Review

Why Honey Bees Are Dying: A Forgotten Cause

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Why Honey Bees Are Dying: A Forgotten Cause

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Abstract: This article explains how resistance alleles have become rare or have disappeared from honey bee populations in Europe (and probably also in North America). Honeybees have the highest recombination frequency of all animals, indicating that pathogenic bacteria, viruses, fungi and microsporidia are an important source of selection. To respond to new virulent strains of pathogens, honeybees need to have access to rare alleles that could foster immunity against 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 with continued selection. In addition, selection for polygenic behavioural traits results in runs of homozygosity and hampers the role of recombination in the creation of 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 among bees, despite chemical treatment. This raises the question why natural selection has not resulted in Europe-wide resistance against the mite of most managed bee colonies. The question is even more compelling by the fact that natural selection has quickly resulted in countrywide resistance in South Africa and South America, but not so in the Northern Hemisphere.

In 1997, the Varroa mite was first identified in South Africa. Mike Allsopp (1) investigated 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 and reached high densities, up to 50,000 mites per bee colony, but already 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 an arbitrary presence". The high infection rate with the mites, without often leading to mortality of a colony and later research on the Deformed Wing Virus (3) , show that bees in South Africa had also quickly developed resistance to the DW virus. In addition, it has been found that in a population of honeybees under natural selection, the DW virus evolves a lower virulence (4)

In South America African *A. m. scutellata* and *A.m. adamsoni* bees were imported from South Africa and Tanzania into Brazil to improve honey production in tropical South America. They escaped from an experimental apiary and hybridized with European bees already present (5) .The hybrids rapidly spread in South America and colonized Central America and the southern United States. Soon after the discovery of Varroa in 1979, the levels of infestations detected were a source of concern for Brazilian apiculture, although there were no reports of bee colony deaths (6). It soon became clear that Africanized bees can survive Varroa infestation without treatment (7, 8, 9, 10, 11). Rapid natural selection seems to have resulted in increased resistance and treatment against Varroa is generally not practiced. Losses of Africanized honey bee colonies due to varroosis are not reported and possible negative effects on honey production seem to be negligible (12). This is surprising, as viruses associated with Varroa, like Deformed Wing Virus are widespread in South America (13, 14, 15) and Africanized bees are not resistant against the virus (16, 17), although in one study, the rate of

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virus increase was lower in Africanized bees than in European bees (18). The Africanized bee is now the common race of honeybee in Brazil.

An important prerequisite for the rapid evolution of Varroa resistance was the enormous number of feral colonies of Africanized honey bees in Brazil. Even in natural rainforest ecosystems without any beekeeping activities, the honey bee is the predominant pollinator. Thus, colonies managed by beekeepers represent only a small percentage of Brazil's honey bee population. The feral honey bee population is permanently exposed to selection for Varroa resistance. In Mexico, the Africanized honeybee was established for the first time in 1992 and was found to be resistant against Varroa already in 1994 (18).

The main trait providing resistance of honeybees against Varroa mites is Varroa specific hygiene (19), also called Varroa sensitive hygiene by American researchers (VSH) (20). The behaviour is based on the recognition of closed worker cells that contain reproducing Varroa mites. Bees exhibiting VSH remove the wax cover from infested cells and remove the infested pupae. VHS has been found in all populations of Varroa resistant bees. A second behavioural trait involved in resistance against Varroa is grooming to remove and kill adult mites form adult worker bees. Both auto- grooming and allo-grooming play a role. Although other traits may be involved (5), VSH and grooming to remove mites from adult bees are the traits always found in Varroa resistant populations (5, 21). These traits are also involved in the resistance to Varroa of the original host, *A. cerana* (5). Also, in the latter species there are other traits involved. Together they make reproduction by Varroa in worker cells of *A.cerana* impossible.

Initially, I thought the answer to the question why natural selection has not been able 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 honey bees, and kept bees are not as intensively managed there as in the North. Almost all interventions by beekeepers in bee colonies inhibit or prevent natural selection. Moreover, there are far fewer wild bee populations in Europe and large parts of North America. These differences between the southern and northern hemispheres could explain the differences in the evolution of resistance to Varroa.

Yet that does not appear to be the whole story. The Swedish researcher Ingmar Fries (22, 23) decided to investigate in a large experiment the question of whether natural selection would lead to resistance to Varroa and associated viruses when beekeepers no longer intervened in the fate of bee colonies. He collected 150 bee colonies, both Buckfast, *ligustica* and *carnica* to ensure the greatest possible diversity and placed them on an isolated peninsula at the south side of Gotland. The colonies were then infected with Varroa and otherwise left largely to their own devices. The results of the experiment were spectacular but not as expected. In the four years the experiment lasted, 38 swarms were produced. But mortality was so high that eventually only 13 colonies remained. The high mortality and the observation that no alleles for Varroa specific hygienic behaviour were present in the remaining colonies suggests that these alleles also did not occur in the 150 colonies at the start of the experiment and because that is a large sample, that there are very few alleles for VSH in the Swedish population. There was also no evidence of grooming against Varroa in the surviving colonies (24). The surviving population was found to be severely inbred and colonies remained small. They were not resistant to Varroa, but were Varroa tolerant, at least in the short Swedish season, the surviving colonies seemed to have the capacity to partly suppress the reproduction of the mites (25). Female mites in surviving colonies produced fewer offspring than mites in control colonies. The proportion of females that did not reproduce was much larger than in control colonies (26). One might ask if the mites in the surviving colonies had also become inbred, and if the reduced fecundity was a mite trait and not a trait of the bees, but Fries and Bommarco (27) showed that the reduced fecundity occurred irrespective of the origin of the mites. Therefore, it is the bees that limit the reproduction of the mites. The colonies did appear to be more tolerant of DW virus (28)

When these bees were moved elsewhere, their survival was no better than that of unselected bees (29)..

In the more than 40 years of Varroa's occurrence in Western Europe, increased tolerance to DW virus has only been found a few times (29 - 31). Thus, alleles for DW virus resistance are also

 doi:10.20944/preprints202310.1544.v1

extremely rare in breed bees in Europe. The absence or, at least the extreme rarity of such alleles makes of resistance to Varroa by natural selection in European commercial colonies of *ligustica*, *carnica* and Buckfast very difficult, as Fries' experiment shows.

The native black honey bee, which has been less exposed to selection by beekeepers due to its long-term unpopularity, seems 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, 14 out of 32 colonies had a high VSH expression, but 18 showed no sign of resistance. The number of colonies without VSH expression seems too high for a polygenic trait such as VSH and suggests that the alleles for VSH related resistance have been lost from some black bee apiaries.

This article explains how resistance alleles have become rare or disappeared from honey bee populations in Europe (and probably also in North America). To do so, we must first look at the weapons that bees have developed through natural selection against infectious diseases.

How bees defend themselves against infectious diseases

Due to their social lifestyle, honey bees would seem to be more vulnerable to infectious diseases than many other animals. The high nest temperature, the many contacts between bees in a hive and the frequent exchanges of food mean that pathogens must spread quickly in a hive. The first line of defence is that of antibiotics in plant resins collected by the bees to make propolis. A natural bee's nest is surrounded by an envelope of propolis (21). Since nectar and pollen also contain antiseptic and antibiotic substances, this food is at the same time a medicine chest. The main weapon of honey bees against infectious disease and the last line of defence is the bees' immune system.

Bees are attacked by a variety of species 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 escape existing immune responses. The question is therefore how bees can arm themselves against new variants of pathogens.

Re-assortment of rare defence alleles and development of new resistant genotypes

Honey bees can defend themselves against new pathogens by virtue of being members of a very large population, which is a reservoir of, often rare, variants of genes. The following paragraphs describe how bees can recruit those rare hereditary variants and bring them to bear in new defence. Moreover, honey bees have very efficient methods of combining such variants with other genes, into new resistant genotypes. These capacities relate to two unusual features of honey bees, polyandry and an exceptionally high rate of genetic recombination

Population Structure

Natural selection has shaped the mating behaviour of bees. Drones and queens meet in drone assemblies during favourable weather conditions. Emmanuelle Baudry (32) investigated where the drones in such assemblies come from. The meeting site she investigated was found to be populated by drones from as many as 240 different colonies and as many as 12,000 drones were present. The Ruttner brothers (33) showed that the drones from one colony visit different drones' meeting places. Annette Jensen (34) showed that the drones a queen mates with can come from as far as 15 km from the queen's nest. This shows that alleles can travel far in natural honeybee populations, but most matings between queens and drones come from animals that travel less far: Ninety percent of the matings occurred within a distance of 7.5 km, and fifty percent within 2.5 km. The 10 % matings between bees that live more than 7,5 km apart, are important for access to rare variants of genes. The 90% that mates with partners from shorter distances allow local selection and adaptation to occur.

This evidence shows that under natural conditions, bees are part of a very large panmictic population (A panmictic population is one in which each individual has an equal chance of mating with every other individual). In a large healthy bee population, there are several variants of most genes, called alleles, and some genes can have as many as 37 alleles (35). Some alleles are common, while others are less common or downright rare.

 doi:10.20944/preprints202310.1544.v1

In small populations, rare alleles are often lost by chance, when 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. Therefore, they are probably important for population vitality at this time. Alleles become rare during a period when they provide no selective advantage. When it comes to alleles of genes involved in the immune system, a rare allele may again become important to fight a new pathogen. An example of a rare allele that confers tolerance to Varroa and became almost fixated by natural selection was found in the surviving colonies of the Gotland experiment (25)

Mating and swarming

The behaviour of mating with many males is called polyandry. In honey bees, there is extreme polyandry: young queens mate with as many as 10 to 20 different drones. The sperm is mixed well in the spermatheca and randomly used. Hence queens use sperm from all those drones to fertilise their eggs (36). This ensures that the workers in a colony are the children of many different fathers and thus differ in hereditary traits. The consequent variation is one of the factors that must limit the spread of pathogens in a population.

Honeybee colonies reproduce by fission. Honeybees are exceptional in that the old queen leaves the colony with the first, and often only swarm. A daughter queen takes over the old nest, with the result that the genetic composition of the colony changes for a large part. The young queen carries 50 % of the old queen's genes, but she mates with a large number of drones, which then changes the fathers of the workers. During the presence of the old queen, the genetic composition of the bee colony does not change. However, the pathogens, having large populations and very short generation times, can evolve and become more virulent during this period. Selection within a colony over such a relatively short time period favours more virulent pathogens as they have more offspring than less virulent pathogens. When the old queen leaves and is replaced by a young mated queen, new alleles arrive in the colony, possibly providing resistance to the pathogens present in the nest. The old queen migrates to a new environment and so escapes from a large fraction of the pathogens. Within colony selection for increased virulence of pathogens does not contradict the theory that vertical transmission favours the evolution of a-virulence whereas horizontal transmission (the spread of disease among unrelated individuals) favours the evolution of virulence (37). Selection for decreased virulence occurs over a longer timespan by inter-colony selection: colonies with highly virulent pathogens will die, while colonies with less virulent pathogens have a high probability to survive. This form of selection is stronger than the short time intra-colony selection for increased virulence.

Making new genotypes

The second weapon bees have in the fight against new pathogens is the ability to make new genotypes. This happens during the production of oocytes: cells with two sets of chromosomes (diploid cells) then produce the haploid (with one set of chromosomes) oocytes. In that process, the corresponding chromosomes inherited from the queen's two parents end up next to each other. They break in certain places and the fragments are exchanged between the chromosomes. In this way, new combinations of alleles are then created. The process is called recombination and occurs in all organisms that reproduce sexually.

The extreme recombination frequency of honey bees

No animal species has such a high genetic recombination frequency as the honeybee, although other eusocial insects have also high recombination rates. Recombination in honey bees happens more than 10 times more often than in mammals (38-40). This raises the question of what this high frequency is good for. Recombination frequency has been shown to be a heritable trait, exposed to natural selection. It allows favourable alleles of different genes to be brought together on the same chromosome, enabling them to be inherited together (41). Since it can also happen that an existing

 doi:10.20944/preprints202310.1544.v1

good combination is actually broken by recombination, an optimal recombination frequency exists. Akira Sasaki and Yoh Iwasa (42) investigated how the optimal recombination frequency depends on the strength of selection by pathogens. They found that recombination frequency increases with increasing threat by pathogens. Based on this, we can conclude that honey bees are more threatened by pathogens than other animals. And that is exactly what we expected based on their lifestyle.

A double-edged sword

The panmictic population structure and extremely high recombination frequency combine to form a double-edged sword in honey bees' fight against new bacterial or viral infections. Through mating behaviour, new rare alleles can be recruited, which can then be combined into new genotypes through recombination with useful alleles of other genes.

In this way, honey bees can probably compensate for slower reproduction and lower population densities compared to those of 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 investigate what goes wrong in the breeding of breed bees. Traits particularly desired by beekeepers are higher honey yield, lower aggression, calm behaviour, reduced tendency to swarm and hygienic behaviour, which are all polygenic behavioural traits.

Professional beekeepers often work with a number of different selection lines, which can then 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. But strict selection comes at a price: that being reduced resistance to new diseases.

The loss of rare alleles

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

This procedure is then repeated with the colonies of the grown queens, and this is repeated for several more generations. Because alleles of genes are not all equally common, the probability of an allele being included in selection is not the same for every allele. Common alleles are more likely to be included in the sample than rare ones. As a result, the rare alleles disappear with continued selection. 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

As a rule, behavioural traits are 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. Thus, selection for such traits results in homozygosity of a large number of genes involved in the trait. Neighbouring genes may also become homozygous when they lift with selected behavioural genes. Selection for desired traits in honeybees leads to homozygous regions, called “runs of homozygosity” (43). In a homozygous region, recombination does not result in new gene combinations. Thus, homozygosity hampers the role of recombination in the creation of new genotypes.

Undesirable mating techniques

To preserve the selected characteristics of breed bees, it is necessary for young queens to mate with drones of the same breed. For this reason, breed bees cannot 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.

 doi:10.20944/preprints202310.1544.v1

Many of the techniques used by beekeepers are also based on small samples taken from a much larger population. For example, the cultivation of queens from a small number of young larvae produced by just one queen and transferred into queen cells, or the use of artificial insemination, sometimes with the semen of only a single drone (SDI, or single drone insemination). SDI is a tool to study heritability in honey bees. This technique is not intended for selection, as it leads to very rapid loss of rare alleles. Many breed bees have been bred in such undesirable ways for decades. The result is bees that, while having the characteristics desired by the beekeeper, no longer have the genetic variation to respond to new pathogens or new variants of pathogens already present.

Other costs of selection

When Anthony Nearman and Dennis van Engelsdorp (43) repeated an experiment from the 1970s, they discovered to their surprise that the median lifespan of honey 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, there is an alternative hypothesis: i.e. that selection for higher honey yield has led to a faster metabolism, which reduces the bees' lifespans. A faster metabolism also makes winter survival more difficult and could therefore contribute to winter mortality.

The drone plague

If the loss of the hereditary variation that could make bees resistant to diseases were limited to breed 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, 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, viruses and other pathogens in the free mating bee population. This is because the exotic drones have lost the alleles that could have provided resistance.

The repeated invasion of exotic drones from breed bee colonies thus is instrumental in rarefying resistance alleles. When resistance genes are rare, the panmictic mating structure of honey bees may prevent local natural selection for resistance, because resistance genes disperse into neighbouring populations at a rate that could be higher or equal to the local rate of recruitment of these genes by selection. Therefore, 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. (34) of a gene with 37 alleles in a 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! The example is a bit misleading, as it considers only one locus and the probability that it becomes heterozygous. The message, that guarding against homozygosity does not prevent the loss of rare alleles, also holds if one considers a large number of genes. When a breeder is using a number of different selection lines to maintain heterozygosity while selecting for a desired trait, rare alleles will be lost in each of the lines.

Why bees are dying

In Europe, winter mortality in the spring of 2023 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 (45), as well as other pesticides such as fungicides. Herbicides

have an indirect negative effect on bees because their application has led to the disappearance of flowering herbs in fields and pastures. In addition, modern agriculture has resulted in habitat loss by changing the landscape: loss of hedges, increasing field size and fragmentation of habitats suitable for pollinating insects. But managed 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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