



A selective sweep in a *Varroa destructor* resistant honeybee (*Apis mellifera*) population



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ABSTRACT

The mite *Varroa destructor* is one of the most dangerous parasites of the Western honeybee (*Apis mellifera*) causing enormous colony losses worldwide. Various chemical treatments for the control of the *Varroa* mite are currently in use, which, however, lead to residues in bee products and often to resistance in mites. This facilitated the exploration of alternative treatment methods and breeding for mite resistant honeybees has been in focus for breeders in many parts of the world with variable results.

Another approach has been applied to a honeybee population on Gotland (Sweden) that was exposed to natural selection and survived *Varroa*-infestation for more than 10 years without treatment. Eventually this population became resistant to the parasite by suppressing the reproduction of the mite. A previous QTL mapping study had identified a region on chromosome 7 with major loci contributing to the mite resistance. Here, a microsatellite scan of the significant candidate QTL regions was used to investigate potential footprints of selection in the original population by comparing the study population on Gotland before (2000) and after selection (2007). Genetic drift had caused an extreme loss of genetic diversity in the 2007 population for all genetic markers tested. In addition to this overall reduction of heterozygosity, two loci on chromosome 7 showed an even stronger and significant reduction in diversity than expected from genetic drift alone. Within the selective sweep eleven genes are annotated, one of them being a putative candidate to interfere with reduced mite reproduction. A glucose-methanol-choline oxidoreductase (GMCX18) might be involved in changing volatiles emitted by bee larvae that might be essential to trigger oogenesis in *Varroa*.

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1. Introduction

The Western honeybee (*Apis mellifera*) is one of the economically most important beneficial insect species (Klein et al., 2007; Watanabe, 1994) providing crop pollination services in addition to apicultural products (Morse and Calderone, 2000). In the United States a phenomenon called colony collapse disorder (CCD) has caused massive losses of honeybee colonies (Cox-Foster et al., 2007; vanEngelsdorp et al., 2008). Potential factors for causing CCD might be the occurrence of *Varroa destructor* in combination with several viruses (Cox-Foster et al., 2007). *Varroa* mites are also the main factor causing losses of colonies during winters in

temperate zones in Europe (Genersch et al., 2010). The mite is associated with Deformed Wing Virus (DWV) (Martin et al., 2012) and has been suggested to act as an important vector for this and other viruses.

V. destructor was found on *A. mellifera* for the first time in Europe almost five decades ago (Matheson, 1995). The details for the host shift from the original host species, *A. cerana*, are not finally clarified (Rosenkranz et al., 2010). Within a few decades the mite spread all over the world. Today only the honeybees in Australia (Anderson and Trueman, 2000; Oldroyd, 1999; Rosenkranz et al., 2010), and some isolated populations in northern Sweden and Norway (SJVFS, 2010), some islands (Tentcheva et al., 2004) and remote oases in deserts (Shaibi and Moritz, 2010) have been reported to be not infected. To control the parasite population usually chemical treatments (acaricides) are used. Without these treatments a colony typically collapses within 2–3 years in temperate climates (Boecking and Genersch, 2008; Rosenkranz et al.,

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2010). Various acaricides are available but unfortunately, *V. destructor* often evolves resistance against these substances within few generations (Elzen and Westervelt, 2004; Lodesani et al., 1995; Pettis, 2004). Additionally, the use of chemicals has been shown to cause residues in the hive products (Martel et al., 2007; Wallner, 1999). Most important however, chemical mite control removes the selective pressure on the host, which precludes any natural selection that might lead to resistant honeybees (Fries and Camazine, 2001).

Selective breeding for resistance to *Varroa* based on phenotypic traits has been considered in the past. Especially the duration of the post-capping stage has been claimed as a superior trait for breeding (Moritz and Jordan, 1992). As the mite is reproducing in the brood comb during the post-capping stage, the number of offspring produced depends on the duration of this stage. Indeed African subspecies south of the Sahara survive *Varroa* infestations without any treatment (Allsopp, 2006) which may be due to natural selection for resistance resulting in many different traits including migratory swarming (Hepburn and Radloff, 1998), enhanced hygienic behaviour (Boecking and Spivak, 1999) and a reduced developmental time (Moritz and Jordan, 1992). Although these traits may contribute towards resistance, it seems inappropriate to use African bees for any breeding purposes outside of Africa, after the experience about the unpredictable and excessive defensive behaviour of Africanized honeybees in the Americas (Collins et al., 1982). Selective breeding for hygienic behaviour resulted in the *Varroa* Sensitive Hygienic Behaviour honeybee strain (Harbo and Harris, 1999) where workers were able to detect infested brood cells and remove the mites (Abdullah et al., 2007). Another approach for producing *Varroa* resistant honeybees is based on natural selection and does not rely on any *a priori* phenotypic information. Such a large scale experiment was initiated in a remote location on the Swedish island Gotland in the year 1999 with the establishment of 150 colonies that had been infested with a defined low number of mites and were then left without any acaricide treatment (Fries et al., 2006). After substantial initial colony losses a stable resistant population of about 10–15 colonies could be established. The surviving colonies showed a significant reduction of the mites' reproductive success compared to colonies where mite control was applied (Locke and Fries, 2011). A cross infection experiment using bees and mites from the selected population as well as control bees and mites from an unselected population showed that the source of the bees was important, but not the source of mites (Fries and Bommarco, 2007). This suggested that indeed the resistance of the bees had been selected and that the trait had a genetic basis. A subsequent QTL-mapping approach (Behrens et al., 2011) identified three QTL regions on chromosomes four, seven and nine that show some additive effects, but also very strong epistatic interactions among the loci.

In this study, a microsatellite scan of the significant candidate QTL regions was used to investigate potential footprints of selection in this population by comparing the study population on Gotland before (2000) and after selection (2007).

2. Materials and methods

2.1. Sampling

The sampling area is located in the southern tip of the Island of Gotland in the Baltic Sea, Sweden (N58°01'–N58°04', E18°09'–E18°15'; a few meters above sea level).

The samples used for this study were collected in the year 2000 (start of the experiment) and in 2007 (after selection). In 2000 two workers per colony ($N = 58$) were sampled directly from the hives. Seven years later 50 drones were collected directly from the hives

and 24 were caught on a drone congregation area (DCA) using a pheromone trap (Yañez et al., 2011). Samples were stored in ethanol until further processing.

2.2. DNA extraction

DNA was extracted from flight muscles by means of a standard solvent extraction method using Phenol–Chloroform (Hunt and Page, 1995). The quantity and quality of extracted DNA was determined by a Nanodrop ND 1000 Spectrometer (peqlab, Erlangen, Germany). Equal amounts of DNA per individual were pooled into 4 pools for each sampling period. Pools were arranged in a way that they contained equal numbers of chromosomal sets for the drone and worker samples. Three pools containing 20 chromosomal sets (either 10 diploid workers (population 2000) or 20 haploid drones (population 2007)), a fourth pool was composed of 14 (7 workers (2000) or 14 drones (2007)) chromosomal sets.

2.3. Genotyping

The pools were genotyped for two of the significant QTL regions detected by Behrens et al. (2011) using 96 polymorphic microsatellite markers. The QTL on chromosome 9 was not considered, as it has only a very small contribution to the overall epistatic effect. 32 markers located on chromosome 4 (average marker spacing: 4.0 cM) and 64 on chromosome 7 (average marker spacing 2.6 cM) were chosen. For further analyses 39 markers were used (average marker spacing on chromosome 4: 4.6 cM; on chromosome 7: 4.9 cM), because the remaining loci either did not amplify or they revealed no polymorphism. Additionally, six reference loci located on several other chromosomes unlinked to the target regions were amplified for all pools.

The microsatellite target sequences were amplified by single locus PCR containing 7.35 µl HPLC-grade water, 1 µl 10× reaction buffer (peqlab, Erlangen, Germany), 0.2 µl dNTP's (10 mmol, diagonal, Münster, Germany), 0.4 µl of a primer pair (10 µmol, metabion, Martinsried, Germany) with the forward primer labelled with a fluorescent dye, 0.25 units *Taq*-Polymerase (peqlab, Erlangen, Germany) and 1 µl of the extracted (pooled) DNA. After an initial denaturation at 94 °C for 4 min 40 cycles of 94 °C for 30 s, 54 °C for 45 s and 72 °C for 45 s were used with a final elongation step at 72 °C for 5 min.

2.4. Fragment analysis

PCR products were separated, including an internal size standard (ET-Rox 400, GE Healthcare Europe GmbH, Freiburg, Germany), using an automated DNA capillary sequencer (MegaBACE 1000, GE Healthcare Europe GmbH, Freiburg, Germany) to determine the length of the amplified products. Electropherograms were analysed using MegaBACE Fragment Profiler Version 1.2. Allele scoring was controlled and inspected manually.

2.5. Statistical data analysis

Assuming that height of a peak in an electropherogram corresponds to the amount of DNA that was used as an input (Moritz et al., 2003) and that peak heights of a pooled sample are additive, the frequency of every allele within a pool was inferred. Since the total number of chromosomes in the pool was known, the total of all peak heights was set equal to 20 alleles (or 14 for the fourth pool). Hence 1/20 (or 1/14) was the contribution of a single allele in the pool. The peaks heights of the various alleles could then be converted into an integer number of alleles with the sum of all alleles corresponding to the number of chromosomal sets within

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