pupae”, not “puppea”.

Seppo Korpela
MTT, Kasvinsuojelu, Jokioinen, Finland

DEE LUSBY:

Been through the small cell book you sent titled: ”Introductionary Study for Breeding Varroaresistant Bees” and find it a very broad and impartial representation of what has been happening with everyone’s thoughts protrayed. It also shows small cell for usage is maybe more widespread then many think for beekeepers looking into this chain of thought for keeping honeybees naturally sized, rather than isolated as many try to make out. Also that the trend is growing. While I don’t agree with some of the points of view as presented, I do agree with the majority as presented, knowing that perhaps differences in our multi-regions far apart from each other, and traditional ways of working bees we each have been individually taught for field management, could be a part of this different point of view for reaching solutions to problems of mites and accompanying secondary diseases. I wholeheartedly feel that the end solution will involve industry wide regression back down to more naturally sized broodcombs, which will then set the stage for better breeding parameters for development of localized strains, and more healthy diet to include more varied propolis sources again for disease control, as more honeybee workers raised in a more compact broodnest, will, by better division of labor, be able to do more tasks necessary to the healthful maintenance of their colonies.

Dee A. Lusby, Commercial Beekeeper, Tucson, Arizona, USA

DORIAN PRITCHARD:

Thank you for showing me your research proposal. I can see you have put a lot of work into it. I have only looked at the papers in English, but I have a few suggestions which I hope are not so obvious that they are not worth making.

I understand the plan is to test a strategy that you hope eventually to recommend to beekeepers in general to help them create a situation in which varroa presents few problems. The people who will assess the final proposal will be experts in the field so you can assume they already know most of the background or can look up any issues with which they are unfamiliar from the references to give. So the overall recommendation is: **keep it brief.**

It seems to me that you have two main ideas here **which need to be treated separately** or the effects of one will undermine your findings on the other. One concerns the development of a strain of bee with a genetically based inbuilt resistance to varroa; the other with sorting out the issue relating to cell size.

1. Cell size.

The findings on cell size are inconsistent. This is possibly because experiments have been carried out on different strains of bee, but also because some observations are anecdotal while others have been subjected to statistical analysis. Statistical analysis is of course meant to remove subjective factors, but it assumes that the population is uniform, so meaningful variation may be swept aside by the statistician in the need to average data from different stocks. What appears to be a more rigorous analysis may have negated real and important variation that may be persuasive to the hands-on operative. On the other hand, the hands-on operative can have an emotional investment in a certain outcome, especially if initial observation seems to favour that outcome.

So one recommendation would be that you identify a fairly uniform stock of bees, perhaps the predominant stock native to your area and repeat the most interesting experiments using different foundations with those bees. This may result in smaller bees and different stocks of

bees may vary in this response. The capacity to change size would be inherited, but not the newly acquired small body size itself. You might find it worthwhile breeding from those stocks that respond best to this treatment, but at the early stages I think you should not confuse this selection with the main one dealt with below. Small size may eventually become entrained by consistent use of small scale foundation; but I think the bees would eventually revert to the size normal for that strain of bees if no foundation were used.

You state that small body size is no disadvantage to bees, but I think you are wrong so far as northern regions are concerned. Bergman's Rule points out that animal species in general, including mankind, get larger the further north you go. This is generally considered to relate to body heat retention, as the ratio of surface area to volume decreases with increasing overall body size. The natural body size for a region should be the most favourable so far as heat retention is concerned, so I question the idea of enforcing smaller body size.

2. Genetic selection experiment.

To have any hope of maintaining a genetically selected strain in a free-mating situation it must be of similar genetic type to the majority strain in that region, otherwise all the good work you do in setting up matings and selecting progeny can be undone rapidly by a few uncontrollable, bad matings. Local *A.m.mellifera* bees have already been selected to survive all local hazards. Native (*mellifera*) bees from other localities may be useful as contributors of genetic material and should be much more suitable than non-native bees such as *A.m.carnica*, *ligustica*, Buckfast, etc.

Another alternative would be to go for an exotic or artificial strain and resign yourself to maintaining it by instrumental insemination. If you work with something like the local bee you should improve the survival of your neighbours' bees as well as your own, as the "good genes" you propagate should spread outwards; but if you use an exotic strain you will help destroy your neighbours' bees by spreading maladaptive genes and also create a zone of aggressive inter-racial hybrids all around you. It would be

extremely difficult to create an artificial strain like Buckfast but if you did the hybrids created by your drones mating with local queens could cause havoc throughout the neighbourhood.

Choosing the local native strain should be acceptable to local beekeepers, would make your research proposal special to your specific area and should appeal to grant awarding bodies, as the conservationists are currently very conscious of the dangers of genetic contamination of native species. So I strongly recommend you work with a bee that is close to native. If you need to introduce a valuable allele (an "allele" is a version of a gene) into your stock from an exotic strain, the most acceptable way would be to send your virgins away to be mated at the distant apiary. This avoids contaminating your home area with maladaptive alleles from foreign drones (in the first generation). You should then concentrate on eliminating possible maladaptive foreign alleles from the descendants, while retaining the specific few beneficial ones. You would need to take special care that the drones produced by subsequent generations of an exotically mated queen do not escape.

One of your ideas that appeals very much to me is that of having your best stocks (i.e. the ones that show greatest resistance to varroa) at a central location, surrounded by others of intermediate resistance. If newly discovered stocks with some resistance are placed in the outer zone they should contribute to the overall gene pool and as superior stocks arise in the outer zone these could be moved towards the centre. I would expect colonies at the centre eventually to acquire resistance based on several favourable characteristics and the assembly of inherited resistance alleles should then spread outwards.

For genetic selection to work you will need to make sure the stocks under selection are actually exposed now and again to varroa mites. If they have greater resistance than average, but are still likely to succumb you could save them by any of several methods, but I think using small-scale foundation would confuse the picture. It would be valuable to have a test apiary within a varroa-infested region to which selected stocks could be transferred for testing.

Selection for specific attributes or for overall survival?

On this point I suggest that you do both: start off by checking that specific attributes you have already identified in parent stocks are actually being taken up by progeny colonies, but always bear in mind that there may be favourable characteristics you have not yet identified. As favourable factors accumulate in your best colonies you may be able to ignore how they actually work, so long as the bees are surviving and not providing a reservoir for reinfection.

I would add the proviso though, that your work will be recognised in a more favourable fashion if you document its progress and report any findings that might explain the bees' resistance. Indeed will probably be required to write reports as a condition of the grant. You also need to identify a comparator control population in a region similar to your own and to record and compare some aspect of varroa infestation and colony survival there with that in your experimental stocks. Alternatively you could monitor the hoped-for progressive improvement in your own stocks as time progresses. If you can do both that would be excellent.

“Scratch” or “on the way” colonies?

If I were you I would found my work on the best local bees available as judged by conventional criteria, and introduce stocks that show some varroa resistance into their vicinity. Put extra drone comb in the latter and if there are no bees locally with any degree of resistance I would send a few virgin queens away to be mated by *mellifera* drones elsewhere in Northern Europe where there are *mellifera* bees that seem to be resistant. If this fails, i.e. your bees do not improve their resistance, you might have to think about sending your queens to more distant apiaries. However, the further away you send them, the greater the work you will have to put in subsequently to eliminate unwanted, undesirable alleles from their descendants.

I hope these suggestions are of use and I wish you all the very best with the project.

*Dr. Dorian Pritchard
Newcastle upon Tyne, UK*

JOB VAN PRAAGH:

Thanks for the book, it gave me an amount of pleasant reading hours. It is a very profound basis to give bee breeders and beekeepers the power and the knowledge, that they should start to select!! themselves. The data you give them are sufficient to allow them to find their own way. As we all know, beekeepers are very individualistically tuned!! They work (nearly all of them) alone on/with their bees. So we can be sure, that the actual genetical variety we have in the populations will stay. Each beekeeper looking for what he thinks is important.

What I missed a bit is the paper by Lodesani, Crailheim and Moritz. And some older works from Kirchhain, both making clear that you must be careful, because of the fact that less Varroas might also mean less bee brood over the season, and as a consequence you might get colonies with lesser “body” to resist bad periods, bad seasons!!

This fact, turned around in a certain way, covers the observations made, that colonies that live in an area with very good pollen supply over the whole season give the impression to be better survivors!!

The EC money was used for a very good purpose!!

With my compliments to Tore Forsman, Per Idestrom and Erik Osterlund for what you did!!

*Prof. Dr. Job van Praagh,
BI-Celle, BRD*

THOMAS RINDERER:

I looked over your report and found it to be very good. I really have no suggestions for its improvement.

Dr. Thomas Rinderer, USDA, USA

MARLA SPIVAK:

I did look through it all, and it is a very interesting collection of research and experiences. My personal feeling (based on our research, and other people's research) is this: Bees of European origin are, in general, highly susceptible to mites. For them to resist mites, they need multiple mechanisms of resistance. The most important mechanism of resistance seems to be the number of viable (=mated) female offspring a mother mite can produce in worker brood. Each mite can lay 4-6 female eggs on a worker pupa, but only 1-2 of the daughters actually mature and mate before the bee emerges from the cell as an adult. Any reduction of this number, for example, instead of producing from 1-2 viable female offspring, if they produced 0.5-1.2 viable offspring, gives the bees a considerable advantage, and slows the reproductive growth of the mites considerably. It is possible to breed bees that reduce the reproductive potential of the mite in this way -- the SMR trait will do this. Please note, that number of viable female offspring is different than mite fertility per se (mite fertility is how many offspring total the mite will produce in one cell), and it is different than mite fecundity (% infestation of the worker brood). We do not know how the SMR trait works -- that is, we do not know HOW the bees reduce the reproductive potential of the mite. My guess is that it is a nutritional problem -- or possibly a chemical difference, where the mite does not obtain sufficient nutrients for reproduction, or the bee larva does not "smell right" and so the mite is not stimulated to lay eggs as quickly -- but it is something physiological.

It is possible, although I have not seen any good research on this, that cell size would reduce the number of total offspring the mite produces (fertility), and possibly the number of viable female offspring. You (or someone) would have to inspect 30 infested cells per colony and count the number of viable offspring (the number of deutonymphs on tan colored pupae -- following the research of Martin, 1994) to know this for sure. It would be a great thing to do!!

Hygienic behavior also helps the bees resist the

mites -- in fact, the SMR bees in the US are also hygienic. Somehow, inadvertently, John Harbo's SMR line is also hygienic, although he didn't select for hygienic behavior at all. But hygienic behavior alone is not the answer. It just helps.. and definitely helps with disease resistance.

Another very important trait is grooming.. number of mutilated mites that fall to a sticky board within 24-48 hours.

It seems from your report that you favor the cell size method for selecting bees for resistance. I will say that the "jury is still out" from my perspective. As for the Lusby's experience: I have not seen any confirmation that the Lusby's bees are actually European bees (not Africanized bees, which are very resistant to the mites on their own.. how? they produce fewer viable female offspring, and are better at grooming).

Anyway, that is my opinion!

Dr. Marla Spivak
University of Minnesota, USA

Some of the subjects brought up in the comments to the preliminary report

We have chosen to make an overview of some of the points in the comments that we find we can add something to, and do so below.

1. Clarifying of the two different sources of bees that can be used when starting a breeding work for Varroa resistance, local or already selected stock. (Erickson, Pritchard)
2. Pointing out the importance of maintaining the obtained result by keeping the area in which virgin queens will mate with desirable drones. (Erickson, Pritchard)
3. Suggestions on the article about how to obtain varroa resistant bees and where it should be placed in the work. (Erickson, Frazier J, Pritchard)
4. The term immunity when used together with bees. (Frazier J)
5. The importance of keeping records and the character of these. (Frazier J, van Praagh)
6. Cellsize (Frazier M, Lusby, Pritchard, Spivak)
7. Non-English articles (Frazier M)
8. Micro fauna (Frazier M)
9. Accumulation of chemicals in wax (Frazier M)
10. Control colonies in the same apiary as test colonies (Korpela)
11. Hygienic behaviour and SMR (Spivak)
12. Maintaining a population (Erickson, Pritchard)

As Marla Spivak points out in her comment, our bees need multiple mechanisms of resistance. Her mentioning of SMR (low or no reproduction of mites in especially worker bee cells) and hygienic behaviour (cleaning out of mites produced, either before they emerge from the cells or from fellow bees) are really complementary characteristics.

One characteristic of many of the experiences is that selection is not made on any one special trait, but on the total survivability of the bee colony. Surely selection is then made on many different traits combined, probably different combinations at different places. A common trait seems to be of hygienic character,

premature uncapping of pupae and cleaning out of such brood infested with mites.

As environment influences performance of the bee colony, a stock doing well in certain respects in one area may not do as well in another area, also concerning varroa resistance. But that of course can be tested also. A main concern with keeping bees is the production of honey. It's therefore of importance to watch out so that better resistance is not the result of low production of brood, which will result in a low bee population and small honey crop. Therefore an important selection criteria of course is not only to survive the Varroa mite, but also to produce honey (as it always is). We can also here mention easy managing of the bees and a good temper. The bees inclination of collecting pollen can here also be of importance to keep a good nutrition standard and high level of the immune system.

A subject of controvers is the cell size in the worker brood area. Some don't think it should be mentioned in a work on selection resistance, while others think it's an important basic consideration. Yes, to use or not to use smaller cell size than what's usual today may have little to do with selection. But it seems it has everything to do with the environment of the bees. And environment is important, that's what every scientist says in this matter. And as many such reports are given that using small cell size in the brood area gives bees with other characteristics than otherwise we find it important to include these experiences here. After all, all historical documents we can find which discriminates between brood and honey area and what was in use in older days points to the fact that smaller cell size in the brood area than what is used today was the norm 100 years ago. And we can find no work showing that enlarged worker brood cells is of no harm or give better performance of the bee colony. As then these good experiences of small cell size do exist and the fact that small cell size is from the beginning more normal for the bee and its environment, we find we would be of no excuse

if we didn't include it here. But of course, it's up to you who will do the actual tests to choose to include it or not.

It is clear from the documents (we treat all of them as true until otherwise is shown, which we have seen no sign of) that it is possible to select for varroa resistance without using small cell size. John Kefuss, Kirk Webster and Alois Wallner are examples of this. Concerning Eric Erickson a minor part of the combs contain smaller cell size than normal of today.

The experiences of those using small cell size (when saying here "using", it means using it in whole apiaries for a couple of years at least) are quite sensational actually, even though problems in the beginning using it is reported. The most sensational are those experiences of Dennis Murrell in Wyoming, USA and Roger White on Cyprus. They have bees surviving and producing crops without any form of treatment AND without any form of selection of the bees. Controversial to say the least. So if their experiences could be repeated by others not much of selection is needed for varroa resistance, but maybe for ability to draw small cell size foundation correct. That doesn't mean that it's easy to get the wanted results with small cell size. Changing to small cell size involve a lot of work too. Maybe a selection for varroa resistance makes our bee survive better while we change our wax (if we want to). We live in a reality world where reinfestation occurs, sometimes quite much. Therefore it's of great advantage to select bees that can handle this. Again, it's you who will make the tests who choose the set up of your test.

Going back to small cell size involves a lot of follow up questions, which also may influence how you consider it. A question that could be normal in the future when discussing research is on what type of bees the research is done, on bees born in small sized cells or in larger cells. To explain the experiences with small cell sized bee colonies (we consider them true) it seems necessary to consider different phenotypes (with resulting somewhat different traits) of the bees as a result of them being born in smaller or bigger cells.

As Seppo Korpela points out it's important to manage all bee colonies in a whole apiary alike. The drifting of bees and robbing occurring, which get a new dimension when mites are involved; you evidently get an evening out of mite populations, or anyway change, that you have little control over. Also an evening out of bees with different qualities between colonies takes place. The apiary may be treated more or less like a unit, thus the importance of alike management and use of bee type and/or cell size. This may be an help to explain why certain tests do not give results in line with experiences given in documents in this work. To keep control colonies in the same apiary as test colonies is quite common still. You have to consider this fact when judging the results of tests. With control colonies that produces many mites in a test apiary you may not see the colonies that would have survived on their own. But you may see colonies that can thrive in spite of a high mite population which is the result of mites coming from neighbouring colonies, or colonies that effectively keep bees with mites on them from entering the hive. We judge it probable that you would loose less colonies and will see differences in varroa resistance easier if susceptible colonies are not allowed to produce high mite populations.

When making selection and getting your new queens mated it is also of great importance, to avoid "reinvansion" of wrong genes through not wanted drones. This is stressed by several comments. This can be solved by using a central area for mating purpose and surrounding area with apiaries of colonies with queens mated in the central area and the use of instrumental insemination or isolated mating stations, such as islands.

To be able to choose between colonies which to breed from, to make selection, you have to have made notes of some kind, brief or more detailed. We who have done this study are used to make a lot of notes, necessary and certainly many times unnecessary. With some frustration we see that many of those giving their experiences of success do very little of notes. The main concern is survivability. Has the colony survived or has it died? Is it producing a good crop? Is it easy to handle?

Kirk Webster is an example of this “method”. He simply has to work this way as he is working alone with limited amount of time to his disposal and make his living of his bees and their products. Erickson and Kefuss keep track of the varroa population by counting mites on the bees, not just one time during the season, but several times. Kefuss is also counting mite infestation in brood. Erickson put a limit for mite percentage on bees above which the queen was shifted to daughters from colonies with low number of mites. This limit together with the performance of the bee colony (not showing varroa and/or virus effects and giving a crop) is the selection criteria for Erickson. Alois Wallner uses somewhat different methods, the percentage of infested worker brood and the so called Varroa killer factor, the percentage of damaged mites in the natural downfall. Those with the best figures are chosen for breeders. Today those breeders are kept in a apiary of its own and they are not treated with any chemical of any kind.

The importance of the micro fauna is of course speculative. Plants can grow in completely “dead” soil without any micro life like for example earth worms, when given fertilizers and sprayed against bugs. But we do know that the micro life is beneficial. If our bees need every little help they can get, the micro fauna may well help. The Russian investigation points to importance concerning resistance to chalkbrood, so why not also in other respects. But Maryann Frazier makes a good point when she suggests that the problems in Germany may well be due to accumulation of chemical residues in the wax in the colonies. As clean wax as possible is certainly essential, avoiding nerve poison residues from the drugs against mites. Such residues may give big problems, especially if synergetic effects takes place with for example residues from drugs used for spraying plant crops or seeds.

To maintain the results achieved are of outmost importance. To do that plans have to be made to keep a resistant stock formed from genetic dilution and to further develop it. This is valid wheather you start with a single geographic race or a population of mixed origin. The result will ultimately be a stock that will be more

and more uniform and adapted to the local environment as it is further selected. This is also a threat in a longer perspective, in that too much inbreeding may occur and vitality lost, also concerning the resistance obtained, as inbreeding and lost vitality often give increased susceptibility to diseases. In the long perspective some sort of interchange of genetics may be needed with other stocks kept and selected in a similar way.

We would have liked to make abstracts in English of articles in non-English languages. The time at our disposal didn't allow us to this. But the articles in English are give though enough explanation of the different experiences to be able for English speaking readers to get the essentials of what is discussed in this study (for those that have access to the preliminary report).

At last, it has been very inspiring doing this study. It has given us much hope and we are convinced that beekeeping will be back to normal, not everywhere at once, but increasingly, at least as far as concerns the Varroa mite.

Tore Forsman, Per Idestrom and Erik Österlund

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- Graph, Fig 1, from Rinderer, Thomas E., de Guzman, Lilia I., Delatte, G.T., Stelzer, J.A., Lancaster, V.A., Kusnetsov, V., Beaman, L., Watts, R., Harris, J.W., *Resistance to the parasitic mite Varroa destructor in honey bees from far-eastern Russia*, *Apidologie*, 2001, vol 32, p 381-394. Showing decline of amount of mites in Primorsky

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Basic environmental management influence on the honey bee colony.

Cell size.

"Ferals"

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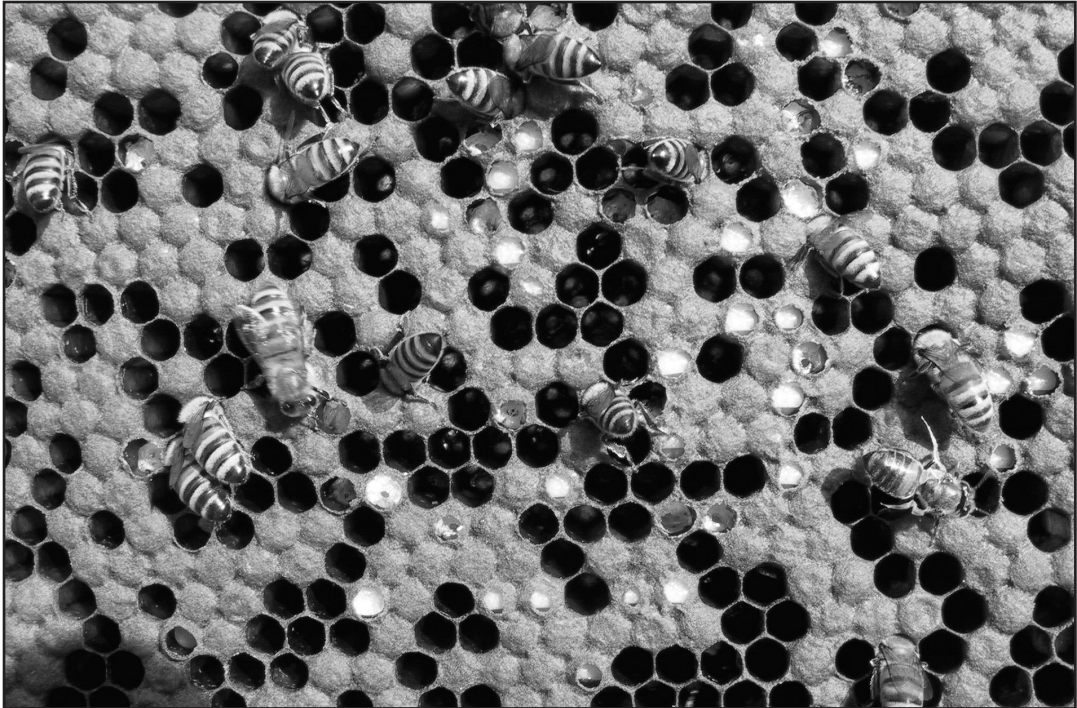
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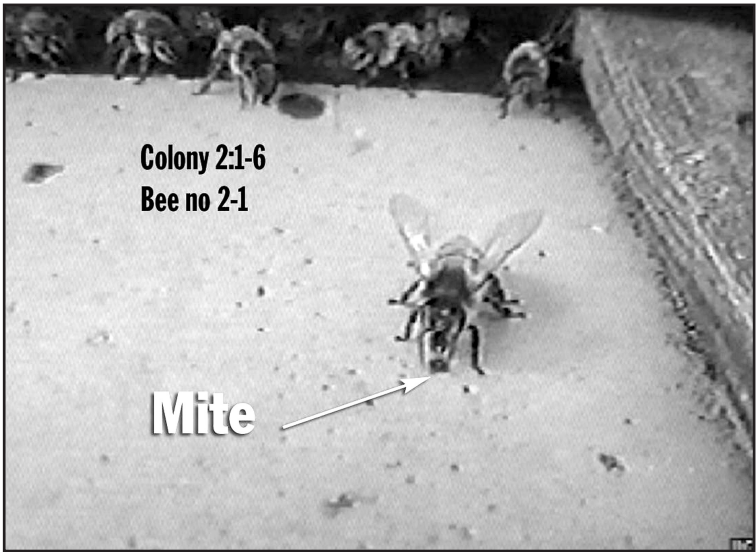
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Two good traits for varroaresistance



Premature uncapping and chewing out of brood with mites at the end of season in a survivor colony in Arizona.



Mitebiter in a survivor colony in Finland.