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Jon Swenson and Bjørn Dahle

Resistance mechanisms towards the ectoparasitic mite *Varroa destructor* in two naturally-surviving honey bee (*Apis mellifera*) populations from Scandinavia

Julia Celine Cuypers
General Ecology
Faculty of Environmental Sciences and Natural Resource Management

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1 Abstract

The invasion of the ectoparasitic mite *Varroa destructor* has caused massive problems for the apicultural industry in Europe and North America. The varroa mite has also contributed to the extinction of most wild and feral honey bee colonies in Europe. However, there are certain managed and feral *A. mellifera* populations scattered around the world that can survive infestation by *V. destructor* without being treated with miticides. Previous studies indicate that colonies from these surviving populations have multiple behavioural and physiological traits that lead to supressed mite reproductive success. Breeding varroa resistant honey bee colonies that do not require chemical treatments could be a potential solution to the varroa epidemic, and the surviving populations may hold the answer to how this can be achieved. In this study, I used honey bee brood cells that were artificially infested with varroa mites to compare mite reproductive success in three honey bee populations; a Norwegian varroa-surviving population, a Norwegian varroa- susceptible population, and a Swedish varroa-surviving population that had been transferred to Norway. I also compared the mite infestation rates, colony size, and colony mortality rates in each population. Although varroa mite reproductive success was significantly lower in the surviving Swedish population compared to the varroa-susceptible population, the mite infestation rates were similar and reached lethal levels in both experimental groups. My results indicated that suppression of varroa mite reproductive success as a resistance mechanism may be insufficient to ensure colony survival if the infestation pressures from surrounding colonies exceed a certain threshold. Despite their ability to supress mite reproductive success, colonies from the Swedish honey bee population, and the Norwegian honey bee population that were examined in this study may not be able to survive varroa infestation in Central Europe, where the density of honey bee colonies is substantially higher than in Scandinavia. I also tested the effect of wax cell size on mite reproductive success in the surviving Norwegian population. My results revealed a non-significant trend, indicating that varroa mite reproductive success was lower in the experimental colonies that were kept on 4.9 mm wax cell foundation compared to the experimental colonies kept on 5.3 mm wax cell foundation. Colony mortality and infestation levels were also significantly lower in the 4.9 colonies. More research is needed to establish whether mite reproductive success is negatively affected by reduced wax cell size.

2 Sammendrag

Den ektoparasittiske midden *Varroa destructor* har spredt seg over hele verden, og den skaper enorme problemer for birøktindustrien i Europa og Nord-Amerika. Varroamidden er også en av hovedårsakene til at ville og forvillede *A. mellifera* bifolk i praksis er utryddet i Europa. Enkelte tamme og forvillede *A. mellifera* populasjoner har imidlertid utviklet resistens mot varroamidden. Bifolk fra disse resistente populasjonene blir også infisert med varroamidd, men de er i stand til å overleve uten at de blir behandlet med kjemiske preparat som dreper midden. Tidligere studier viser at bifolk fra disse overlevende populasjonene har utviklet ulike fysiologiske mekanismer og adferd som hindrer optimal reproduksjon hos varroamidden. For å stanse varroaepidemien ønsker man å avle frem varroaresistente bifolk, men i Europa har dette fungert dårlig så langt. Studier av varroaresistente populasjoner av honningbier kan derfor resultere i kunnskap som gjør det mulig å avle frem resistente bifolk. I denne studien brukte jeg kunstig infisering av yngelceller til å sammenlikne varroamiddens reproduksjonsevne i tre ulike populasjoner av honningbier: en svensk resistent populasjon, en norsk resistent populasjon, og en norsk ikke-resistent kontrollpopulasjon. Jeg sammenliknet også populasjonsstørrelse, infiseringsgrad, og dødelighet i disse tre populasjonene. Selv om middreproduksjonen var lavere i den svenske resistente populasjonen sammenliknet med kontrollpopulasjonen, var det ingen forskjell i infiseringsgrad mellom de to gruppene. Resultatene mine antyder at redusert middreproduksjon som resistensmekanisme ikke er tilstrekkelig for å sikre bifolkets overlevelse dersom smittepresset fra andre bifolk overskrider en viss grense. Det betyr i praksis at bifolk fra de to svenske og norske varroaresistente populasjonene som ble undersøkt her, sannsynligvis ikke vil være i stand til å overleve i Sentral-Europa, eller andre områder der det drives intensiv birøkt og tettheten av bifolk generelt er høyere enn i Skandinavia. I denne studien undersøkte jeg også om redusert størrelse på bienes yngelceller påvirker varroamiddens reproduksjonsevne i den norske resistente populasjonen. Resultatene mine viste en ikke-signifikant trend som indikerte at varroamiddens reproduksjonsevne var lavere hos bifolkene som fikk rammer med små voksceller (4,9 mm), sammenliknet med bifolkene som fikk rammer med store voksceller (5,3 mm). Vinteroverlevelsen var også høyere i 4,9 mm gruppen. Ytterligere studier er nødvendige for å fastslå om redusert cellestørrelse har en reell effekt.

3 Introduction

The native host of the ectoparasitic mite *Varroa destructor* is the Asian honey bee *Apis cerana.* These two species coexist in a stable host-parasite relationship, resulting from a long evolutionary process shaped by natural selection. Trough this process, *A. cerana* colonies have evolved behavioural traits and population dynamics that prevent the infesting mite populations from reaching dangerously high levels (Locke et al. 2012; Rath 1999; Zheguang et al. 2017). However, its host shift to the Western honey bee *Apis mellifera*, has enabled the varroa mite to extend its range dramatically. The rapid expansion of the mite has been mediated by apicultural practices such as migratory beekeeping, and bee shipments. Today, *V. destructor* is present on every continent and is regarded as the most destructive apicultural disease in the world (Boecking & Genersch 2008). The invasion of *V. destructor* has caused more economic loss than any other apicultural disease. It also poses a significant threat to global food security, because pollination by managed honey bees is essential in many crop cultivation systems (Boecking & Genersch 2008; Calderone 2012; Rosenkranz et al. 2009)

The varroa mite is considered as the main biological cause of the recent large- scale losses of managed *A. mellifera* colonies in Europe and North America. The number of wild and feral colonies in these parts of the world have also dwindled (Büchler et al. 2010). Mite populations in untreated *A. mellifera* colonies will grow exponentially and in temperate climates untreated colonies will usually die in 2-3 years after infestation (Rosenkranz et al. 2009). By feeding on hemolymph the varroa mites weaken worker bees and impair organ formation in developing pupae (Schneider & Drescher 1987) as cited in Boecking and Genersch (2008). According to Rosenkranz et al. (2009), loss of hemolymph during the brood stage leads to lower hatching weights, shorter life spans, discoloration, and reduced cognitive abilities. The actual breakdown of the colony is caused by viral infections transmitted by the mites, which function as biological and mechanical vectors of multiple honey bee RNA viruses (de Miranda & Genersch 2010; Genersch & Aubert 2010). During the final stages before colony breakdown, the bee population decreases steadily. Typical symptoms seen in colonies collapsing from varroosis include scattered brood nests and crippled bees suffering from Deformed Wing Virus (DWV). Lack of coordinated social behaviour will ultimately lead to colony disintegration, as the colony can no longer function as an entity (Rosenkranz et al. 2009).

Deformed Wing Virus (DWV) is one of the most well studied honey bee viruses, and its occurrence is closely correlated to the global spread of the varroa mite (Baker & Schroeder 2008; de Miranda & Genersch 2010; Genersch & Aubert 2010; Martin et al. 2012; Tentcheva et al. 2004). Prior to the rapid mite invasion, DWV and other honey bee RNA viruses would rarely produce overt infections. Consequently, these viruses were considered harmless, although low concentrations of viral particles could be detected in most colonies (de Miranda & Genersch 2010; Genersch & Aubert 2010; Yue & Genersch 2005).

Female varroa mites alternate between two distinct stages in their life cycle, a phoretic stage and a reproductive stage. During the phoretic stage, female varroa mites cling to the exterior of worker bees while feeding on hemolymph. Drifting forager bees, foragers robbing of weakened infested colonies, and swarms will spread the mites between colonies. Mite reproduction happens inside capped brood cells (Rosenkranz et al. 2009). In *A. cerana* successful mite reproduction is only possible in drone brood, but in *A. mellifera* the mites can reproduce both in drone and worker brood (Zheguang et al. 2017). Female mites enter the brood cells shortly before they are capped. Approximately three days after capping the mother mite produces the first egg, which normally develops into a male. The males are short lived and can only exist inside the capped brood cells. The next eggs will normally develop into female mites. The mother mite can produce up to four female eggs at thirty-hour intervals in worker brood. Both the male and female offspring undergo a protonymph and a deutonymph stage before they become mature and mate inside the brood cell. One or two mated female offspring will usually reach maturity in worker brood and leave the cell along with the emerging bee and the foundress mite (Dieteman et al. 2013; Rosenkranz et al. 2009).

Since the arrival of varroa mites in Europe, beekeepers have used a wide variety of miticides to limit the devastating effects of the varroa mite and the viruses it vectors (Rosenkranz et al. 2009). Varroa resistance to miticides is an increasing problem and cases have been documented in several countries (Rodríguez-Dehaibes et al. 2005). The use of miticides is also problematic to the apicultural industry because they often have negative side effects on the bees, they are expensive, and traces of these substances are detectable in hive products. Breeding mite resistant strains of bees is therefore a more sustainable long-term solution (Boecking & Genersch 2008; Rinderer et al. 2010; Rosenkranz et al. 2009). Paradoxically, miticide treatments and other apicultural practices such as the combination of weak colonies will remove selective pressures, thereby inhibiting the genetic ability of the bees to develop intrinsic tolerance or resistance mechanisms (Locke et al. 2012). Tolerance is defined as the

ability of the host to reduce the effect of the parasite, and host resistance is defined as the ability to reduce the fitness of the parasite (Råberg et al. 2009). According to Rinderer et al. (2010), resistance mechanisms towards varroa can be classified as either behavioural or physiological. Physiological resistance mechanisms are related to brood attractiveness, the length of the phoretic phase of the mites, or duration of the post capping stage. Classic behavioural resistance mechanisms include varroa sensitive-hygiene (VSH), social grooming (removal of phoretic mites from nest mates), or self-grooming. VSH is the ability of worker bees to suppress mite reproduction by detecting and removing infested brood (Boecking & Spivak 1999). Recapping behaviour is another resistance mechanism that was recently found to disrupt the mite reproduction process and increases mortality of the mite offspring (Oddie et al. In press). The caps of infested cells are removed and the cells are left open for a brief period, before they are resealed with a new wax capping (Boecking & Spivak 1999; Harris et al. 2012). All of the aforementioned behavioural traits are common in *A. cerana*, and they are expressed more frequently than in *A. mellifera* colonies. In addition, *A. cerana* workers prevent new mites from emerging by entombing infested drone brood (Rath 1999). VSH is a heritable trait in *A. mellifera* and can be enhanced through selective breeding (Boecking et al. 2000). Several American breeding programs have succeeded in producing strains that suppress mite reproduction and require fewer miticide treatments compared to "conventional" strains. One of the most promising breeding programs uses genetic material based on imported stock with high levels of resistance to varroa from the Primorsky region in Eastern Russia (Rinderer et al. 2001; Rinderer et al. 2010). Selection for resistant European colonies has proved to be more difficult and importing resistant stock from outside Europe has not worked well so far. Primorsky hybrids that were imported from North America did not express mite resistant characteristics to the same extent when they where imported to Germany. The colonies also had other traits making them unsuitable for commercial beekeeping, such as low honey yield, low brood production, and a bad temperament (Büchler et al. 2010; Rosenkranz et al. 2009).

In addition to the Primorsky population in Russia, there are other managed and feral *A. mellifera* populations scattered around the world that continue to persist despite not being treated with miticides. The existence of these populations supports the theory that breeding for varroa resistance or tolerance is possible. Such populations can be found in Europe and some of them have survived for more than a decade without varroa treatments (reviewed by Locke 2016). Interestingly, the resistance mechanisms enabling these colonies to survive

varroa infestation have seemingly evolved through natural selection and can differ among populations. Some surviving populations are characterized by increased grooming and hygienic behaviour when compared to local susceptible colonies, *e.g.,* the surviving population of *A. m. intermissa* in Tunisia, the Primorsky population, and the French Avignon population. Supressed mite reproductive success has been reported in a surviving Norwegian population and a surviving Swedish population, although these populations do not express increased hygienic behaviour (Locke et al. 2012; Oddie et al. 2017). Reduced colony size and brood production is an important characteristic of the Swedish population (Locke & Fries 2011; Locke et al. 2012). According to Locke (2016), this variation in resistance mechanisms implies that there are multiple ways of achieving resistant colonies, and that resistance is not dictated by one single trait alone. Furthermore, there is also considerable evidence indicating that colony health and disease resistance is strongly affected by genotype x environment interactions (GxE-interactions) (Büchler et al. 2014; Francis et al. 2014). The aim of most current apicultural management practices and breeding strategies is to create gentle and highly productive colonies. Through this process, introgression and hybridization has led to the loss of genetic diversity, resilient subspecies, and ecotypes with specific local adaptations. Large colonies that do not swarm and produce high numbers of brood are favourable in an apicultural and economic perspective (Dukku 2016; Meixner et al. 2014; Tarpy 2003). However, according to the principles of ecology and parasitology, such colonies have all the traits making them more susceptible to varroa and other epidemics. A common characteristic of the varroa-surviving colonies is that they are not subject to intense management or they are feral. Colony size is usually small and productivity is generally low (Locke et al. 2012; Locke 2016).

A possible solution to the varroa epidemic could be altered management practices, and to breed colonies that are productive, but also exhibit varroa resistant traits. The varroa-surviving populations might provide insight into how this can be accomplished. An important question related to this issue is to what extent varroa resistance in these populations is affected by GxE effects. If the influence of environment is substantial, resistant strains must be bred locally, based on “native” ecotypes. If the effect of environment is minimal, it is possible to breed a single honey bee strain that will be resistant to varroa, regardless of environmental variation. This study is part of a large- scale ring test called the Ricola ring test and uses European *A. mellifera* populations that are surviving without varroa treatments. The purpose of this ring test is to examine the role of GxE interactions in relation to varroa resistance in

European varroa surviving honey bee populations. The objective of this study is to compare the survival and varroa resistance mechanisms of a surviving Swedish population that was relocated to Norway, with a local surviving Norwegian population. A local Norwegian varroa-susceptible population was used as a control group. The effect of wax cell size on mite reproductive success was also tested. Reducing the size of the cells in the wax foundation from the conventional 5.3mm has been suggested as a method that negatively affects varroa population growth (Oddie et al. 2018a). Because the surviving Norwegian population is kept on small (4.9 mm) cell wax foundation, the effect of wax cell size on mite reproductive success was also tested as part of the same experiment. Specifically, I aimed to answer the following questions:

1. **Is mite resistance in the European varroa-surviving populations influenced by genotype- environment interactions?**
2. **Does the size of the wax cell foundation affect mite reproductive success?**

Figure 1: Honey bees (*Apis mellifera*) with phoretic *Varroa destructor* mites and deformed wings resulting from infection by deformed wing virus (DWV) vectored by the mites.
(Photo credit Bjørn Dahle)

4 Methods

4.1 Ring test

The Ricola ring test is based on three different European varroa-surviving *A.mellifera* populations, originating from Norway (Gjerdrum), France (Avignon), and Sweden (Gotland). In July 2016, 36 test colonies founded by queens from these surviving populations were established at six different test sites: Avignon, Bern, Hohenheim, Ghent, Uppsala, and Oslo (Fig.2). In each varroa-surviving population, 2-3 unrelated mother queens were used to produce the daughter queens, that after mating within each surviving population, were distributed to the test sites. At each test site, 12 colonies founded by young mated queens from each of these 3 surviving populations as well as from a local susceptible population were established. The 48 test colonies at each test site were established at 2 apiary locations (<60 km apart) to avoid strong competition for food resources that could impair colony development. At each apiary location, colonies were spatially arranged to reduce drifting of bees between colonies.

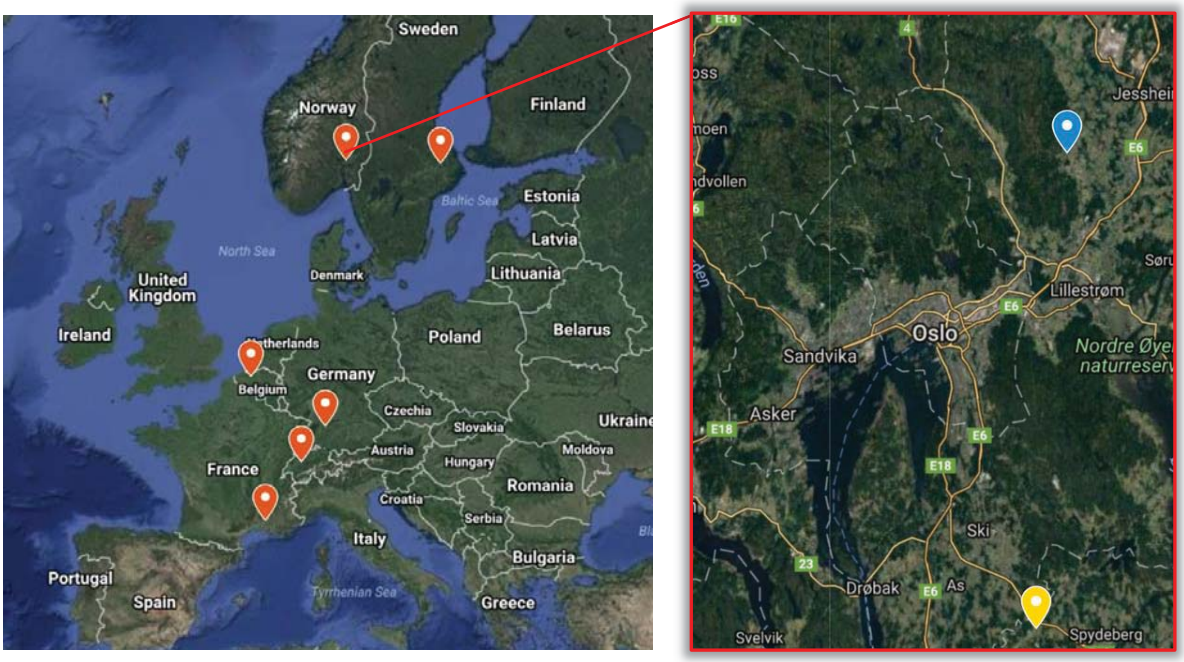

Figure 2: The red markers indicate the six test sites in the Ricola ring test on European *Varroa destructor* - surviving honey bee populations. The yellow marker indicates the Hobøl apiary and the blue marker indicates the Gjerdrum apiary at the Norwegian test site in the Oslo area in Southern Norway.

Prior to shipping of the queens, samples of wax debris from breeder colonies were collected and analysed for the presence of *Melissococus plutonius* and *Paenibacillus larvae*, to avoid the import of honey bee bacterial diseases. *M. plutonius* and *P. larvae* are the causative agents of the two honey bee diseases European foulbrood and American foulbrood, respectively. Due to the presence of *M. plutonius* bacteria in the French wax debris samples, it was not possible to take French queens to Norway. Consequently, 12 French queens and their colonies where replaced by colonies founded by Norwegian surviving queens kept on 4.9 mm wax comb foundation (compared to standard 5.3 mm in the other test colonies at the Norwegian test site). The 5.3 colonies were founded using varroa susceptible bees, whereas the 4.9 colonies were founded using bees from the Norwegian surviving population. These colonies where used to examine the effect of smaller wax cell size on mite reproductive success and mite population growth. The honeybee colonies in which the experimental queens were introduced were bought from 5 different beekeepers. In September 2016, mite infestation levels in the test colonies were estimated by the powdered sugar method (Dieteman et al. 2013). Rather than using a miticide with high efficacy on all test colonies, 8% lactic acid was sprayed on the bees in colonies with higher mite infestation level in an attempt to harmonize mite infestation levels among test colonies at the start of the experiment. In this way, all colonies would be challenged by mites during the experimental season 2017, making it easier to detect differences in mite resistance.

The number of test colonies declined before the start of the experiment because some colonies did not survive the winter of 2016/2017. Some colonies also swarmed during the experimental period. In some cases, queen supersedure could not be prevented (the colonies changed their old queen for her daughter), and these colonies where excluded from further tests. Colonies with population sizes of less than 4000 adult bees, and frequent symptoms of DWV infection in September 2017 were considered to be dead or unable to survive the coming winter. These colonies where removed from the test apiaries to prevent domino effects by transmission of mites to neighbouring colonies by drifting bees and through hive robbing. Both the surviving Swedish and Norwegian populations are of mixed origin from several *A. m*. subspecies, but their genetic composition was not analyzed in this study. The control colonies that were used are of Buckfast origin, a planned hybrid breed. They had been treated annually with oxalic acid for varroa mites for more than 10 years. Colony winter mortality (2017/2018) was assessed in mid - April 2018.

4.2 Study populations in Norway

The surviving Norwegian population had not been treated against varroa since 1997. The losses of colonies due to varroa and secondary virus infections were high (>30%) during the first 5-8 years and population size (about 200-250 colonies) were maintained by splitting surviving colonies (T. Reinertsen pers. com). Oddie et al. (2017) found significantly lower mite infestation levels and reduced mite reproductive success in these colonies, when compared to local susceptible colonies. However, there were no differences in rate of grooming and VSH. Annual colony losses are also low compared to the national average of approximately 10% (Oddie et al. 2017). The surviving Norwegian colonies have been subject to some artificial selection, mainly regarding colony size and productivity.

The surviving Swedish population was the result of a natural selection experiment called the Bond Project ("live and let die") and had not been treated against varroa since 1999. Originally 150 isolated test colonies from several locations in Sweden were established on the island of Gotland in the Baltic Sea. Mites were introduced to the colonies, and they were left unmanaged and free to swarm. More than 80% of the colonies were lost over the course of the first three years. Swarming rates were also high during the first two years, until the infestation levels reached the point where the colonies were too weak to swarm. After these first three years, the population gradually recovered. Mite infestation levels dropped, winter losses became less frequent, and swarming rates increased again (Fries et al. 2003, Locke 2012). Locke and Fries (2010) found lower mite reproductive success and reduced colony size in the Bond population compared to local susceptible colonies, but no differences in rate of VSH and grooming.

4.3 Test sites

Six colonies from each experimental group were established in each of the two test apiaries. The first apiary is situated in Gjerdrum municipality, approximately 25 km north-east of Oslo, while the second apiary is situated in Hobøl municipality approximately 31 km south-east of Oslo (Fig.1). In Hobøl the distance to the closest known neighbouring apiary is approximately 2 kilometers. The distance to the closest known neighbouring apiary in Gjerdrum is also 2 km, and this is an apiary with varroa-resistant colonies. The climate in Southern Norway is temperate. The average temperature in January is – 9.0 ºC, and the average temperature in

July is 10.8 ºC (Yr 2018), so mite reproduction is restricted to the brood rearing period from March to October.

4.4 Colony size and varroa mite infestation levels

The population size of the test colonies was estimated using the Liebefelt method (Delaplane et al. 2013). The percentage of adult bees, capped brood, open brood, and food on each frame was visually estimated and recorded to calculate the area of wax combs they covered. The population size of each colony was calculated based on the data and estimated number of bees and brood cells/cm^2 (Delaplane et al. 2013). At the same time, samples of approximately 400 bees where collected from the outermost brood comb in each hive. Thirty bees form each sample was frozen at -80°C for later virus analyses. The remaining bees were frozen at -18°C, and later washed according to the "soapy water method" (Dieteman et al. 2013). The dislodged mites where collected in a sieve and counted, and the number of mites per hundred bees was recorded. Because the soapy water method only accounts for the phoretic mites present in each colony, the mite infestation data where corrected using the estimated amount of capped brood in each colony from the Liebefelt meassurements, and data on mite distribution in colonies with different amounts of capped brood (Al Toufailia 2016). Finally, the estimated total infestation rates (mites per 100 bees and capped brood) were calculated based on these data. All of the aforementioned measurements where performed three times over the course of the bee season (May/June, July, and September) in 2017. Some colonies in the Gjerdrum apiary were so weak that they could not be sampled in May. For this reason, the bee samples were collected a month later than in Hobøl (mid June). The infestation rates were divided in half to compensate for this difference, as varroa population size doubles every month during the brood-rearing season (Fries & Kristiansen 2009). In September 2016, no measurement of population size was made, but it was assumed to be 10, 000 adult bees and no capped brood.

4.5 Varroa mite reproductive success, VSH, and recapping

Mite reproductive success, VSH, and recapping rates were analyzed by dissecting brood cells artificially infested with a single varroa mite. Due to logistic constraints, the artificial infestation of the colonies in Hobøl was performed at the end of June/beginning of July 2017, whereas artificial infestation of the Gjerdrum colonies was performed in August 2017. Mites used for artificial infestation were harvested using the powdered sugar method described in Dieteman et al. (2013). Brood from one test colony was infested using mites taken from a neighboring test colony and vice versa, so all colonies were infested with foreign mites

In order to identify recently capped brood, frames with highly developed (but still uncapped) larvae were removed from the colonies. The positions of these larvae were marked on transparent plastic sheets and placed on top of the frames. The frames were inserted back into their respective colonies and retrieved after approximately three hours. Brood that had been capped during the last three-hour period was used for infestation. A scalpel was used to cut a small opening in the side of the cell caps. The mites were carefully slipped into the brood cells with a small paint brush. Finally, the caps were closed, and the location of the infested cells were marked again. After infestation, the brood frames were placed back into their respective colonies. Some of the queens from the Gjerdrum colonies were caged and released after approximately 36 hours, to maximize the chances of finding larvae with a suitable age for infestation. However, most colonies were infested without queen caging. Approximately 9 days after artificial infestation, the frames were removed from their colonies and stored in a freezer. We managed to infest 1019 brood cells (9 colonies in the control group, 8 colonies in the Norway 4.9 group, 6 colonies in the 5.3 group, and 10 colonies in the Swedish group). Because the amount of brood with a suitable age for infestation was limited, we were not able to artificially infest an equal number of colonies in each apiary. The number of cells infested per colony is also highly variable. This resulted in small and unequal sample sizes, which complicated the statistical analysis.

The artificially infested brood cells were dissected the following fall. Each cell was opened using a scalpel, and the contents of the cells were carefully removed with a small paintbrush. The brood stage of the pupa was recorded, and the mites were inspected with the aid of a stereomicroscope and LED illumination. Mite reproductive parameters, such as number of female offspring, number of male offspring, and number of foundress mites, were recorded according to the method described in the RNSSB protocol for investigation of supressed mite reproductive success and recapping (Büchler et al. 2017). The inside of each cell cap was inspected for signs of recapping. The number of empty brood cells resulting from VSH behavior was also registered. Brood cells containing multiple mite foundresses were not included in the statistical analysis. Mite fecundity was defined as the number of deutonymphs per foundress, if at least one male offspring was present in the cell (if the foundress produced no male offspring, fecundity was recorded as zero). Viable offspring was calculated as the number of mated deutonymphs that could potentially reach maturity before the host bee emerges (Dieteman et al. 2013) (P. Rosenkrantz pers.com.).

4.6 Statistical analysis

All statistical tests were performed in R; the glme4 package was used to create generalized linear models (GLM). The proportion of cells removed in each test group (VSH) was extracted and analyzed separately to reduce the skew of the dataset. The proportions of VSH cells and recapped cells in each group were compared using a chi-square test. A chi-square test was also used to test differences in mortality rates. A Bonferroni correction was applied to account for multiple tests performed on the same data set. The effects of population, cell size, and apiary on number of viable offspring and fecundity was tested by creating GLMs. Stepwise removal of non-significant terms was used, and the dispersion parameter of the models was assessed to compare the quality of the models. The Multcomp package was used for post-hoc Tukey tests. Colony size in each population and apiary was compared using a two-way ANOVA. Sample size was unequal, but this was not accounted for because the differences were relatively small. The means of each population were compared with a Tukey HSD test.

5 Results

5.1 Varroa mite reproductive success and fecundity

Due to small and uneven numbers of artificially infested cells in some colonies, it was not possible to add colony as a random factor in any of the models. When VSH cells were excluded from the analysis, population was the only variable that had a significant effect on mite fecundity and reproductive success ($p < 0.01$ for both models). Both the average fecundity and average number of viable offspring per foundress was significantly lower in the Swedish population compared to the control population. Cell size was borderline significant $p= 0.0498$ (fecundity) and $p= 0.0538$ (viable offspring). This was a non-significant trend suggesting reduced mite fecundity and reproductive success in the Norwegian 4.9 population relative to the control population and the 5.3 population (Table 1). The frequency of failed mite reproduction was high in the Swedish population and the Norwegian 4.9 population (Fig. 3).

Table 1: Mean mite fecundity per foundress and average number of viable offspring per foundress in each honey bee population (± SE). Mite fecundity was defined as the number of deutonymphs per foundress if at least one male offspring was present in the cell. Viable offspring was calculated as the number of mated deutonymphs that could potentially reach maturity before the host bee emerges.

Population	Fecundity	Viable offspring	Infested cells (N)
Control	0.64 ± 0.09	0.60 ± 0.08	271
Norway 4.9	0.48 ± 0.06	0.46 ± 0.06	270
Norway 5.3	0.64 ± 0.09	0.62 ± 0.09	162
Sweden *	0.40 ± 0.05	0.39 ± 0.05	317

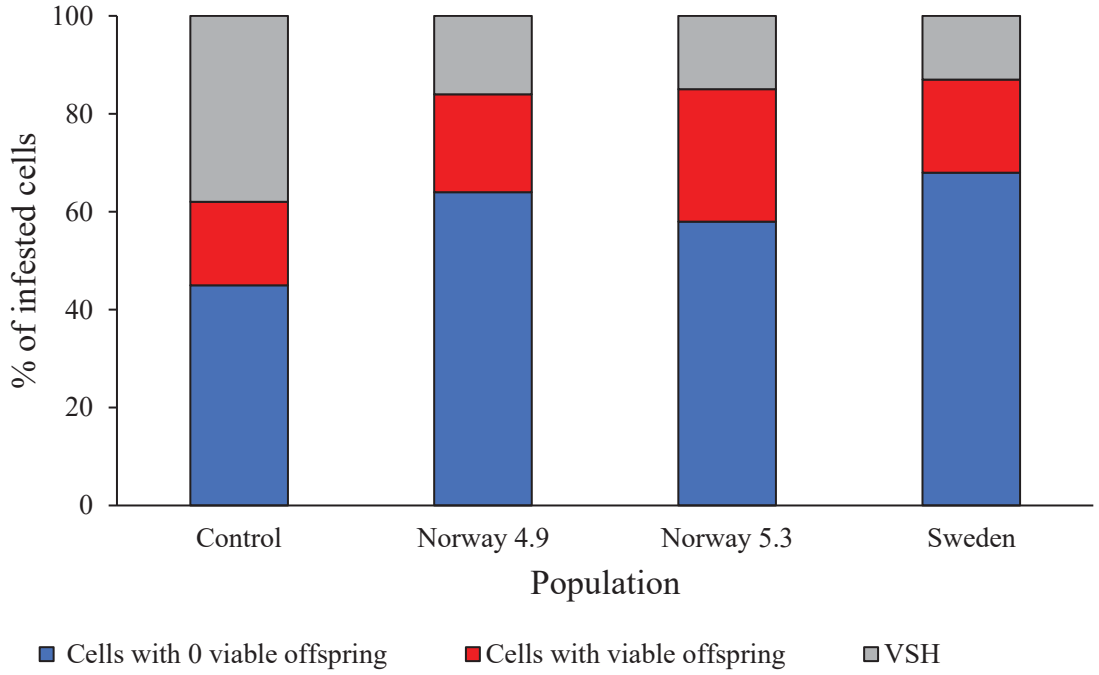

Figure 3. The proportion of artificially infested brood cells in each honey bee population containing no viable varroa mite offspring, viable mite offspring, and the proportion cells where bees have performed varroa-sensitive hygienic behavior (VSH). Number of artificially infested cells in each honey bee population: Control N= 271, Norway 4.9 (small cell foundation) N= 270, Norway 5.3 (standard cell foundation) N= 162, Sweden N= 317

5.2 Varroa sensitive hygiene (VSH)

The proportion of artificially infested cells that were emptied by the worker bees was significantly higher in the control group compared to the surviving populations $p < 0.01$ ($\chi^2 = 65.052$, df = 1) (Fig.4). No significant differences in VSH were found among the surviving populations.

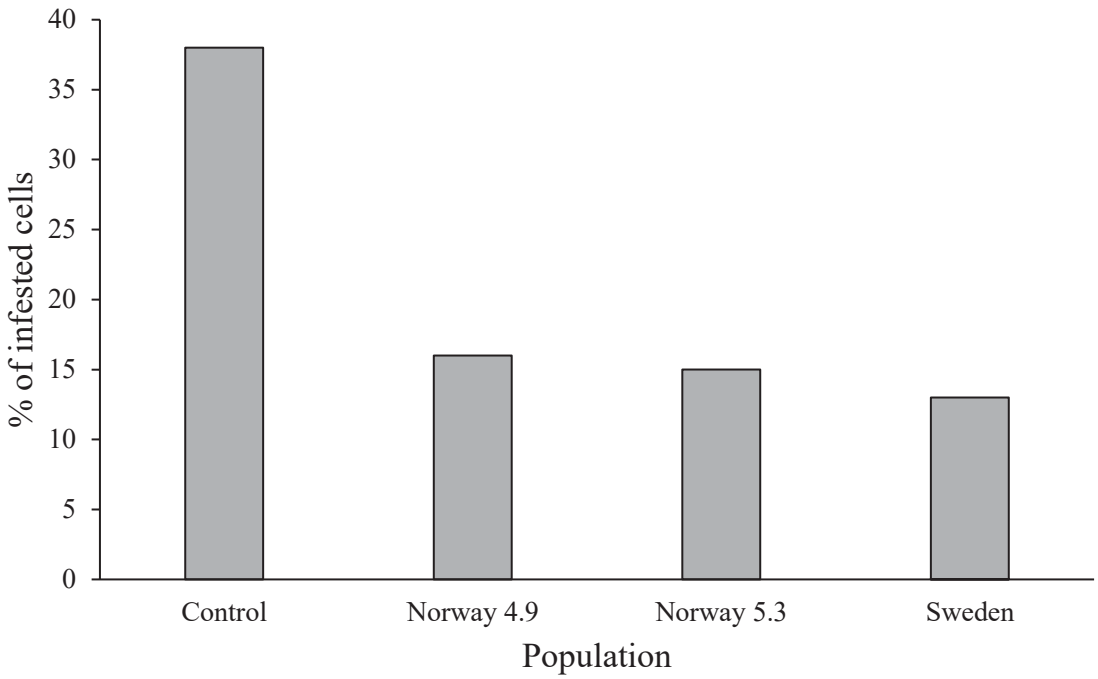

Figure 4: The proportions of artificially infested brood cells that were subject to varroa- sensitive hygiene in each honey bee population. Number of artificially infested cells in each population: Control N= 271, Norway 4.9 (small cell comb foundation) N= 270, Norway 5.3 (standard cell foundation) N= 162, Sweden N= 317

5.3 Recapped brood cells

The proportion of recapped cells was lower in the Swedish honey bee population compared to the control population $p < 0.01$ ($\chi^2 = 16.005$, $df = 1$) and the two Norwegian populations $p < 0.01$($\chi^2 = 11.572$, $df = 1$), (Fig.5). No significant differences were found between the Norway 4.9 and Norway 5.3 population. There were no significant differences between the two Norwegian groups and the control population. The proportion of cells that did not contain viable offspring was approximately 75% in both the recapped and the untouched cells in all populations.

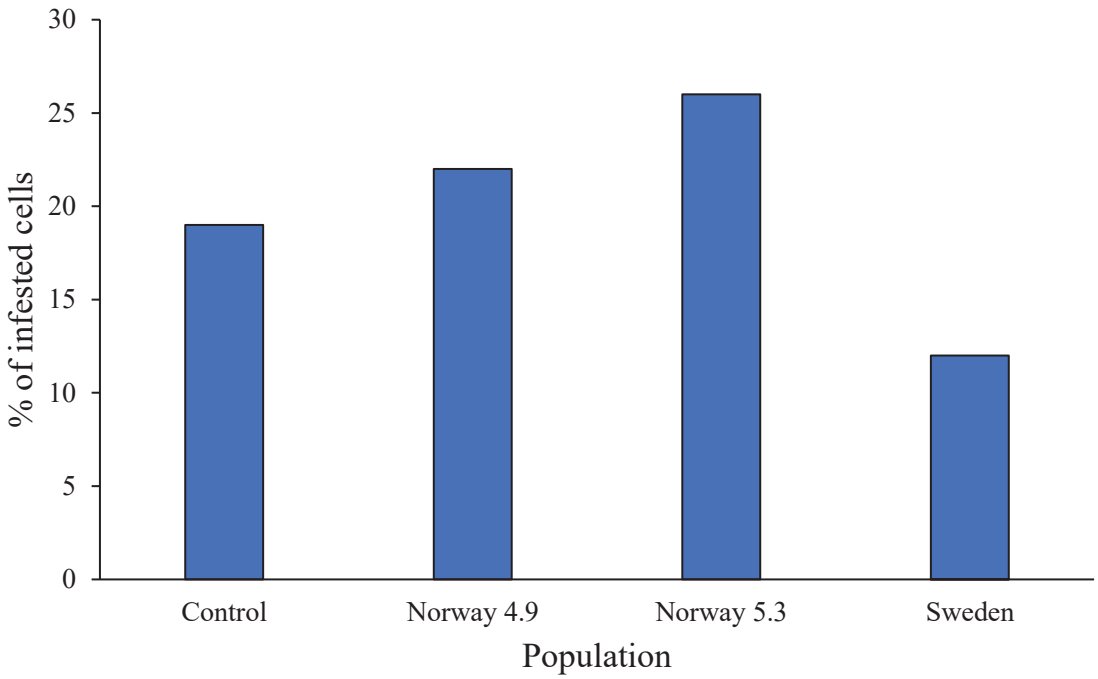

Figure 5: The proportion of honey bee brood cells that were infested with varroa mites and recapped in each population. Number of artificially infested cells in each population: Control N= 271, Norway 4.9 (small cell comb) N= 270, Norway 5.3 (standard cell comb) N= 162, Sweden N= 317.

5.4 Varroa mite infestation levels

The infestation levels were relatively similar in all honey bee populations and in both apiaries in September 2016, after the treatment of highly infected colonies with 8% lactic acid (Fig. 6). In May/June 2017, however, the infestation rates were higher in the Hobøl apiary, except in the 4.9 population (Fig. 7). The relative differences among the populations in the Gjerdrum apiary were small. In Hobøl, the 4.9 population had lower infestation rates compared to the other populations. In July 2017 the infestation rates were all higher in Hobøl, except in the 5.3 population (Fig. 8). All infestation rates were relatively similar in Gjerdrum, but in Hobøl the control population and the Swedish group had higher infestation rates. The infestation rates in July 2017 were analyzed as a function of apiary and population. This was to reduce the distorting apiary effect observed in September 2017 (Fig.7). Apiary had a significant effect on the infestation rates in July 2017 ($p < 0.01$). Population also had a significant effect on the infestation rates among the colonies in July 2017. The infestation rates in the Norwegian 4.9 population were significantly lower than in the control population in July 2017 ($p < 0.01$) (Table 3). The other populations were not significantly different from each other. In September 2017 the infestation rates were higher in Hobøl, and the 4.9 population had the lowest infestation rates in this apiary (Fig. 9). In Gjerdrum, the Swedish population and the

4.9 population had the lowest infestation rates. Both the GLM and the graphic representation of the data indicated a clear apiary effect, which became more apparent over the course of the summer.

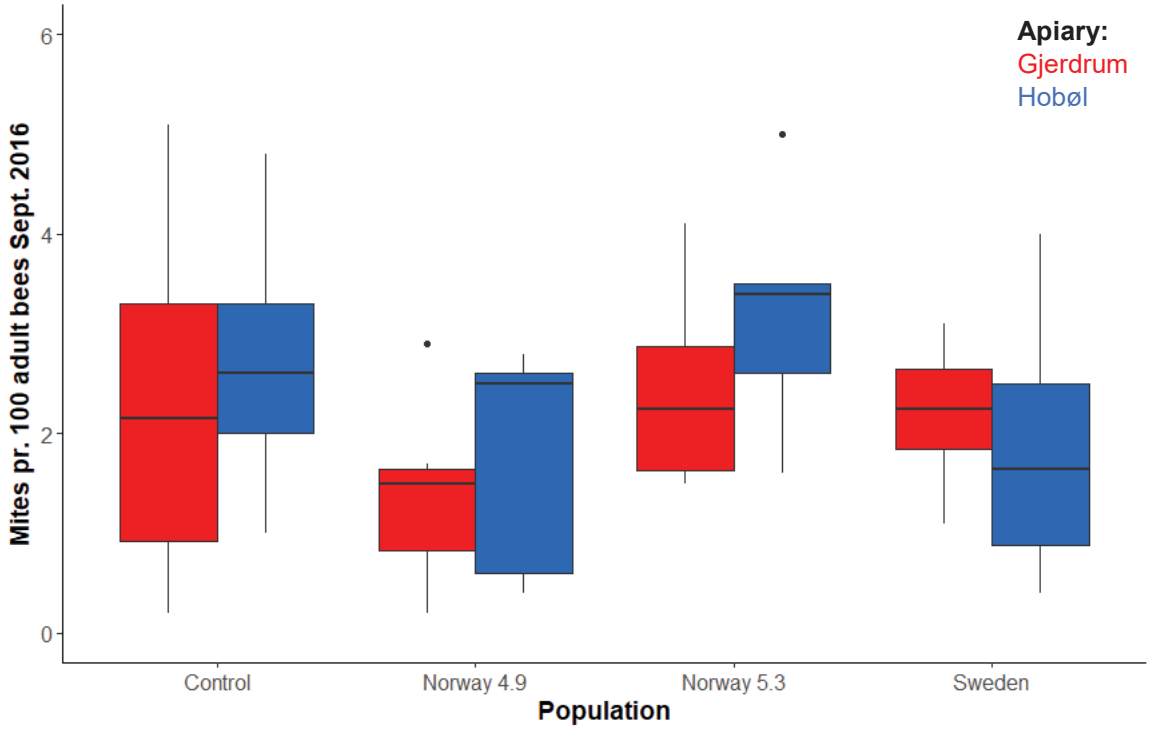

Figure 6: Varroa mite infestation levels in the three honey bee populations in September 2016 after the colonies were established and received lactic acid treatments to even out the infestation levels. Interquartile ranges are included, and the medians are indicated by the black horizontal lines. The whiskers indicate the maximum and minimum values excluding outliers which are indicated by black dots.

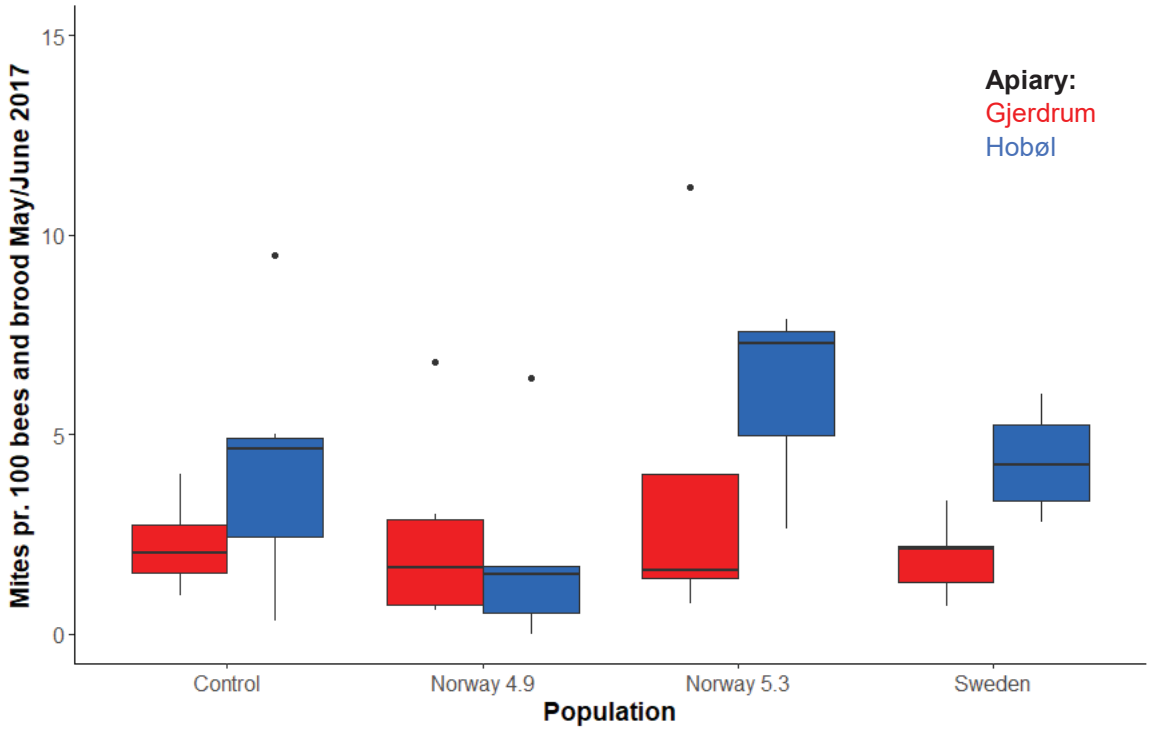

Figure 7: Varroa mite infestation rates in the three honey bee populations in May/ June 2017. The infestation rates in Gjerdrum were divided by two to account for the fact that the samples were taken one month later. Interquartile ranges are included, and the medians are indicated by the black horizontal lines. The whiskers indicate the maximum and minimum values excluding outliers which are indicated by black dots.

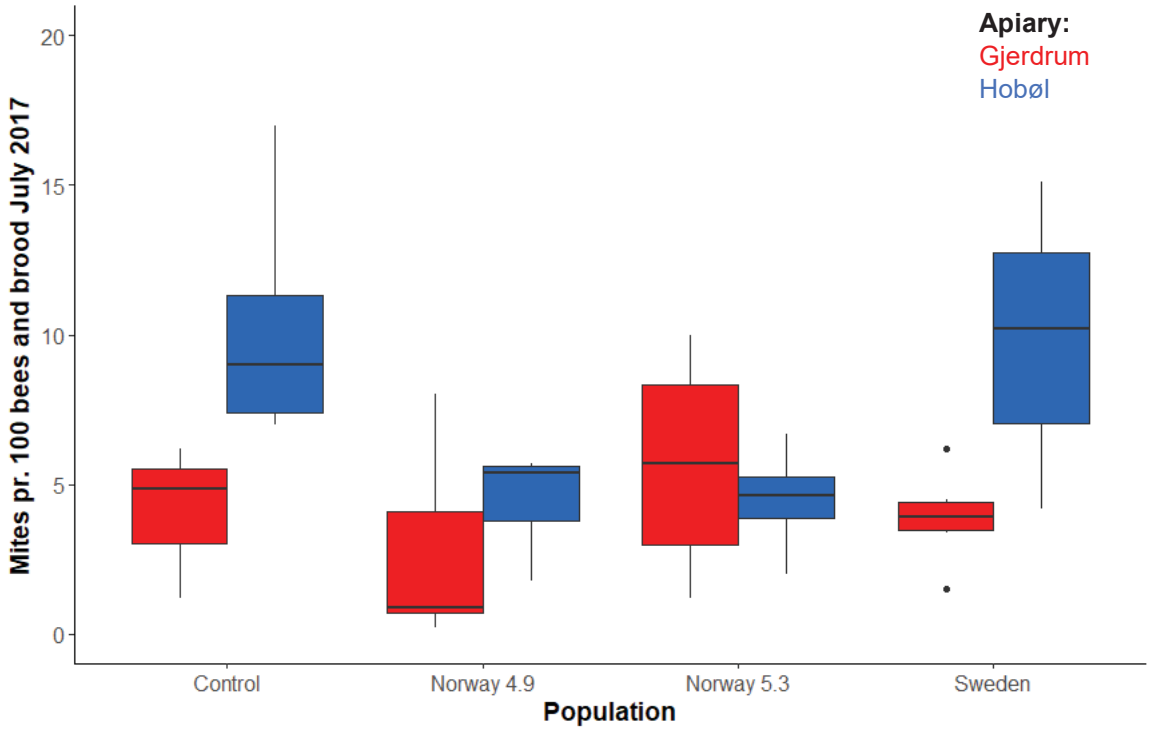

Figure 8: Varroa mite infestation levels in the three honey bee populations in July 2017. Interquartile ranges are included, and the medians are indicated by the black horizontal lines. The whiskers indicate the maximum and minimum values excluding outliers which are indicated by black dots.

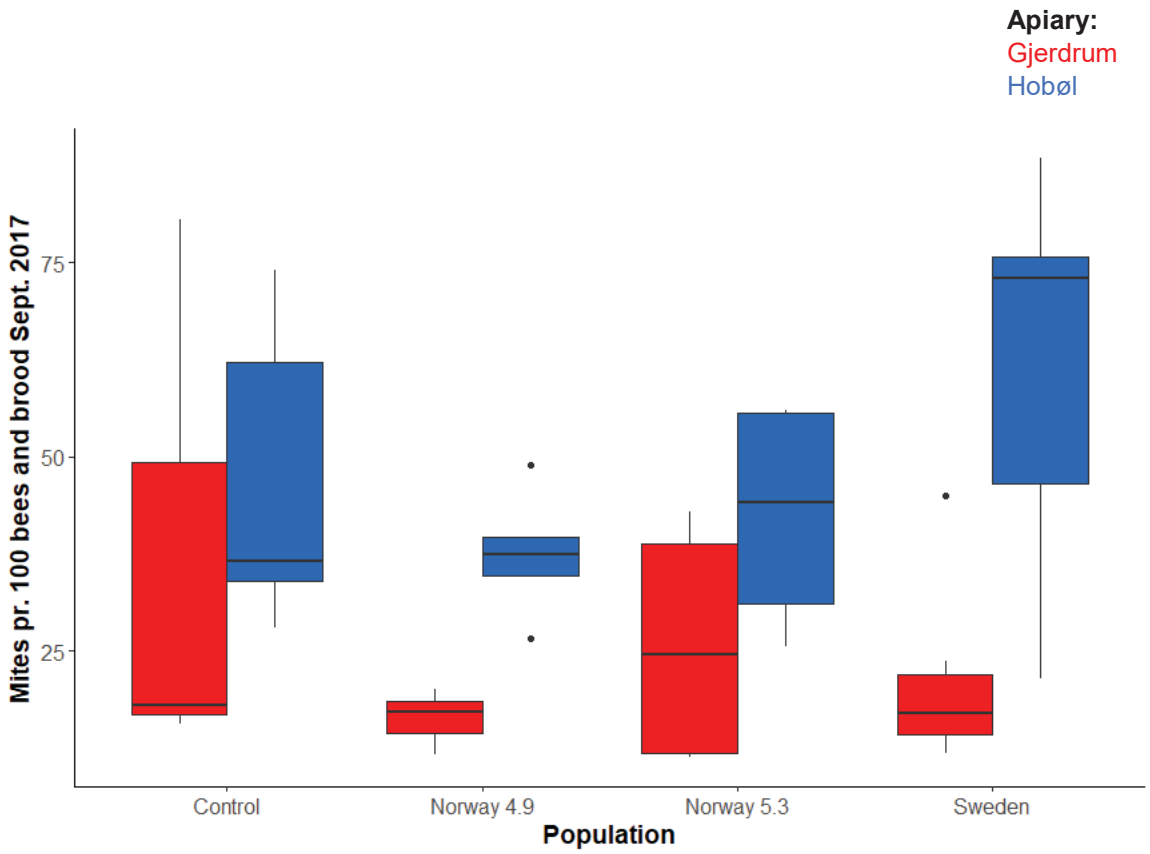

Figure 9: Mite infestation levels in September 2017. Interquartile ranges are included, and the medians are indicated by the black horizontal lines. The whiskers indicate the maximum and minimum values excluding outliers which are indicated by black dots.

Table 2: The mean infestation rates in each honey bee population (mites per 100 bees and brood) ± SE in July 2017. Control Gjerdrum N =3, Control Hobøl N= 6, Norway 4.9 Gjerdrum N= 5, Norway 4.9 Hobøl N= 5, Norway 5.3 Gjerdrum N= 4, Norway 5.3 Hobøl N= 4, Sweden Gjerdrum N= 6, Sweden Hobøl N= 6.

Population	Mean mites per 100 bees and capped brood cells	Number of colonies (N)
Control	9.50 ± 6.28	9
Norway 4.9	3.62 ± 2.62	10
Norway 5.3	5.08 ± 2.96	8
Sweden	6.89 ± 4.33	12

5.5 Colony size

The mean number of adult bees was significantly higher in the Gjerdrum apiary compared to the Hobøl apiary ($p < 0.01$) (Fig. 10). The mean number of adult bees was also significantly lower in the Swedish population than the Norway 4.9 population ($p = 0.02$). The mean number of adult bees in the other populations were not significantly different. The mean amount of capped brood was relatively similar in each population, but tended to be higher in Gjerdrum than in Hobøl (Fig. 11).

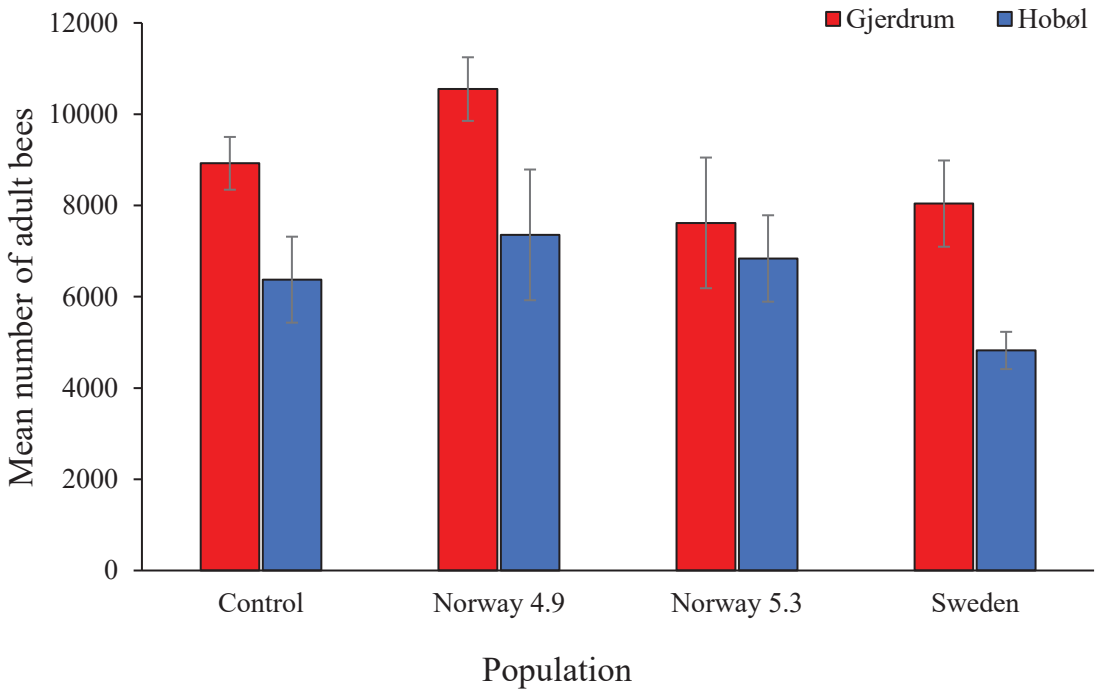

Figure 10: Mean number of adult honey bees (±SE) based on three Liebefeldt measurements (May, July, and September). Gjerdrum N= 16, Hobøl N= 20. Control N=8, Norway 4.9 N= 8, Norway 5.3 = 8, Sweden = 12

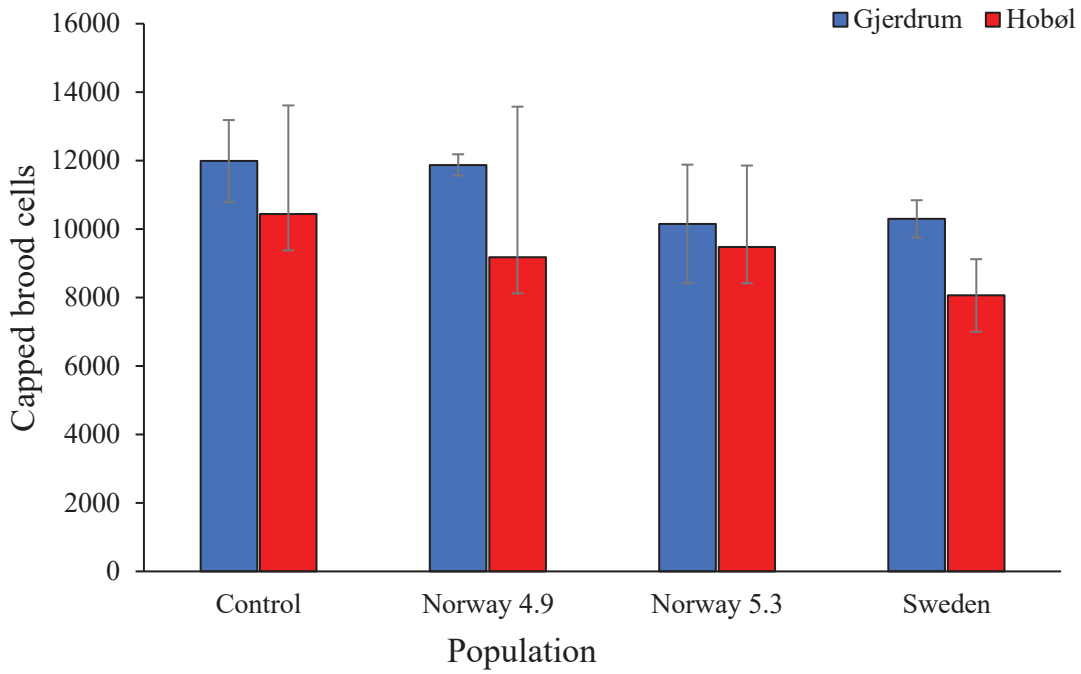

Figure 11: Mean number capped brood in each honey bee population (±SE) based on three Liebefeldt measurements (May, July, and September). Gjerdrum N= 16, Hobøl N= 20. Control N=8, Norway 4.9 N= 8, Norway 5.3 = 8, Sweden = 12

5.6 Colony mortality

Seven colonies in the Hobøl apiary were deemed to be unable to survive the winter of 2017/2018, because of small colony size (< 4000 adult bees) combined with mite loads of ≥ 30 mites per 100 bees. Consequently, these colonies were removed from the apiary and killed to avoid further redistribution of mites from collapsing colonies. All colonies in the Gjerdrum apiary were wintered. During winter 2017-2018, all colonies in Hobøl died, except 3 Norway 4.9 colonies, and 1 Norway 5.3 colony. The dead colonies showed signs of late brood rearing in the fall of 2017 and had used much of their winter food. In Gjerdrum, 3 control colonies, 1 Swedish, and 1 Norway 5.3 colony died during the winter. In September 2017 signs of DWV were clearly visible in several of the Hobøl colonies. No clinical symptoms of other pests and pathogens were observed in the experimental colonies, except in one of the Swedish colonies which, was strongly infested with chalk-brood (*Ascosphaera apis*) to an extent that it probably had a strong negative impact on colony development. The results from the Chi- square tests revealed that there were significant differences in mortality rates among the populations in both apiaries, $p=0.05$, $\chi^2= 7.813$, $df = 3$ (Hobøl), and $p=0.02$, $\chi^2= 9.133$, $df = 3$ (Gjerdrum). The 4.9 colonies had the highest survival rate in Hobøl. In Gjerdrum, all of the surviving populations had colonies that were alive in April 2018.

Table 3: Honey bee colonies still alive after the winter of 2017/2018 of the total number of remaining colonies in each group that did not swarm or change queens during the summer of 2017. Winter mortality was assessed in mid - April 2018.

Apiary	Control	Norway 4.9	Norway 5.3	Sweden
Gjerdrum	0/3 Colonies	3/3 Colonies	4/5 Colonies	5/6 Colonies
Hobøl	0/5 Colonies	3/5 Colonies	1/4 Colonies	0/6 Colonies

6 Discussion

The resistance mechanisms towards varroa mites of the Norwegian and Swedish honey bee populations that have survived for more than a decade without chemical treatments are well documented. However, there is little knowledge about how these resistance mechanisms are influenced by environmental factors. The aim of this study was to test whether colonies from the Swedish surviving population would continue to express resistance traits in Norway. I also wanted to know more about the potential effects of wax cell size on mite reproductive success. My results revealed that varroa mite reproductive success and fecundity were significantly lower in the Swedish colonies than in the control colonies. There was also a non-significant trend indicating that mite fecundity and reproductive success were lower in the Norwegian 4.9 (small cell) population. However, over the course of the experiment the varroa population increased rapidly in all colonies and reached high levels, especially in the Hobøl apiary, where 7 colonies collapsed in August/September. Worker bees that were disfigured by DWV were frequently observed in the dying colonies. After the winter of 2017/2018 only four colonies survived in Hobøl, all from the Norwegian resistant population. Apiary effects that likely resulted from drifting bees, robbing of weak hives, or dissimilar infestation pressures from surrounding apiaries probably overruled the limited differences in mite reproduction, and made it difficult to discern cause and effect relationships. Coinciding apiary effects and high colony mortality rates were also observed at the other European test sites (P.Neumann pers.com).

6.1 Varroa mite reproduction:

The lowered mite fecundity and mite reproductive success observed in the Swedish population were in line with the results of Locke and Fries (2011), who studied the varroa resistance mechanisms of this population in Gotland, Sweden. Contrary to expectations, no general differences in mite fecundity and reproductive success could be demonstrated between the control population and the Norwegian populations, except that mites in the Norwegian colonies that were kept on small cells possibly expressed a reduced fecundity and mite reproductive success compared to the control population and the Norwegian population kept on conventional 5.3 mm cells. Reduced mite reproductive success has previously been identified as the main resistance mechanism of both the surviving Norwegian and the Swedish populations, as well as the surviving French population in Avignon. These (naturally selected) populations reduced mite reproductive success by 10-30% compared to local susceptible control populations (Oddie et al. In press). At present, the actual mechanisms by which these surviving populations supress mite reproductive success are not fully understood. These mechanisms could also differ among the populations, because suppression of mite reproductive success is a characteristic that has evolved independently in populations that are geographically and genetically secluded (Locke et al. 2012). A recent study on the surviving Norwegian population revealed a significant reduction in the post-capping period of worker brood, compared to the worker brood of local susceptible colonies (Oddie et al. 2018b). This trait has not been described in the European honey bee subspecies before, and the study provides insight into an underlying mechanism which may be contributing to supressed mite reproductive success in the surviving Norwegian population. Oddie et al. (In press) suggest that mite resistance in this population is the result of shorter post-capping time, in combination with other resistance traits, such as recapping. Mite reproduction is closely synchronized with the development of the host bee. Oogenesis in the foundress mite is activated by chemical cues such as different cuticular compounds emitted by the recently capped larvae (Garrido & Rosenkranz 2004), but this mechanism has not been studied in the resistant populations in the present study.

Mite fecundity and the average number of viable offspring per foundress was substantially lower in our study using artificially infested cells compared to the results of studies based on the same surviving populations that used naturally infested cells (Locke & Fries 2011; Oddie et al. 2017; Rosenkranz et al. 2009). This effect was likely a direct consequence of the artificial infestation technique which was used. The method was invasive and could cause

damage to the cell cap and the bee larvae if it is not performed correctly. It is also possible that these manipulated brood cells could influence the rate of hygienic behaviour. Hygienic bees detect abnormal or diseased brood through olfactory signals (Gramacho & Spivak 2003) and the artificially infested cells could have alien scents that can influence the behaviour of the bees. There was also no way of ensuring that the mites used for artificial infestation were at the correct stage in their life cycle for reproduction. Still, artificial infestation is less time consuming, especially if mite infestation rates are low. The low mite reproductive success is likely a systematic error, as there is little reason to suspect that it has affected any of the populations differently. Therefore, analysis of the relative differences between the populations should still have produced reliable results. Some colonies did not have enough brood with a suitable age for infestation and it was not possible to infest as many colonies in the Gjerdrum apiary as in the Hobøl apiary. This lowered the sample size considerably, reduced statistical power, and prevented the use of colony ID as a random variable. The results from the models on mite fecundity and reproductive success should therefore be interpreted with caution. Additional data could potentially have revealed significant differences in mite reproductive success between the surviving Norwegian population and the control population. Still, the artificial infestation data indicate that the surviving Swedish population continues to express resistance traits after being relocated to Norway.

6.2 Varroa sensitive hygiene (VSH):

The reduction in mite reproductive success in the Swedish honey bee population did not appear to be linked to VSH. This was similar to the results of Locke and Fries (2011), and their conclusion that VSH is not a relevant factor leading to reduced mite reproductive success in the surviving Swedish population. The same conclusion was also drawn by Oddie et al. (2017) based on studies of the surviving Norwegian population. However, my results indicate that the proportion of infested cells that were emptied by worker bees was higher in the control population than in the surviving populations. Locke and Fries (2011) used the removal rate of pin-killed brood as an indicator for VSH (the brood was killed by inserting a needle through each cell cap), whereas Oddie et al. (2017) analysed the removal rate of naturally infested brood. Therefore, these results may not be comparable to mine, which were obtained using artificially infested brood. The control populations may also have differed in behavioural characteristics, as some *A. mellifera* genotypes and subspecies are more inclined towards emptying infested cells than others (Boecking et al. 2000; Büchler et al. 2010; Moretto et al. 1993; Uzunov et al. 2014). The control population used by Oddie et al. (2017)

was also a different subspecies (*A. m.carnica*). Furthermore, VSH is influenced by local conditions and temporal variation in the available plants, nectar flow, and weather. If the nectar flow and foraging conditions are favourable, worker bees will remove dead and infested brood more rapidly to make space for nectar (Uzunov et al. 2014). According to Panasiuk et al (2009) measurements of hygienic behaviour are correlated with nectar flow the day before, but I did not have information on the nectar flow during the experimental manipulations. Expression of hygienic behaviour at the colony level is also determined by the age distribution of the colony, because most hygienic bees are younger than three weeks (Arathi et al. 2000; Panasiuk et al. 2010).

6.3 Recapped brood cells:

The analysis of the artificially infested cells revealed no significant differences in recapping between the Norwegian population and the control population. The recapping rate in the Swedish population was also significantly lower compared to the control population and the Norwegian population. This result was surprising and was not in line with the findings of (Oddie et al. In press), who demonstrated that the recapping rates in the surviving Swedish, Norwegian, and French populations were significantly higher than in the local varroa-susceptible control populations. This conflicting result could be a consequence of the artificial infestation technique, or the timing of the experiment. The recapping study by Oddie et al (In press) was conducted in August and September, when mite infestation rates are known to be at their peak. In my study, most of the artificial infestation was performed in the end of June and start of July. As the brood infestation rates increase, more worker bees will start expressing hygienic behaviour (Harris et al. 2012). This flexible division of labour in honey bees and other species of social insects is described by response threshold models. Response threshold models are based on the idea that all workers have the ability to perform a specific task, but that the degree of stimulus needed for the worker to start performing the task varies among individuals in the colony (Beshers & Fewell 2001). Gramacho and Spivak (2003) found differences in olfactory sensitivity and behavioural responses in honey bees bred for hygienic behaviour. At low brood infestation rates only the most sensitive hygienic worker bees will uncap and recap infested cells or empty the cells completely. At higher infestation levels, the number workers performing hygienic behaviour will increase, because the probability that less sensitive workers encounter enough stimuli from mite infested cells increases (Harris et al. 2012). Therefore, it is possible that the higher recapping rates in the surviving populations only become apparent when infestation rates exceed a certain threshold.

According to Oddie et al. (In press) the recapping workers in the surviving populations also specifically target cells with fertile mites. Artificially infested brood cells may not provide realistic conditions for testing this type of behavior, because the number of offspring per foundress mite was lower than normal. Also, recapping frequencies can be underestimated in populations that perform this behaviour at an early stage after the after the artificial infestation. Recapping that took place shortly after the artificial infestation would pass unnoticed during the analyses of the infested brood cells, because the prepupal stage and spinning of the cocoon which makes it possible to determine whether the cell has been recapped lasts for 3-5 days (Winston 1987). Infested cells that contained no foundress mites when they were dissected could therefore either be the result of poorly performed artificial infestation or recapping during the prepupal stage.

6.4 Varroa mite infestation rates and colony mortality:

In general, honey bee colonies in Southern Europe survive with higher mite infestation rates than in Northern Europe (Meixner et al. 2014). In Germany colonies with a mite infestation rate exceeding 10% of the adult bees are likely to have reduced winter survival (Genersch et al. 2010). Based on the observed infestation rates and the results from a previous genotype x environment interaction experiment by Meixner et al. (2014), I expected high rates of winter mortality. In the Hobøl apiary the average infestation rate measured in September 2017 was 45 mites/100 bees, which is more than 3 times higher than the average infestation rate in Gjerdrum. Interestingly, three out of the four colonies that survived the winter of 2017/2018 in Hobøl were Norwegian 4.9 colonies. This group also had significantly lower infestation rates in July 2017 than the other populations. Thirteen of the 17 wintered colonies survived in Gjerdrum, all colonies of which were from the resistant populations. As mentioned earlier, the infestation data are likely biased by unwanted effects, such as hive robbing, and the proximity to colonies with high infestation rates. DeGrandi-Hoffman et al (2016) demonstrated that the proportion of phoretic mites and the proportion of infested cells was correlated with the number of infested foragers entering and leaving the hive. This was true for varroa-susceptible colonies, but also for colonies from Russian hygienic stock (DeGrandi-Hoffman et al. 2017). As the proportion of infested workers entering and leaving the hive increased in fall, models used to estimate mite infestation rates based only on mite reproduction predicted infestation rates that were much lower than what was actually observed in the colonies. Despite the high infestation rates and mortality among the Swedish colonies in Hobøl, they continued to supress mite reproductive success. If anything, the results from my study clearly

indicated that suppression of mite reproductive success as a resistance mechanism is insufficient to keep the mite population at bay if the infestation pressures from other colonies exceed a certain level. Auto- and allogrooming are the only known mechanisms by which worker bees can dispose of adult mites originating from sources outside the colony (Boecking & Spivak 1999) and this behaviour is not expressed to a high extent in the Swedish and Norwegian populations (Locke & Fries 2011; Oddie et al. 2017). There was no way of controlling for or quantifying the infestation pressures from surrounding apiaries in my study, and some of the most heavily infested hives probably acted as potent infestation sources to other colonies. However, the observed infestation and mortality rates in each apiary implied that the infestation pressures likely were much higher in the Hobøl apiary. The viral samples taken from each colony could not be analysed in time before my study was completed, but the viral loads and viral strains vectored by the mites at each location could also have been different. It would also have been easier to compare the infestation rates in each population if the colonies had been treated with oxalic acid at the start of the experiment. This treatment would have killed most of the mite population in each colony, thereby ensuring that all colonies started the experiment with infestation levels that were as equal as possible. However, at that point in time in my study, it was not decided whether the analysis of mite reproduction should be performed using naturally or artificially infested cells. Oxalic acid treatments would have reduced the chance of finding naturally infested cells considerably. Nevertheless, the results indicated that resistant colonies, which supress mite reproductive success, are still vulnerable if they are exposed to colonies with high mite infestation levels.

6.5 Colony size:

The mean number of adult bees was higher in Gjerdrum than in Hobøl, and the mean number of adult bees was also higher in the 4.9 population compared to Swedish population. This result corresponded well with the observed differences in mite infestation rates and mortality rates in each apiary. The estimated number of adult bees was relatively low compared to the results from similar studies (Locke & Fries 2011; McLellan 1978). Colony size was estimated during the middle of the day, when most foragers were outside the hive. This has likely contributed to the lowered estimates of colony size. Handling honey bee colonies in the afternoon and evening is generally more difficult than during the day because the hives contain more bees and are more aggressive. Some of the experimental colonies were also demanding to work with because of their defensive behaviour. Again, analysis of the relative

differences between colonies should not have been a problem, because colony size was estimated approximately at the same time of the day in both apiaries.

6.6 Cell size:

There were no significant differences in varroa mite fecundity and reproductive success among the surviving Norwegian honey bee colonies reared 4.9 (small cell), and 5.3 (standard cell) wax comb foundation. However, both models indicated a non-significant trend suggesting that mite fecundity and reproductive success was lower in the 4.9 population than in the 5.3 population. Interestingly, Oddie et al. (2018a) found a significant reduction in the number of viable female offspring in varroa-susceptible colonies reared on 4.9 mm wax foundation, compared to susceptible colonies reared on standard 5.3 mm foundation. No such difference in mite reproductive success was found in colonies from the surviving Norwegian population reared on small and conventional cells. If small cell wax foundation impairs mite reproductive success, it would be a very simple and cost-effective apicultural management practice that would not involve chemical treatments. This solution could be used alone or in combination with resistance breeding. However, the potential effects of reduced cell size on mite reproductive success are much disputed (Berry et al. 2010; Coffey et al. 2010; Piccirillo & De Jong 2003).

African honey bees *A. m. scutellata* and Africanized honey bees (European and African hybrid crosses) are resistant to varroa and produce cells that are smaller than cells drawn from commercial wax cell foundation. Several studies on African and Africanized honey bees suggest that foundress mites prefer larger brood cells (Message & Gonçalves 1995; Piccirillo & De Jong 2003). Furthermore, Piccirillo and De Jong (2003) claim that beekeepers make bees bigger than they would be naturally, by providing their colonies with wax foundation stamped with cell patterns that are bigger than the cells they would build on their own. This theory is supported by the study of Berry et al. (2010), who reported that the average mass of bees reared on 4.9 mm foundation were approximately 12 mg less than that of bees reared on 5.3 mm foundation. The same study also indicated that a reduced cell size had no negative effects on mite reproduction. Based on their study on mite reproduction in worker brood of the *A. m. capensis* clone, Martin and Kryger (2002) claim that less space within the brood cells can have a negative impact on the survival of male deutonymphs and mother mites, because it increases the chance that they get trapped in the upper part of the brood cell. However, Martin and Kryger (2002) also predict that smaller cell size would not be an effective method for controlling mite population growth in apiculture, because smaller cells

normally lead to smaller bees. On the other hand, Mcmullan and Brown (2006) claim that a reduction in cell size does not produce proportionally smaller bees in all *A. mellifera* subspecies. Some subspecies also have morphological differences that influence the amount of available space within the brood cell. According to Mcmullan and Brown (2006) the ratio of thorax -width to cell -width (fill -factor) is considerably higher in the European dark bee *A. m. mellifera* than in American strains, which leaves less available space for mite reproduction. The decrease in size of the bees reared on small cells was also less than 20 %, which is low compared to other strains. Still, Coffey et al. (2010) concluded that cell size had no effect on mite reproductive success in the European dark bee. Small cell wax foundation could be a solution for some subspecies and strains, depending on their morphometry and the extent to which the size of the bees is reduced in response to smaller cell size. This could potentially explain the differences in mite reproduction between the surviving Norwegian population and the control population observed by Oddie et al (2018a). However, no studies so far have examined the change in the size of bees from the surviving European populations reared in small and conventional cells.

At present it is not clear whether the potential difference in mite reproduction, and colony survival between the Norwegian surviving bees at 4.9 mm cells and 5.3 mm cells were related to cell size per se, or if this difference was related to socially transmitted mite resistance behaviour because the 4.9 mm population was established using both resistant queens and resistant worker bees. Social learning mechanisms in honey bees and other species of social insects is well documented (Leadbeater & Dawson 2017). Honey bee colonies have also evolved one of the most intricate communication systems documented among social insects. This communication system involves chemical cues and a dance language which is used to locate food, nest sites, or other resources. It also enables the individuals of the colony to respond collectively and rapidly in harmful situations (Winston 1987). However, because there were no differences in the rate of recapping or VSH between these two groups a social learning effect also appears unlikely.

6.7 Genotype-environment interactions:

Because the environmental conditions of Gotland, Sweden and Southern Norway are not very different in terms of climate and seasonality, I did not expect to find any large differences in varroa resistance and survival between the colonies from these two populations. Adding colonies from the surviving French population to the experiment would have provided more insight into this matter, but this was not practically feasible. Genotype × environment

interactions (GEI) can be defined as a change in the relative performance of two or more genotypes measured in two or more environments. In general, GEI arise when the performance of the different genotypes is not equally influenced by the different environments (Falconer & Mackay 1996). In addition to variation in biotic and abiotic environmental factors (e.g, climate, other pathogens, or pesticides), different apicultural practices can also produce genotype- environment interactions that can potentially affect varroa resistance (Büchler et al. 2013; Rosenkranz et al. 2009). My results suggested that foragers infested with mites from external sources contribute substantially to colony infestation levels. Therefore, the density of surrounding hives and apiaries may be an important factor affecting the survival of the two populations that were tested. Nolan and Delaplane (2017) demonstrated that the distance between individual colonies has a significant effect on mite infestation levels. These authors also point out that the distances between honey bee colonies in nature can range from 304 to 4848 m (Seeley & Smith 2015), while the distance between the hives of managed colonies can be less than 1 m.

Norway and Sweden have some of the lowest densities of bee honey bee colonies in Europe (1 hive per km^2 or less) (Chauzat et al. 2013). Migratory beekeeping is also restricted in Norway (Dahle 2010). More research is needed to find out whether specific resistance traits such as reduced post capping time and recapping behaviour are affected by climatic variables or apicultural practices. However, my study indicated that, even if the surviving colonies continue to express traits that reduce mite reproductive success in southern Europe, this may not be enough to ensure their survival in areas with highly intensified apiculture. The high losses of Swedish and Norwegian colonies at test sites in Southern Europe further substantiate this point. Finally, it is important to keep in mind that bee genotypes are only one part of a more complex system, which also involves mite and virus genotypes. Studies based on host-parasite systems in other species indicate that environment has a significant effect on host-parasite coevolution (Wolinska & King 2009). Mite associated virus infection dynamics have been studied in the Swedish surviving population, but not in the other European surviving populations (Locke et al. 2014). Nevertheless, epidemiological theory predicts that vertical transmission selects for less virulent parasites because the host is needed for parasite reproduction. If parasites are transmitted horizontally, natural selection may favour virulent parasites over avirulent parasites (Bull 1994). Therefore, preventing horizontal transmission of mites between colonies could select for mites that are less virulent (Nolan & Delaplane 2017).

7 Conclusion and implications for apiculture

The Swedish colonies continued to express resistance traits after being moved to Norway. Despite the high mortality and infestation rates observed in one of the test apiaries, I can not conclude that the colonies from the Swedish surviving populations are no longer resistant to varroa after being moved to Norway. However, the results also indicate that the surviving colonies may be vulnerable to infestation by mites from other colonies. This means that they may not be able to survive in Southern/Central Europe because the density of honey bee colonies in this area is much higher than in Scandinavia. Selecting for traits that enable the colonies to dispose of mites that enter the colonies via infested foragers could be a potential solution to this issue. Even if the environmental influence on traits that supress mite reproductive success in the surviving populations is minimal, resistance breeding based on a single “alround” resistant strain is probably not sustainable in the long term. This strategy would lead to loss of genetic diversity and local adaptations, which in turn would lead to less resilience towards new pests and pathogens. Cell size may have a supressing effect on mite reproduction in the Norwegian surviving colonies, but more research is needed. Studies on the size reduction of bees from the surviving populations reared in smaller cells, or quantification of the available space within small and large cells could perhaps provide more insight into this matter.

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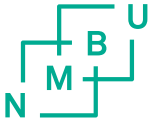

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway