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

H. Michael G. Lattorff[1,2]; Josephine Buchholz[3]; Ingemar Fries[4]; Robin F.A. Moritz[2,3,5]

[1] Institut für Biologie, Tierphysiologie, Martin-Luther-Universität Halle-Wittenberg, Domplatz 4, 06099 Halle (Saale), Germany.

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

[3]Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany.

[4] Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, Uppsala 750-05, Sweden

[5]Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa.

Corresponding author:

HMG Lattorff

Institut für Biologie, Tierphysiologie,

Martin-Luther-Universität Halle-Wittenberg

06099 Halle (Saale), Germany

Phone: +49-345-5526389

Fax: +49-345-5527264

Email: lattorff@zoologie.uni-halle.de

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 (GMCOX18) might be involved in changing volatiles emitted by bee larvae that might be essential to trigger oogenesis in *Varroa*.

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, *Apis 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 & 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., 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).

Material and Methods

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.

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.

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 10x reaction buffer (peqlab, Erlangen, Germany), 0.2 µl

dNTP's (10mmol, diagonal, Münster, Germany), 0.4 µl of a primer pair (10µmol, 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.

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.

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 the respective pool. These allele counts were used to calculate the overall mean allele frequency in the replicate pools.

The pools were used as replicates in order to assess the reliability of the method for estimating allele frequencies by means of a Friedman test in order to evaluate the differences in allele frequency estimates amongst pools for a given locus.

The expected heterozygosity (H_E) for every locus and sample was calculated using the following formula:

$$H_E = 1 - \sum p_i^2 \quad (1)$$

with p_i – allele frequency of the i^{th} locus.

In order to exclude locus-specific effects $R\theta$ was calculated using the H_E of the workers from 2000 (Pop_0) and the drones from 2007 (Pop_7) (Wiehe et al., 2007).

$$R\theta = \frac{\left(\frac{1}{1-H(\mathrm{Pop}_7)}\right)^2 - 1}{\left(\frac{1}{1-H(\mathrm{Pop}_0)}\right)^2 - 1} \quad (2)$$

Thus, $lnR\theta$ values that are negative show a reduction of the H_E from 2000 to 2007.

The $lnR\theta$ values of the candidate regions were standardised using the mean and the variance of the $lnR\theta$ values of the reference loci. The probability of the $lnR\theta$ values of the target loci being in the range of the reference loci was calculated assuming a *t*-distribution with $df = 5$ for the reference loci. Probability values were calculated for the target loci to fit this cumulative *t*-distribution. A Bonferroni correction was used in

order to adjust the *p*-value for multiple testing. All calculations were done using standard spread sheet software.

The calculation of the effective population size (N_e) was done using the software TempoF$_s$ (Jorde and Ryman, 2007). This program estimates genetic drift (F_S) using observed allele frequencies of samples from different time points. The effective population size (N_e) can be calculated by the following formula, where *t* is the number of generations between the sampling populations, F_S is taken as an unbiased estimator:

$$N_e = \frac{t}{2F_S} \quad (3)$$

Candidate genes

The genome assembly *Apis mellifera* version 4.5 and the official gene set OGSv3.2 (Elsik et al., 2014) was used to determine candidate genes within the potential selective sweep. This region was restricted to the loci that showed probability values within the lower 5% tail of a cumulative t-distribution after Bonferroni adjustment for multiple tests. A core region was chosen for the main marker with a range of 0.5 cM to each side translating into 50 kb each (100 kb in total) based on the genome-wide recombination rate of 22 cM/MB (Solignac et al., 2007). This region was extended to include complete annotation of genes to finally 104 kb. We further broadened this region to include a second marker using the same approach resulting in a region of 282 kb. All annotated genes contained in the official gene set were used for a BLAST2GO (Conesa et al., 2005) search in order to find GeneOntology (GO) annotations for homologous genes or GO annotations for characteristic protein domains.

Results

The genotyping was started with 96 markers (32 on chromosome 4 and 64 on chromosome 7). Finally, 39 markers (5 and 34 on chromosomes 4 and 7, respectively) did successfully amplify and were used for further analyses. Chromosome 4 was excluded from selective sweep analysis, because of a too low marker density.

Reduction of genetic diversity

The honeybee population of Gotland shows a significant reduction in genetic diversity from 2000 to 2007 for the majority of screened polymorphic markers on chromosome 7, indicated by negative $lnR\theta$ values (mean ± s.d.: -0.72 ± 1.12), significantly lower than zero (single sample t-test, $t = 3.74$, $df = 33$, $p < 0.001$).Also the six reference loci, which are unlinked to the resistance trait, yield negative $lnR\theta$ values (mean ± s.d.: -1.08 ± 0.75; single sample t-test, $t = 3.51$, $df = 5$, $p = 0.02$). This effect can also be seen in the number of alleles, which showed a clear reduction over time (median number of alleles, 2000: 6; 2007: 4), which was close to significance (Wilcoxon matched pairs test, $z = 1.826$; $p = 0.07$) and the effective number of alleles (mean ± s.d.; 2000: 4.7 ± 1.6; 2007: 2.8 ± 0.7) was highly significantly reduced (paired t-test, two-tailed; $p = 0.01$).

Also on chromosome 4, the number of alleles was reduced from 4 alleles in 2000 to 3 alleles in 2007. However, this difference was not statistically significant (Wilcoxon matched pairs test, $z = 1.603$; $p = 0.11$) nor was the reduction in the effective number of alleles (paired t-test, $p = 0.997$).

In contrast the marker loci on chromosome 7 did show very highly significant differences for both, the number of alleles (median number of alleles, 2000: 5; 2007: 4;

Wilcoxon matched pairs test, z = -3.514; p = 0.0004) and the effective number of alleles (mean ± s.d.; 2000: 3.7 ± 1.7; 2007: 2.8 ± 1.2; paired t-test, two-tailed; p=0.0006).

Although there is an overall decrease in the number of alleles, also new alleles might occur, either due to mutation or to non-detection at the earlier time-point. In total, 25 loci did not gain new alleles, but 18 of the observed loci did gain at least one previously undetected allele. On the contrary, at 9 loci no losses of alleles were observed, while 34 loci lost at least one allele. This difference in gain vs. loss of alleles is highly significant (two-tailed Fischer's exact test, p=0.0008). The net effect of gain and loss of alleles showed for 13 loci a compensation of the loss by gain of new alleles, whereas only four loci gained at least one allele and 26 loci lost at least one allele. The difference in net gain/loss is also highly significant (two-tailed Fischer's exact test, p=0.0048).

The estimates of allele frequencies are very robust. Tests for deviations of allele frequency estimates amongst pools using a Friedman test revealed no significant deviations and indicate the robustness of the procedure.

Selective sweep

Two regions were found on chromosome 7with a reduction of heterozygosity significantly higher than in the flanking regions or reference loci (Fig. S1).

In the first region two loci, K0718 (position: 75.5 cM) and UN378 (76.4 cM), showed highly significant differences in their $lnR\theta$ values. The cumulative probability for the standardized *t* scores of these two loci to be sampled out of the distribution given by the reference loci is extremely low ($t_{(K0718)}$ = -6.41; $p_{(K0718)}$ = 1.2 x 10^{-12}; $t_{(UN378)}$ = -6.36; $p_{(UN378)}$ = 1.9 x 10^{-12}; cut-off value for a nominal 0.05 lower tail (Bonferroni corrected for 34 independent tests: 0.0015): -3.34; Fig. 1a). These two loci are separated by 1 cM

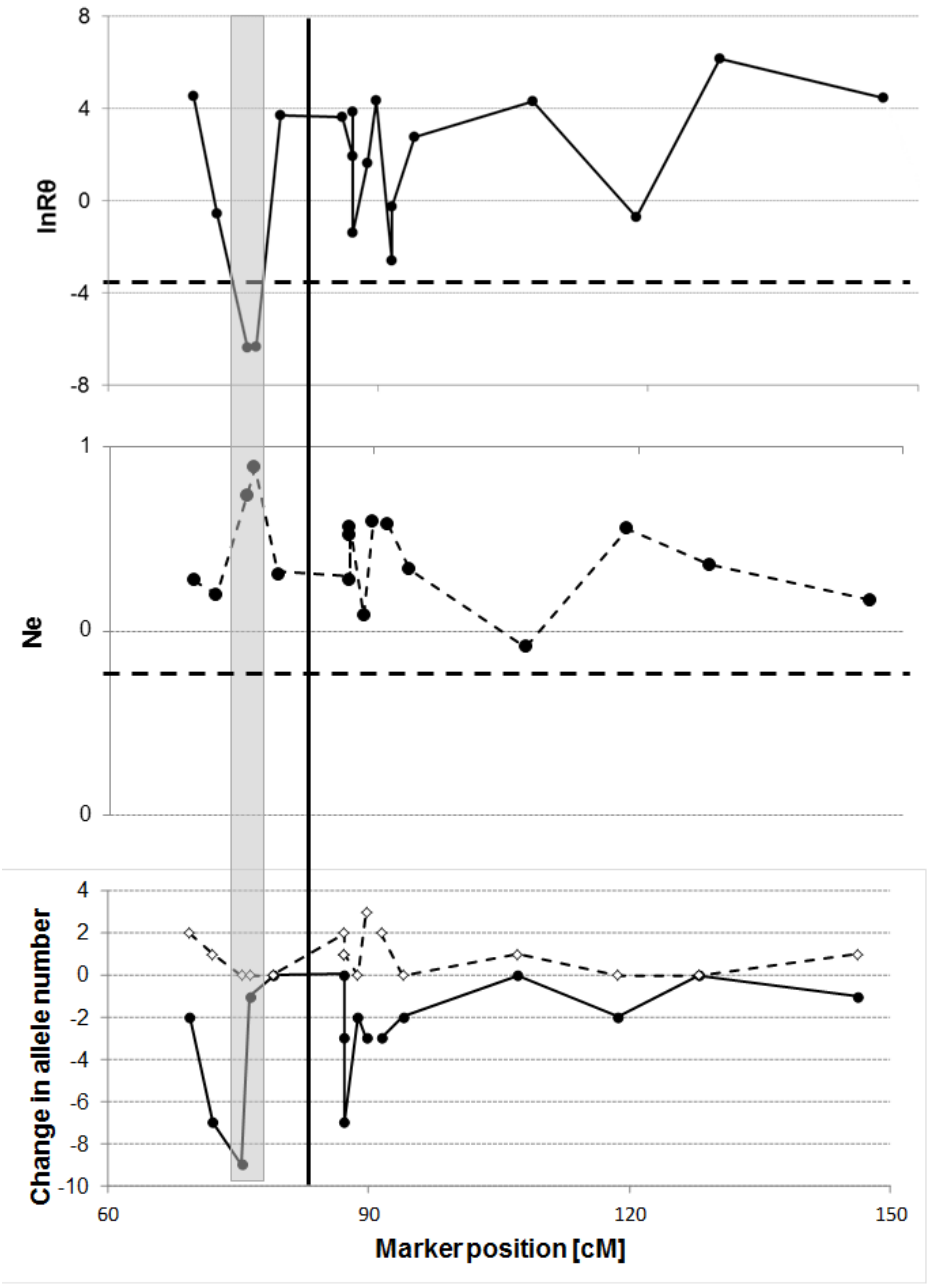

Fig. 1 Parameters for the 95% confidence interval region of the QTL on chromosome 7: in all three diagrams the location of the selective sweep is marked (greyshaded) as well as the QTL position (black vertical line). **A** shows the differences in the *lnR*θ values (scaled to reference loci) within the QTL region.The significance level (nominal level: 0.05; Bonferroni adjusted: 0.0015) is marked by the dashed line.In **B**the effective population size of every locus is shown and in **C** visualizesthe gain (dashed) and loss (full) of alleles for every marker is illustrated.

and about 12 cM away from the hot spot of the QTL mapping, but are located within the 95% confidence interval region for the QTL (Behrens et al., 2011). At locus K0718 the heterozygosity was reduced from 0.81 (2000) to 0.33 (2007) with 12 alleles detected in the year 2000 sample and only 3 alleles in 2007. The number of effective alleles decreased from 5 to about 1.

At locus UN378 the distinctions of the heterozygosity are grave (2000: H_E= 0.46, 2007: H_E= 0.05) and the number of alleles decreased from three (2000) to two (2007). The number of effective alleles was reduced by one (2000: 1.85; 2007: 1.05). At this locus evidence for a selective sweep is clearly visible (Fig. 2). Before selection three alleles

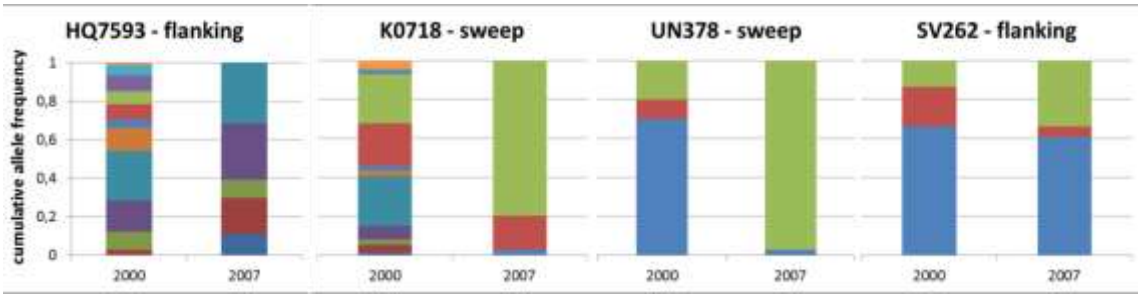

Fig. 2 Allele frequencies of flanking and sweep loci. Stapled bars indicate allele frequencies of the two flanking and the two sweep loci before and after selection. Per locus same colors represent same alleles.

were found (107, 116 and 119). The allele 119 was rare at this time-point with a frequency of 0.16, whereas the allele 107 was the most frequent one showing a frequency of 0.57. After seven years of selection only two alleles were left (107 and 119). Allele 107 became the rarest one (frequency: 0.03) whereas allele 119 had spread close to fixation (frequency: 0.97). Taking this information into account, it is obvious that locus UN378 is the locus, which is in more close proximity to the selective sweep than the second locus, K0718. More precisely, the allele 119 (UN378) is physically linked to a selected allele at another gene. Assuming linkage equilibrium for the two

loci before selection had started, most alleles of the highly diverse locus K0718 were physically linked to the both frequent alleles (107 and 116) of locus UN378, which were subsequently lost during the selection procedure as well as all other linked alleles. Thus, the increase of an allele (186) at locus K0718 from an initial frequency of 0.26 to 0.80 is a pure hitch-hiking effect caused by physical linkage.

The effective population size (N_e) was calculated for all loci with a 95% confidence interval ranging between N_e= 7 and N_e= 11. Locus UN378 shows the lowest value of all with a value of N_e= 2. Locus K0718 is also outside of the confidence interval (N_e= 6) (Fig. 1b).

The second region that shows signatures of a selective sweep is 69.1 cM away from the hotspot marker (UN391, highest LOD score) of the QTL mapping (Behrens et al., 2011) and therefore unlinked to the region detected by the QTL mapping. Furthermore, this region is well outside of the 95% confidence interval for the QTL, which has a range of 5 MB, ranging from the markers K0718 (75.5 cM) to K0711 (128 cM) (Fig. 1). This region includes two loci that show significant differences in heterozygosity UN327 (t= -5.06; p= 8.7 x 10^{-08}) and AT171 (t= -3.86; p = 0.0001). These loci are separated from each other by about 15 cM. Furthermore, there is one non-significant marker located in between them. At locus UN327 the heterozygosity decreased from 0.74 (2000) to 0.29 (2007) and also the number of alleles (median: 2000: 5; 2007: 2) as well as the number of effective alleles (2000: 3.87; 2007: 1.41) did show regression. The same effect on the heterozygosity can be seen at the second locus, AT171 (2000: 0.64; 2007: 0.23). The number of alleles at this locus declined from five in 2000 to two in 2007, as well as the number of effective alleles (2000: 2.81; 2007: 1.31).

Candidate genes

Eleven candidate genes were detected in the core region of 104 kb (Table 1), defined around the core locus of the selective sweep region UN378. Ten genes are protein coding. The remaining gene is a long non-coding RNAs (lncRNA) that is involved in regulation of gene expression of other genes. The target of the lncRNA is unknown. Amongst the protein coding genes, seven have homologs in other species and for five a function can be inferred based on the GO annotation of orthologs.

Amongst the protein coding genes with known function three are involved in basic metabolic and cellular processes. Dipeptidyl peptidase 9-like (GB53802) shows properties to cleave dipeptides from the N-terminal end after a proline. From mammalian models it is known that this protein is involved in processes like cell behaviour, in the biology of tumors and the adaptive immune system. The mitochondrial ribosomal protein L51 (mRpL51, GB53770) is a structural component of 39S subunit of mitochondrial ribosomes and hence involved in translational processes. The gene LOC100577875 (GB53769) shows homology to a low density lipoprotein receptor that might be involved in iron ion transport processes. The fourth gene, GMC oxidoreductase 18 (GMCOX18, GB53768) is a member of the glucose-methanol-choline oxidoreductase family (GMC oxidoreductases). These enzymes are FAD dependent oxidoreductases showing a range of catalytic properties. Finally, there is a gamma-aminobutyric acid (GABA) B receptor subunit 2 (GABBR2, GB53807). This forms, together with subunit 1, a transmembrane receptor within the nervous system inhibiting neuronal activity through G-protein coupled second messenger systems. The potential involvement in resistance to *Varroa* of the lncRNA and five of the protein coding genes without inferred, cannot be predicted so far.

Discussion

Over the seven years of isolation and the strong selection pressure by the *Varroa* mite, the honeybee population on Gotland showed high losses (95 %) of colonies (Fries et al., 2006). This also led to a strong overall reduction of the genetic diversity measured in terms of heterozygosity, the number or effective number of alleles, respectively. Nevertheless, it was possible to detect a small genomic region within a QTL candidate region (Behrens et al., 2011) that shows strong evidence for the occurrence of a selective sweep. One of the two markers located within this region (UN378) shows an extremely strong signature of a selective sweep and a formerly rare allele (119) has spread nearly to fixation (Fig. 2). The second locus (K0718), which showed 12 different alleles before selection, lost most alleles of low frequency, which were linked to the frequent allele 107 (UN378) that showed a drastic decrease of the frequency.

Detection limit

The detection of selective sweeps in honeybees generally is complicated due to the high recombination rate of 22.04 cM/Mb (Solignac et al., 2007; HGSC, 2006). This can be overcome by using a high marker density within the region of interest. Selective sweep mapping for the verification of previously mapped QTLs has been used for a study of *Nosema* resistant bees (Huang et al., 2014a, b). This study has shown that a signature of selection can be inferred with increasing marker coverage within the QTL region. However, Huang et al. (2014b) used a selected and an unselected population for the selective sweep study, both of them with an overall substantial amount of genetic variation. Here, we use a similar approach using previous knowledge about the location

of the candidate region derived from the QTL mapping (Behrens et al., 2011). A high marker density (1.83 cM), being 10 times higher than the average marker density of the QTL study, was chosen for the mapping of the potential selective sweep region. The main difference to the study of Huang et al. (2014) is that the population chosen for the study in our case was the same population sampled before the selection had started and after a severe selection pressure had taken place.

As the detection of selective sweeps depends on an accurate estimation of the population wide allele frequencies, there will be an uncertainty due to finite sample sizes. However, the sampling strategy for the current study was based on two criteria, 1) a similar sample size between temporal sampling points and 2) the most rigorous estimation for the second sample, that might have suffered from an overall reduction of genetic diversity due to genetic drift. Therefore, the second sampling period was accomplished by a full sampling of all available colonies (7 colonies in 2007). This results in sampling of 25 % of the colonies present at the beginning of the experiment in 2000 (39 of 150) using a similar sized sample. Thus, the estimates of the allele frequencies for the second sampling are based on the total population, whereas the estimates for the first sampling are based on 25 % of the population resulting in a detection of alleles with a frequency of 0.05 with a probability higher than 95%.

Tolerance and resistance

In a previous study, dealing with honeybee/*Varroa*-mite interactions, the up- and down-regulation of several genes that might be involved in this interaction was determined. Navajas et al. (2008) compared *Varroa*-parasitized and non-parasitized full-sister pupae from two different genetic stocks, one susceptible and one resistant. The main

conclusion of this study was that differences in gene expression are associated with both, the presence of the parasite and *Varroa*-resistance. The experiments did show that there are significant differences in the expression of genes responsible for neuronal excitability, the proliferation of neuroblasts within the mushroom-body and olfactory cognition. This leads to the assumption that the mite-resistant individuals are more sensitive to external stimuli than susceptible ones. Thus, olfaction and neuronal sensitivity may play an important role for the detection of infested brood cells within the hive. Furthermore, the combination of these two features seems to be associated with behaviours like hygienic behaviour and grooming. However, it was never investigated if there were any behavioural differences in the studied populations (Navajas et al., 2008).

The Gotland population did show significant differences in the colony size and the reproductive success of *Varroa destructor* (Locke and Fries, 2011) compared to non-selected honeybee colonies. However, hygienic behaviour and grooming were not increased (Locke and Fries, 2011).

Therefore it appears that the survival of the Gotland population as well as the French population studied by Navajas et al. (2008) is not a matter of tolerance, but one of true resistance, because it is based on a significant reduction of *Varroa* reproduction (Locke et al., 2012). On Gotland the fecundity and reproductive output of the mite was impaired, whereas the proportion of infertile female mites were much higher in the selected French population (Locke et al., 2012). In the latter case it is still unclear if the high proportion of infertile mites were due to selective removal of reproducing mites or an actual reduction in mite fertility. There are several possible reasons for the reduction in reproductive capacity. Since the parasitic mite reproduces in sealed brood cells, resistance due to reduced mite reproduction might be based on changes in the post-

capping developmental process of the honeybee. The resistant phenotype in the QTL mapping study was the failure of the *Varroa* mites to reproduce on drone pupae suggesting that the mites suffered some deficiency that failed to initiate their ovary activation.

Regulation of vitellogenesis and oogenesis

Several hypotheses have been proposed to explain the initiation of vitellogenesis and hence oogenesis in *Varroa* mites. Based on experimental manipulations it has been suggested that chemical signals of the 5th instar spinning larvae initiate oogenesis in *Varroa* mites when entering the cell shortly before capping (Garrido and Rosenkranz, 2004) within a very short time window (Frey et al, 2013). Since mites introduced into cells containing larvae that are too old do not activate their ovaries (Frey et al., 2013) it has been suggested that the smell of a larva encountered by a *Varroa* mite when entering a brood cell is sufficient to trigger oogenesis (Garrido and Rosenkranz, 2004). Oogenesis activation induced by volatiles of bee larvae does not require the specific uptake of any substance.

Contrary to this, it has been stated that oogenesis in *Varroa* is initiated by sucking hemolymph of bee larvae (Steiner et al., 1994). The latter hypothesis predicts that mites have to take up substances from their hosts in order to activate oogenesis.

The most common mechanism in blood feeding arthropods is nutritional sensing of amino acids after a blood meal. Studies on ticks (Umemiya-Shirafuji et al., 2012) and mosquitoes (Hansen et al., 2004) showed that the *vitellogenin* (*vg*) synthesis is activated by a blood meal. *Vitellogenines* are proteins produced in the fat body and are taken up by the maturing oocytes. During embryogenesis vitellin (glycosylated vitellogenin)

contained in the yolk is a very important source of nutrients (Cabrera Cordon et al., 2013). The sensing of nutrients taken up due to a blood meal occurs via the target of rapamycin (TOR) signalling pathway, which is a regulatory network that controls cellular activity according to nutrient availability and is essential for the yolk protein precursor (YPP) expression in mosquitoes (Hansen et al., 2004) and ticks (Umemiya-Shirafuji et al., 2012). Nevertheless, it might be that other substances have to be taken up due to a blood meal. However, large molecules like proteins are unlikely to be such substances as they are enzymatically digested in the midgut of blood feeding arthropods. Smaller molecules and / or hydrophobic substances that might be able to pass the membranes of epithelial midgut cells are prime candidates.

Potentially, oogenesis is activated by both, chemical signals emitted by larvae and substances taken up during hemolymph sucking (Garrido and Rosenkranz, 2004). While volatiles of the larvae might prime the mites, molecules from bee hemolymph might act to finally complete oogenesis. Resistance of honeybees in form of reducing mite reproduction might occur in two different ways. Volatiles emitted by larvae are altered in resistant bees and do not act to initiate *Varroa* oogenesis. Otherwise, factors of the hemolymph might be changed, so that factors essential for oogenesis of the mite in addition to the TOR signalling pathway are absent in resistant honeybees. Alternatively, the hemolymph of the resistant honeybees might contain additionally a factor that inhibits TOR signalling, *vitellogenin* synthesis or uptake of *vitellogenin* into oocytes.

Candidate genes

At this stage, the analyses of the candidate genes within the selective sweep region does not allow for a conclusive decision for one of the hypotheses, as none of the annotated genes is directly involved in *vitellogenesis*, oogenesis or TOR signalling.

Within the sweep region, eleven candidate genes are predicted based on genome assembly 4.5 of *Apis mellifera* and the gene annotations of the official gene set 3 (OGSv3.2) (Elsik et al., 2014). None of the candidate genes seems to be directly involved in reproduction with a potential as an essential component that has to be taken up by *Varroa* mites in order to trigger vitellogenesis and oogenesis. However, potential candidates are an lncRNA that might be involved in the down-regulation of certain genes essential for *Varroa* reproduction and a GABA (γ-amino-butyric acid)-receptor, which might be responsible for inhibitory signals within the central nervous system of the host.

A promising candidate is a glucose-methanol-choline (GMC) oxidoreductase 18 (GMCOX18, GB53768), a member of a large family of oxidoreductases with a total of 21 genes in *A. mellifera* (Kunieda et al., 2006) with diverse functions in sperm storage, glucose metabolism and cuticle biosynthesis (Kunieda et al., 2006). Gene expression studies have shown that several GMC oxidoreductases are expressed during larval and pupal stages in *Drosophila*, but also in epidermal cells in adults with similar expression patterns in the honeybee (Iida et al., 2007). Studies in chrysomelid beetles have shown that members of the GMC oxidoreductases are involved in chemical defences (Michalski et al., 2008; Rahfeld et al., 2014).

Taken together this might indicate that the chemical properties of honeybee larvae might be changed during the selection process in the Gotland population. If indeed initiation of *Varroa* oogenesis is dependent on chemical cues from larvae and these had been changed in the Gotland population this might explain the generally reduced mite reproduction in colonies from Gotland irrespective of the mite origin (Fries and Bommarco, 2007).

Acknowledegment

We are grateful to P. Leibe for technical assistance with genotyping. Financial support was granted by the BEEDOC research network (EU FP7 to RFAM and IF, EU contract number: FP7-KBBE-2009-3 244956 CP-FP).

References

Abdullah, I., Reuter, G. S., Spivak, M., 2007. Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa destructor*. Apidologie 38, 67–76.

Allsopp, M., 2006. Analysis of *Varroa destructor* infestation of southern African honey bee populations. MS Dissertation, University of Pretoria, Pretoria.

Anderson, D.L., Trueman, J.W., 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp. Appl. Acarol. 24, 165–189.

Behrens, D., Huang, Q., Geßner, C., Rosenkranz, P., Frey, E., Locke, B., Moritz, R.F.A., Kraus, F.B., 2011. Three QTL in the honey bee *Apis mellifera* L. suppress reproduction of the parasitic mite *Varroa destructor*. Ecol. Evol.1, 451–458.

Boecking, O., Genersch, E., 2008. Varroosis – the Ongoing Crisis in Bee Keeping. J. Verbrauchersch. Lebensmittelsich. 3, 221–228.

Boecking, O; Spivak, M., 1999. Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. Apidologie 30, 141-158

Cabrera Cordon, A.R., Shirk, P.D., Duehl, A.J., Evans, J.D., Teal, P.E.A. 2013. Variable induction of vitellogenin genes in the varroa mite, *Varroa destructor* (Anderson & Trueman), by the honeybee, *Apis mellifera* L, host and its environment. Ins. Mol. Biol. 22, 88–103.

Collins, A.M., Rinderer, T.E., Harbo, J.R., Bolten, A.B., 1982. Colony defense by Africanized and European honey bees. Science 218, 72-74

Conesa, A., Götz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., Robles, M., 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674-3676.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A, Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S., Lipkin, W.I., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science, 318, 283–287.

Elsik, C.E., Worley, K.C., Bennett, A.K., Beye, M., Camara, F., Childers, C.P., de Graaf, D.C., Debyser, G., Deng, J., Devreese, B., Elhaik, E., Evans, J.D., Foster, L.J., Graur, D., Guigo, R., HGSC production teams, Hoff, K.J., Holder, M.E., Hudson, M.E., Hunt, G.J., Jiang, H., Joshi, V., Khetani, R.S., Kosarev, P., Kovar, C.L., Ma, J., Maleszka, R., Moritz, R.F.A., Munoz-Torres, M.C., Murphy, T.D., Muzny, D.M., Newsham, I.F., Reese, J.T., Robertson, H.M., Robinson, G.E., Rueppell, O., Solovyev,

V., Stanke, M., Stolle, E., Tsuruda, J.M., Van Vaerenbergh, M., Waterhouse, R.M., Weaver, D.B., Whitfield, C.W., Wu, Y., Zdobnov, E.M., Zhang, L., Zhu, D., Gibbs, R.A., 2014. Finding the missing honey bee genes: lessons learned from a genome upgrade. BMC Genomics 15, 86.

Elzen, P., Westervelt, D., 2004. A scientific note on reversion of fluvalinate resistance to a degree of susceptibility in *Varroa destructor*. Apidologie 35, 519–520.

Frey, E., Odemer, R., Blum, T., Rosenkranz, P., 2013. Activation and interruption of the reproduction of *Varroa destructor* is triggered by host signals (*Apis mellifera*). J. Inv. Pathol. 113, 56–62.

Fries, I., Bommarco, R., 2007. Possible host-parasite adaptations in honey bees infested by *Varroa destructor* mites. Apidologie 38, 525–533.

Fries, I., Camazine, S., 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie 32, 199–214.

Fries, I., Imdorf, A., Rosenkranz, P., 2006. Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. Apidologie 37, 564–570.

Garrido, C., Rosenkranz, P., 2004. Volatiles of the honey bee larva initiate oogenesis in the parasitic mite *Varroa destructor*. Chemoecology 14, 193–197.

Genersch, E., von der Ohe, W., Kaatz, H.H., Schroeder, A., Otten, C., Buchler, R., Berg, S., Ritter, W., Muhlen, W., Gisder, S., Meixner, M., Liebig, G., Rosenkranz, P., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41: 332-352.

Hansen, I., Attardo, G.M., Park, J.H., Peng, Q., Raikhel, A.S., 2004. Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc. Natl. Acad. Sci. USA 101, 10626–10631.

Harbo, J.R., Harris, J.W., 1999. Selecting honey bees for resistance to *Varroa jacobsoni*. Apidologie 30, 183-196.

Hepburn, H.R., Radloff, S.E., 1998. Honeybees of Africa. Springer.

Honeybee Genome Sequencing Consortium (HGSC), 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature 443, 931–949.

Huang, Q., Kryger, P., Le Conte, Y., Lattorff, H.M.G., Kraus, F.B., Moritz, R.F.A., 2014a. Four quantitative trait loci associated with low *Nosema ceranae* (Microsporidia) spore load in the honeybee *Apis mellifera*. Apidologie 45, 248-256.

Huang, Q., Lattorff, H.M.G., Kryger, P., Le Conte, Y., Moritz, R.F.A., 2014b. A selective sweep in a microsporidian parasite *Nosema*-tolerant honeybee population, *Apis mellifera*. Anim. Genet. 45, 267-273.

Hunt, G.J., Page, R.E., 1995. Linkage Map of the Honey Bee, *Apis mellifera*, based on RAPD Markers. Genetics 139, 1371–1382.

Iida, K., Cox-Foster, D.L., Yang, X., Ko, W.Y., Cavener, D.R., 2007. Expansion and evolution of insect GMC oxidoreductases. BMC Evol.Biol. 7, 75.

Jorde, P.E., Ryman, N., 2007. Unbiased estimator for genetic drift and effective population size. Genetics 177, 927–935.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. P. Roy. Soc. B 274, 303–313.

Kunieda, T., Fujiyuki, T., Kucharski, R., Foret, S., Ament, S.A., Toth, A.L., Ohashi, K., Takeuchi, H., Kamikouchi, A., Kage, E., Morioka, M., Beye, M., Kubo, T., Robinson, G.E., Maleszka, R., 2006. Carbohydrate metabolism genes and pathways in insects: insights from the honey bee genome. Ins. Mol. Biol. 15, 563–576.

Locke, B., Fries, I., 2011. Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. Apidologie 42, 533–542.

Locke, B., Le Conte, Y., Crauser, D., Fries, I., 2013. Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honeybee populations. Ecol. Evol. 2, 1144-1150.

Lodesani, M., Colombo, M., Spreafico, M., 1995. Ineffectiveness of Apistan treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy). Apidologie 26, 67–72.

Martel, A., Zeggane, S., Aurières, C., Drajnudel, P., Faucon, J., Aubert, M., 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar or Asuntol 50. Apidologie 38, 534–544.

Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., Schroeder, D.C., 2012. Global honey bee viral landscape altered by a parasitic mite. Science 336, 1304–1306.

Matheson, A. 1995. First documented findings of *Varroa jacobsoni* outside its presumed natural range.Apiacta 30, 1-8.

Michalski, C., Mohagheghi, H., Nimtz, M., Pasteels, J., Ober, D., 2008. Salicyl Alcohol Oxidase of the Chemical Defense Secretion of Two Chrysomelid Leaf Beetles. Molecular and functional characterization of two new members of the glucose-methanol-choline oxidoreductase gene family. J. Biol. Chem. 283, 19219–19228.

Moritz, R.F.A., Jordan, M., 1992. Selection of resistance against *Varroa jacobsoni* across caste and sex in the honeybee (*Apis mellifera* L., Hymenoptera: Apidae). Exp. Appl. Acarol.16, 345–353.

Moritz, R.F.A., Scharpenberg, H., Lattorff, H.M.G., Neumann, P., 2003. A technical note for using microsatellite DNA analyses in haploid male DNA pools of social Hymenoptera. Insectes Soc. 50, 398-400.

Morse, R.A., Calderone, N.W., 2000. The Value of Honey Bees As Pollinators of U.S. Crops. Bee Culture 128.

Navajas, M., Migeon, A., Alaux, C., Martin-Magniette, M.L., Robinson, G.E, Evans, J.D., Cros-Arteil, S., Crauser, D.,Le Conte, Y., 2008. Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. BMC Genomics 9, 301.

Oldroyd, B.P., 1999. Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. Trends Ecol. Evol. 14, 312–315.

Pettis, J.S., 2004. A scientific note on *Varroa destructor* resistance to coumaphos in the United States. Apidologie 35, 91–92.

Rahfeld, P., Kirsch, R., Kugel, S., Wielsch, N., Stock, M., Groth, M., Boland, W., Burse, A., 2014. Independently recruited oxidases from the glucose-methanol-choline oxidoreductase family enabled chemical defences in leaf beetle larvae (subtribe Chrysomelina) to evolve. Proc. R. Soc. B 281: 20140842. http://dx.doi.org/10.1098/rspb.2014.0842

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Invert.Pathol.103, S96–119.

Shaibi, T., Moritz, R.F.A., 2010. 10,000 years in isolation? Honeybees (*Apismellifera*) in Saharan oases. Cons. Genet. 11, 2085–2089.

Solignac, M., Mougel, F., Vautrin, D., Monnerot, M., Cornuet, J.M., 2007. A third-generation microsatellite-based linkage map of the honey bee, *Apis mellifera*, and its comparison with the sequence-based physical map. Genome Biol. 8, R66.

Steiner, J., Dittmann, F., Rosenkranz, P., Engels, W., 1994. The first gonocycle of the parasitic mite (*Varroa jacobsoni*) in relation to preimaginal development of its host, the honey bee (*Apis mellifera carnica*). Invert.Repr.Dev. 25, 175–183.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M.E., Bergoin, M., 2004. Prevalence and Seasonal Variations of Six Bee Viruses in *Apis mellifera* L .and *Varroa destructor* Mite Populations in France. Appl. Environ. Microbiol. 70, 7185–7191.

Umemiya-Shirafuji, R., Boldbaatar, D., Liao, M., Battur, B., Rahman, M. M., Kuboki, T., Galay, R.L., Tanaka, T., Fujisaki, K., 2012. Target of rapamycin (TOR) controls vitellogenesis via activation of the S6 kinase in the fat body of the tick, *Haemaphysalis longicornis*. Int. J. Parasitol. 42, 991–998.

VanEngelsdorp, D., Hayes, J., Underwood, R.M., Pettis, J., 2008. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PloS One 3, e4071.

Wallner, K., 1999. Varroacides and their residues in bee products. Apidologie 30, 235–248.

Watanabe, M.E., 1994. Pollination worries rise as honey bees decline. Science 265, 1170.

Wiehe, T., Nolte, V., Zivkovic, D., Schlötterer, C., 2007. Identification of Selective Sweeps Using a Dynamically Adjusted Number of Linked Microsatellites. Genetics 175, 207–218.

Yañez, O., Jaffé, R., Jarosch, A., Fries, I., Moritz, R.F.A., Paxton, R.J., Miranda, J.R., 2011. Deformed wing virus and drone mating flights in the honey bee (*Apis mellifera*): implications for sexual transmission of a major honey bee virus. Apidologie 43, 17–30.

SUPPLEMENTARY MATERIAL

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

H. Michael G. Lattorff, Josephine Buchholz; Ingemar Fries, Robin F.A. Moritz

Table S1. Characteristics for microsatellite loci on chromosome 7; 4 and for control loci (on chromosomes 1; 2; 3; 6 and 8) spread over several chromosomes. Chr – chromosome, cM – genetic position on chromosome, H_E – expected heterozygosity, lnR – according to formula (2)

						No. of alleles		No. of effective alleles	
Primer	Chr	cM	H_E(2000)	H_E(2007)	lnR	2000	2007	2000	2007
K0720	7	69.5	0.787	0.817	0.31497	10	10	4.69	5.45
HQ7593	7	72.1	0.869	0.758	-1.26968	11	5	7.19	4.13
K0718	7	75.5	0.811	0.333	-3.07371	12	3	5.29	1.50
UN378	7	76.4	0.460	0.0526	-3.05824	3	2	1.85	1.06
SV262b	7	79.1	0.502	0.5131	0.058754	3	3	2.01	2.05
HQ7624	7	86	0.760	0.764	0.035747	6	8	4.16	4.23
A107	7	87.2	0.789	0.735	-0.49086	5	4	4.75	3.77
UN315b	7	87.2	0.477	0.634	0.891855	2	3	1.91	2.73
UN391	7	87.2	0.856	0.703	-1.52051	10	4	6.95	3.37
BE007b	7	88.8	0.577	0.470	-0.5839	4	2	2.37	1.89
HQ7671	7	89.8	0.703	0.736	0.251966	5	5	3.37	3.79
AP256	7	91.5	0.881	0.704	-1.90029	14	9	8.40	3.38
UN334d	7	91.5	0.822	0.692	-1.16747	7	6	5.62	3.24
BI300	7	94	0.715	0.683	-0.23251	6	4	3.51	3.16
K0716B	7	107.2	0.601	0.641	0.24774	3	4	2.50	2.78
A080	7	118.7	0.633	0.394	-1.31406	4	2	2.72	1.65
K0711	7	128	0.339	0.494	0.814195	2	2	1.51	1.98

UN023	7	146.2	0.721	0.756	0.291088	8	8	3.58	4.10
UN327	7	156.3	0.741	0.290	-2.65439	5	2	3.87	1.41
AC066	7	158.5	0.721	0.406	-1.85973	4	3	3.58	1.68
AT171	7	171.6	0.644	0.234	-2.28401	5	2	2.81	1.30
A024	7	182.1	0.692	0.482	-1.25537	4	2	3.25	1.93
SV187	7	189.6	0.767	0.612	-1.12968	6	4	4.30	2.58
BI111	7	190.6	0.751	0.566	-1.24932	7	4	4.01	2.31
K0703	7	196.7	0.531	0.626	0.550485	4	4	2.13	2.68
K0757	7	210.4	0.406	0.579	0.925313	2	3	1.68	2.37
BI080	7	213.3	0.790	0.793	0.029296	7	9	4.76	4.83
K0702B	7	216.4	0.665	0.497	-0.98522	5	2	2.98	1.99
BI217	7	220.1	0.817	0.805	-0.13207	6	6	5.45	5.12
BI235	7	221.2	0.580	0.651	0.435978	4	4	2.38	2.87
AC205	7	229.2	0.690	0.398	-1.67458	6	3	3.22	1.66
AC174	7	230.8	0.368	0.339	-0.15536	2	2	1.58	1.51
AT035	7	235.7	0.825	0.671	-1.35145	10	5	5.73	3.04
K0763	7	237.9	0.610	0.736	0.873047	8	5	2.57	3.79
K0430	4	58.4	0.708	0.657	-0.36177	4	4	3.43	2.92
K0429	4	69.4	0.386	0.518	0.69013	4	3	1.63	2.07
AT047	4	75.7	0.485	0.614	0.723083	3	3	1.94	2.59
K0428	4	79.6	0.604	0.606	0.010962	5	3	2.53	2.54
BI106	4	81.6	0.561	0.406	-0.82319	3	2	2.28	1.68
A079	1	32.8	0.861	0.719	-1.47538	13	5	7.20	3.56
A033	2	85.6	0.836	0.589	-1.99011	8	3	6.08	2.43
A008	2	192.8	0.722	0.709	-0.09774	5	4	3.59	3.44
UN351B	3	250.3	0.765	0.674	-0.71387	7	5	4.26	3.06
A113	6	58.6	0.716	0.420	-1.75377	4	4	3.52	1.72
A014	8	60.4	0.716	0.646	-0.49151	4	4	3.52	2.82

Table S2. Results of a Friedman-test for estimation of the reliability of allele frequency estimates using different pools.

Locus	chi-square	p-value	df
A107	0.000	1.00	2
A113	0.539	0.91	3
A14	0.375	0.95	3
A24	0.000	1.00	3
A33	0.269	0.97	3
A79	1.971	0.58	3
A8	1.800	0.61	3
A80	1.077	0.58	2
AC174	0.000	1.00	3
AC205	0.623	0.89	3
AC66	0.091	0.99	3
AP105	0.395	0.94	3
AP256	2.638	0.45	3
AT171	0.273	0.97	3
AT35	0.174	0.98	3
AT47	0.333	0.95	3
BE007	2.676	0.44	3
BI106	0.536	0.91	3
BI111	0.153	0.98	3
BI217	0.231	0.97	3
BI235	0.618	0.89	3
BI300	0.660	0.88	3
BI80	4.359	0.23	3
HQ7593	0.281	0.96	3
HQ7623	4.132	0.25	3
HQ7624	0.780	0.85	3
HQ7671	0.073	0.99	3
K0428	2.707	0.44	3
K0429	1.100	0.78	3
K0430	0.711	0.87	3
K0702	0.929	0.82	3
K0703	1.286	0.73	3
K0711	0.000	1.00	3
K0716	1.000	0.80	3
K0718	6.663	0.08	3
K0720	1.560	0.67	3
K0757	1.105	0.78	3
K0763	5.067	0.08	2
SV187	0.474	0.92	3
SV262	1.000	0.80	3

UN023	0.783	0.85	3
UN315	0.000	1.00	3
UN327	3.128	0.37	3
UN334	0.915	0.82	3
UN351	3.000	0.39	3
UN378	0.182	0.91	2
UN391	1.398	0.71	3

FigS1. Comparison of the effective number of alleles for the loci in the selective sweep (K0718 and UN378) and the flanking loci (HQ7593 and SV262) for the two different time points.