Digging into the Genomic Past of Swiss Honey Bees by Whole-Genome Sequencing Museum Specimens

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Abstract

Historical specimens in museum collections provide opportunities to gain insights into the genomic past. For the Western honey bee, *Apis mellifera* L., this is particularly important because its populations are currently under threat worldwide and have experienced many changes in management and environment over the last century. Using Swiss *Apis mellifera mellifera* as a case study, our research provides important insights into the genetic diversity of native honey bees prior to the industrial-scale introductions and trade of non-native stocks during the 20th century—the onset of intensive commercial breeding and the decline of wild honey bees following the arrival of *Varroa destructor*. We sequenced whole-genomes of 22 honey bees from the Natural History Museum in Bern collected in Switzerland, including the oldest *A. mellifera* sample ever sequenced. We identify both, a historic and a recent migrant, natural or human-mediated, which corroborates with the population history of honey bees in Switzerland. Contrary to what we expected, we find no evidence for a significant genetic bottleneck in Swiss honey bees, and find that genetic diversity is not only maintained, but even slightly increased, most probably due to modern apicultural practices. Finally, we identify signals of selection between historic and modern honey bee populations associated with genes enriched in functions linked to xenobiotics, suggesting a possible selective pressure from the increasing use and diversity of chemicals used in agriculture and apiculture over the last century.

Key words: *Apis mellifera mellifera*, museum genomics, genetic diversity, selection signatures, haplotype phasing, biodiversity.

Significance

Little is known about native honey bees' genetic diversity and structure preagricultural and apicultural revolutions during the 20th century—the beginning of commercial bee breeding and decline of wild honey bees following the arrival an invasive ectoparasite. We find no reduction in genetic diversity of a historic honey bee population compared with its contemporary conspecifics. We further identify genes enriched in functions linked to immunity, and the detoxification of possible agrochemicals. The results do not only reveal novel insights into the honey bee genomic past, but also provide valuable baseline genomic data of native populations aiming at making improved conservation management decisions. In addition, our approach to sequence honey bee museum samples serves as a case study for the sequencing of other precious museum specimens.

Introduction

For the most important pollinator of wild and cultured plants, the Western honey bee (*Apis mellifera*) (Klein et al. 2007; Gallai et al. 2009; IPBES 2016), much has changed in the last decades. Today, it is under pressure globally from new invasive parasites and emergent pathogens, increased use of pesticides, habitat loss, and climate change (Neumann and Carreck 2010; Potts et al. 2010; Vanengelsdorp and Meixner 2010), culminating in major losses of managed honey bee colonies worldwide (Liu et al. 2016; Maggi et al. 2016; Gray et al. 2019; Morawetz et al. 2019).

One of the primary factors driving colony losses is the ectoparasitic mite, *Varroa destructor*, and its associated viruses (Guzmán-Novoa et al. 2010; Dainat et al. 2012). In the late 1970s, this parasite, native to Asia, spread throughout Western Europe and North America decimating wild *A. mellifera* colonies. Such is the scale of the threat that today the majority of honey bees cannot survive without human intervention (Rosenkranz et al. 2010). It is thus generally accepted that feral honey bee colonies nearly became extinct after the arrival of the mite (Moritz et al. 2007; De la Rúa et al. 2009), although there are reports of wild honey bee populations persisting in large woodlands (Seeley 2017; Kohl and Rutschmann 2018).

The widespread colony losses and associated population size decline might have potentially resulted in a genetic bottleneck for the remaining wild European honey bees. Such population collapses can lead to loss of genetic diversity and thereby threaten long-term adaptive potential to future environmental changes (Frankham et al. 2002; Allendorf et al. 2013). Honey bees, due to their haplodiploid mating system and social organization, are particularly sensitive to inbreeding depression (Zayed 2009). Studies have demonstrated that high intracolony diversity decreases pathogen load (Desai and Currie 2015) and enhances productivity (Oldroyd et al. 1992; Mattila and Seeley 2007), survivorship (Tarpy et al. 2013), thermoregulation (Jones et al. 2004), and homeostasis (Oldroyd and Fewell 2007). Moreover, it has also been shown that locally adapted honey bees have higher survival (Büchler et al. 2014; Burnham et al. 2019) and lower pathogen levels (Francis et al. 2014), from which follows that there is a need to conserve the underlying genotypic variation (Frankham et al. 2002).

In the second half of the 20th century, Europe underwent large-scale agricultural intensification associated with drastic land-use changes, triggering a significant decline in insect biodiversity (Robinson and Sutherland 2002; van Lexmond et al. 2015). One of the major drivers of this decline is the increasing use of pesticides (Le Féon et al. 2010; Goulson et al. 2015). The late 20th century also witnessed an intensification of apiculture with beekeepers beginning to apply chemicals inside the colony to control pests and pathogens (Johnson 2015). Chemicals applied within the hive, such as miticides and antibiotics, as well as agrochemicals acquired externally can persist for many years in beeswax and affect honey bee colonies in the long-term (Mullin et al. 2010). In the same time frame, apiculture has further experienced rapid professionalization including migratory beekeeping, increased breeding efforts, and importations of non-native subspecies and selected stock. For instance, throughout large parts of its distributional range the native European M-lineage honey bee subspecies, *Apis mellifera mellifera*, has been replaced by C-lineage bees, mainly *Apis mellifera carnica*, *Apis mellifera ligustica* and Buckfast bees preferred by beekeepers (Pinto et al. 2014; Parejo et al. 2016).

Past and contemporary populations differ by natural and human-mediated factors and circumstances, such as beekeeping practices, prevailing pathogens, or the pesticide regime on crops. Modern honey bee populations rely heavily on human management, and their genetic composition is therefore influenced by commercial trade (Vanengelsdorp and Meixner 2010) and artificial selection (Wragg et al. 2016; Parejo et al. 2017). Moreover, they are much more exposed to the drastic land-use changes and prevailing agricultural practices of recent times. Throughout much of Europe until the 1950s, honey bees were much less intensively managed, more closely reflecting natural conditions with little or no human selection, and mostly kept by swarm beekeeping, and thereby in constant gene flow with the wild population. Gaining a greater understanding of the genetic diversity in the past can inform our understanding of the impact of the agricultural and apicultural revolutions on honey bee populations.

One powerful way to investigate the changes between past and modern populations is by analyzing samples that predate the drastic environmental and human-induced transformations. Museum specimens, therefore, offer an excellent opportunity by providing a window into the past (Lister 2011). Comparing historic and contemporary allelic frequencies is the most direct and powerful way to detect microevolutionary change (Mikheyev et al. 2015). The main caveat of museum samples, however, is the difficulty of obtaining high-quality DNA for molecular genetic analysis (Staats et al. 2013), although improvements in DNA extraction protocols continue to be developed (Tin et al. 2014; Sproul and Maddison 2017). Until recently the majority of studies using museum specimens have been based on PCR-amplification of specific genes or mitochondrial DNA (Habel et al. 2009; La Haye et al. 2012). However, due to DNA degradation, fragments which are shorter than the PCR target region cannot be amplified (Tin et al. 2014). The recent advances in high-throughput sequencing enable us now to overcome the challenges of extracting genomic information from museum specimens, as most methods are designed for short fragmented DNA (Staats et al. 2013; Burrell et al. 2015). In the field of human evolution, protocols for high-throughput sequencing applied to ancient DNA from archaeological sites are well established

(reviewed by Slatkin and Racimo 2016). However, fewer efforts have been made in the application to historical museum collections from animals and plants (e.g., sequencing of the mitogenome as in Miller et al. 2008; Hung et al. 2013; Besnard et al. 2016), and only most recently high-density genome-wide analyses of museum specimens have been reported (Mikheyev et al. 2015; Linck et al. 2017; Sánchez Barreiro et al. 2017; Cridland et al. 2018).

To date, for honey bees, there have been two studies published using whole-genome sequence data of museum specimen, both of which concerned the introduced range of A. mellifera in North America. Mikheyev et al.(2015) investigated the genomic changes over a 33-year period of a wild honey bee population in Ithaca following the introduction of V. destructor. The authors found evidence of a mitochondrial bottleneck but with little loss of nuclear genetic diversity and population size. Cridland et al. (2018) documented the temporal genetic changes in Californian populations with northern populations experiencing a shift in genetic ancestry from M- to C-lineage since the 1960s and southern populations undergoing Africanization.

Here, we hypothesize that the drastic changes regarding population decline, agricultural and apicultural intensification during the last decades have had profound effects on the genetic diversity and ancestry of native honey bee populations and left signatures of selection on their genomes. Using the Swiss dark honey bee population as a case study, we sequenced museum specimens dated between 1879 and 1959 that were provided by the Natural History Museum in Bern, Switzerland, to investigate the genomic past of the native A. m. mellifera bees. Native Swiss honey bees have suffered from a severe population size decline in recent decades due, in part, to Varroa mites, as well as introductions and replacements of non-native stocks. Together with whole-genome sequence data from a previous study of contemporary Swiss bees (Parejo et al. 2016), we investigated genetic diversity, mitochondrial DNA haplotypes, admixture, and selection signatures. To our knowledge, this is the first study to whole-genome sequence historic honey bee specimen from their native range.

Materials and Methods

Samples

Museum Samples

Twenty-two Swiss A. m. mellifera museum specimens dated between 1879 and 1959 (61–141 years old) were obtained from the Natural History Museum in Bern, Switzerland (fig. 1). Specimen consisted of dried and pinned worker bees (diploid) stored by the museum but originating from several private collections of Swiss entomologists. Samples have been assigned with a QR-code deposited in the Natural History

Museum in Bern (supplementary table S1, Supplementary Material online).

Modern Samples

Whole-genome sequence data were available from a previous study (Parejo et al. 2016) of which we selected 40 pure Swiss A. m. mellifera drones (haploid). These samples cover a slightly larger geographical range than the available museum bees, but due to breeding activities they are very similar to each other representing the current population. In addition, to investigate overall genetic structure and admixture proportions, 36 honey bees from a different evolutionary lineage widely employed by beekeepers in Switzerland were included in some analyses. These included 24 A. m. carnica and 12 A. m. ligustica drones (supplementary table S1, Supplementary Material online) from Parejo et al. (2016) and Henriques et al. (2018).

DNA Extraction and Sequencing

Genomic DNA was extracted from the hind legs of museum specimen (fig. 1) carefully rinsed with Ringer solution, using a phenol–chloroform–isoamyl alcohol (25:24:1) method (Ausubel 1988). Pair-end (2 × 125 bp) libraries (kit) were prepared following manufacturers protocol using the NEBNext Ultra II kit (New England Biolabs, Inc) and sequenced on the Illumina HiSeq3000 platform with 20 samples per lane.

Mapping, Variant Calling, and Single Nucleotide Polymorphism Sets

Mapping

Raw sequence data from modern and historic samples were processed using Cutadapt v1.8 (Martin 2011) to remove Illumina universal adaptors and keep only reads with minimum lengths of 20 bp and a minimum base quality score of 20. Trimmed reads were then mapped against the dark honey bee reference genome INRA_AMelMel_1.0 (www.ncbi.nlm.nih.gov/assembly/GCA_003314205.1, last accessed September 5, 2020) using bwa mem 0.7.10 (Li and Durbin 2009). PCR duplicates were marked using PICARD 2.18.23 (http://broadinstitute.github.io/picard/, last accessed September 5, 2020). Mapping statistics including depth of coverage and percentage of mapped reads were calculated using samtools 1.7 (Li et al. 2009) and GATK v4.1.0.0 (Mckenna et al. 2010; Van Der Auwera et al. 2013). DamageProfiler (https://damageprofiler.readthedocs.io/en/latest/index.html, last accessed September 5, 2020) was used on museum samples to generate damage profiles of the mapped DNA reads caused by deamination of cytosine over time which leads to misincorporations of G→A at the 5′ and C→T at the 3′ ends (Briggs 2010; Sawyer et al. 2012).
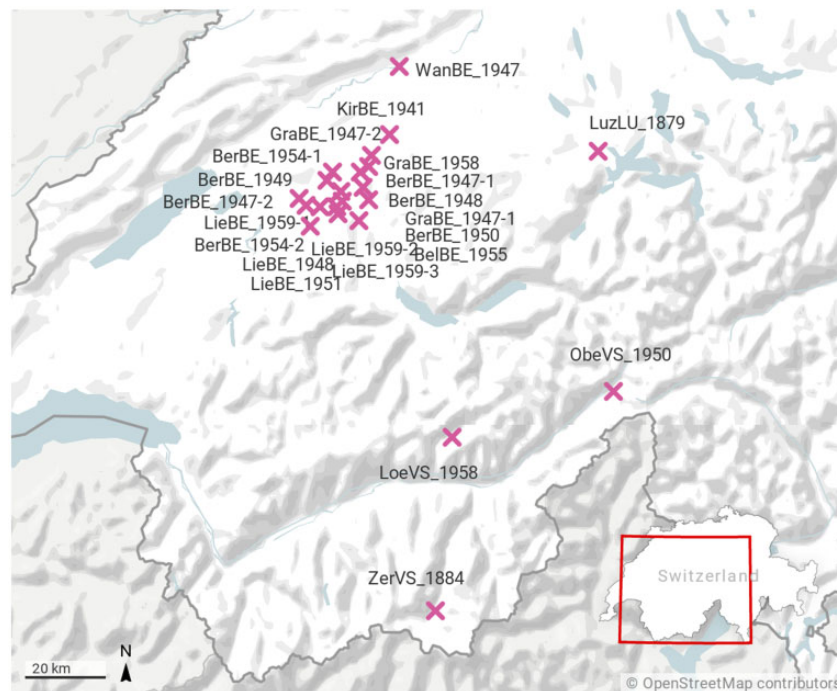
Fig. 1.—Sampling sites of the 22 *A. m. mellifera* museum specimens. Most samples originate from the region around Bern dating between 1941 and 1959, but some are from mountain areas. The oldest sample is from Luzern (1879), Central Switzerland. The second oldest sample (1884) is from Zermatt, Valais, in the Southern Alps. Map created with Datawrapper (www.datawrapper.de, accessed February 2020).

Variant Calling

To increase variant confidence, single nucleotide polymorphism (SNP) calling for the *A. m. mellifera* (N_{MUSEUM}=22 workers, N_{MODERN}=40 drones) was performed using two different software tools (GATK's Haplotypecaller and SAMtools mpileup). First, following GATK's best practices, individual GVCFs were produced using Haplotypecaller with parameters: minimum mapping quality = 20, max alternate alleles = 2, minimum quality score = 20, and sample-ploidy = 2 for museum (diploid workers) and sample-ploidy = 1 for modern samples (haploids drones). Subsequently, GVCFs were combined and genotyped to produce a VCF-file containing raw variants for all *A. m. mellifera* samples. Variants were filtered according GATK's hard-filtering recommendation (MQ < 40.0, FS > 60.0, QUAL < 30.0, MQRankSum < −12.5, ReadPosRankSum < −8.0) except for quality by depth (QD), where a stricter filter was applied (QD > 5) after QD distribution was investigated before and after filtering (supplementary figs. S1 and S2, Supplementary Material online). Second, multisample SNP calling was also performed using SAMtools/BCFtools mpileup 1.7 (Li et al. 2009) with parameters mapQuality ($q > 30$), baseQuality ($Q > 20$), and filtering low-quality variants (QUAL < 30). Both call sets (GATK and SAMtools) were filtered on depth (minimum 5×, maximum 3*average DP) and to include only biallelic SNPs on chromosomes 1–16. Finally, the variants from both call sets were merged using BCFtools

isec to keep only SNPs identified in both sets. Variant calling statistics were calculated with BCFtools *stats*.

Annotation

No published annotation is available for the dark honey bee reference genome (*A. m. mellifera*; INRA_AMelMel_1.0). Thus, annotation files (gtf, annotation release 104) from the latest honey bee genome Amel_HAv3.1 (Wallberg et al. 2019) were remapped onto the INRA_AMelMel_1.0 genome using NCBI's remapping service (www.ncbi.nlm.nih.gov/genome/tools/remap, last accessed September 5, 2020). Finally, a custom database for SnpEff4.3t (Cingolani et al. 2012) as per software instructions was generated to annotate the variants and predict their potential effects excluding intergenic, up- and downstream annotations.

Haplotype Phasing

Phasing genotypes into haplotypes is a fundamental requirement of some analyses, such as that of extended haplotype homozygosity (see below), which seek to exploit linkage disequilibrium (LD) between markers. Statistical phasing can be performed with or without a reference panel, and generally the use of an external reference panel has been shown to increase phasing accuracy (Delaneau et al. 2013). However, using haplotypes from the modern drone (haploid) data set to phase the historic worker (diploid) data set risks potentially

creating artifactual museum haplotypes that are modern recombinants of ancestral variation. We therefore tested the impact of phasing both with and without the use of the modern bees as a reference panel on the museum bees.

First, SNPs for all samples were filtered on call rate >0.95 using VCFtools (Danecek et al. 2011) leaving 2,651,904 SNPs and missing SNPs were imputed within SHAPEIT4 (Delaneau et al. 2019). Preliminary analyses masking 5% of the SNPs with 100% call rate revealed that imputation using this approach is highly accurate (>90% accuracy, supplementary fig. S6, Supplementary Material online) while keeping a larger number of SNPs. Subsequently, the data were phased without a reference panel in museum and modern data sets independently, and using the self-imputed modern bees as references we phased the unphased museum data once more. Phasing was performed with SHAPEIT4 (Delaneau et al. 2019) with the sequencing flag, a minimum window size of 0.1 Mb (the minimum permitted by SHAPEIT4) and an effective population (Ne) size of 150,000 which approximates the Ne calculation of Wallberg et al. (2014) for the Northern A. m. mellifera samples they studied. Genetic maps for phasing were generated by lifting over the Amel4.5 reference genome physical positions in the crossover data generated by Liu et al. (2015) to those of INRA_AMelMel_1.0, and used the crossover information to estimate genetic positions for each SNP. The combined phased data sets including either reference or self-phased museum worker bees were filtered on minor allele frequency (MAF > 0.05), each leaving 1,828,439 SNPs for signatures of selection analyses and linkage decay estimation.

Mitochondrial DNA

Variants in the mitochondrial genome of the A. m. mellifera bees were called using SAMtools mpileup 1.7 with the—ploidy 1 option and keeping only SNP variants with high quality (QUAL >30). The resulting 254 SNPs were genotyped in the C-lineage samples using GATK's Haplotypecaller with mode genotype-given-alleles. C-lineage and A. m. mellifera samples were combined and SNPs with >20% missing calls were removed. This left 205 SNPs to perform Median-Joining network analysis (Bandelt et al. 1999) in PopART (Leigh and Bryant 2015).

Population Structure Analyses

Combining Haploid Drones to Diploid Individuals

Population structure analyses are sensitive when haploid and diploid data sets are analyzed together (Dufresne et al. 2014; Wragg et al. 2016). To get the most informative and unbiased results for population structure analyses, we therefore randomly combined the haploid genotypes of two modern A. m. mellifera drones using a custom script to generate in silico diploids following the procedure in Wragg et al. (2016).

For population structure analyses, the data set with the diploid museum samples and "diploidized" modern samples of A. m. mellifera was filtered and pruned on LD using PLINK1.9 (Chang et al. 2015). We applied –indep-pairwise 50 10 0.1 to filter out variants with a correlation of >0.1 in each window of 50 variants as recommended by Alexander et al. (2009) and kept only SNPs with a 100% call rate. This left 59 K independent SNPs, which were then genotyped in the C-lineage drones using GATK's Haplotypecaller with mode genotype-given-alleles. Finally, similar to the modern A. m. mellifera samples, the haploid C-lineage drones were randomly combined into diploid individuals using a custom script.

The combined data set for the population structure analyses comprised 59 K SNPs genotyped in 60 samples (22 museum A. m. mellifera, 20 "diploidized" modern A. m. mellifera, 12 "diploidized" A. m. carnica, and 6 "diploidized" A. m. ligustica). This data set was used to estimate the average genome-wide divergence, model-based ancestry, and in principal component analysis (PCA).

Ancestry, PCA, and Population Differentiation

To infer the genetic ancestry of each individual, we performed model-based clustering as implemented in ADMIXTURE (Alexander et al. 2009). We ran the analysis unsupervised with 10,000 iterations for 1–5 hypothetical ancestral (K) clusters. Cross-validation error was estimated for each cluster and used to determine the optimal number of K clusters. We also performed PCA to assess the population structure in the absence of a model (Price et al. 2006). PCA was applied to the pairwise genetic relationships between all individuals ($N = 60$) according to their identity-by-state values computed in PLINK 1.9 (Chang et al. 2015). Admixture and PCA results were processed and plotted in R (R Development Core Team 2013). Based on these results, a single admixed museum bee was identified, which was subsequently excluded from downstream analyses (population differentiation, genetic diversity, linkage, and selection signatures).

Population differentiation was estimated as mean pairwise F_{ST} (Weir and Cockerham 1984) per site as implemented in VCFtools (Danecek et al. 2011). The mean and confidence intervals were calculated from 10 randomly selected bootstrap samples of 10 modern A. m. mellifera, 10 historic A. m. mellifera, and 10 C-lineage bees.

Genetic Diversity

To infer the adaptive potential within the modern and historic A. m. mellifera populations two genetic diversity measures were employed: 1) Expected heterozygosity (H_{Exp}) for each individual was calculated using the data set of 59 K unlinked SNPs; and 2) nucleotide diversity (π; Nei 1982), which was calculated from the whole-genome data for each population

in window sizes of 10 kb with 5 kb overlap using VCFtools (Danecek et al. 2011). The mean and confidence intervals for H_{Exp} and π were calculated with 10 randomly selected samples for each of 10 modern and 10 historic *A. m. mellifera*.

LD Estimation

LD between each pair of SNPs in the modern and historic populations was calculated using the self- and reference-phased museum bees ($N = 21$ samples $\rightarrow N = 42$ haplotypes) and the haploid modern drones ($N = 40$) by estimating the Pearson's squared correlation coefficient (r^2) in Plink v1.09 (Chang et al. 2015). Pairwise LD (as r^2 values) were calculated between all the pairs of SNPs within each chromosome based on the exact solution of the Hill equation (Hill 1974; Gaunt et al. 2007) and applying a MAF filter of 0.05. The extent of LD decay was estimated based on physical distance between each SNP pair. LD decay curves were calculated as the average r^2 within bins of 200 base pairs, up to a distance of 10 kbp and plotted in R.

Selection Signatures Analyses

Genome Scan

Several measures are available to infer signatures of selection, employing a range of test statistics each with its own limits and merits (reviewed in Vitti et al. 2013). Here, we employed cross-population extended haplotype homozygosity (XP-EHH) described by Sabeti et al. (2007). The XP-EHH test statistic is based on extended haplotype length which has been shown to be most suitable to infer recent selection and is especially useful for identifying hard and soft selective sweeps with causative alleles that have not reached fixation (Sabeti et al. 2007; Vitti et al. 2013). The XP-EHH analysis is based on haplotype length and is, thus, sensitive to accurate phasing. Hence, we performed the analysis twice—once comparing the self-phased museum to modern bees, and secondly comparing museum bees phased with the modern bees as a reference panel to the modern bees. The analyses were performed using selscan v1.2.1 (Szpiech and Hernandez 2014) with default MAF and EHH truncation values of 0.05. The obtained XP-EHH scores for each SNP were normalized (Z-transformed) by subtracting genome-wide mean XP-EHH and dividing by the standard deviation (supplementary fig. S4, Supplementary Material online). SNPs with absolute Z-transformed XP-EHH values in the 99th percentile were considered significant. A similar approach has been employed by other studies investigating selection signatures in the honey bee genome (Wallberg et al. 2016; Montero-Mendieta et al. 2019). Finally, genes associated with SNPs annotated with SNPeff as intronic, exonic, 3'UTR, 5'UTR, and splice variants, that were identified in both genome scans (self-phased and reference-phased worker bees) are considered as putative candidates under selection.

Gene Ontology Enrichment Analysis

Gene Ontology (GO) annotations provide a convenient means of grouping genes by their known functions and predicted biological roles, enabling enrichment analyses to be conducted. The online resource DAVID v.6.8 (Database for Annotation, Visualization, and Integrated Discovery; Huang et al. 2007) was accessed in June 2020 to test if the candidate genes identified demonstrated enrichment for any particular function (Huang et al. 2009). We used as a background gene set all honey bee genes associated with at least one SNP in our analyses. Functionally related genes were clustered using the gene functional classification tool set to highest stringency. Enrichment for GO category terms was performed with the functional annotation analysis tool using the GO categories of Biological Process, Cellular Component, Molecular Function, and KEGG pathway. The functional annotation clustering tool was subsequently used to cluster similar GO terms.

Results

Mapping and SNP Calling

In total, 686,376,114 sequencing reads were generated from the 22 museum samples. A summary of alignment statistics of these sequence reads in addition to those of the 76 modern samples is provided in supplementary table S1, and figures S1 and S2, Supplementary Material online. The average mapping rate across the museum samples was 93.7%, with a mean depth of coverage of 13.9×, ranging from 5.55× to 27.39×, in comparison with the modern *A. m. mellifera* drones with mean 10.3× and range 7.3–21.2. Only 34% of reads for sample KirBE_1941 mapped to the reference genome, possibly indicating contamination, nevertheless, the depth of coverage was 7.8× and the sample retained in downstream analyses. On average for museum and modern samples, respectively, 85% and 92.2% of the genome per sample was callable, having a depth of coverage >4×, whereas the lowest breadth of coverage was observed in BerBE_1947-2 (57.7%). The oldest sample, LuzLU_1879-2, returned a depth and breadth of coverage of 9.02× and 69.6%, respectively.

Analysis of DNA degradation by DamageProfiler (supplementary table S2 and fig. S5, Supplementary Material online) indicated only minor 5' (2.1% ± 0.6 SD) and 3' (2.4% ± 0.5 SD) misincorporations compared with studies of ancient DNA (e.g., ∼8%, Peltzer et al. 2018). The misincorporations of two the oldest museum samples dating from 1884 to 1879 were estimated at 3% and 4.5% for G→A at the 5' end, whereas for the C→T at the 3' end it was 2.5% and 4.3%, respectively. However, because mismatches at the ends of the sequence reads are soft-clipped by *bwa mem* during alignment, no additional read filtering or clipping was performed.

Overall, with except for the two oldest samples and BerBE_1947-2 with the lowest breath of coverage, the sequence quality of the museum samples can be considered

comparable with those of the modern samples (supplementary tables S1 and S2, Supplementary Material online).

Variant calling was performed using GATK's Haplotypecaller and SAMtools mpileup, resulting in 4,611,541 and 4,145,606 SNPs, respectively (supplementary table S3, Supplementary Material online). Applying filters to remove low-quality variants and intersecting both variant sets, left 3,252,197 genome-wide SNPs. The average genotyping call rate was 0.94 in the museum samples and 0.97 in the modern bees, with all samples except for three exceeding 90% call rate (supplementary table S3A, figs. S6 and S7, Supplementary Material online). The museum sample with the lowest genotyping rate of 0.78 was BerBE_1947-2, which was also the sample with the lowest mapping rate potentially due to contamination.

Different additional filters were applied for subsequent analyses (supplementary table S3, Supplementary Material online): For estimating nucleotide diversity and for phasing the data set was additionally filtered on call rate 0.95 leaving 2,651,904 SNPs, for linkage and XP-EHH a MAF filter of >0.05 was applied leaving 1,828,439 SNPs, and for PCA, model-based admixture, F_{ST}, and H_{Exp} the data set was filtered for unlinked SNPs with a call rate of 100% leaving 59,320 SNPs.

Mitochondrial Haplotype Networks

Mitochondrial network analysis revealed evidence of mitonuclear discordance. The native dark honey bee of Switzerland belongs to the M-lineage, but we found that one individual sampled in Liebefeld in 1959 (LieBE_1959-1) and another sampled in the Swiss Alps in 1958 (LoeVS_1958) possess mitochondrial haplotypes that cluster with C-lineage bees (fig. 2). A phylogenetic analysis of SNPs identified on the complete mitochondrial genome showed that all but two modern and historic *A. m. mellifera* samples cluster in two M-lineage clades (supplementary fig. S8, Supplementary Material online). The two historic samples that are placed in a distant branch in the phylogenetic analysis are the same samples that cluster within the C-linage of the haplotype network.

Population Structure

Population structure inferred by the model-based ADMIXTURE and PCA each revealed four (sub-) populations representing historic *A. m. mellifera*, modern *A. m. mellifera*, *A. m. carnica* and *A. m. ligustica* bees (fig. 3). The optimal number of clusters identified from the lowest cross-validation error is $K = 2$ (supplementary fig. S9, Supplementary Material online), which correspond to the two major evolutionary lineages: M- (*A. m. mellifera*) and C-lineage (*A. m. carnica* and *A. m. ligustica*). One of the museum bees, LieBE_1959-1 shows evidence of genetic admixture with the C-lineage (60.5% M-lineage and 39.5% C-lineage ancestry), as indicated by the dual-color genetic background in the ADMIXTURE plot (fig. 3A) and its

intermediate placement along PC1 in the PCA plot (fig. 3B). LieBE_1959-1 is one of two bees identified from the mitochondrial analyses to possess C-lineage mtDNA (fig. 2).

When estimating population differentiation (F_{ST}) using the 59 K SNP data set, the museum bee identified as being admixed was excluded from the analysis. The F_{ST} analysis revealed a high divergence between C-lineage bees and the historic and modern *A. m. mellifera* populations ($F_{ST}>0.4$), and, whereas the divergence between the modern and museum samples was expectedly very low, it was significantly different from 0 based on random subsampling ($F_{ST}=0.007$, 95% CI 0.004–0.009).

Genetic Diversity

To investigate differences in genetic diversity and population histories between modern and museum *A. m. mellifera*, we calculated expected heterozygosity (H_{Exp}) and nucleotide diversity (π). Heterozygosity differed significantly between both populations ($H_{E(Museum)}=0.243$, 95% CI 0.241–0.245, $H_{E(Modern)}=0.258$, 95% CI 0. 257–0.259). The formula of H_{Exp} is based on allele frequencies and is therefore not influenced by ploidy, nor the generation of in silico diploids (as we tested in preliminary analyses). Moreover, we also estimated nucleotide diversity which is calculated on haploid sequences (phased genotypes) and, thus, insensitive to ploidy. Similarly to H_{Exp} also nucleotide diversity was slightly higher in the modern *A. m. mellifera* population ($\pi=0.00241$, 95% CI 0.00240–0.00243) compared with the historic ($\pi=0.00227$, 95% CI 0.00224–0.00230). Both measures of genetic diversity being larger in the modern population suggest enhanced adaptive potential. Observed heterozygosity in the identified admixed museum bee (LieBE_1959-1) was considerably higher ($H_{Obs}=0.42$) than the expected heterozygosity ($H_{Exp}=0.26$), and also higher than for all other *A. m. mellifera* samples, suggesting the admixture to be recent.

LD Decay

LD decay between SNPs in the modern and historic *A. m. mellifera* populations as measured using r^2 over increasing distances between pairwise SNPs is shown in figure 4. The maximum average LD for SNPs less than 200 bps apart was 1.5 times as high in the modern population ($r^2=0.43$) compared with the historic population ($r^2\sim0.28$). LD decays quickly for both populations, but long-range LD was found to be considerably lower for the historic ($r^2\sim0.02$) than the modern population ($r^2\sim0.12$) potentially reflecting the recent population history of a small, inbred or admixed population. There is a slight, but insignificant tendency for LD to be higher in museum bees phased with the drones as references (fig. 4).
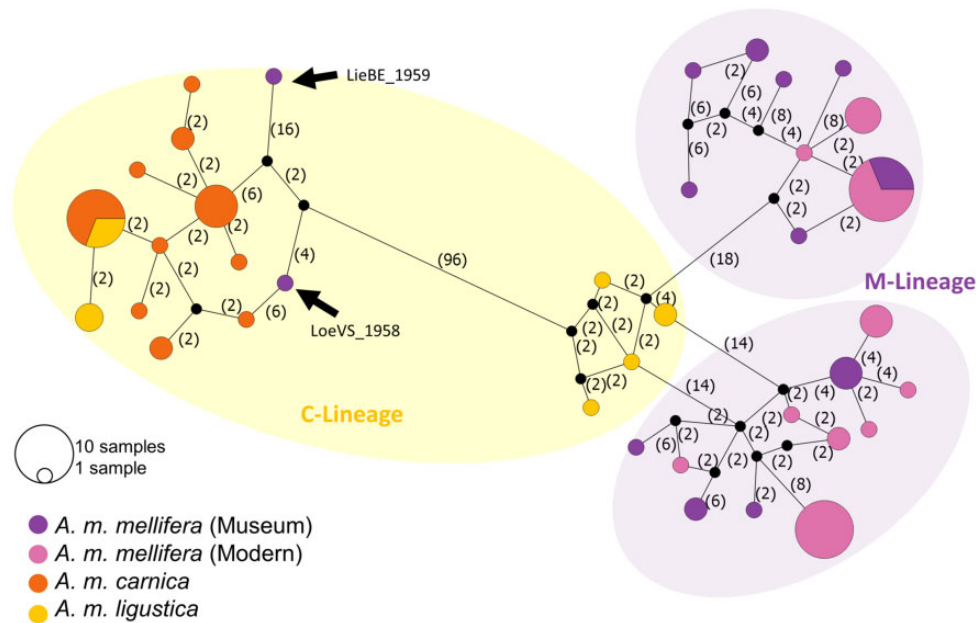
Fig. 2.—Median-joining network inferred from 205 mtDNA SNPs and 60 samples ($N = 22$ museum *A. m. mellifera*, $N = 20$ modern *A. m. mellifera*, $N = 12$ *A. m. carnica*, and $N = 6$ *A. m. ligustica*). Hypothetical (unsampled or extinct) haplotypes are denoted as filled black circles. The values in brackets indicate base pair differences between haplotypes. M-lineage samples including modern and historic *A. m. mellifera* are grouped into two clades, with the exception of two museum samples (LieBE_1959-1 and LoeVS_1958) which cluster with C-lineage bees (denoted by the arrows).
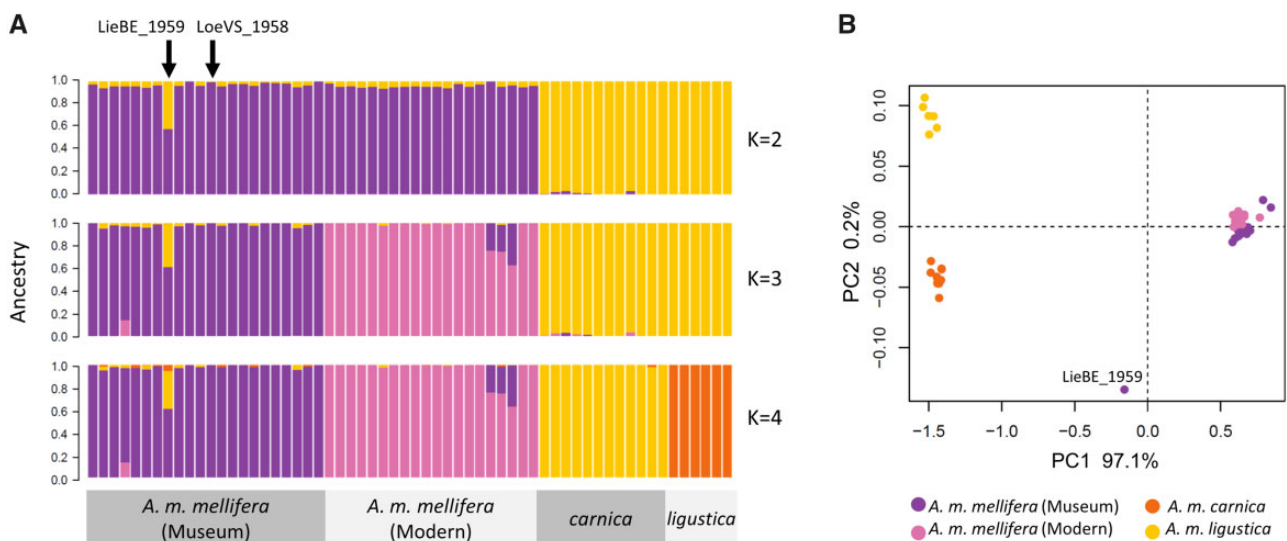

Fig. 3.—Population structure inferred from the LD-pruned 59 K SNPs and 60 samples ($N = 22$ museum *A. m. mellifera*, $N = 20$ "diploidized" modern *A. m. mellifera*, $N = 12$ "diploidized" *A. m. carnica*, and $N = 6$ "diploidized" *A. m. ligustica*). (*A*) Genetic ancestry as calculated with ADMIXTURE for $K = 2$ to 4 hypothetical ancestral populations. Each color represents one of K clusters. Each individual is represented by a horizontal bar and colored according to the proportion of the genome that was derived from each cluster. The optimal number of clusters identified by cross-validation is $K = 2$. (*B*) PCA of genetic distance between individuals. The first principal component (PC1) explains 97% of the variation indicating strong divergence between M- and C-lineage honey bees, whereas PC2 accounts only for 0.2% of the variance.

Selection Signatures between Historic and Modern *A. m. mellifera* Populations

We investigated the presence of signatures of selection between the historic and modern *A. m. mellifera* populations

using the XP-EHH method (Sabeti et al. 2007) and with two different data sets including either the self-phased or reference-phased museum worker haplotypes. There was an 84% overlap of the SNPs (15,354 SNPs) falling within the 99th percentile in both scans (supplementary fig. S10,

Supplementary Material online) which were considered as being associated with putative signatures of selection (fig. 5 and supplementary table S4, Supplementary Material online). In a first screening, we identified an extreme peak on chromosome 11 (supplementary fig. S11, Supplementary Material
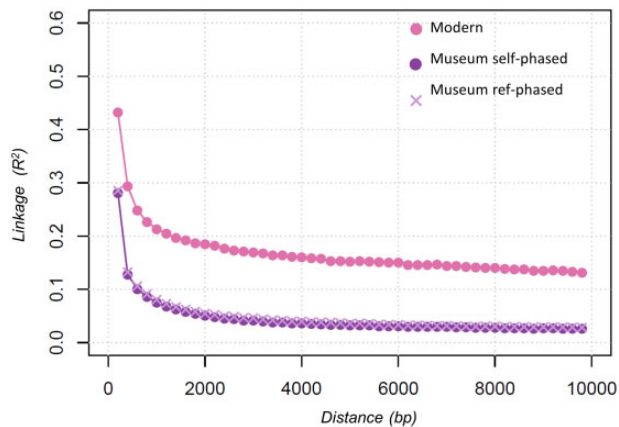
online), which on further investigation was identified to most likely be a duplication not captured in the reference genome due to 1) the average depth of coverage in the region being ~1.6 times the chromosome average, and 2) the presence of reads with both alleles (reference and alternate) in the haploid drones. Furthermore, this peak lies within a low-complexity centromere and no gene is annotated within ±15 kb. We have thus excluded it from further analyses. The remaining SNPs are associated with 644 candidate genes (supplementary table S5, Supplementary Material online). Many of the genes identified are uncharacterized loci (29.8%) as the annotation of the honey bee genome is far from complete. Of the 15,354 SNPs, 275 and 13 SNPs were predicted by SNPeff to have MODERATE and HIGH impact, respectively (supplementary table S6, Supplementary Material online). These included mostly nonsynonymous base pair changes, and also splice region variants as well as stop lost/ gained variants.

GO analysis is a strategy to identify the most important biological processes of candidate regions identified in whole-genome selection scans. However, the power of these analyses depends on the number and quality of annotated genes available for the focal organism (Yon Rhee et al. 2008). We conducted a GO analysis using the online resource
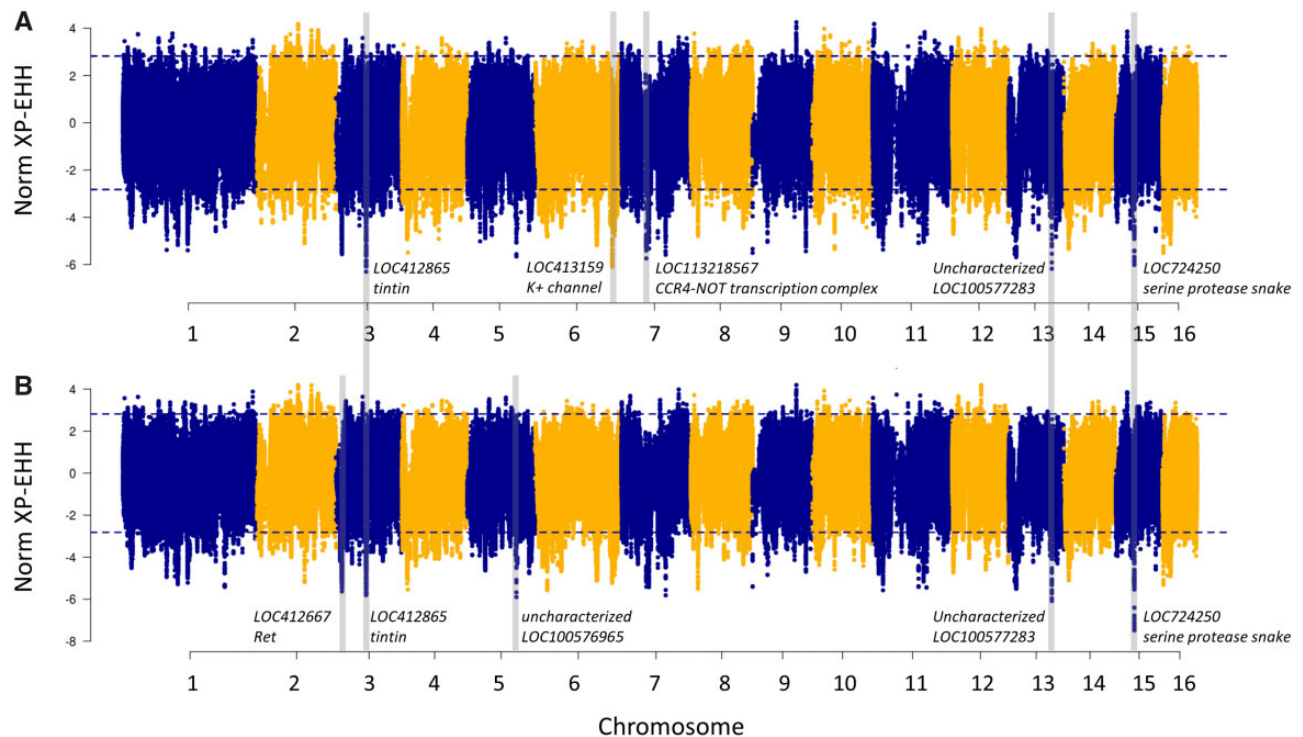

Fig. 4.—LD between SNPs as measured by r^2 (*y* axis) for increasing distance between SNPs (*x* axis) for *A. m. mellifera* modern drones ($N = 40$) and *A. m. mellifera* museum bee haplotypes ($N = 42$ haplotypes) self-phased and phased using the drones as a reference panel.

Fig. 5.—Signatures of selection between historic and modern *A. m. mellifera* from Switzerland. XP-EHH was performed using 42 haplotypes derived from 21 museum samples (diploid) (*A*) self-phased and (*B*) reference-phased, and 40 haplotypes derived from modern drones (haploid). XP-EHH scores are plotted along the 16 honey bee chromosomes with negative values indicating selection in the modern population. The dashed lines denote SNPs in the 99th percentile of the absolute XP-EHH scores. This figure excludes the false positive peak on chromosome 11 (4945317-4945798), which can be seen in figure S11, Supplementary Material online. The five highest peaks of each analysis are labeled with their putative genes under selection.

Table 1

Significantly Enriched Gene Groups (Enrichment Score > 3) between Historic and Modern *A. m. mellifera* as Inferred from the XP-EHH Whole-Genome Scan and Based on the Functional Classification Tool of DAVID v.6.8

Groups	Gene ID	Gene Name
Gene group 1 enrichment score: 10.805	LOC413215	Lachesin
	LOC409546	Lachesin
	LOC726655	Uncharacterized LOC726655
	LOC725803	Slit homolog 1 protein-like
	Abscam	Dscam family member AbsCAM
	LOC410662	Lachesin-like
	LOC725091	Lachesin-like
	LOC411176	Neurotrimin-like
	LOC725840	Lachesin-like
	LOC724195	B-cell receptor CD22
	LOC410563	Uncharacterized LOC410563
	LOC410888	Lachesin-like
	LOC724847	Uncharacterized LOC724847
	LOC100577522	Uncharacterized LOC100577522
	LOC412813	Neuronal growth regulator 1
	LOC412859	Hemicentin-2
	LOC410696	ADAMTS-like protein 3
	LOC725924	Leucine-rich repeat-containing protein 24
	LOC726017	Lachesin
	LOC409707	Cell adhesion molecule 4-like
	LOC725264	Uncharacterized LOC725264
	LOC412855	Down syndrome cell adhesion molecule-like protein Dscam2
	LOC725543	Chaoptin-like
	LOC725870	Slit homolog 3 protein-like
	LOC409701	Uncharacterized LOC409701
	LOC411158	Leucine-rich repeat-containing protein 24
	LOC413555	Lachesin-like
	LOC411345	Neurotrimin
Gene group 2 enrichment score: 3.790	LOC100577743	Uncharacterized LOC100577743
	Or30	Odorant receptor 30
	LOC413829	Diuretic hormone receptor
	LOC100578724	Uncharacterized LOC100578724
	LOC411760	Metabotropic glutamate receptor 7
	LOC100576984	Odorant receptor 4-like
	LOC102655559	Uncharacterized LOC102655559
	DopR2	Dopamine receptor 2
	5-ht7	Serotonin receptor 7
	LOC412883	Allatostatin C receptor
	LOC552142	Latrophilin Cirl-like
	Akhr	Adipokinetic hormone receptor
	LOC100578662	Cadherin EGF LAG seven-pass G-type receptor 1-like
	LOC724760	G-protein coupled receptor Mth2
	LOC724853	Probable G-protein coupled receptor Mth-like 1
	Or57	Odorant receptor 57
	LOC724237	Prostaglandin E2 receptor EP3 subtype
	LOC102656567	Uncharacterized LOC102656567
	LOC100578739	Neuropeptide Y receptor-like
	LOC412994	Octopamine receptor beta-3R
	LOC412570	Tachykinin-like peptides receptor 99D
	LOC100577231	Uncharacterized LOC100577231
	LOC724602	FMRFamide receptor
	LOC100577888	Uncharacterized LOC100577888
	LOC100576383	Uncharacterized LOC100576383

(continued)

Table 1 Continued

Groups	Gene ID	Gene Name
Gene group 3 enrichment score: 3.377	nAChRa6	nAChR alpha6 subunit
	LOC412993	Glutamate receptor ionotropic, kainate 2-like
	nAChRa3	nAChR alpha3 subunit
	nAChRa7	nAChR alpha7 subunit
	LOC408645	Glutamate receptor, ionotropic, kainate 2
	nAChRa1	nAChR alpha1 subunit
	Grd	GABA-gated ion channel
	nAChRa4	nAChR alpha4 subunit

DAVID. Of the 644 candidate genes for selection, 617 were present in the DAVID knowledgebase (v6.8) (supplementary table S5, Supplementary Material online). For the background gene list of 10,848 genes associated with at least one SNP in our analysis, 10,174 genes were present in the DAVID knowledgebase. Functional classification clustered 105 candidate genes into 10 groups (supplementary table S7, Supplementary Material online), of which 3 had an enrichment score >3 (table 1). The most enriched of these, gene group 1 (enrichment score = 10.8) entails genes related to the immunoglobulin superfamily (IgSF), amongst them are two Down syndrome cell adhesion molecules (Dscam) family members (Abscam and Dscam2) and eight homologs of lachesin, another cell-adhesion molecule. The second most enriched group (enrichment score = 3.8) are transmembrane signaling receptors involved in sensory perception of smell, odorant binding, response to stimulus and sensory transduction. The third most enriched group 3 (enrichment score = 3.8), includes five nicotinic acetylcholine receptors (nAChRs) (nAChRa7, nAChRa6, nAChRa4, nAChRa3, and nAChRa1), two ionotropic glutamate receptors (LOC408645, LOC412993) which act as neurotransmitter, as well as Grd, a gamma-aminobutyric acid (GABA) channel. To highlight is also gene group 6 (enrichment score = 2.4; supplementary table S7, Supplementary Material online), which entails five tyrosine-protein kinases. Furthermore, GO analysis revealed that clusters of GO terms of the candidate genes are significantly enriched (enrichment score > 2) for terms related to regulation, membrane, synapses, ion channels, and signaling (supplementary table S8, Supplementary Material online).

Discussion

Honey bees are our most important pollinators but they are currently facing a plethora of threats worldwide. Among these, genetic threats to population viability require special attention for honey bees due to their haplodiploidy and complementary sex determination (Zayed 2009). It is, thus, critical to preserve the diverse genetic resources of locally adapted bees. However, in a world where honey bees are largely managed what, precisely, defines diverse and locally adapted honey bees? Museum specimen collections offer a great opportunity to gain insight into the genomic past of honey bee populations before the advent of modern apicultural and agricultural practices. Here, we elucidate the historic genetic composition and diversity of a native dark bee population, and investigate how environmental and anthropogenic transformations have left signals on their genomes. To this end, we successfully sequenced and examined the whole-genomes of 22 samples dated between 1879 and 1959 provided by the Natural History Museum, Bern, Switzerland.

Mito-Nuclear Discordance in One Museum Sample Dating from 1958

Discordant patterns between nuclear and mitochondrial markers have been found in numerous studies (Toews and Brelsford 2012). Different processes have been proposed to explain such findings, among others these include adaptive introgression of mtDNA, differences in demographic histories, and sex-biased dispersal (Prowell et al. 2004; Walton et al. 2008; Toews and Brelsford 2012; Wragg et al. 2018). In A. mellifera, patterns of mito-nuclear discordance were previously identified in the Italian bees (C-lineage) which held M-lineage haplotypes in their mitochondrial DNA (Franck et al. 2000). Similarly, in the M-lineage, south and west Iberian honey bees (M-lineage) carry A-lineage mitotypes potentially from secondary contact with African subspecies (Miguel et al. 2011). In our study, we identified one museum specimen from 1958 having C-type mtDNA while being of complete M-lineage ancestry in the nuclear genome. This sample has been collected in Lötschental, a valley that lies on the traditional north–south transit route through the Swiss Alps, and is connected with Italy through two major tunnels built at the beginning of the last century (supplementary fig. S12, Supplementary Material online). It is, therefore, possible for a C-lineage colony or queen to reach Lötschental by transhumance or natural dispersal. Either way, the most probable hypothesis for the mito-nuclear discordance would be that of a relict C-lineage mitochondria which, in subsequent generations, was progressively replaced in the nuclear genome by the prevailing native A. m. mellifera genetic background. In the absence of selection, a migrant offspring typically loses 50% of its ancestral genetic background with each successive generation, thus, after six generations less than 2% of the

genome remains of introduced ancestry. Assuming a generation time for honey bees between 2 and 3 years (Winston et al. 1981), detecting a migrant lineage background in the nuclear genome becomes difficult after 12–18 years (6 generations). In our case, this means that the C-lineage introduction, natural, or human-mediated, we observed must have occurred much earlier than 1958 in order for its admixture signal to have eroded in the nuclear genome.

Nuclear-Admixed Honey Bee from 1959

Another individual sampled in Liebefeld in 1959 also contained C-lineage mitotype, and was also found to be admixed in the nuclear genome. Higher observed than expected heterozygosity in this sample suggests a recent hybridization. Liebefeld is home of the national Swiss Bee Research Center, which, starting in the late 1950s, maintained testing apiaries to conduct research projects on breeding and selection with different honey bee lineages. This would likely explain the admixed individual found in that area. Anecdotal reports of C-lineage bee importations can be found in the Swiss beekeeping journals much earlier (e.g., H. Schneider, head of breeding, Swiss beekeeping association). Nevertheless, all other samples in this study show no signs of admixture indicating that introductions were not widespread, or given the much higher *A. m. mellifera* densities, likely never posed a significant threat to the genetic integrity of native bees.

In contrast, recent studies have evidenced strong signs of admixture in Swiss honey bees (Pinto et al. 2014; Parejo et al. 2016). Our finding that none of the museum samples before 1959 displayed signs of admixture, supports the hypothesis that the present day genetic structure is due to human management, and not, for instance, through a natural contact zone given the close geographic proximity to C-lineage bees. It was not until 1965, when the Beekeepers Association of Western Switzerland decided to give preference to the *A. m. carnica* breed, that large-scale introductions and replacements started, culminating in today's rather admixed population.

No Evidence of Loss of Genetic Diversity

For honey bees, high intracolony diversity has been shown to be essential to colony health and vitality (Oldroyd et al. 1992; Mattila and Seeley 2007; Tarpy et al. 2013; Desai and Currie 2015). In our study, we expected the modern *A. m. mellifera* population to carry lower genetic diversity compared with historic populations due to the decline of the number of wild colonies after the arrival of *V. destructor* and because of increased breeding efforts which typically reduces genetic diversity in managed livestock. Contrary to our expectations, we observed higher genetic diversity in modern bees. Thus, the reduction in census population size has not led to a genetic bottleneck in the current Swiss *A. m. mellifera*

population, which thereby still holds the adaptive potential to face future anthropogenic and environmental changes. Similarly, Mikheyev et al. (2015) found no loss of genetic diversity in a wild honey bee population in a forest in Ithaca, despite a massive reduction in effective population size. Based on its similarity with managed populations in the surrounding area, the authors interpret that the modern wild population has presumably received immigrants that have escaped from managed apiaries. In California, Cridland et al. (2018) attribute the increased diversity in modern populations to the introduction of Africanized bees. Both studies investigated honey bee populations in North America, the introduced range of *A. mellifera*. Hence, their estimates of genetic diversity only reflect the species' invasive range and conclusions drawn from them can therefore not readily be extrapolated to the native range harboring inherent subspecies diversity.

In this study, it is not conclusive what the possible reasons are to explain the identified higher diversity estimates in the modern population. First, it is possible that mechanisms such as the extreme polyandrous mating system with its long-distance mating flights (Adams et al. 1977), the haplodiploid sex determination (Wilson 1905), and the high recombination rate (Liu et al. 2015) maintain an intrinsic high level of genetic variation in honey bee populations, as has been suggested elsewhere (Wallberg et al. 2014; Mikheyev et al. 2015). This hypothesis is supported by a recent study on an inbred population of clonal (thelytokous) honey bees, *Apis mellifera capensis* (Smith et al. 2019). In spite of inbreeding, the authors found substantially high levels of heterozygosity maintained by heterozygous advantage. Second, extrinsic factors such as increased human management can increase diversity in the honey bee (Harpur et al. 2012). Until the last few decades, apiculture in Switzerland was characterized by traditional swarm-beekeeping, and selection for favorable traits predominantly occurred at the beekeeper's own apiary. As a consequence, the honey bee's genetic composition was still largely driven by natural forces. Today, the movement of hives and introductions of *A. m. mellifera* from different regions are likely promoting increased levels of genetic diversity. Moreover, low levels of C-lineage introgression (undetected by model-based Admixture) in the modern population can result in higher diversity levels than in the unadmixed progenitor population (Harpur et al. 2013).

Despite stringent filtering and quality control, there might be some bias in estimating diversity measures from museum specimens for instance due to limited sampling or lower sequence data quality, which potentially leads to underestimating historic diversity. Nevertheless, the fact that we do *not* find markedly reduced genetic diversity in contemporary native *A. m. mellifera* compared with their historic ancestors may suggest that loss of genome-wide neutral genetic diversity might not be one of the major drivers for colony losses. This does not exclude, however, that specific locally adapted genetic variants may be lost jeopardizing colony survivability (De la Rúa

et al. 2013). In fact, there is a recent paradigm shift in conservation genomics that genome-wide averages of "genetic" diversity might not reflect the actual adaptive potential, and rather the conservation focus should be on "functional" diversity (Hoffmann et al. 2017). Numerous studies identifying quantitative trait loci (QTLs), such as hygienic behavior, in honey bees (Oxley et al. 2010; Tsuruda et al. 2012), highlight the role of additive genetic variance in shaping important phenotypes. It is variation at such fitness-related QTLs that are crucial for future adaptive potential (Zayed 2009). The conservation of the genetic diversity of native subspecies remains important to maintain within-population variation (De la Rúa et al. 2013).

Increased LD in the Modern Population

Genome-wide patterns of LD are affected by population subdivision, changes in population size, drift and admixture (Slatkin 2008). Thus, the analysis of LD decay curves can help to elucidate population history (Slatkin 2008). In our study, the historic population displays a low and quickly falling LD curve reflecting an unstructured and large population. In contrast, we found considerably higher LD in the modern than in the historic population, despite the higher genetic diversity in the former. Increased levels of LD are usually found after a population bottleneck, due to inbreeding, or after admixture of individuals with very different allele frequencies (Slatkin 2008). Although we find no direct evidence of inbreeding as evidenced by the higher diversity measures, the modern population is characterized by a few small-scale conservation areas on the one hand, and a breeding population of commercially selected breeding lines on the other hand. The maintenance of a closed breeding program combined with low levels of admixture from C-lineage bees could explain the contrasting patterns of diversity and LD in the modern population.

Little Difference between Self- and Reference-Based Haplotype Phasing

Phase information is important for understanding phenotypic expression and haplotype diversity (reviewed in Tewhey et al. 2011), and accurate phasing is crucial for analyses based on haplotype length (Delaneau et al. 2019). Statistical phasing can be performed with or without a reference panel. Whole-genome sequence data in contrast to SNP array data and reference-based phasing is generally known to improve accuracy of statistical phasing (Delaneau et al. 2013), and we thought that this was particularly true for the honey bee with its known high LD decay. Surprisingly, we found only negligible differences in LD structure between the self-phased and referenced-phased museum worker bees. Similarly for the XP-EHH genome scan, the results were comparable: Three out of the top five peaks in each analysis were overlapping, and overall most peak signals were shared among

the two genome scans, albeit with differences in the signal intensity (fig. 5). Although our data does not allow estimating the switch error rates, a study in *Drosophila melanogaster* revealed low error rates with increasing numbers of reference haplotypes—particularly when the reference haplotypes are from the same population (Bukowicki et al. 2016).

Selection Signatures Potentially Associated with Immunity and Chemicals in Agriculture and Apiculture

By comparing the contemporary and historic populations using whole-genome sequencing, it is not only possible to investigate population structure and genetic diversity, but also to identify genes under selection—whether this be due to natural or artificial selection pressures. Overall, we find on average that XP-EHH values are negative, indicating selection in the modern population and corroborating the presence of a historic "wild type" population. The genes associated with these signals of selection have multiple important molecular and biological functions. GO enrichment analyses revealed the genes identified to be significantly enriched in functions related to cell membrane, synapses, signaling, and regulation of biological and cellular processes. The most enriched gene group in our GO analysis contains members of the IgSF. IgSF proteins, well-known to precisely recognize and adhere to cells, are involved in a diverse range of functions including cell–cell recognition, muscle structure, cell-surface receptors, and the immune system (Vogel et al. 2003; Watson 2005). *Dscam*, a well-studied IgSF gene of *Drosophila* and of which two orthologs are represented in gene group 1, is able to generate large numbers of different molecules by alternative splicing making (Watson 2005; Ng and Kurtz 2020). *Dscam* is upregulated after infection and expressed in both the fat body and hemocytes of different insect species (Watson 2005), and there is a growing body of evidence that this hypervariable region is an important player in immunity (reviewed in Armitage et al. 2015).

The enrichment found in immune-related genes in our study could be related due to the emergence of novel parasites and pathogens. Most importantly *V. destructor* and associated viruses could have imposed high selection pressure due to its high mortality, and also the gut parasite *Nosema ceranea*, relatively recently introduced to Europe (Botías et al. 2012), and/or a bacterial disease, European foulbrood, whose etiological agent is *Melissococcus plutonius*, and which has caused numerous heavy outbreaks in Switzerland since the 1990s (Roetschi et al. 2008). Although the correlation with these novel pathogens and the enrichment in IgSF genes in this study remain speculative, it is clear that the immune system is a key fitness-related function and is likely to have evolved in Swiss honey bees over time.

One of the enrichment groups comprises tyrosine kinases, which act as on-off switch of other enzymes and are thereby critical for regulatory processes (Lemmon and Schlessinger

2010). In the potato beetle, *Leptinotarsa decemlineata*, Shi et al. (2016) found that receptor tyrosine kinase genes respond transcriptionally to five different insecticides by a mode-of-action independent way, and conclude that these mechanisms may, in part, be responsible for sublethal effects of insecticides in insects. Moreover, three *D. melanogaster* orthologs (*Drl*, *Src64b*, and *Abl*) of our identified tyrosine kinases have been found to affect learning and memory (Dura et al. 1995; Moreau-Fauvarque et al. 2002; Akalal et al. 2011), characteristics that are often described as sublethal effects in honey bees exposed to pesticides (Thompson 2003).

The third most enriched gene group is associated with the nervous system and contains ion channels and neurotransmitter genes, that is five nAChRs, a GABA channels and two glutamate receptors. These proteins are targets of several pesticides widely used in agriculture (Johnson 2015): neonicotinoids (e.g., Clothianidin, Imidacloprid, Thiamethoxam) bind to the nAChRs. GABA channels are target sites of organochlorides (e.g., DDT, dieldrin) as well as for Fipronil, a phenylpyrazole, who also targets glutamate receptors. In Switzerland, DDT was banned in 1972 and organochlorides are no longer in use, but were heavily applied in agriculture in the 1950s and 1960s because of their effectiveness, and could therefore potentially exert strong selection pressures. The first generations of neonicotinoids were employed in agriculture in the 1990s, and were until recently the most widely used insecticides in Switzerland and the world (Sgolastra et al. 2020). The EU and Switzerland banned the use of the three most commonly used neonicotinoids in 2018, with partial exceptions (Sgolastra et al. 2020). Neonicotinoids and Fipronil have similar properties causing nervous stimulation at low concentrations but receptor blockage, and paralysis at higher concentrations (Simon-Delso et al. 2015). In honey bees, sublethal doses lead to impaired learning and navigation, higher mortality and susceptibility to disease via impaired immune function and lower fecundity (van Lexmond et al. 2015). Such sublethal effects have been observed in numerous field-realistic studies (reviewed in Desneux et al. 2007; Blacquiere et al. 2012) and the above-mentioned pesticides have been widely used over prolonged periods over the last decades in Switzerland. It is plausible, therefore, that exposure to insecticides could lead to selection pressures in nontarget species, such as that which we have observed comparing modern to historical Swiss honey bees.

However, organochlorides are not the only substance to target GABA channels, thymol has also been shown to interact with GABA receptors in insects (Priestley et al. 2003; Tong and Coats 2010; Waliwitiya et al. 2010). Thymol, a monoterpene, is a major component of the essential thyme oil and is part of the recommended integrated pest management treatment against *V. destructor* in Switzerland (Imdorf et al. 1999). As thymol is applied in-hive, honey bees are in direct contact with the product, and so this may also exert a selection pressure.

Ultimately, genome scans for signatures of selection cannot directly reveal which selection pressures have led to the identified signatures. Yet, collectively, our findings suggest that the increased use of chemicals in modern agriculture and apiculture has left a legacy on genomes of the *A. m. mellifera* population under study.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

Author Contributions

Conceived and designed the study, collected data: M.P. Analyzed and interpret the data: M.P. with input from D.W., A.E., D.H. M.P. wrote the manuscript with input from D.W., A.E., D.H., and J.-D.C. All authors approved the final version of the manuscript.

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