



Review Article

The Downside of Selection: A Forgotten Cause of Honeybee Decline

Jacques J.M. van Alphen

Abstract

This article explains how resistance alleles have disappeared from honeybee populations in Europe and North America. Honeybees have the highest recombination frequency of any animal, suggesting that pathogenic bacteria, viruses, fungi and microsporidia are an important source of selection. To respond to new virulent strains of pathogens, honeybees need access to rare alleles that could confer immunity to a new pathogen. By mating in a large panmictic population, new rare alleles can be recruited, which can then be combined into new genotypes through recombination with useful alleles of other genes. Selection for desirable traits typically involves taking a small sample from a larger population. As a result, rare alleles are under-sampled and disappear from the selected population as selection continues. In addition, selection for polygenic behavioural traits leads to runs of homozygosity and hampers the role of recombination in creating new genotypes. Restoring large panmictic populations of native subspecies of honeybees can provide a reservoir from which lost alleles can be recovered.

Keywords: Honeybees; Genetic recombination; Panmictic; Loss of genetic variation; Resistance against pathogens; Natural selection

Introduction

More than 40 years after its invasion of Western Europe and North America, the Varroa mite and associated viruses are still causing high mortality in bees, despite chemical treatments. This raises the question of why natural selection has not been able to solve the problem. The question is made all the more compelling by the fact that natural selection quickly solved the problem in South Africa and South America, but not in the northern hemisphere. The Varroa mite was first identified in South Africa in 1997. Mike Allsopp (1) studied the spread of the mite. As a result, we now have detailed knowledge of the early years of the Varroa invasion in South Africa (2). The Varroa mite spread rapidly, reaching high densities of up to 50,000 mites per colony, but after a few years the infection rate declined rapidly until, after 3 to 5 years for *A.m. capensis* and 5 to 7 years for *A.m. scutellata*, the Varroa was, in Allsopp's words, "no more than a random presence". The high rate of infection with the mites, often without colony mortality, and later research on Deformed Wing Virus (3), show that bees in South Africa had rapidly developed resistance to the DW virus. In South America, African honeybees of the subspecies *A. m. scutellata* were imported from South Africa and Tanzania to Brazil to improve honey production in tropical South America. They escaped from an experimental apiary and hybridised with European bees already present (4) and the hybrids spread throughout South America, colonising Central America and the southern United States. Soon after the discovery of Varroa in 1979, the levels of infestation detected became a source of concern for Brazilian

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apiculture, although there were no reports of colony losses (5). It soon became clear that Africanised bees could survive Varroa infestation without treatment (6, 7, 8, 9, 10). Rapid natural selection seems to have led to increased resistance and treatment against Varroa is generally not practised. Losses of Africanized honeybee colonies due to varroosis are not reported and possible negative effects on honey production appear to be negligible (5). This is surprising because viruses associated with Varroa, such as Deformed Wing Virus, are widespread in South America (11, 12, 13), and Africanized bees are not resistant to the virus (14, 15), although in one study the rate of virus increase was lower in Africanized bees than in European bees (16). The Africanised bee is now the most common breed of honeybee in Brazil.

An important factor in the rapid evolution of Varroa resistance was the huge number of feral colonies of Africanised honeybees in Brazil. Even in natural rainforest ecosystems where there is no beekeeping, the honey bee is the dominant pollinator. The colonies managed by beekeepers therefore represent only a small percentage of the Brazilian honeybee population. The wild honeybee population is under constant selection for Varroa resistance. In Mexico, the Africanised honeybee was first introduced in 1992 and was found to be resistant to Varroa in 1994. At first, I thought the answer to the question of why natural selection had failed to solve the Varroa problem in Europe and North America was rather trivial. After all, both South Africa and South America have high densities of wild honeybees, and domestic bees are not as intensively managed as in the north. Almost all beekeepers' interventions in colonies inhibit or prevent natural selection. There are also far fewer wild bee populations in Europe and much of North America. These differences between the southern and northern hemispheres could explain the differences in the evolution of resistance to Varroa. However, this does not seem to be the whole story. The Swedish researcher Ingmar Fries (17,18) decided to investigate in a large-scale experiment whether natural selection would lead to resistance to Varroa and related viruses when beekeepers no longer intervened in the fate of the colonies. He collected 150 colonies, both Buckfast, *ligustica* and *carnica* to ensure the greatest possible diversity, and placed them on an isolated peninsula on the south side of Gotland. The colonies were then infected with Varroa and left largely to their own devices. The results of the experiment were spectacular, but not as expected. During the four years of the experiment, 38 swarms were produced. But the mortality rate was so high that only 13 colonies remained. The high mortality and the observation that the remaining colonies did not carry the Varroa-specific Hygienic Behaviour (VHS) alleles suggested that these alleles were not present in the populations from which the 150 colonies were derived. The 13 colonies were found to be highly inbred and remained small. They were not resistant to

Varroa but were Varroa tolerant, at least in the short Swedish season. When these bees were moved elsewhere, their survival was no better than that of unselected bees (19). However, the colonies appeared to be resistant to DW virus. In the more than 40 years that Varroa has been present in Western Europe, resistance to DW virus has only been found once elsewhere, apart from the Gotland experiment: in Belgium (20,21). Alleles for DW virus resistance are therefore extremely rare in breeding bees. The absence, or at least extreme rarity, of such alleles makes resistance to Varroa by natural selection very difficult in European commercial *ligustica*, *carnica* and Buckfast colonies, as the Fries experiment shows. The native black honeybee, which has been less subject to selection by beekeepers due to its long-standing unpopularity, appears to be in a slightly better position. At the start of a Varroa resistance selection programme of bees from the Mellifica group around Chimay, Belgium, 18 out of 32 colonies showed no signs of resistance. Of the remaining 14 colonies, most had high VSH expression. The number of colonies without VSH expression is too high for a polygenic trait like VSH. This means that the alleles for VSH-related resistance must also have been lost from some black bee colonies. This article explains how resistance alleles have disappeared from honey bee populations in Europe (and probably North America). To do this, we first need to look at the weapons that bees have developed through natural selection against infectious diseases.

How bees defend themselves against infectious diseases

Because of their social lifestyle, honeybees appear to be more susceptible to infectious diseases than many other animals. The high nest temperature, the many contacts between bees in a hive, and the frequent exchange of food mean that pathogens must spread rapidly in a hive. The first line of defence is the antibiotics in the plant resins that bees collect to make propolis. A natural bee nest is surrounded by a coat of propolis. As nectar and pollen also contain antiseptic and antibiotic substances, this food is also a medicine chest. The honeybee's main weapon against infectious diseases and last line of defence is its immune system. Bees are attacked by a wide range of bacteria, fungi, microsporidia and viruses. All of these have short generation times and populations many times larger than those of bees. As a result, they often produce new mutants, some of which can evade existing immune responses. The question is how bees can protect themselves against new variants of pathogens.

Reassortment of rare defence alleles and development of new resistant genotypes

Honeybees are able to defend themselves against new pathogens because they are part of a very large population, which is a reservoir of often rare gene variants. The following

describes how bees can recruit these rare genetic variants and use them in new defences. In addition, honeybees have very efficient ways of combining such variants with other genes to create new resistant genotypes. These capabilities are related to two unusual features of honey bees, polyandry and an exceptionally high rate of genetic recombination.

Mating behaviour

Natural selection has shaped the mating behaviour of bees. Drones and queens meet in drone congregation areas under favourable weather conditions. Emmanuelle Baudry (22) investigated where the drones in such gatherings come from. She found that drones from up to 240 different colonies were present at the congregation area she studied, and that up to 12,000 drones were present. The Ruttner brothers (23) showed that drones from one colony visit several drone congregation areas. Annette Jensen (24) showed that the drones a queen mates with can come from up to 15 km away from the queen's nest. This evidence shows that, under natural conditions, bees are part of a very large panmictic population (a panmictic population is one in which every individual has an equal chance of mating with every other individual). In a large healthy population of bees, there are several variants of most genes, called alleles, and some genes can have as many as 37 alleles (25). Some alleles are common, while others are less common or even rare. In small populations, rare alleles are often lost by chance if they are not passed on to the next generation. In large panmictic populations, rare alleles can be conserved for a very long time. Common alleles have become common through natural selection. They are therefore likely to be important for the vitality of the population at that time. Alleles become rare at a time when they no longer confer a selective advantage. For alleles of genes involved in the immune system, a rare allele may become important again to fight a new pathogen. The behaviour of mating with many males is called polyandry. In honeybees, there is extreme polyandry: young queens mate with up to 10 to 20 different drones, and they use the sperm of all these drones to fertilise their eggs. This ensures that the workers in a population are the offspring of many different fathers and therefore have different genetic traits. The resulting variation is one of the factors that must limit the spread of pathogens in a population.

Making new genotypes

The second weapon bees have in the fight against new pathogens is the ability to create new genotypes. This happens during the production of egg cells: cells with two sets of chromosomes (diploid cells) then produce haploid egg cells (with one set of chromosomes). During this process, the corresponding chromosomes inherited from the queen's two parents end up side by side. They break at certain points and the fragments are swapped between the chromosomes. This creates new combinations of alleles. This process is called

recombination and occurs in all organisms that reproduce sexually.

The extreme recombination frequency of honey bees

No animal species has a higher frequency of genetic recombination than the honeybee. Recombination in honeybees is more than 10 times higher than in mammals (26, 27, 28). This raises the question of what this high frequency is good for. Recombination frequency has been shown to be a heritable trait that is subject to natural selection. It allows favourable alleles of different genes to be brought together on the same chromosome and be inherited together. Since it can also happen that an existing good combination is actually broken by recombination, there is an optimal recombination frequency. Akira Sasaki and Yoh Iwasa (29) investigated how the optimal recombination frequency depends on the strength of selection by pathogens. They found that recombination frequency increases as the threat of pathogens increases. From this we can conclude that honeybees are more threatened by pathogens than other animals. This is exactly what we expected from their lifestyle.

A double-edged sword

The panmictic population structure and extremely high recombination frequency are a double-edged sword in the honeybee's fight against new bacterial or viral infections. Through mating, new rare alleles can be recruited, which can then be combined with useful alleles from other genes to form new genotypes. In this way, honeybees may be able to compensate for slower reproduction and lower population densities compared to bacteria and viruses.

The artificial selection of breed bees

Armed with this understanding of how a natural population of honey bees can develop resistance to a new pathogen, we can look at what goes wrong when selecting breed honey bees. Traits that are particularly desirable to beekeepers include increased honey yield, reduced aggression, calm behaviour, reduced tendency to swarm and hygienic behaviour, all of which are polygenic behavioural traits. Professional beekeepers often work with a number of different selection lines, which can later be combined to ensure hereditary variation. Selection for desirable traits is quite possible. We are all familiar with examples of highly selected Carnica or Buckfast colonies that meet desired traits. However, strict selection comes at a price: reduced resistance to new diseases.

The loss of rare alleles

Selection for desirable traits usually involves taking a small sample from a large population. For example, to increase honey yield, queens are propagated from colonies that already have a higher honey yield than the other colonies

in the population. This process is then repeated with the colonies of those queens, and so on for several generations. Because alleles of genes are not all equally common, the probability of an allele being included in selection is not the same for each allele. Common alleles are more likely to be included in the sample than rare ones. As a result, the rare alleles will disappear as selection continues. This applies not only to the alleles of genes involved in the traits being selected for, but also to the rare alleles of all other genes.

Runs of Homozygosity

Behavioural traits are usually influenced by many (i.e. >100) different genes, they are so-called polygenic. Many of the genes involved in a behavioural trait are recessive and are only expressed when homozygous. Selection for such traits therefore results in homozygosity for a large number of genes involved in the trait. Neighbouring genes may also become homozygous if they are linked to selected behavioural genes. Selection for desired traits in honeybees results in homozygous regions called "runs of homozygosity" (30). Recombination does not lead to new gene combinations in a homozygous region. Thus, homozygosity inhibits the role of recombination in creating new genotypes and so affects the evolution of resistance against new pathogens.

Undesirable mating techniques

To maintain the selected characteristics of breed bees, it is necessary that young queens mate with drones of the same breed. Breed bees cannot therefore be part of a panmictic population, and the limited size of the population at the mating station means that rare alleles can still be lost by chance. Many of the techniques used by beekeepers are also based on small samples of a much larger population. For example, the breeding of queens from a small number of young larvae produced by a single queen and transferred to queen cells, or the use of artificial insemination, sometimes using seed from a single drone. (SDI, or single drone insemination) are used to study heritability in honeybees. This technique is not intended for selection as it leads to very rapid loss of rare alleles. Many bee breeds have been bred in this undesirable way for decades. The result is bees that have the traits desired by the beekeeper but lack the genetic variation to respond to new pathogens or new variants of existing pathogens. In fact, Themudo et al (31) found that genetic variation in European honeybees declined during the 20th century.

Other costs and benefits of selection

When Anthony Nearman and Dennis van Engelsdorp (32) studied the lifespan of worker bees under different feeding regimes, they were surprised to find that the bees in the experiment lived much shorter than expected. They then searched the literature for similar experiments in the past and

found 64 experiments conducted in the USA over a period of 50 years. Taken together, these experiments showed that the lifespan of worker bees in the US has decreased since the 1970s, from an average of 34.3 days to 17.7 days. They attributed the difference to the Varroa mite and its associated viruses, and the negative effects of chemical control of Varroa. However, in Europe, a population of bees exposed to Varroa and pesticides, but not selected for high honey yield, had a lifespan comparable to that of 50 years ago (33). Therefore, an alternative hypothesis is more likely, i.e. that selection for higher honey production has led to a faster metabolism, which reduces the lifespan of bees. Nearman and van Engelsdorp (32) studied the effects of shorter lifespan on honey production. The model they used predicts that honey yield decreases with the lifespan of adult workers. Data on honey production in the USA show that honey yield has declined since the 1970s. This evidence suggests that selection for higher honey production in the US has, paradoxically, resulted in a decrease in honey production and shows that selection for desirable traits has a cost. Faster metabolism also makes winter survival more difficult and may therefore contribute to winter mortality of breeding bees.

The drone plague

If the loss of the genetic variation that could make bees resistant to diseases were limited to race bees, the damage would be acceptable. Unfortunately, in Western Europe, wild honey bees have become very rare, so that the wild populations can no longer function as reservoirs of hereditary variation. Keepers of breed bees send their virgin queens to a mating station but simply let all the drones they produce fly free. As a result, in Europe, the native black honey bee's survival is threatened by genetic contamination. The massive invasion of exotic drones repeated year after year also hinders natural selection for resistance to bacteria and viruses in the free mating bee population. This is because the exotic drones have lost the alleles that could have provided resistance. Keepers of breed bees should prevent their drones from mating uncontrollably

Heterozygosity is not a good measure of healthy bees

Heterozygosity *per se* is sometimes considered a good measure of a colony's "health", but this is not so. To explain why, we use the example reported by Hassett et al. (25) of a gene with 37 alleles in the population of Irish black bees. Now suppose that 27 rare alleles are lost through selection and the 10 that remain all occur at a frequency of 0.1. Then only one in 10 drones has the same allele as the queen. The probability is then 1/10 that a fertilised egg in that situation is homozygous, so the probability of heterozygosity is then 0.9. That is a high value, while 73% (27/37 * 100) of the alleles are lost during selection!

Why bees are dying

In Europe, winter mortality in 2023 and 2024 of honeybee colonies exceeded by far the 10 % that is considered normal. There is no doubt that the mortality of honey bees is largely due to the use of insecticides such as neonicotinoids, as well as other pesticides such as fungicides (34). Herbicides have an indirect negative effect on bees because their application has led to the disappearance of flowering herbs in fields and pastures. But bees are also dying because large panmictic honeybee populations no longer exist and because the alleles needed for resistance to new diseases, such as the varroa mite and associated viruses, have largely disappeared as a result of selection by beekeepers.

A solution

If beekeepers kept only the native subspecies, it would be possible to restore the panmictic population structure needed to keep bees resilient to new diseases. Then hobby beekeepers would have to let their bees make natural nuptial flights

Moreover, if we can ensure that the native honey bees return to our forests as wild species, we will have not only the bees of hobby beekeepers but also the wild bees that will then be part of a large panmictic population. Professional beekeepers can then use bees selected for the traits desired for their profession, as long as they use selections from the native subspecies. If the selected bees of the professional beekeepers become vulnerable to diseases, they can recover the lost alleles from the panmictic population.

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