Status and Conservation of the Nordic Brown Bee

Final report

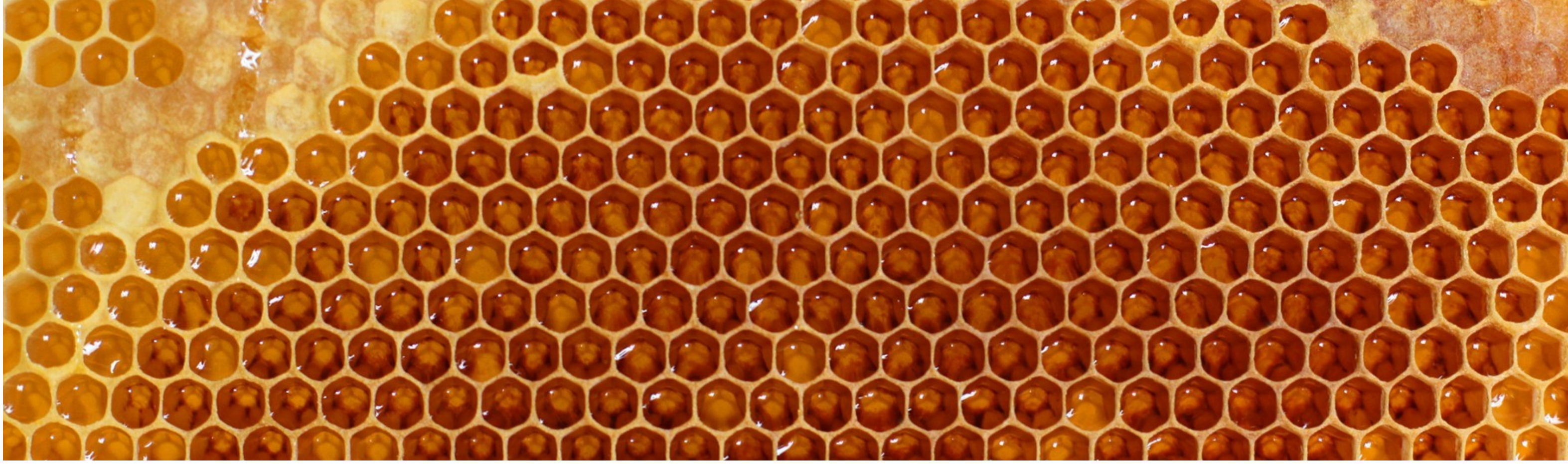
Ruottinen, Lauri; Berg, Peer; Kantanen, Juha; Kristensen, Torsten Nygaard; Praebel, Anne; F. Groeneveld, Linn

Publication date:
2014

Document Version
Early version, also known as pre-print

Link to publication from Aalborg University

Citation for published version (APA):
Ruottinen, L., Berg, P., Kantanen, J., Kristensen, T. N., Praebel, A., & F. Groeneveld, L. (2014). *Status and Conservation of the Nordic Brown Bee: Final report*. NORDIC GENETIC RESOURCE CENTER. NordGen publication series 2014:02



Status and Conservation of the Nordic Brown Bee: Final report

L Ruottinen, P Berg, J Kantanen, TN Kristensen & A Præbel

NordGen

Lauri Ruottinen, MTT Agrifood Research Finland, 31600 Jokioinen, Finland

Peer Berg, NordGen – The Nordic Genetic Resource Center, P.O. Box 115, 1431 Ås, Norway

Juha Kantanen, MTT Agrifood Research Finland, 31600 Jokioinen, Finland and University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

Torsten Nygaard Kristensen, Fredrik Bajers Vej 7H, 9220 Aalborg East, Denmark

Anne Præbel, NordGen – The Nordic Genetic Resource Center, P.O. Box 115, 1431 Ås, Norway

Layout: **Linn F. Groeneveld**, NordGen – The Nordic Genetic Resource Center, P.O. Box 115, 1431 Ås, Norway

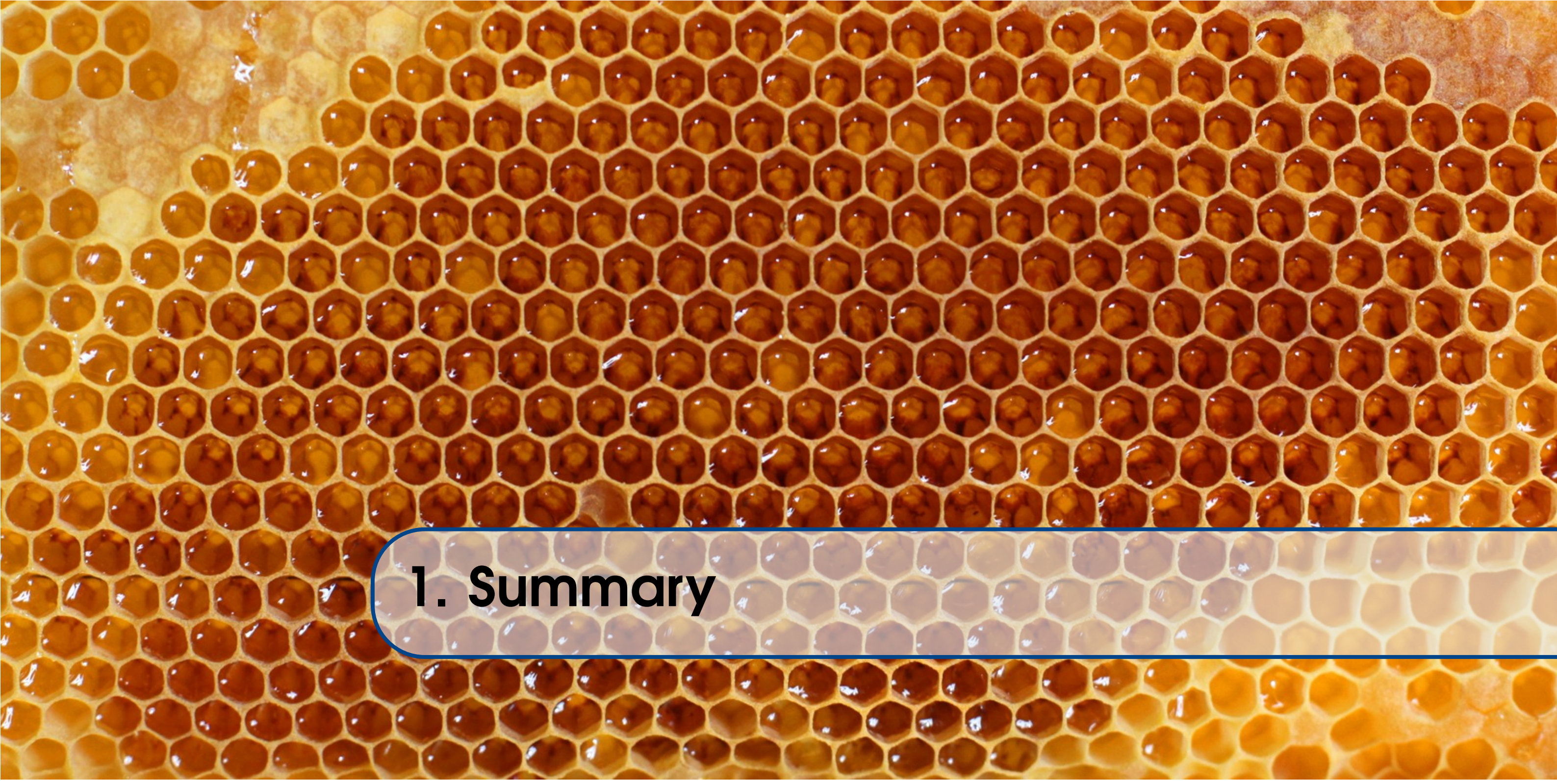
NordGen publication series 2014:02

PUBLISHED BY THE NORDIC GENETIC RESOURCE CENTER

WWW.NORDGEN.ORG

First printing, November 2014

Contents



1. Summary

Apis mellifera mellifera, the Nordic brown bee, was the first honeybee subspecies to colonize the Northern European region and honey has been collected and consumed in this region for about 8000 years. The Nordic brown bee displays excellent characteristics, such as high winter hardiness, strong drive to collect pollen, high longevity of the worker bees and the queen, and flight strength even in cold weather. However, from a beekeeping perspective, some colonies have undesirable characteristics, such as showing a high swarming tendency, being runny on the comb, and being relatively aggressive and defensive.

During the 20th century, *A. m. mellifera* thus has been introgressed or replaced by Southern European or synthetic subspecies, to the degree that it is currently endangered with only few original *A. m. mellifera* populations left in the Nordic and Baltic countries. Conservation of genetic diversity is imperative for maintaining the future adaptive potential of *A. m. mellifera* and populations in general, and for obtaining products with potentially unique characteristics.

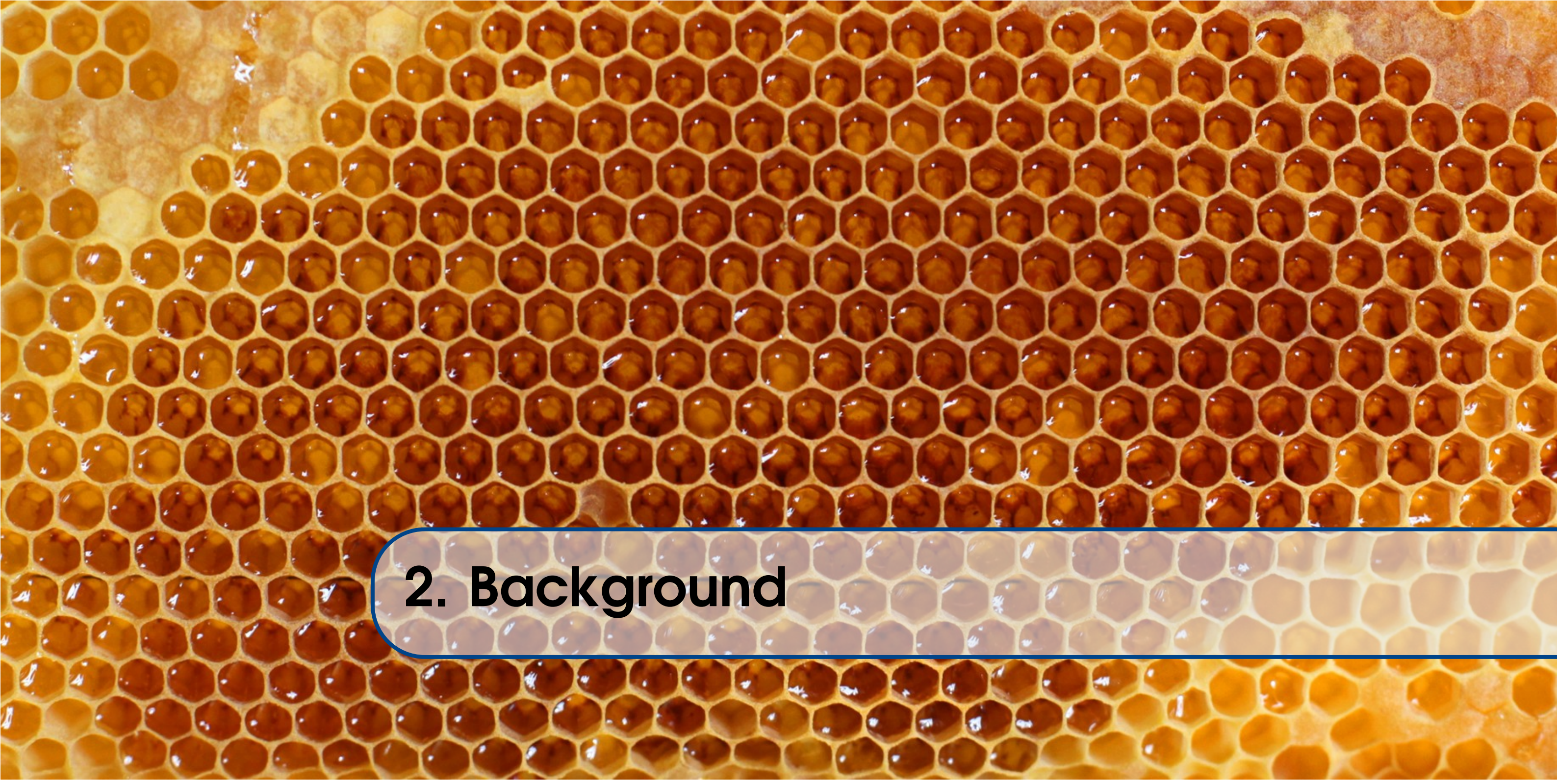
In 2011, the Nordic Genetic Resource Center (NordGen) established an *ad hoc* working group to clarify the current status of the Nordic brown bee in the Nordic and Baltic countries, to summarize the current *in situ* and *ex situ* conservation of *A. m. mellifera* and to provide suggestions for future research activities and initiatives.

A main result of this work was that the Nordic brown bee suffers from a bad reputation within the beekeeping community. It is not clear which of the perceived negative characteristics are actually found across all *A. m. mellifera* populations and which of them can be remedied by *A. m. mellifera* specific management. A contributing factor may also be that many populations have low effective population sizes, which may lead to inbreeding and inbreeding depression. Additionally, due to their sex determination mechanism, small populations of these haplodiploid bees are at a higher risk of extinction than comparable diploid populations.

Future conservation and sustainable use of *A. m. mellifera* calls for comprehensive phenotypic and genetic characterisation, and if possible, performance testing and selective breeding for genetic improvement. Additionally, more effort should be put into the development and re-adoption of management techniques suitable for *A. m. mellifera*, especially those concerning queen rearing. Efficient *in situ* conservation work should be combined with research activities, education and practical beekeeping.

The *in situ* conservation work of *A. m. mellifera* in Nordic and Baltic countries has been carried out by public organizations and private people. Enhancement of conservation and

expansion of the existing populations should include international cooperation, first and foremost coordinated exchange of genetic material. Financing of the conservation efforts ought to be diversified to include funding from national and/or international research grants, governmental agencies and private businesses. However the most essential component is coordination of the national and international resources, and cooperation between actors. Based on the results of this project, we propose the establishment of a Nordic-Baltic network for *in situ* conservation of *A. m. mellifera*.



2. Background

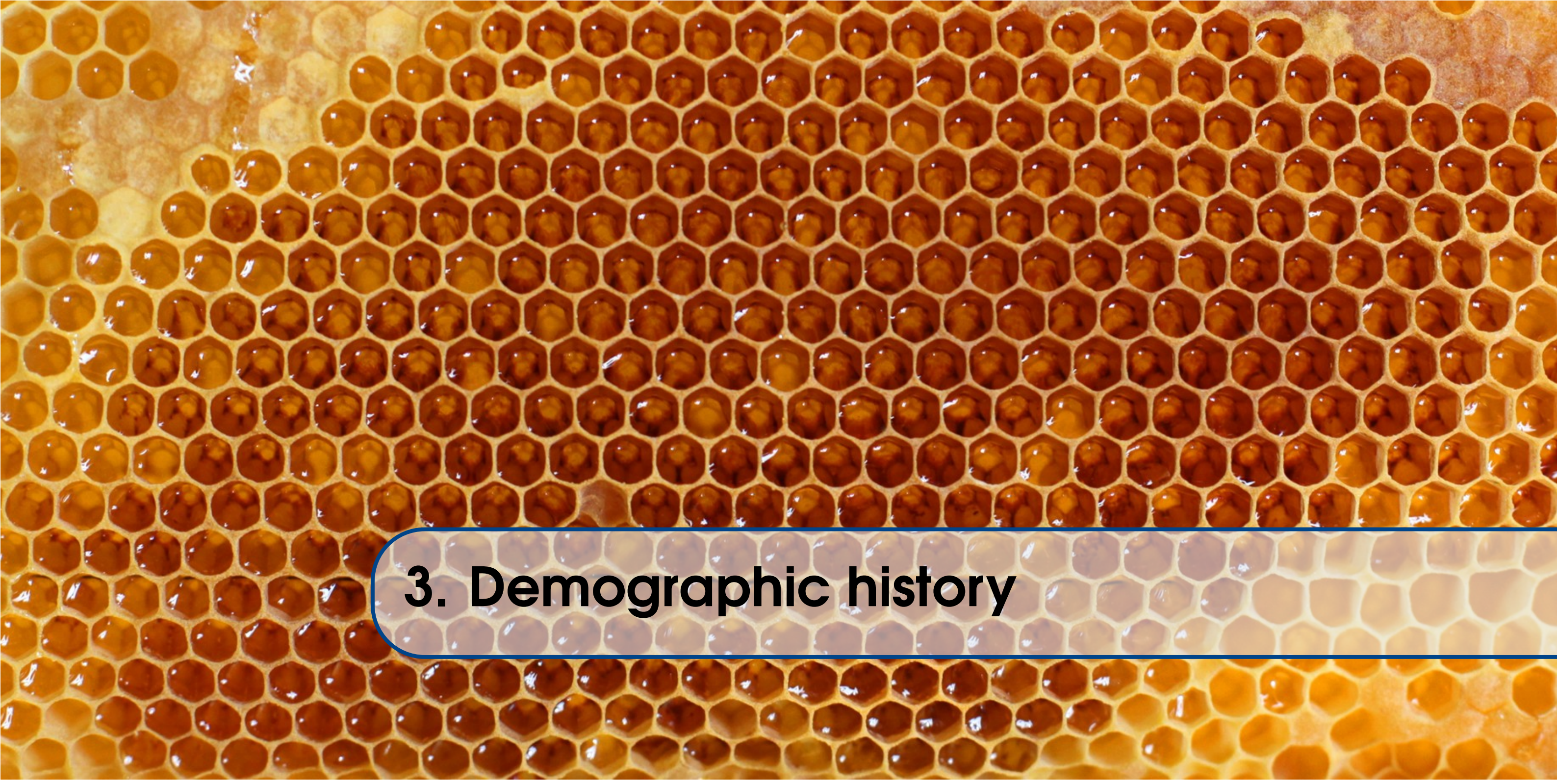
Honey has been collected, produced and consumed in the Nordic and Baltic countries since the last ice age, approximately 8000 years ago [Crane, 1999]. *Apis mellifera mellifera*, often termed the Nordic brown bee or the Nordic dark bee, was the first honeybee subspecies to colonize the Northern European region, and also the first subspecies to be farmed for honey production. *Apis mellifera mellifera* has adapted to the climatic conditions in the Nordic-Baltic region, including short summers and cold winters [Ruttner, 1988]. Significant winter hardiness, a strong drive to collect pollen, high longevity of the worker bees and queens, good ability to take care of the brood area with a comparatively low number of worker bees, and flight strength even in cold weather and strong wind are some of the documented adaptations to Nordic-Baltic environmental conditions [Ruttner, 1988].

During the 20th century *A. m. mellifera* has been replaced by Southern European or synthetic subspecies in the Nordic and Baltic countries [Jensen et al., 2005]. *Apis mellifera mellifera* is now endangered in Europe [De la Rúa et al., 2009]. Maintaining locally adapted honeybee populations is important for the same reasons as conserving farm animal breeds [FAO, 2007]. Conservation of genetic diversity is imperative for maintaining the future adaptive potential of species and populations in general, and for obtaining different products with potentially unique characteristics. Specifically, climate change is likely to bring upon several challenges, e.g., by increasing the incidence of diseases. Genetic diversity is a prerequisite for the evolutionary response to these challenges. Moreover, *A. m. mellifera* populations are of scientific interest in several disciplines such as physiology, ecology, conservation genetics, and animal breeding. Last but not least, cultural reasons advocate for conservation of *A. m. mellifera* [Ruttner, 1988; Jensen et al., 2005; Soland-Reckeweg et al., 2009].

In February 2011, the Nordic Genetic Resource Center (NordGen) initiated an inventory project aiming to document the current status and conservation activities of *A. m. mellifera* in the Nordic and Baltic region. The work involved providing recommendations for future work within this area. The four-month project, run during summer and fall 2012, consisted of interviews of stakeholders, ten visits to *A. m. mellifera* breeders and *in situ* conservation sites, literature reviews, and attendance at meetings and conferences. In addition, an *ad hoc* group consisting of *A. m. mellifera* experts from Nordic and Baltic organisations was established. The members of the *ad hoc* group were:

- Bjørn Dahle, Senior Adviser, Norwegian Beekeepers' Association, Norway
- Per Ideström, Chairman of Association and Project NordBi, Sweden
- Armands Krauze, Chairman of Latvian Beekeepers' Association, Latvia
- Per Kryger, Senior Researcher, Aarhus University, Denmark
- Torbjörn Andersen, Iceland
- Lauri Ruottinen, Reseacher, MTT Agrifood Research Finland, Finland

Members of the group provided information about the current status of *A. m. mellifera* in Norway, Sweden, Latvia, Denmark and Finland.



3. Demographic history

3.1 The historical distribution of the Nordic brown bee

The western honeybee, *A. mellifera*, is distributed in a wide range of environments. Following the expansion of its range after the last ice age it developed into a number of geographically isolated and morphologically and genetically distinct subspecies. Within each of these subspecies there are distinct geographic populations each consisting of a number of colonies. Europe is home to 10 of the 27 currently recognized honeybee subspecies [Ruttner, 1988; Whitfield et al., 2006; Meixner et al., 2007; Costa et al., 2012a].

Following the last ice age, approximately 8000 years ago, *A. m. mellifera* was distributed from the British Isles and Middle Europe up to the Ural Mountains (Figure 3.1). The colonies were feral and lived mainly in holes in tree trunks. People began to collect honey and brood from the wild honeybee colonies [Ruttner, 1988; Garnery et al., 1998; Crane, 1999; Jensen et al., 2005; Meixner et al., 2007; Strange et al., 2007; Carreck, 2008; Soland-Reckeweg et al., 2009]. Tribes had their own honey collecting areas. Such ancient forest beekeeping culture still exists in South-Western Ural areas (Figure 3.2). Siberian forest beekeeping is part of the conservation work of the local bee populations in Russia [Hyttinen, 2012].

Forest beekeeping was common in many European countries up until the 19th century. People also carried parts of tree trunks with bees to their villages (Figure 3.3). This made it possible to keep the bee colonies in the proximity of the homes instead of collecting honey in the forests. Later, bees were kept in artificial straw nests [Huotari, 2007]. Crane [1999] summarises the domestication of the honeybees in the Nordic and Baltic countries from the Middle Ages to the 19th century. The domestication process was relatively uniform in all Nordic countries; first bees were kept in log hives, then in straw skeps and finally in wooden boxes. This development took 200-300 years, while both the *A. m. mellifera* bees and knowledge of beekeeping were slowly moving from Central Europe to the Nordic and Baltic countries.

Invention of wooden hive boxes and movable frames in the 19th century started modern beekeeping. Since then, it has been possible to move bees over long distances. A unit including a bee swarm with a queen in a wooden portable box was called a bee package. Producing package bees led to intense transportation of bees and thereby distribution of different honeybee subspecies around the world [Seeley, 1985; Crane, 1999].

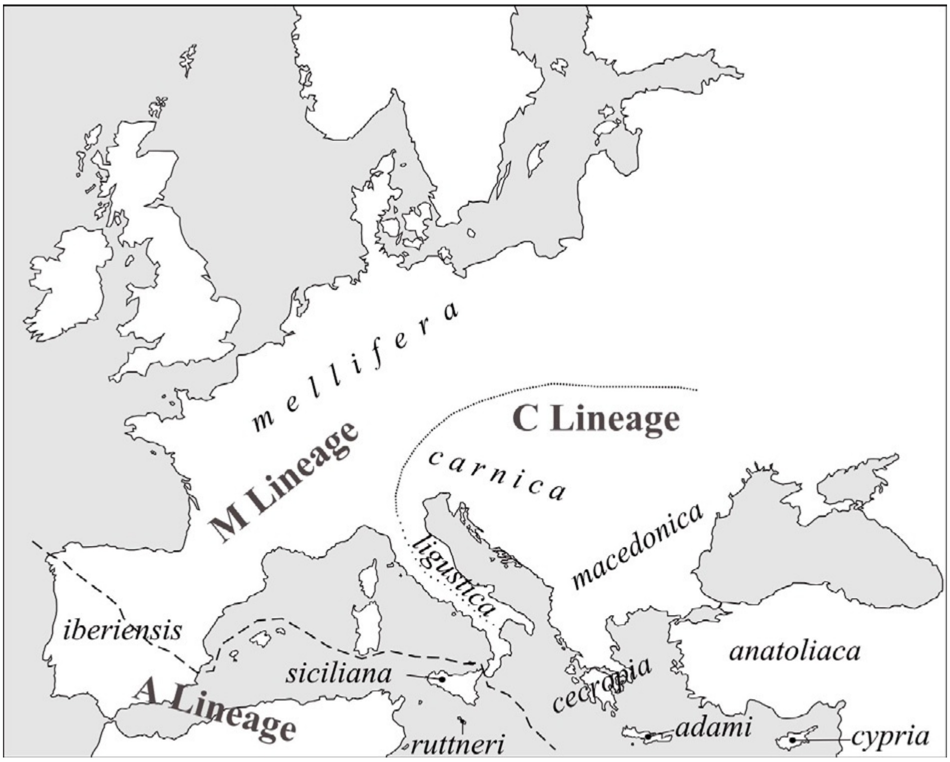


Figure 3.1: Approximate natural distribution of the *Apis mellifera* evolutionary lineages and subspecies in Europe [original figure from De la Rúa et al., 2009].

3.2 Causes for the Nordic brown bee becoming endangered

From being the native bee and the bee used in honey production, *A. m. mellifera* is now endangered in all Nordic and Baltic countries. This section is a summary of factors that have contributed to this, and thus also factors that have to be considered to ensure a successful conservation of *A. m. mellifera* and its diversity. Five major factors can be identified and they are described in the following sections.

3.2.1 Replacement of the Nordic brown bee with other honeybee subspecies

During the 19th century replacement of *A. m. mellifera* with other bee subspecies was already ongoing in the central European regions, where *A. m. mellifera* was endemic. It has been estimated that at the end of the 19th century more than 100 000 Carniolan *Apis mellifera carnica* bee colonies were sent from Slovenia all over the world. The bees were mainly exported to middle Europe, north of the Alps, where *A. m. mellifera* occurred natively [Ida Gnilšak, Curator, Museum of Apiculture, Radovljica, Slovenia, personal communication]. The Italian yellow coloured bee, *Apis mellifera ligustica*, was also sent around the world [Seeley, 1985]. A fast process of replacing *A. m. mellifera* began in both the original areas and in the areas where *A. m. mellifera* was introduced in the 17th and 18th century [Crane, 1999].

The fact that honeybee colonies can easily be transferred over long distances forms a common threat to all of the native honeybee populations around the world [De la Rúa et al., 2009]. New bee subspecies were replacing and crossed with the endemic *A. m. mellifera* populations also in all Nordic countries [Jensen et al., 2005]. For example, in 1990 more than 95% of approximately 40 000 bee colonies in Finland were *A. m. ligustica* and the original *A. m. mellifera* had practically disappeared. However, transporting queens and colonies can also be used for reintroducing *A. m. mellifera* to areas where it has disappeared.

Only a few studies have compared honey production of the different *A. mellifera* subspecies and populations within subspecies. A study conducted on populations of *A. m. ligustica* showed that colonies produced most honey when kept in their areas of origin [Costa et al., 2012b]. This demonstrates that *A. m. ligustica* populations are locally adapted and there is no reason to believe that the same does not apply to the Nordic brown bee. Anecdotal evidence confirms that competitiveness of *A. m. mellifera* is largely dependent on the ecosystem where the colony is situated.

Swarming behaviour makes the handling of the colonies laborious and easily affects the producer's preference of bee subspecies. The popularity of *A. m. mellifera* has decreased in the Nordic and Baltic countries due to a strong swarming tendency. Beekeepers prefer subspecies with lower swarming tendencies such as *A. m. ligustica*, *A. m. carnica* and *Apis mellifera caucasica*. Ruttner [1988] describes that the high swarming tendency originates from a local ecotype of *A. m. mellifera* bees from Northern Germany and the Netherlands. The generalisation of *A. m. mellifera* as an extremely swarming subspecies is based on experience with the Northern German "moor bee" ecotype, earlier named *Apis mellifera lehzeni* [Hämäläinen et al., 1978]. However, practical bee breeding in Sweden has shown that *A. m. mellifera* expresses large variation in this trait, which makes it possible to select against high swarming tendency [Ingvar Arvidsson, personal communication, see also Uzunov et al. 2014]. Also, the aggressiveness, restless and runny behaviour, difficulties in finding a queen, and sensitivity to brood diseases have been suggested to be reasons why *A. m. mellifera* has lost its popularity among beekeepers. The unfavourable traits are often caused by inadequate selection or poor management routines and/or procedures. A summary of the reasons for the negative reputation of *A. m. mellifera* bees is presented in Table 3.1.

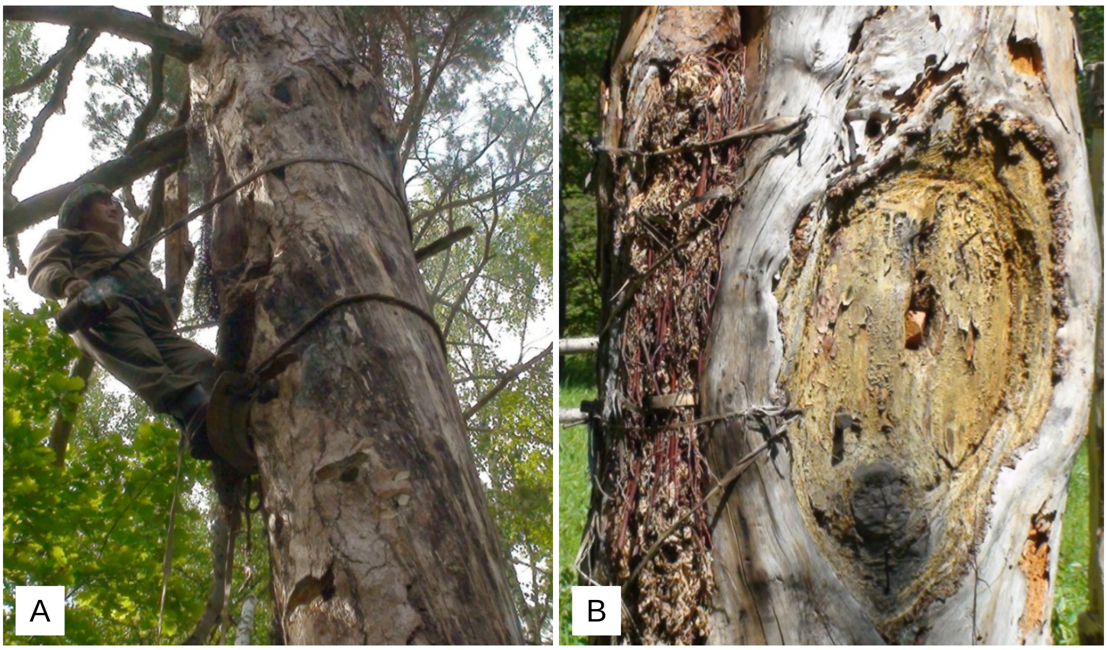


Figure 3.2: Forest beekeeping of Borsa bees in Shulgan-Tash National Park, Southern Ural in Russia: some of the trees in the park have been used to produce honey for more than one hundred years. The trees are marked with identity marks showing to whom the tree belongs. A) Traditional harvesting methods are still practised by Finnish-Ugrian beekeepers. B) The little stick in the flight entrance prevents bears and smaller animals from entering the cavity [Photos: Veikko Hyttinen].

Figure 3.3: Logs containing bee hives moved from the forest to the home yard [Photo: Veikko Hyttinen].

3.2.2 Commercial production of honeybee queens

When both the biological and technical knowledge of queen rearing increased, beekeepers were able to begin large scale queen production. The commercial queen market was opened in the late 19th century. Technological developments enabled the production of hundreds of virgin queens from a single colony. A single queen could be sent in a small cage with a few nursery bees to anywhere in the world. A new colony could then be created with this queen or the queen could be placed in an existing bee colony. The genes from the new queen were thereby introgressed into the original population surrounding the new queen. The industrial production of honeybee queens led to massive gene flow from favoured bee subspecies and strains to local Nordic and Baltic populations of *A. m. mellifera*. Strong sales promotion and fashion to favour only, for instance, certain Italian yellow *A. m. ligustica* or grey Slovenian *A. m. carnica* bees, reduced the diversity of the original bee breeds and local populations dramatically.

In Finland more than half of the 10 000 honeybee queens sold annually were imported during the last three decades [Ruottinen, 2005]. At present, around 20 queen producers rear practically the entire one million honeybee queens sold annually in USA [van Engelsdorp and Meixner, 2010]. Mass production and transfer of honeybee queens is the fastest way to replace original bee populations but this practice may diminish the genetic diversity in populations in a short time span [van Engelsdorp and Meixner, 2010; Kryger, 2012].

Unfortunately, the natural behaviour of *A. m. mellifera* has made it less suitable for industrial queen production compared to many other subspecies. The queen is timid and is hard to find among the worker bees. Additionally, bees run fast on combs. Mass production of queens also requires a lot of worker bees and highly populated colonies. *Apis mellifera mellifera* colonies do not grow particularly large and the population growth during the season is not suitable for strong worker bee production. *Apis mellifera mellifera* therefore needs customised queen rearing techniques.

3.2.3 Local outbreaks of fatal bee diseases & dispersal of disease resistant bees

An aggressive strain of the European foul brood bacteria (*Melissococcus plutonius*) broke out in the breeding stock of the Norwegian gene bank area in 2010-2011. The bacteria destroyed one third of the breeding material of *A. m. mellifera* [Bjørn Dahle, personal communication].

Table 3.1: Summary of the phenotypic and genetic factors contributing to the negative image of *Apis mellifera mellifera*, and the impact of beekeeping techniques on the reputation.

Phenotypic feature degrading the image of *A. m. mellifera* bees	**Genetic basis**	**Unsuitable beekeeping techniques strengthening the negative features**	**Recommended beekeeping techniques to improve the image of *A. m. mellifera***
Aggressiveness and timid behaviour	Crossings with other bees	Uncontrolled mating of queens, unsuitable and hard handling of bees	Selection for gentleness, isolated mating areas for queen rearing, training and improvement of management skills
Runny behaviour	Original feature	Heavy use of smoke instead of water spray to settle down the bees	Training and improvement of management skills to handle the *A. m. mellifera* bees
High swarming tendency	Continuously producing new queens from swarm queen cells promotes genetic selection for swarming	Increase of swarming tendency through false queen breeding techniques and selection	Relaxing selection for high swarming tendency, training and improving special beekeeping techniques for *A. m. mellifera* especially before midsummer when swarming occurs
Low productivity and unsatisfying population development	Wrong ecotype for the area / maladaptation	Using queen material with unknown performance and G*E adaption, false rhythm in beekeeping actions	Selecting ecotypes that are adapted to local conditions, using continental and coastal ecotypes in right ecosystems, committing performance tests to find suitable material for the local conditions
Difficulty to find the queen when needed for the management purposes	Black colour and timid behaviour	Wrong time to check the queen, using unmarked queens, hard handling	Right timing to search and handle the queen during the season, using white number tags to mark the queen, new technology (RFID signs), using queen excluder to place the queen in certain part of the hive, using techniques where queen does not have to be found
Sensitivity to brood diseases	Low number of adults compared to brood area in spring, unknown genetic sensitivity to pathogens	Unsuitable hive construction, unnecessary disturbance of the colony homeostasis, lack of disease control, infection pressure from equipment	Use of techniques that support the bee colonies' natural resistance against pathogens, improved disease control, selection for disease resistance, reducing the rate of inbreeding

During the last years, European foul brood has caused a serious threat in conservation areas of *A. m. mellifera* in Switzerland. It is still not clear whether *A. m. mellifera* are especially sensitive to the *M. plutonius* bacterium.

In 2003, the Riga University bee yard in Latvia practically lost its whole population of *A. m. mellifera* due to an American foul brood (*Paenibacillus larvae*) breakout [Armands Krauze, personal communication]. Such small *A. m. mellifera* populations are very vulnerable to sudden hazards.

Apis mellifera mellifera bees went almost extinct in the British Isles in the beginning of the 20th century due to a disease named "Isle of Wight Disease". Later it was concluded that it was the tracheal mite *Acarapis woodi* that had destroyed over 90% of the *A. m. mellifera* populations in Great Britain [Bailey, 1958]. Monk Brother Adam (1898-1996) crossed *A. m. ligustica* bees from Northern Italy with the remaining *A. m. mellifera* strains resulting in the so-called "Buckfast bee", which is resistant to tracheal mites. The Buckfast bee has replaced numerous local bee populations of *A. m. mellifera* for example in Denmark, Sweden and Finland. Attempts to combat the tracheal mite by replacing local subspecies with *A. woodi* resistant bees are devastating to the local bee populations.

The honeybee parasite varroa mite (*Varroa destructor*), with associated viruses, destroys bee colonies. Beekeepers can fight the varroa mite by importing varroa resistant bees. This strategy might not be effective in the long run as the resistance mechanism against bee parasites is usually a result of a co-evolution in local ecosystems. Additionally, importation is not recommendable from a conservation point of view, as it easily leads to extinction of the local bee populations. The potential for natural selection can be maintained by protecting the original bee populations like *A. m. mellifera*. Instead of measuring only one or a few phenotypic features to select bees for varroa resistance, more attention is now paid to integrated Gene-Environment-Parasite interactions in a breeding program aiming at increasing resistance towards the varroa mite in local bee populations [Costa et al., 2012a].

At the NordBi meeting in Fränsta, Sweden, 2011, many of the beekeepers expressed their concern regarding the sensitivity of *A. m. mellifera* to chalk brood disease (*Ascosphaera apis*). This susceptibility has also been observed by beekeepers both in South-Western Finland and in Sweden [Ingvar Arvidsson, Inger Bengtsson, Lassi Kauko, Aimo Nurminen, Calle Regnell, Per Thunman, personal communication]. Chalk brood disease is a fungal infection of bee larvae, causing them to die and mummify in the nest brood area. Sensitivity towards this disease might have a genetic basis or it might be a consequence of incorrect beekeeping techniques or a combination of these. *Apis mellifera mellifera* bees take care of a large brood area with a small number of adult bees in cold spring weather. The growth of the *A. apis* spores is temperature dependent, so that low temperatures trigger the growth of the chalk brood disease spores. This should be taken into account in management practices, so that especially warm beehive constructions should be used in the spring time.

3.2.4 Inbreeding and genetic drift

Many of the local *A. m. mellifera* populations have low effective population sizes compared to the introduced subspecies [Kryger, 2012]. Whilst the importation of queens of foreign subspecies can result in introgressive hybridization of native subspecies, large scale queen breeding and the widespread propagation of selected stock will ultimately reduce the effective population sizes, increasing the rates of inbreeding [reviewed in Zayed, 2009]. Inbreeding leads to increased homozygosity and potentially expression of deleterious recessive alleles, leading to inbreeding depression. Genetic drift reduces genetic variation and thereby the ability to adapt to environmental changes through evolutionary responses, a process which is especially pronounced in small populations [Frankham et al., 2002; Kristensen and Sørensen, 2005].

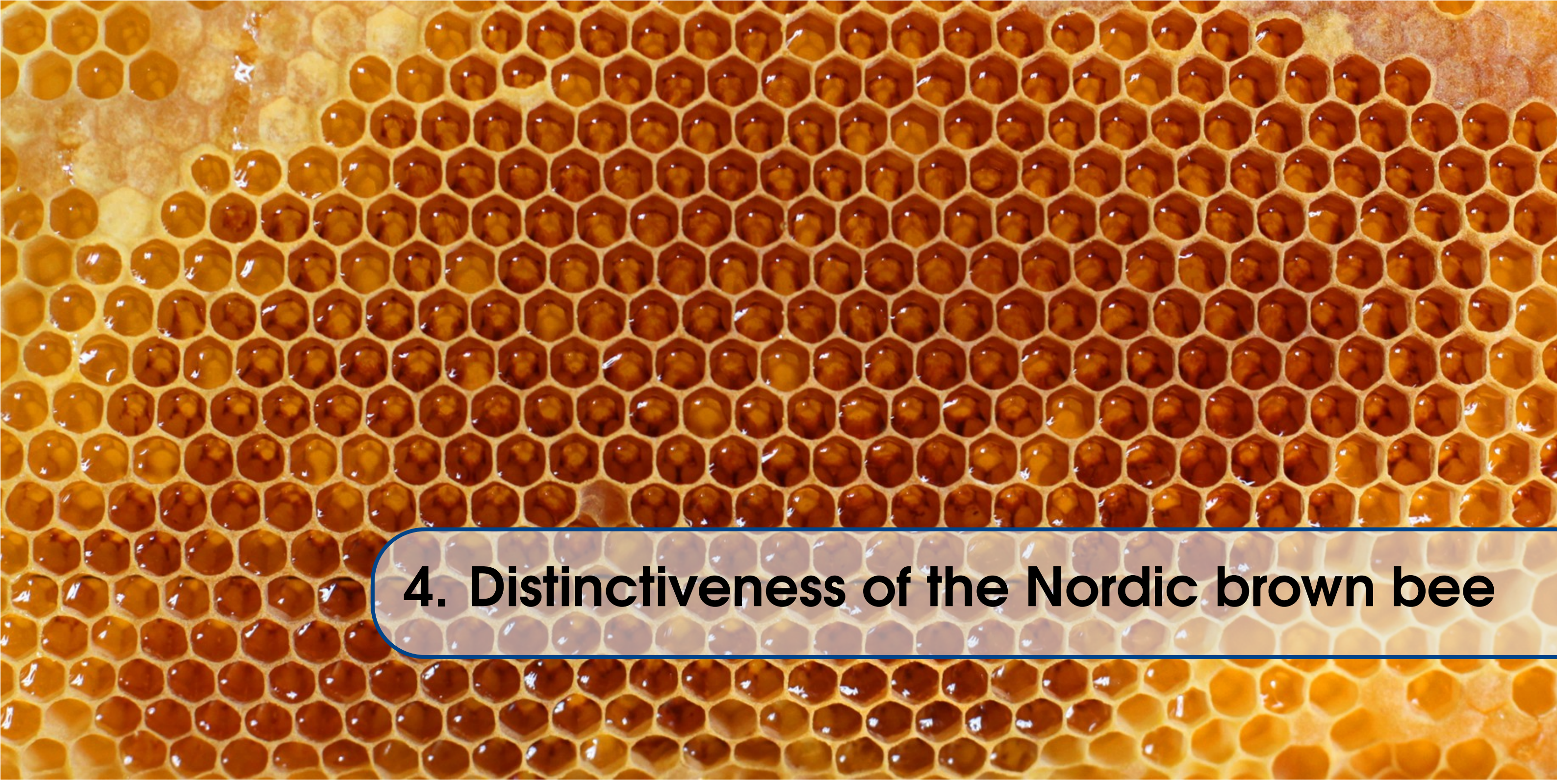
The genetic diversity of a honeybee population is affected by the number of new queens that are introduced into the population and by the number of different populations that these queens originate from. In honeybees sex is determined by heterozygosity at a single locus (the Sex Determination Locus, SDL) harbouring the complementary sex determiner gene (cds) [Gempe and Beye, 2009]. The cds locus has been recognized as highly variable with 16 distinguishable alleles [Hyink et al., 2013]. An embryo becomes male by default unless it has two different forms of cds. Thus, all unfertilized eggs (hemizygous) develop into males, fertilized eggs heterozygous at SDL develop into females, and those homozygous at SDL develop into males. Using a very narrow breeding material diminishes the sex allele diversity of the population in a short time period. This causes undesirable changes in the demographics of the colonies through an increased number of male broods and it lowers the general productivity of the colony. A detailed description of sex determination in honeybees is available in the literature [e.g. Gempe et al., 2009; Gempe and Beye, 2009; Hoff, 2009; Hyink et al., 2013].

The number of *A. m. mellifera* bee colonies does not tell us very much about the genetic diversity of the populations in each country. To maintain genetic diversity, more attention should be paid to queen rearing strategies, including adoption of suitable queen rearing techniques and number of reared queens. Additionally, actions should be taken towards coordinated exchange of genetic material within the Nordic countries as beekeepers from Denmark, Sweden and Finland regard inbreeding as one of the main challenges in *A. m. mellifera* beekeeping.

Conservation concepts emphasising maximum avoidance of gene flow between colonies quickly lead to strong inbreeding and genetic drift. In the long run, conservation of strictly closed small populations is likely to be unsuccessful. An example of a small closed conservation population is the Læsø bee population in Denmark, which has been reported to show clear signs of inbreeding depression [Kryger, 2012].

3.2.5 Sub-optimal management techniques for the Nordic brown bee

Modern beekeeping techniques were developed simultaneously with the expansion of the most popular bee subspecies *A. m. ligustica* and *A. m. carnica*. Standardisation of the frame size and hive boxes led to universal management techniques in professional bee operations, first in the New World and then in many European countries. Courses, education materials, handbooks and journals promoted the new techniques. The whole management concept was optimised for *A. m. ligustica*, *A. m. carnica* and *A. m. caucasica* bees. It included rough handling of frames and colonies, avoidance of early splitting of the colonies before the main nectar flow, large room for overwintering, demand of low swarming tendency, removing the queen cells for swarm control and heavy usage of smoke during management (Table 3.1). Many of these methods are too harsh for *A. m. mellifera* bees. Beekeepers keeping *A. m. mellifera* had to continue using more old fashioned and less efficient techniques or simply develop unique methods and equipment to handle hives [Manner, 1925]. Physiology, behaviour and the annual life cycle of *A. m. mellifera* differ from other bees and need to be taken into account in management techniques. Surprisingly, this is not commonly known by beekeepers [Ingvar Arvidsson, personal communication].



4. Distinctiveness of the Nordic brown bee

A full description of standard methods for characterising subspecies and ecotypes of *A. mellifera* has been published in the open source COLOSS1 Beebook 2013 found under: `http://www.coloss.org/beebook/I/subspecies-ecotypes` [Dietemann et al., 2013].

4.1 Morphometry

Measuring the morphological properties of the honeybee body is a classical method to describe the different subspecies of honeybee (*A. mellifera*). Evaluation of the colour pattern and measuring the dimensions of different organs, such as tergites, wing venation and tongue length, can be used in morphological analysis of honeybee subspecies [Meixner et al., 2013].

Most of the morphological measurements are based on wing venation. The indexes describing the properties of tested honeybee ecotypes are based on the proportions of different wing vein lengths and angles from the wing samples. This characterisation can be carried out by using graphical tools and suitable software. The most commonly used indexes are the cubital index and discoidal index. The purity or hybridisation level of the worker bees can be evaluated by comparing the measurements with expected standard values [Ruttner, 1988].

Beekeepers use the wing index analyses to reveal possible hybridization of their breeding populations. Measuring cubital and discoidal indexes is common in Sweden, Austria, Switzerland and Czech Republic. The data is mostly used on the local level to detect the purity of local populations or to test single beekeeper's colonies. The results are used directly by beekeepers to evaluate their own bee populations or to support network members in choosing appropriate material for breeding.

The NordBi organisation in Sweden quantifies cubital and discoidal indexes every year from different breeding stocks. NordBi gives the results about the purity of different local *A. m. mellifera* populations to their members to support the breeding and conservation work of *A. m. mellifera* (`http://www.nordbi.org/Information.html`). Beekeepers also look at the colour pattern of the abdomen to find possible hybridisation among the worker bees, but there is no standardised protocol [Ingvar Arvidsson, personal communication].

Ruttner [1988] names unique morphometric characteristics for *A. m. mellifera*. The length of the abdominal cover hair is double the length compared to other *A. mellifera* subspecies (up to 0.5 mm), and the body size is larger with a broad abdomen, compared to bees from a warmer climate. The cubital index is lower than 1.8, which is much lower than in *A. m. ligustica* and *A. m. carnica*, where the cubital index is above 2.5.

Morphometric analyses can be used as a cost effective and time saving method for preliminary scanning of possible hybridization/introgression of local *A. m. mellifera* populations. Promising bee material can be collected for more detailed DNA analyses. DNA techniques are currently replacing morphological methods, although some morphometric measures still provide useful and cost effective results.

4.2 DNA Analyses

Microsatellites are suitable markers for testing the genetic origin of honeybee populations. More than 500 microsatellites have been identified for population genetic studies in honeybees. Depending on the question being asked, the results from DNA analyses and wing index measurements do not always correlate well with each other. DNA tests are typically more expensive and time consuming than morphometric analyses. However, they provide the most reliable information in regard to identifying purebred *A. m. mellifera* populations [Soland-Reckeweg, 2006]. Results from DNA analyses could be used for guiding breeding decisions in an international conservation network aiming at reducing e.g. inbreeding and genetic drift.

In recent years there have been numerous studies which employed Single Nucleotide Polymorphism (SNP) panels and even whole genome sequences to address various research questions on honeybees [Honeybee Genome Sequencing Consortium, 2006; Chávez-Galarza et al., 2013; Elsik et al., 2014; Harpur et al., 2014; Pinto et al., 2014]. Some of these markers could possibly be useful in future assessments of diversity and level of purity.

Only a few DNA studies have been carried out on Northern European *A. m. mellifera* populations [Jensen et al., 2005; Soland-Reckeweg, 2006; Pinto et al., 2014]. These studies showed that some of the Nordic populations represent pure *A. m. mellifera* bees and could be used as a comparative standard of pure *A. m. mellifera* bees worldwide. The studied populations were significantly differentiated. However, some of the British populations were quite similar to each other. The two island populations from Læsø (Denmark) and Colonsay (Scotland) were quite distinct. The Læsø population in Denmark has been studied continuously and hybridization with *A. m. ligustica* in this population is well documented [Jensen et al., 2005; Kryger, 2012; Pinto et al., 2014]. DNA technologies have also provided information on variation between Nordic and middle European populations of *A. m. mellifera*. For example Soland-Reckeweg [2006] showed that the variation among *A. m. mellifera* populations in Norway and Sweden is larger than among Nordic and Swiss *A. m. mellifera* populations. Genetic variation among the southern Swiss and the Scandinavian *A. m. mellifera* populations was 4.7%, and the genetic variation between Norwegian and Swedish populations was 10.7%.

For breeding purposes, routine testing with DNA techniques is too expensive for beekeepers. Commercial honeybee DNA analyses are provided by the company Apigenix, Switzerland (`www.apigenix.com`). The price to analyse the extent of hybridization of a single bee colony (representing one queen) is around 100 €. The analysis is based on 30 individual drones per bee colony. Because the drones are present only in summer time, the samples should be taken during the period from June to August [Soland-Reckeweg et al., 2009].

4.3 Performance tests

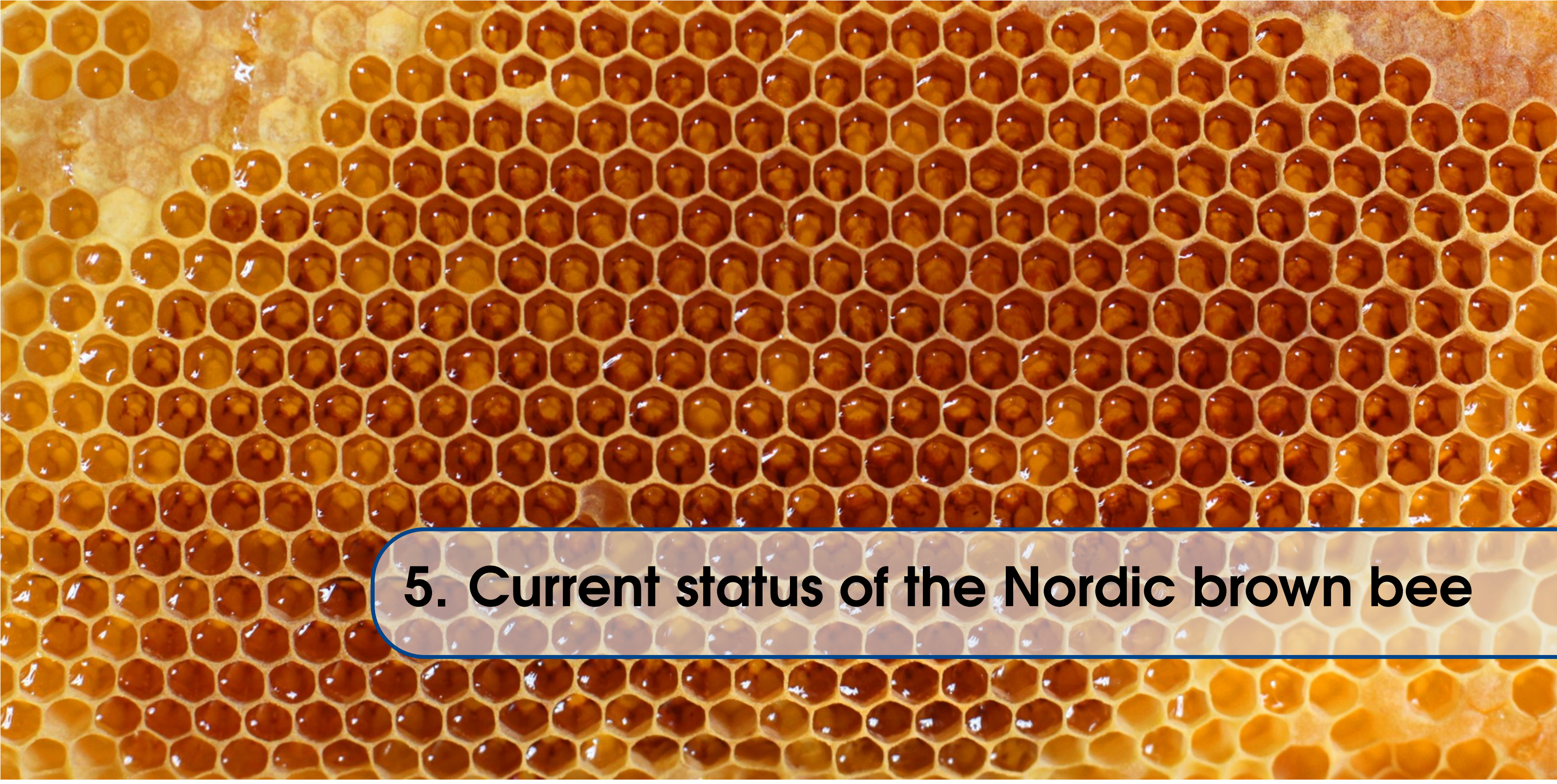
Morphological or genetic measures based on neutral markers are not necessarily informative when it comes to explaining variation in performance traits like honey production, overwintering ability, aggressiveness, swarming tendency of the bee colony, and disease resistance. These phenotypic traits can be tested during the inspection of the beehive or in controlled laboratory studies. A methodology for comparative performance evaluation was developed and tested in COLOSS network working group 4 [Costa et al., 2012a].

Suitable temper and moderate swarming tendency are two of the key factors for successful and sustainable *in situ* conservation work of *A. m. mellifera*. Beekeepers are able and willing to work with moderately gentle and slowly swarming bees. The natural diversity of *A. m. mellifera* bees also includes gentle but fearful bees with reasonable swarming tendency. Gentle *A. m. mellifera* bees are found in Bengtfors in Sweden and Rymättylä in Finland [Ingvar Arvidsson and Aimo Nurminen, personal communication; field studies 2012].

Very strong overwintering performance and good ability to collect nectar from heather (*Calluna vulgaris*) are unique properties of *A. m. mellifera* that distinguish this subspecies from southern subspecies. Adaptation to the Atlantic climate in Western Europe and to the continental climate in Eastern Europe can be observed as a slow or fast brooding rhythm during the season. Performance tests also show higher defence behaviour and swarming tendency in *A. m. mellifera* when compared to Italian (*A. m. ligustica*) and Carniolan (*A. m. carnica*) bees [Ruttner, 1988].

The integrated and coordinated use of morphological, DNA based, and performance information should be used to support the conservation activities of *A. m. mellifera* bees. All of these tools have their pros and cons, and none of them alone can provide the information needed to conserve the endangered populations and subspecies. Future developments in genomics and identification of markers under selection will without doubt also aid in:

- identifying candidate genes explaining variation in functional phenotypes [Spötter et al., 2012]
- identifying the origin of *A. m. mellifera* [Soland-Reckeweg et al., 2009]
- better quantification of the extent to which populations of *A. m. mellifera* are purebred [Kryger, 2012]



5. Current status of the Nordic brown bee

5.1 Methods

One aim of this project was to investigate the current status of *A. m. mellifera* in the Nordic and Baltic countries and make suggestions for future *in situ* and *ex situ* conservation of the Nordic brown bee. The project was carried out between the 1st of February 2011 and 31st of December 2012 and included literature studies, *ad hoc* group work, interviews, fieldwork during the summer 2012, and presenting the results at international seminars. The *ad hoc* group kick-off meeting was held in Oslo in April 2012. Presentations, either oral or poster, were held at the following international meetings:

- Nordic-Baltic Apicultural Research Symposium, Tartu, Estonia, February 2011. Kantanen, J. and Ekström H. Towards a strategy for the conservation of the Nordic brown bee.
- COLOSS (Prevention of honeybee COlony LOSSes) working group 4, Workshop, Jokioinen, Finland, January 2012, Bee book and data analyses of ecotype-environment interaction experiments, Ruottinen, L. and Kantanen, J. Conservation of the Nordic Bee (*Apis mellifera mellifera*).
- Nordic-Baltic Apicultural Research Symposium, Riga, Latvia, January 2012, Ruottinen, L., Kettunen-Præbel, A. and Kantanen, J. Conservation of the Nordic brown bee (*Apis mellifera mellifera*).
- NordBi Conference 2012, Fränsta, Sweden, March 2012, Ruottinen, L., Kantanen, J. and Kettunen-Præbel, A. Conservation of the Nordic brown bee *Apis mellifera mellifera*.
- COLOSS (Prevention of honeybee COlony LOSSes) working group 4, Workshop Puławy, Poland, April 2012, Ruottinen, L., Kettunen-Præbel, A. and Kantanen, J. The status and future of *Apis mellifera mellifera* in Nordic-Baltic countries.
- SICAMM (International Association for the Protection of the European Dark Bee) conference 2012, Landquart, Switzerland September 2012, Ruottinen, L., Kettunen-Præbel, A. and Kantanen, J. Conservation of the Nordic brown bee (*Apis mellifera mellifera*).
- EurBee, 5th European Conference of Apidology, Halle an der Saale, Germany, September 2012, ad hoc consultations.
- "Genotype-by-Environment Interactions and Adaptation of Farm Animals on Phenotypic and Molecular Levels", Tuusula, Finland, November 2012, Costa, C., Büchler, R., Berg, S., Bienkowska, M., Bouga, M., Bubalo, D., Charistos, L., Le Conte, Y., Drazic, M., Dyrba, D., Fillipi, J., Hatjina, F., Ivanova, E., Kezic, N., Kiprjanovska, H., Kokinis, M.,

Korpela, S., Kryger, P., Lodesani, M., Meixner, M., Panasiuk, B., Pechhacker, H., Palmen, P., Oliveri, E., Ruottinen, L., Uzunov, A., Giacomo, V. and Wilde, J. A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality of honey bee colonies.

- Nordic-Baltic Apicultural Research Symposium, Oslo, Norway January 2013, Ruottinen, L., Kettunen-Præbel, A. and Kantanen, J. The status and future of *Apis mellifera mellifera* in Nordic-Baltic countries.

Thirteen interviews with people from other than Nordic and Baltic countries were carried out during the seminars and fieldwork. Interviews included questions of the national status, testing methods and distribution of the *A. m. mellifera* in Ireland, France, Poland, Switzerland, Austria, United Kingdom and Germany. Representatives from other countries were also contacted, but no detailed information was obtained.

The interviews yielded the following information. The largest populations of *A. m. mellifera* are in Norway, Sweden and presumably in Russia. Populations of potential conservation interest exist in Ireland, Scotland, Sweden, Norway and Denmark. Some pure *A. m. mellifera* bees have also been identified in Austria, Switzerland and France (Figure 5.1). The distribution data is incomplete, and new local populations for conservation work can still be found. One of the main problems is to find suitable pairing sites for *A. m. mellifera* bees in the areas where the density of honeybee colonies is very high. In the Nordic and Baltic countries the density is less than 0.5 hives/km^2 (Finland, Norway and Sweden 0.2 hives/km^2), while in Central Europe it can be 10 to 20 hives/km^2 or even more. There are good possibilities in the Nordic countries to establish isolated mating areas for conservation purposes. The Nordic material could be used as an international comparative standard in analysing the purity of *A. m. mellifera* populations in other countries. [Ingvar Arvidsson, Veikko Hyttinen and Jerzy Wilde, personal communication, Soland-Reckeweg et al. 2009; Oleksa et al. 2011; Kryger 2012; SICAMM conference; `www.nordbi.org`].

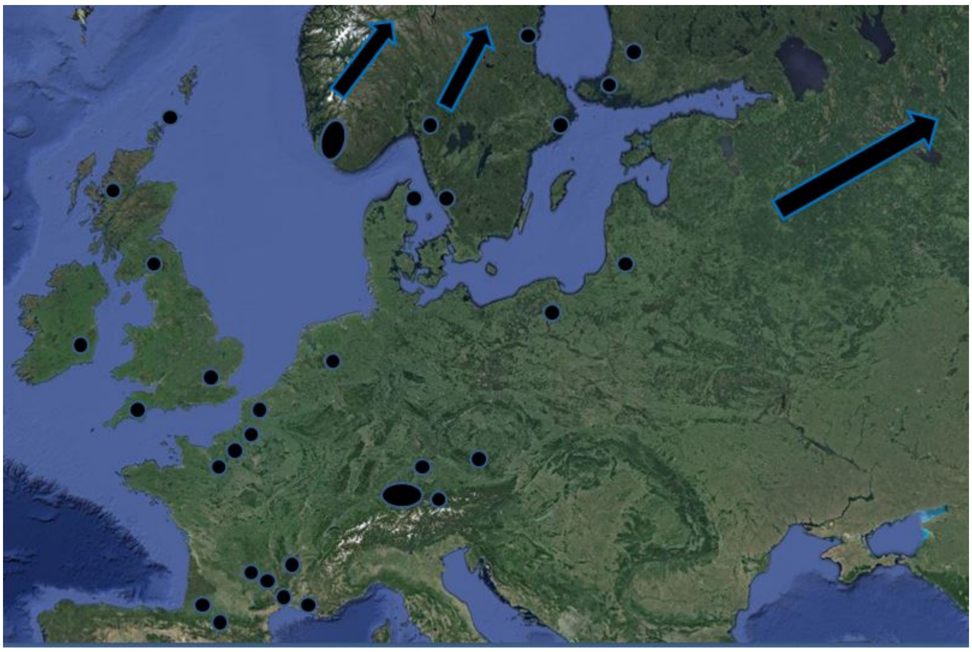


Figure 5.1: Distribution of *A. m. mellifera* in Europe in 2012. Black circles represent areas with *A. m. mellifera* colonies, with a few additional colonies present further north and east as indicated by the arrows.

Table 5.1: Contact list of *A. m. mellifera* beekeeping organisations.

Country	Link	Target group	Content
Sweden	www.nordbi.nu	400 NordBi association members	General information about the organisation and activities
	www.nordbi.org/Information.html	Printed NordBi-journal for members. Two free issues available on NordBi website per year	Recent information and seasonal reports. Information about morphometric measurements and activities of the pairing sites and queen sale announcements
Norway	www.norges-birokterlag.no	Norwegian Beekeepers Association's homepage, 3000 members	The association administrates the registered *A. m. mellifera* beekeepers in Norway and coordinates the breeding strategy of Norwegian bee populations
Finland	www.mehiläishoitajat.fi	Finnish Beekeepers Association's homepage, 3000 members	General information about the organisation and activities. Private *A. m. mellifera* beekeepers in Finland can be contacted via the association
Denmark	www.brunbi.dk	Homepage for Læsø beekeepersapea	General information and contact to private beekeepers at Læsø
Latvia	www.strops.lv	Latvian Beekeepers Association's homepage	Contact to *A. m. mellifera* beekeepers in Latvia
Estonia	www.mesinikeliit.ee	Estonian Beekeepers Association's homepage	General information and contact to private beekeepers
Lithuania	www.bitininkusajunga.lt	Lithuanian Beekeepers Association's homepage	General information and contact to private beekeepers
Other	www.sicamm.org	International Association for the Protection of the European Dark Bee	Information on executive committee and members. General information about the organisation and activity links to other countries

Field work was done in South-West Finland and across Mid-Sweden from Stockholm towards the Norwegian border. During the fieldwork five key persons in Sweden (Per Thunman, Calle Regnell, Ingvar Pettersson, Ingvar Arvidsson, Inger Bengtsson), and three key persons in Finland (Lassi Kauko, Aimo Nurminen and Veikko Hyttinen) were interviewed. The information from group discussions and personal communication during the NordBi meeting in Fränsta, Sweden, March 2012, has also been included in the list of research needs and other activities needed to improve the conservation of the *A. m. mellifera* bees (see Chapter 6). The National beekeepers' associations in Finland and Norway were contacted during the study. Please refer to Table 5.1 for contact details of the respective national beekeepers' associations. Furthermore, five private bee farms in Finland and Sweden were also visited.

5.2 National status of the Nordic brown bee in the Nordic region

The status of *A. m. mellifera* differs within the Nordic-Baltic region. Figure 5.2 gives an overview of bee yards used for conservation and breeding work in the Nordic region and Table 5.2 lists the number of *A. m. mellifera* colonies per country.

Table 5.2: Summary of current number of colonies of *A. m. mellifera* in the Nordic and Baltic countries. The asterisk denotes that the number of colonies for queen production and pairing can be smaller.

Country	Colonies total	Colonies in effective conservation work	Conservation measures
Denmark	300	200*	protected area on Læsø
Estonia	not available	not available	not available
Finland	300	100*	private actions by beekeepers
Iceland	0	0	no *A. m. mellifera*, only Buckfast bees
Latvia	100	100	the Latvian University of Agriculture works with private beekeepers' assistance, public support
Lithuania	not available	not available	not available
Norway	8000	900*	protected area in Vest-Agder and Rogaland counties, national support for beekeepers
Sweden	2000	1000	NordBi association since 1990, supported by the NordBi project

Norway and Sweden form a region where *A. m. mellifera* exists, but is threatened by other subspecies. Both countries have their own permanent populations in several locations. The Norwegian and Swedish populations have been shown to differ from each other, based on results from molecular analyses [Jensen et al., 2005; Soland-Reckeweg, 2006]. In contrast, Finland, Denmark and the Baltic countries, only have a few *A. m. mellifera* colonies left and the original populations are nearly extinct. Populations in these countries are extremely small. There is no natural gene flow between these colonies, but the exchange of breeding queens between beekeepers is increasing.

The following country summaries are based on the interviews conducted during this project. For comparison see Bouga et al. [2011], which contains country data for Norway and Denmark.

5.2.1 Norway

There are a total of 45 000 honeybee colonies in Norway. Out of these, 5000-8000, belonging to three populations, have been recognized as *A. m. mellifera* colonies. There is an isolated breeding area in Vest-Agder and Rogaland counties (Figure 5.3). It was established and protected by law in 1987. It includes Flekkefjord, Lund, Sokndal and Sirdal municipalities with a total area of 3500 km^2. The breeding area is restricted such that:

1. Only *A. m. mellifera* can be kept in the area.
2. Migratory beekeeping in and out of the area is prohibited.
3. It is prohibited to take bees or breeding material (queens, egg, larvae or semen) into the area.

The number of beekeepers and colonies in the area has been steadily declining from 150 beekeepers with a total of 2700 colonies in 1985 to 30 beekeepers with 977 colonies in 2011, when a sugar tax was introduced.

The Norwegian Beekeepers Association (NBA) is coordinating a breeding program, where the *A. m. mellifera* colonies are performance tested for temperament, swarming tendency and honey production.

Some of the Norwegian populations have been characterized with DNA techniques. A study based on 1183 SNP loci, which included samples from 10 Norwegian colonies from the isolated breeding area showed these to be pure *A. m. mellifera* [Pinto et al., 2014]. It is expected that many different populations can be found in isolated areas in the counties of Hedmark, Aust-Agder, Vest-Agder, Rogaland, Hordaland, and Sogn and Fjordane, which have a long tradition in

Figure 5.2: *Apis mellifera mellifera* bee yards used for conservation and breeding work in the Nordic and Baltic countries. The blue markers show conservation and breeding sites. The red marker denotes the founder population of the current *A. m. mellifera* bees in Finland. Norwegian information has not been updated on the map. The green marker indicates the Norwegian Beekeepers Association, which keeps a register of *A. m. mellifera* beekeepers. This map, with additional information, is available at `http://goo.gl/maps/ghVHN`.

beekeeping.

The NBA executes several activities to promote conservation work of *A. m. mellifera* bees in Norway. A pure breeding area in Vest-Agder and Rogaland counties produces breeding material for the NBA. Approximately one third of the beekeepers get their material from the NBA's breeding population. Wing indexes are used in Vest-Agder and Rogaland counties to ensure the purity of the breeding material. The cubital/discoidal index, Dawino method and DNA analyses are used for scanning the quality of the NBA material. Also, the mating station at Rødberg in Buskerud County has been shown to be safe from foreign gene flow.

Data from the performance testing of *A. m. mellifera* queens in the breeding program of the NBA are entered into the German database for estimation of breeding values (`www.beebreed.eu`). This information can be utilised by all *A. m. mellifera* beekeepers. The NBA is trying to encourage beekeepers to join this system, but interest has been limited. Many beekeepers currently prefer other subspecies like *A. m. carnica* and Buckfast bees.

The situation for the breeding population of the NBA is critical at the moment. This is due to several factors. Hybridization occurred at NBA's mating station in 2005, but remained undiscovered until 2009. This resulted in a drastic culling of queens that were not found to be pure *A. m. mellifera*. In 2010 an outbreak of European foul brood (*M. plutonius*) destroyed one third of the remaining breeding material of *A. m. mellifera* from the NBA's breeding program,

Figure 5.3: Pure breeding area in Vest-Agder & Rogaland counties, Norway, marked in orange.

as well as most of the standing population of *A. m. mellifera* in the counties of Aust-Agder and Vest-Agder. The NBA is considering several measures to increase the breeding population, among them import of material from other countries.

In 2012 the total budget for running the whole national breeding program, including *A. m. mellifera*, *A. m. carnica* and limited Buckfast bee breeding, was 165 000 €. The NBA is funding the *A. m. mellifera* conservation efforts in the protected area with 7000-8000 €/year. Beekeepers in Norway, who have more than 24 colonies, can apply for a 50 € subsidy/colony. During the fall 2013 a Norwegian organisation for the protection of *A. m. mellifera* has been established under the umbrella of the NBA.

5.2.2 Denmark

The small, Danish population of *A. m. mellifera* bees consists of approximately 200 colonies. It is situated on Læsø island (Figure 5.4). There is a protection area for *A. m. mellifera* bees on the island and pure mating is ensured by physical distance to other subspecies. None the less, a recent study based on analyses of 1183 SNPs from 10 colonies from Læsø island showed an introgression level of about 14% for these Danish colonies [Pinto et al., 2014].

A few colonies are also kept on Bornholm Island and some colonies are on Christiansø, Skarø, Agger Tange and at Flakkebjerg research station, Aarhus University. In 2013 a new island, Endelave, has joined the conservation efforts, but the number of colonies there is very small and colonies at Endelave and Læsø are not likely to be genetically differentiated.

The Læsø population has a status of a protected population. It is managed by private beekeepers that are responsible for managing and breeding the bee colonies. The queen rearing is pedigree based and the population is continuously monitored by DNA analyses performed at Aarhus University. Special attention is given to a current chalk brood outbreak on Læsø, mainly affecting the *A. m. mellifera* bees.

Conservation of *A. m. mellifera* on Læsø is facing serious problems. Small population size and total isolation has led to increased rates of inbreeding and genetic drift. Genetic drift and inbreeding have been observed in the Læsø bee population both on the phenotypic (inbreeding depression) and genetic (loss of rare alleles) level [Kryger, 2012]. There is an urgent need to

Figure 5.4: Conservation on Læsø island also includes the conservation of the habitat: The heather on Læsø is invaded by birch and pine trees, thus reducing the available forage for the bees [Photo: Per Kryger].

increase the population size of this endangered population.

5.2.3 Sweden

NordBi association, with 400 members, is a well organised society for *A. m. mellifera* beekeepers in Sweden. Around 2000 colonies are considered to be *A. m. mellifera* colonies. Half of them can be used as a gene reserve according to cubital and discoidal index measurements. Special software (CBeeWing `http://www.cybis.se/cbeewing/indexs.htm`) is used for the wing index analysis. Performance evaluations include swarming tendency, temperament, comb behaviour, honey yield and sensitivity to chalk brood. Inspections are done three times every season and the traits are evaluated on a scale from 1 to 5.

Around 100 colonies are used for breeding queens, which are used to establish the next generation. This means that less than 10% of the population is actively managed. Besides this, queen rearing from swarm cells is common and wider variation is possible in practical beekeeping of *A. m. mellifera* bees.

For Swedish *A. m. mellifera* beekeepers genetic diversity is the most important prerequisite for protection and development of *A. m. mellifera* lines. For these purposes NordBi started using isolated mating areas where several local populations can be kept in the same mating area, to allow for gene exchange between subpopulations. Insemination and isolated mating stations are used to select and purify specific *A. m. mellifera* lines.

The ectoparasite *V. destructor* has infected practically all European colonies, except those found in the Åland Islands in Finland and the northern part of Sweden and Norway. There are still thousands of colonies without the Varroa mite infection in both Sweden and Norway. This restricts bee material transport from Varroa positive areas in the south to Varroa free areas in the north.

A common management method is to split the colony when the first swarm cells appear. In many places, the new queens are reared from swarm cells and it can be expected that this kind of management keeps the swarming tendency high. It has been noted that swarming behaviour is strongly fixed and cannot be easily stopped once it has begun.

NordBi's members have carried out a large range of conservation measures including collecting original gene material, arranging pure mating places and running a mating station with queen rearing on Lurö in the lake Vänern. The work is partly financed by the Swedish Ministry of Agriculture.

5.2.4 Finland

Apis mellifera mellifera was kept in many places in Finland until the 1970s. *Apis mellifera mellifera* was commonly crossed with *A. m. ligustica* bees and many crossbred colonies existed at the end of 20th century. The crossed *A. m. mellifera* bees were particularly aggressive and this resulted in an unfavourable reputation. At the end of 1990s more than 90% of the 40 000 colonies found in Finland were *A. m. ligustica* and there was only one place in Muhos known to have *A. m. mellifera* bees. This Väinö Mäki's population was taken to the apiaries of three private queen breeders and Finnish Beekeepers' breeding station at the end of the millennium. Later the station was closed and now only one commercial *A. m. mellifera* beekeeper is breeding *A. m. mellifera* queens in South-West island Rymättylä (Figure 5.5).

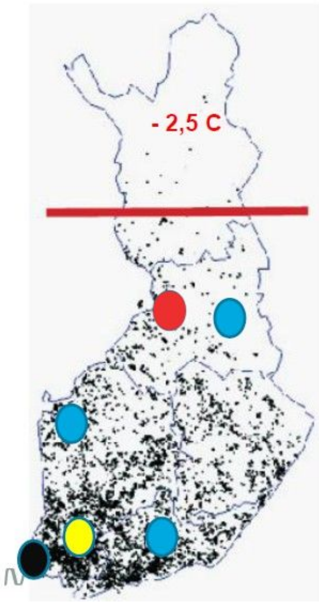


Figure 5.5: The history of *A. m. mellifera* bees in Finland. The small black spots show bee yards of all *A. mellifera* subspecies present in 2003. The red line is the polar circle N 66°33'. The most northern *A. m. mellifera* bee colonies are permanently kept in Lapland where the annual average temperature is -2.5 C°. The light blue circles indicate *A. m. mellifera* populations present in 1980. The red circle indicates Väinö Mäki's population and the yellow circle indicates the conservation site (including the Finnish Beekeepers' breeding station) to which some of the bees from Väinö Mäki's population were brought at the end of the 1990s. The black circle represents the Rymättylä commercial queen breeding operation.

There is no systematic analysis of purity or properties of *A. m. mellifera* bees in Finland, nor is any financial support offered to conserve these populations. Some genetic material has been imported from Sweden to Finland during the last ten years and there has been some export of queens to Latvia.

5.2.5 Latvia

In 1971 only two colonies of *A. m. mellifera* were found during a research expedition in Latvia. During the next 20 years around 60 colonies were kept in Teiču National Park Conservation Area. The state beekeeping institute was reorganized in 1991 and the colonies were moved from the conservation area to a private company. Artificial insemination was also started by a private company. Since 2000 *A. m. mellifera* conservation work has been organized by Latvia University of Agriculture. The Latvian Beekeepers' Association has been involved in conservation work since 2004 and the association's volunteers and advisers help Latvia University of Agriculture in practical beekeeping work.

In 2006 and 2007, there was an American foul brood (*P. larvae*) breakout in the apiary at Latvia University of Agriculture. Many of the valuable colonies were lost. In 2008, queens were imported from Finnish breeders to compensate for these losses, and insemination was started again. Many breeding queens were lost due to swarming in 2009, followed by heavy winter losses the next winter, but queens were still inseminated in 2010.

New queens of the "Olecko – Augustowska un Polnocka" line were imported from Poland to compensate for the loss during the disease outbreak and overwintering losses. The imported population was excluded from breeding as morphometric measurements revealed that it poorly represented *A. m. mellifera* bees. Work with existing queens continued with artificial insemination. Some colonies showed to be up to 98% *A. m. mellifera* based on wing index measurements. In 2011 queens with good morphometric purity indexes were imported from a Swedish NordBi breeder.

In 2012, colonies were moved to the conservation area in Teiču National Park and they overwintered very well. The apiaries at Latvia University of Agriculture are now located in four different places and they are managed by the Latvian Beekeepers' Association. In 2011, the Ministry of Agriculture supported the conservation work, comprising 60 colonies, with 8000 €.

6. Challenges for conservation

Problems associated with the conservation and potential solutions to conserve *A. m. mellifera* in the Nordic and Baltic countries are listed in the following sections and stakeholder tasks and possible funding sources are summarized in Table 6.1.

6.1 Lack of a comprehensive database

There is no common database for *A. m. mellifera* beekeepers, genetic material and colonies in the Nordic and Baltic countries. This project constituted an attempt to perform a regional assessment of the distribution and abundance of *A. m. mellifera* in the region. A comprehensive database of beekeepers contributing to the conservation work of *A. m. mellifera* would be a very useful tool for conservation work. Information through a web-based database could be easily shared with all beekeepers who are interested in *A. m. mellifera* bees. The administration rights could be given to national key stakeholders. Basic pedigrees, recommendations regarding exchange of genetic material and links to `http://www.beebreed.eu` could easily be added to the database content. Agreeing on the rules and providing training needed to use such a database could be one of the items for an international Nordic and Baltic training school or a symposium. This symposium could be hosted by some of the existing organisations like NordBi, NordGen, Nordic Baltic Bee Research Symposium or national beekeeping organisations or research units.

6.2 Lack of comparative studies of the Nordic Brown bee

There is not enough knowledge about the genetic origin and genetic differentiation of *A. m. mellifera* populations, morphometric properties and phenotypic performance of the local populations in the Nordic and Baltic countries. Despite this, the Nordic material has been used as a standard in international comparative studies of the purity of *A. m. mellifera* populations in other countries. The most powerful tool is to use a combination of all three assessment methods, namely genetic analyses, morphometric studies and performance evaluations. Only the combined information enables successful and sustainable conservation work. The Nordic local populations should be scanned as soon as possible to strengthen the status as an international standard for pure *A. m. mellifera*. This endeavour could be an excellent task for a co-operative Nordic-Baltic research network. It is also possible that more valuable *A. m. mellifera* populations for conservation work might be found, especially in Norway and Sweden.

Table 6.1: Summary of stakeholder tasks and possible funding sources for the establishment of an *A. m. mellifera* bee conservation network in the Nordic and Baltic countries.

	Beekeepers	**Queen breeders**	**Supporting organisations**
Roles, tasks and tools	New *A. m. mellifera* beekeepers Learning successful beekeeping techniques Primary evaluation of the bee material Sampling the bee material	Evaluation of bee material Establishing isolated pairing stations Learning the evaluation techniques	Advisers and teachers: training new *A. m. mellifera* beekeepers Network administration and project management Producing research data
Funding sources	Course fees Using public education resources	Profitable business Need financial support for conservation work	Membership fees Directing public money to *A. m. mellifera* Responsibility for information work Scientific projects

6.3 Need for additional isolated mating areas and larger populations

Conservation of *A. m. mellifera* populations is challenged by genetic threats associated with populations of small size, namely inbreeding and genetic drift. In Denmark, the Læsø population shows signs of inbreeding depression [Kryger, 2012]. Some of the Norwegian and Swedish populations are also threatened by inbreeding, because of the small number of queen producers. The queen breeders tend to select their breeding material from a very narrow genetic origin, representing only few colonies. In some cases it is prohibited to import any kind of new genetic material into the conservation areas. This can reduce the number of alleles and lead to diminished vitality in a few generations. The bee colony density is low in the Nordic and Baltic countries. It is fairly easy to find suitable isolated mating areas for *A. m. mellifera* bees. New mating stations for conservation purposes could be established in collaboration with national parks and other nature reserves. A sustainable gene exchange program should be planned between the conservation areas in order to minimize inbreeding and genetic drift. How to arrange this could be one of the topics in courses held for *A. m. mellifera* beekeepers in the Nordic and Baltic countries.

6.4 Lack of beekeepers, correct information & management guidelines

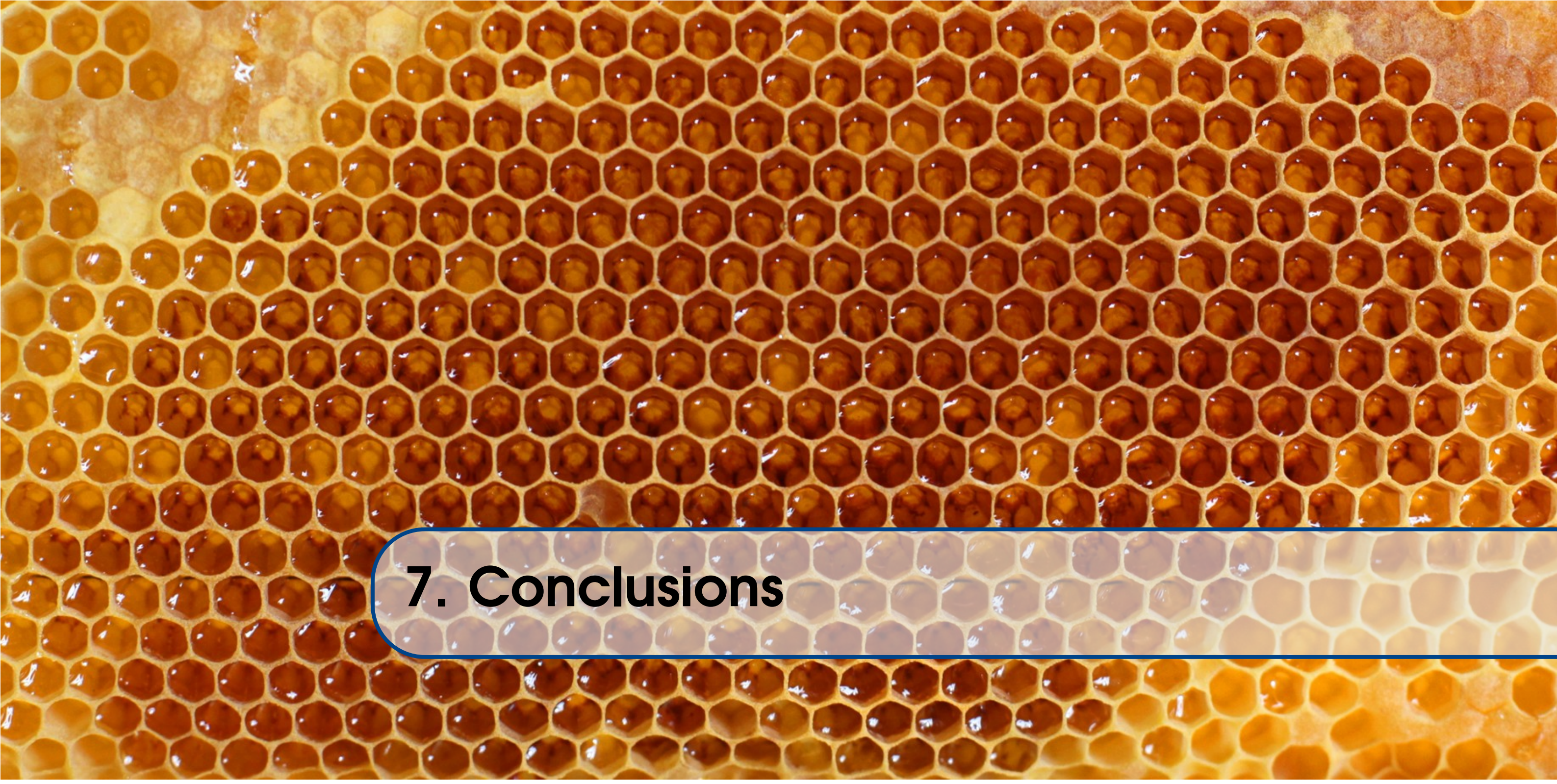
The number of *A. m. mellifera* beekeepers and colonies is small and has been declining during the last decades. Although some *A. m. mellifera* beekeepers state that there are no big differences in beekeeping techniques used for *A. m. mellifera* and other subspecies, the interviews revealed that this might not be true. Procedures used for handling of the bees, timing of the management work, controlling swarming, finding the queen, rearing new queens, and maintaining purity differ between *A. m. mellifera* and other bee subspecies.

The reputation of the *A. m. mellifera* bees among beekeepers is poor and partly based on insufficient knowledge. Opinions are mainly based on the “old bad reputation” of *A. m. mellifera* as being aggressive, fast swarming and a non-productive crossbred black bee. The intrinsic value of the conservation work should be stressed in addition to the good productive properties that *A. m. mellifera* bees may exhibit when managed properly.

It is possible to increase the number of colonies, reduce genetic drift and inbreeding, improve skills of beekeepers and encourage interaction of *A. m. mellifera* beekeepers in Nordic and Baltic countries for example by planning and running Nordic-Baltic *A. m. mellifera* training courses. The basis of beekeeping is similar in these countries and therefore a Nordic-Baltic training network would be beneficial for all parties.

The aims of the training courses could be:

- to teach the basic and special management of *A. m. mellifera* bees, especially for beginners
- to educate towards improved production and delivery of the *A. m. mellifera* material for *in situ* conservation purposes
- to encourage the existing organisations to be committed to the conservation of *A. m. mellifera*
- to support the research activities connected to conservation work
- to increase the number of active queen breeders



7. Conclusions

There are several original *A. m. mellifera* populations left in the Nordic and Baltic countries. Future conservation and sustainable use of *A. m. mellifera* calls for comprehensive phenotypic and genetic characterisation, and if possible, performance testing and selective breeding for genetic improvement. Additionally, more emphasis should be put on the development and re-adoption of management techniques suitable for *A. m. mellifera*, especially those concerning queen rearing. Efficient *in situ* conservation work should combine research activities, education and practical beekeeping.

The *in situ* conservation work of *A. m. mellifera* in Nordic and Baltic countries has been done by public organizations and private people. Enhancement of conservation and expansion of the existing populations should include international cooperation, first and foremost coordinated exchange of genetic material. Financing of the conservation efforts ought to be diversified to include funding from national and/or international research grants, governmental agencies and private businesses. However the most essential component is good coordination of the national and international resources, and cooperation between actors. Based on the results of this project, we propose establishment of an open Nordic-Baltic network for *in situ* conservation of *A. m. mellifera*.

Acknowledgements

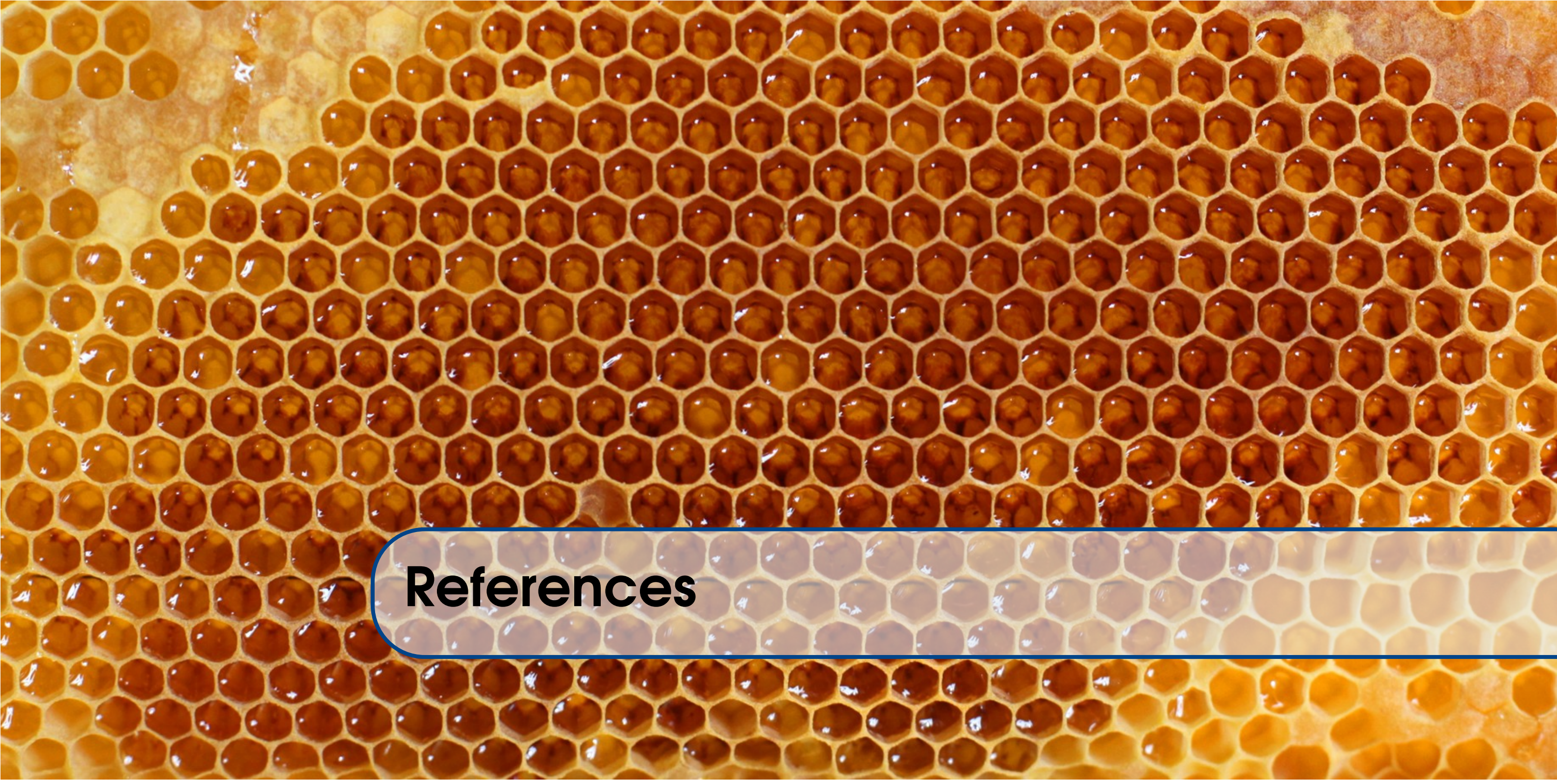
The authors would like to thank Ingvar Arvidsson, Per Thunman (vice president of the executive comittee of SICAMM), Ingvar Pettersson, CalleRegnell, and Inger Bengtsson from NordBi, Sweden, for the *in situ* demonstrations of Swedish *A. m. mellifera* conservation actions. Lassi Kauko, a member of the SICAMM committee, from Köyliö, Finland, is thanked for valuable materials and comments on the manuscript and *A. m. mellifera* beekeeper Aimo Nurminen from Rymättylä, Finland, for the demonstration of the Finnish *in situ* conservation work.

We would like to express our sincere thanks to the *ad hoc* group members, who gave detailed information on the national status and conservation actions of *A. mellifera* in each country:

- Bjørn Dahle, Senior Adviser, Norwegian Beekeepers' Association, Norway
- Per Ideström, Chairman of Association and Project NordBi, Sweden
- Armands Krauze, Chairman of Latvian Beekeepers' Association, Latvia
- Per Kryger, Senior Researcher, Aarhus University, Denmark
- Torbjörn Andersen, Iceland

We thank Veikko Hyttinen and Per Kryger for providing photos used in this report.

This work was funded by the Nordic Genetic Resource Center.



References

Bailey, L.
1958. The epidemiology of the infestation of the honeybee, *Apis mellifera* L., by the mite *Acarapis woodi* Rennie and the mortality of infested bees. *Parasitology*, 48:493–506.

Bouga, M., C. Alaux, M. Bienkowska, R. Büchler, N. L. Carreck, E. Cauia, R. Chlebo, B. Dahle, R. Dall'Olio, P. De la Rúa, A. Gregorc, E. Ivanova, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, P. Kozmus, P. Kryger, Y. Le Conte, M. Lodesani, A. M. Murilhas, A. Siceanu, G. Soland, A. Uzunov, and J. Wilde
2011. A review of methods for discrimination of honey bee populations as applied to European beekeeping. *Journal of Apicultural Research*, 50:51–84.

Carreck, N.
2008. Are honey bees (*Apis mellifera* L.) native to the British Isles? *Journal of Apicultural Research*, 47:318–322.

Chávez-Galarza, J., D. Henriques, J. S. Johnston, J. a. C. Azevedo, J. C. Patton, I. Muñoz, P. De la Rúa, and M. A. Pinto
2013. Signatures of selection in the Iberian honey bee (*Apis mellifera iberiensis*) revealed by a genome scan analysis of single nucleotide polymorphisms. *Molecular Ecology*, 22:5890–5907.

Costa, C., R. Büchler, S. Berg, M. Bienkowska, M. Bouga, D. Bubalo, L. Charistos, Y. Le Conte, M. Drazic, W. Dyrba, J. Fillipi, F. Hatjina, E. Ivanova, N. Kezic, H. Kiprijanovska, M. Kokinis, S. Korpela, P. Kryger, M. Lodesani, M. Meixner, B. Panasiuk, H. Pechhacker, P. Petrov, E. Oliveri, L. Ruottinen, A. Uzunov, G. Vaccari, and J. Wilde
2012a. A Europe-Wide Experiment for Assessing the Impact of Genotype-Environment Interactions on the Vitality and Performance of Honey Bee Colonies: Experimental Design and Trait Evaluation. *Journal of Apicultural Science*, 56:147–158.

Costa, C., M. Lodesani, and K. Bienefeld
2012b. Differences in colony phenotypes across different origins and locations: evidence for genotype by environment interactions in the Italian honeybee (*Apis mellifera ligustica*)? *Apidologie*, 43:634–642.

Crane, E.
1999. *The world history of beekeeping and honey hunting*. Taylor and Francis Group, New York, N.Y.

De la Rúa, P., R. Jaffé, R. Dall'Olio, I. Muñoz, and J. Serrano
2009. Biodiversity, conservation and current threats to European honeybees. *Apidologie*, 40:263–284.

Dietemann, V., J. D. Ellis, and P. Neumann
2013. *The COLOSS BEEBOOK Volume I, Standard methods for* Apis mellifera *research: Introduction*. International Bee Research Association, Cardiff, Wales.

Elsik, C. G., K. C. Worley, A. K. Bennett, M. Beye, F. Camara, C. P. Childers, D. C. de Graaf, G. Debyser, J. Deng, B. Devreese, E. Elhaik, J. D. Evans, L. J. Foster, D. Graur, R. Guigo, K. J. Hoff, M. E. Holder, M. E. Hudson, G. J. Hunt, H. Jiang, V. Joshi, R. S. Khetani, P. Kosarev, C. L. Kovar, J. Ma, R. Maleszka, R. F. a. Moritz, M. C. Munoz-Torres, T. D. Murphy, D. M. Muzny, I. F. Newsham, J. T. Reese, H. M. Robertson, G. E. Robinson, O. Rueppell, V. Solovyev, M. Stanke, E. Stolle, J. M. Tsuruda, M. V. Vaerenbergh, R. M. Waterhouse, D. B. Weaver, C. W. Whitfield, Y. Wu, E. M. Zdobnov, L. Zhang, D. Zhu, and R. a. Gibbs
2014. Finding the missing honey bee genes: lessons learned from a genome upgrade. *BMC Genomics*, 15:86.

FAO
2007. *Global plan of action for animal genetic resources and the Interlaken declaration*. Commission on Genetic Resources for Food and Agriculture, FAO, Rome, Italy.

Frankham, R., D. A. Briscoe, and J. D. Ballou
2002. *Introduction to Conservation Genetics*. Cambridge University Press.

Garnery, L., P. Franck, E. Baudry, D. Vautrin, J. M. Cornuet, and M. Solignac
1998. Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). II. Microsatellite loci. *Genetics Selection Evolution*, 30:S49–S74.

Gempe, T. and M. Beye
2009. Sex determination in honeybees. *Nature Education*, 2:1.

Gempe, T., M. Hasselmann, M. Schiøtt, G. Hause, M. Otte, and M. Beye
2009. Sex determination in honeybees: Two separate mechanisms induce and maintain the female pathway. *PLOS Biology*, 7:e1000222.

Harpur, B. a., C. F. Kent, D. Molodtsova, J. M. D. Lebon, A. S. Alqarni, A. a. Owayss, and A. Zayed
2014. Population genomics of the honey bee reveals strong signatures of positive selection on worker traits. *Proceedings of the National Academy of Sciences of the United States of America*, 111:2614–9.

Hoff, M.
2009. Male or female? for honeybees, a single gene makes all the difference. *PLOS Biology*, 7:e1000186.

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

Huotari, K.
2007. *Mehiläishoidon kotiutuminen Suomeen ja vaiheet järjestäytymiseen asti itsenäisyyden ajan alulla [History of the introduction of apiculture to Finland, and its adaptation to suit Finnish conditions, in the early years of Independence].* Jyväskylä University Printing House and Sisäsuomi Oy, Jyväskylä, Finland.

Hyink, O., F. Laas, and P. K. Dearden
2013. Genetic tests for alleles of complementary-sex-determiner to support honeybee breeding programmes. *Apidologie*, 44:306–313.

Hyttinen, V.
2012. Mustan mehiläisen alkulähteillä [Fountainhead of the black bee]. *Maatiainen*, 2:10–12.

Hämäläinen, E., S. Korpela, and K. Långfor
1978. *Mehiläishoitajan käsikirja, [Hand book of beekeeping].* Kustannusosakeyhtiö Otava, Helsinki, Finland.

Jensen, A. B., K. a. Palmer, J. J. Boomsma, and B. V. Pedersen
2005. Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. *Molecular Ecology*, 14:93–106.

Kristensen, T. N. and A. C. Sørensen
2005. Inbreeding – lessons from animal breeding, evolutionary biology and conservation genetics. *Animal Science*, 80:121–133.

Kryger, P.
2012. New threats for honeybee conservation on the island of Læsø. In *Proceedings of the 5th European Conference of Apidology, Halle an der Saale, Germany*, number 5 in EurBee, P. 134.

Manner, O.
1925. *Luonnonmukainen mehiläishoito [Natural Beekeeping].* Kustannusosakeyhtiö Otava, Helsinki, Finland.

Meixner, M., M. Worobik, J. Wilde, S. Fuchs, and N. Koeniger
2007. *Apis mellifera mellifera* in eastern Europe—morphometric variation and determination of its range limits. *Apidologie*, 38:191–197.

Meixner, M. D., M. A. Pinto, M. Bouga, P. Kryger, E. Ivanova, and S. Fuchs
2013. Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. *Journal of Apicultural Research*, 52:1–28.

Oleksa, A., I. Chybicki, A. Tofilski, and J. Burczyk
2011. Nuclear and mitochondrial patterns of introgression into native dark bees (*Apis mellifera mellifera*) in Poland. *Journal of Apicultural Research*, 50:116–129.

Pinto, M. A., D. Henriques, J. Chávez-Galarza, P. Kryger, L. Garnery, R. van der Zee, B. r. Dahle, G. Soland-Reckeweg, P. de la Rúa, R. Dall' Olio, N. L. Carreck, and J. S. Johnston
2014. Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: a genome-wide assessment using SNPs and mtDNA sequence data. *Journal of Apicultural Research*, 53:269–278.

Ruottinen, L., ed.
2005. *Mehiläishoitoa käytännössä osa 2. [Beekeeping in practice].* AO-Paino, Mikkeli, Finland.

Ruttner, F.
1988. *Biogeography and Taxonomy of Honeybees*. Springer-Verlag, Berlin, Germany.

Seeley, T.
1985. *Honeybee Ecology: A Study of Adaptation in Social Life.* Princeton University Press, Princeton, New Jersey.

Soland-Reckeweg, G.
2006. *Genetic differentiation and hybridization in the honeybee (*Apis mellifera *L.) in Switzerland*. PhD thesis, University of Bern.

Soland-Reckeweg, G., G. Heckel, P. Neumann, P. Fluri, and L. Excoffier
2009. Gene flow in admixed populations and implications for the conservation of the Western honeybee, *Apis mellifera*. *Journal of Insect Conservation*, 13:317–328.

Spötter, A., P. Gupta, G. Nürnberg, N. Reinsch, and K. Bienefeld
2012. Development of a 44K SNP assay focussing on the analysis of a varroa-specific defence behaviour in honey bees (*Apis mellifera carnica*). *Molecular Ecology Resources*, 12:323–332.

Strange, J. P., L. Garnery, and W. S. Sheppard
2007. Persistence of the Landes ecotype of *Apis mellifera mellifera* in southwest France: confirmation of a locally adaptive annual brood cycle trait. *Apidologie*, 38:259–267.

Uzunov, A., C. Costa, B. Panasiuk, M. Meixner, P. Kryger, F. Hatjina, M. Bouga, S. Andonov, M. Bienkowska, Y. Le Conte, J. Wilde, D. Gerula, H. Kiprijanovska, J. Filipi, P. Petrov, L. Ruottinen, H. Pechhacker, S. Berg, W. Dyrba, E. Ivanova, and R. Büchler
2014. Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *Journal of Apicultural Research*, 53:248–260.

van Engelsdorp, D. and M. D. Meixner
2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology*, 103:S80–S95.

Whitfield, C. W., S. K. Behura, S. H. Berlocher, A. G. Clark, J. S. Johnston, W. S. Sheppard, D. R. Smith, A. V. Suarez, D. Weaver, and N. D. Tsutsui
2006. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science*, 314:642–645.

Zayed, A.
2009. Bee genetics and conservation. *Apidologie*, 40:237–262.