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REVIEW ARTICLE

Conserving diversity and vitality for honey bee breeding

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Summary

Beekeepers in Europe, North America and other parts of the world have repeatedly been afflicted by elevated and sometimes unexplained colony losses. Multiple factors have been considered in connection with increased winter losses. In addition to national programmes investigating possible causes for increased honey bee mortality, scientists collaborate at an international level on different aspects of bee health within the COLOSS network. Within this network, Working Group 4 explores aspects of genetic diversity in relation to the vitality and health of honey bee populations. In this paper, we briefly review the genetic diversity of honey bees in Europe, discuss the effects of beekeeping and selective breeding on honey bee populations under the aspect of genetic diversity and bee health, and review the current status of EU legislation with respect to protection of native bee populations. We introduce and discuss recent approaches in honey bee selective breeding to improve disease resistance by introducing traits related to colony vitality. Finally, we present the aims of WG4 within the COLOSS network and briefly introduce our experimental approach.

El rol de la vitalidad y la diversidad genética en la pérdida de colonias.

Resumen

Recientemente, apicultores de Europa, Norteamérica y otras partes del mundo han sido afectados por la pérdida alarmante de colonias a una magnitud sin precedente. Múltiples factores pueden estar involucrados en este fenómeno. Además de los proyectos de investigación a nivel nacional, ha sido establecida la red científica internacional COLOSS para identificar los factores que a nivel individual (de abeja) y de colonia provocan la severa pérdida de las colonias e investigar efectos sinérgicos entre ellas. Dentro de la red COLOSS, el WG 4 se enfoca a cuestiones de vitalidad y diversidad de la abeja en relación con la pérdida de colonias. Este artículo de revisión presenta una descripción sobre el estado actual de la diversidad genética de la abeja melífera en Europa, los efectos de crianza en las poblaciones, el marco jurídico de la protección de las subespecies, y el concepto de vitalidad en protocolos de crianza. También se dan a conocer las metas del grupo de trabajo "diversidad y vitalidad" con la red COLOSS.

Keywords: honey bees, *Apis mellifera*, genetic diversity, breeding, environment, disease resistance, vitality

Introduction

Although honey bees (*Apis mellifera* L.), in contrast to other livestock, are only semi-domesticated, their populations in Europe have nonetheless been seriously affected by human activities. Between 1970 and 2007, the number of honey bee colonies in Europe gradually decreased from over 21 million to about 15.5 million (FAO, 2009; Aizen and Harder, 2009). In addition, beekeepers in Europe and also in North America have repeatedly been afflicted by elevated and sometimes unexplained winter losses (Higes *et al.*, 2006; Oldroyd, 2007; vanEngelsdorp and Meixner, 2009). A multitude of factors that may contribute to increased winter losses have been discussed comprehensively in the recent literature. Most prominently among them were: diseases and parasites, including the mite *Varroa destructor*, virus diseases, and the microsporidian *Nosema* spp. (Higes *et al.*, 2006; Cox-Foster *et al.*, 2007; Rosenkranz *et al.*, 2009; de Miranda and Genersch 2009; de Miranda *et al.*, 2009; Johnson *et al.*, 2009); the extended use of pesticides in agricultural systems including the use of genetically modified organisms (Desneux *et al.*, 2007; Nguyen *et al.*, 2009; Frazier *et al.*, 2009; vanEngelsdorp *et al.*, 2009; Chauzat *et al.*, 2009; vanEngelsdorp and Meixner, 2009); but also socio-political aspects such as trade or economics that may have contributed to colony decline (vanEngelsdorp and Meixner, 2009). It is unlikely that one single factor will account for all losses, but instead several factors may occur simultaneously, or influence each other.

Several national programmes, such as the German Honey Bee Monitoring (http://www.ag.bienenforschung.de) or the US research team on CCD have been initiated to collect data on honey bee mortality, comprehensively analyze representative bee samples for diseases and abnormalities, and result in an assessment of potential risk factors for colony overwintering. On an international level, the European Union is currently funding a network of scientists who collaborate on different aspects of bee health: COLOSS (prevention of COlony LOSSes), COST (European Cooperation in Science and Technology) Action FA0803. Within this network, Working Groups have been formed to cover different aspects of bee health: 1. monitoring and diagnosis; 2. pests and pathogens; 3. environment and beekeeping; 4. diversity and vitality.

The health of honey bee colonies cannot be understood without taking account of the genetic variability of bee populations, and their adaptation to regional environmental factors such as climate and vegetation, prevailing diseases, and other aspects of regional importance. In this paper, we present a brief overview on the genetic diversity of honey bees in Europe, discuss the effects of beekeeping and selective breeding on honey bee populations with regard to genetic diversity and bee health, and review the current status of EU legislation with respect to the protection of native bee populations. We also introduce and discuss recent modifications of existing approaches to honey bee selective breeding to improve disease resistance by introducing traits related to colony vitality. Finally, we present the aims of WG4 within the COLOSS network and briefly introduce our experimental approach.

Overview of genetic diversity of honey bees in Europe

In its native range of Africa, Europe, Western and Central Asia, the honey bee, *Apis mellifera* L., has adapted to a wide variety of ecosystems. About 26 subspecies and numerous ecotypes have been described, based upon behaviour, morphology, and molecular evidence (Ruttner 1988; Sheppard *et al.* 1997; Sheppard and Meixner, 2003). Europe possesses a wide range of climatic and vegetation zones and, after the last Ice Age, was populated by honey bees from South European and Mediterranean refugia (Ruttner, 1988; Garnery *et al.*, 1998a; b; Franck *et al.*, 1998; 2000; Meixner *et al.*, 2007). Europe is thus home to 10 of the 26 currently existing subspecies, including two of the most frequently used subspecies in commercial beekeeping worldwide. Many European subspecies have been subjected to sometimes intense pressures resulting from human activities, such as breeding strategies and intensive land use. The biodiversity of the honey bees of Europe and the conservation status of the European subspecies have been recently reviewed in detail by De la Rúa *et al.* (2009). In the following paragraphs, we only give a very brief overview.

The original range of *A. mellifera mellifera* extends from the Alps and Carpathians to the latitude of 60°N, from the Atlantic seashore of western Europe eastward to the Urals and beyond. Today the distribution of this subspecies is much reduced: in many Central and Northern European countries (such as Germany, Austria, Denmark and Sweden) beekeepers have almost completely substituted the native honey bee population with subspecies of greater commercial interest, but extensive populations still exist in Britain, France, Switzerland and Poland (Garnery *et al.*, 1998a; b; Jensen *et al.*, 2005; Strange *et al.*, 2007; Meixner *et al.*, 2007; Carreck, 2008; Soland-Reckeweg *et al.*, 2009).

The Iberian honey bee *A. m. iberiensis* is present in its native area of origin: Spain, Portugal and the Balearic Islands (Radloff *et al.*, 2001; De la Rúa *et al.*, 2002; 2003; 2004; 2005), where it is still fairly widespread, although introductions of other subspecies do occur. The natural area of distribution of the Italian honey bee *A. m. ligustica* is the Apennine Peninsula, confined by the Alps and the Mediterranean Sea. Its hybrid origin from different refugial populations has been demonstrated using molecular markers (Franck *et al.*, 2000). The Italian bee is very popular for commercial beekeeping and is currently present wherever professional beekeeping is practiced worldwide.

The "Carniolan bee" *A. m. carnica* originates from Central-Eastern Europe (the area covering Austria, Slovenia, Croatia, Bosnia-Herzegovina, Albania, Serbia, Hungary and Romania) (Ruttner, 1988; Sušnik *et al.*, 2004; Muňoz *et al.*, in press), but due to its commercially attractive traits such as high honey production and gentleness, is now distributed almost worldwide.

A. m. macedonica extends across eastern Europe from the Ukraine and Bulgaria to Greece (Meixner *et al.*, 2007). According to recent studies, at least in the Former Yugoslavian Republic of Macedonia a fairly authentic population exists (Uzunov *et al.*, 2009). The existence of a separate subspecies, indigenous to Bulgaria and named "*A. m. rodopica*" has, however, been hypothesized (Petrov, 1995).

To the south, *A. m. cecropia* is distributed all over southern Greece, including the Peloponnese and the islands (Ruttner, 1988), where, in spite of importations, some areas still possess populations of their native subspecies (Bouga *et al.*, 2005a; b).

Geographical isolation of Mediterranean islands, which are characterized by mild, rainy winters and hot, dry summers, has promoted the differentiation of locally adapted subspecies: *A. m. siciliana* in Sicily (Sinacori *et al.*, 1998); *A. m. ruttneri* in Malta (Sheppard *et al.*, 1997); *A. m. cypria* in Cyprus (Ruttner, 1988; Kandemir *et al.*, 2006) and *A. m. adami* in Crete (Ruttner, 1980). The conservation status of these bees must be regarded as endangered; in particular, the range of *A.m. siciliana* has been reduced to a few islands around the main island of Sicily (Dall'Olio *et al.*, 2008).

Effects of beekeeping and breeding on honey bee populations

In contrast to other regions of its distribution range, virtually the entire honey bee population of Europe today consists of colonies managed by beekeepers (Jaffé *et al.*, 2009). This fact can probably be attributed to factors such as the destruction of natural habitats and the spread of the parasitic mite *Varroa destructor*. The latter in particular can be assumed to have effectively eliminated the wild honey bee population of Europe (Moritz *et al.*, 2007; De la Rúa *et al.*, 2009).

Thus, although the protection and proliferation of the species *A. mellifera* and its subspecies in Europe today entirely depends on beekeeping activities, one consequence of these activities has been to favour the distribution of the commercially most interesting subspecies *A. m. carnica*, *A. m. ligustica* and *A. m. caucasica* through the importation of queens, usually to the disadvantage of the respective native subspecies or ecotypes. Several examples of beekeeper-mediated introgression of foreign subspecies in Europe are known. Apart from the deliberate substitution of *A. m. mellifera* by *A. m. carnica* in Germany and surrounding countries (Dreher, 1946; Maul and Hähnle, 1994), importation and spread of *A. m. carnica*, *A. m. ligustica* and hybrid strains are known from numerous other regions (Garnery *et al.*, 1998a; b; Ivanova *et al.*, 2007; Jensen *et al.*, 2005; Strange *et al.*, 2008). One consequence of this development is the reduction or loss of genetic diversity, since characteristic traits of the autochthonous subspecies may become endangered or lost by introgression of foreign subspecies. For instance, when tested for genetic diversity parameters, honey bees from managed populations in Europe had lower values in comparison to wild African populations (Moritz *et al.*, 2007; Jaffé *et al.*, 2009).

In addition, the population sizes and densities of European honey bee populations have been reported to be comparatively small, making the task of conserving small endemic populations as potential breeding stock for future generations more difficult, and increasingly urgent (Moritz *et al.*, 2005; Moritz *et al.*, 2007). Interestingly, De la Rúa *et al.* (2009) observed a pronounced density gradient across Europe, with population densities in the east and south of Europe being considerably higher than in the north and west, which they attributed to different beekeeping policies in the respective countries.

Another consequence of professional beekeeping is that some beekeepers have specialized in queen breeding. Most breeders produce and distribute large numbers of progeny from few queen mothers, thereby decreasing genetic variability in honey bee populations. In Europe, coordinated national breeding schemes are in place in many countries, especially those with a tradition in the production of the commercially most interesting races (*A. m. ligustica* and *A.m. carnica*). Over generations, a few dozen beekeepers produce thousands of queens, which are sold locally and also exported across Europe and the world (Lodesani and Costa, 2003). Whilst it is known that in the US as few as 500 breeder queens have been used to provide progeny for most of the commercial hives present in the country (Schiff and Sheppard, 1995; 1996; Delaney *et al.*, 2009), little information is available from Europe. Dall'Olio *et al.* (2007) have, however, discussed the possibility that queen breeding routines currently in use have resulted in amalgamation of populations of different geographic origin within Italy with subsequent loss of genetic variability.

At the colony level, genetic variability has been shown to be important for disease resistance, homeostasis, thermoregulation and overall colony fitness (Tarpy, 2003; Jones *et al.,* 2004; Graham *et al.,* 2006; Mattila and Seeley, 2007). If genetic variability is important for immune response and defence against parasites, breeding approaches based on controlled mating of pure stock while neglecting resistance traits against diseases may leave the selected population with a reduced capacity to ward off stressors. Furthermore, genetic similarity amongst colonies in wide areas may also increase the chances of successful disease transmission, accompanied by an increased risk of colony losses. For beekeepers, lost colonies mean less honey, but the more serious risk lies in the loss of potential breeding stock.

The recently sequenced honey bee genome (Honey Bee Genome Sequencing Consortium, 2006) promises new tools for selection of breeding stock. The usage of molecular markers will allow much faster identification of carriers of desirable traits. When using these tools care should, however, be taken to conserve diversity at other genome regions to maintain vitality of the bees.

Legal means of protection of subspecies

Trade of commercial animals, including bees, within the EU is regulated by Council Directive 91/174/EEC. According to this directive, free trade is the general rule, and the introduction of non-indigenous races should not be obstructed on the basis of zootechnical or genealogical rules that apply locally. According to Council Directive 92/65/EEC, TRACES certificates, providing proof of health, are required to accompany any shipments of bees between EU countries. While some European countries have used sanitary reasons as a means to prevent the introduction of non-native bees, several member states that recently joined the EU (e.g. Slovenia) included the subject of conservation of their autochthonous honey bee in the accession negotiations to obtain a better legal basis of protection. Some European countries have initiated special projects and set up protected mating areas with the aim of protecting and conserving their native bees which face the risk of extinction, including *A. m. mellifera* in Denmark and France, or the Sicilian subspecies *A. m. siciliana* (Lodesani and Costa, 2003; Strange *et al.*, 2008; Dall'Olio *et al.*, 2008).

The history of the Danish island of Laeso as a reserve to conserve native honey bees can be used as an example to illustrate the contrasting issues of free trade and conservation of native subspecies. A ban on keeping introduced bees was issued in 1993, but was immediately fought in court by local beekeepers who refused to comply, and were not willing to give up the use of imported bees, thereby preventing conservation measures for the native bee from taking effect. In 2001, however, the EU Court decided that indeed the issue of conservation can preside over that of free trade. Thus, countries do have the right to set aside areas for the conservation of their local subspecies and are allowed to ban importations and use of imported stock. The Laeso example thus illustrates the important issue of obtaining consensus and support for conservation plans from the local beekeeping community. For any conservation effort to be successful, it appears vital that the beekeepers in a proposed conservation area must agree to the plans and support them fully. The enforcement of rules, *i.e.* controlling the origin of each hive in an area, is hardly possible on a large scale, and without local beekeeper support, conservation plans can quickly end up being just plans.

Only in the field of organic beekeeping does a directive exist, in the form of Reg. CE 834/2007, recommending preference of local ecotypes of honey bees. To improve the legal protection of native honey bees, De la Rúa *et al.* (2009) suggested making this indication compulsory and legally binding.

Introducing vitality as a parameter into honey bee breeding

Local adaptation implies that over generations some colonies will do better than others; those that thrive do so because they are well suited to their environment (and their pathogens). The process of adaptation (in the absence of imports) can happen naturally (natural selection), but it can also be influenced by the beekeeper. The evolution of beekeeping as a cultural practice and as a profession (Crane, 1990) has, however, also resulted in the development and use of techniques that may ultimately reduce the vitality of honey bee colonies.

Selective breeding is based on the concept of comparing the performance of colonies in a number of traits that are considered of major apicultural interest, such as honey production, temperament, swarming tendency and quietness on the comb. Technical recommendations based on these traits were published in 1972 (Ruttner, 1972), and are currently widely accepted as a standard method on a European level.

On the other hand, technical measures to compensate for certain selective pressures resulting from environmental conditions are also regularly applied by beekeepers and breeders. Apart from activities such as artificial feeding or protection of the hives against cold weather, the regular use of medication to control parasites and diseases, in particular *Varroa destructor*, will result in the survival of susceptible colonies which are thus given a chance to participate in the reproductive process. To make things worse, these colonies may be stronger, appear healthier and may be able to rear more and healthier drones than non-treated ones (Schneider and Drescher, 1987; Schneider *et al.*, 1988; Duay *et al.*, 2002). In consequence, their chances to mate successfully may be higher than those of non-treated colonies that have been forced to cope with the pathogens on their own (Bubalo *et al.*, 2005). In contrast, Büchler *et al.* (2006) were able to show that in the absence of any treatment with miticides the mating success of colonies in an isolated mating station depends to a large extent on the infestation level, and thus, on the ability of the colonies to cope with the parasite.

The development of natural resistance against parasites has been well characterized in the case of *V. destructor*, where several reports document the survival of colonies in the absence of any treatment (Rinderer *et al.*, 2001; Büchler *et al.*, 2002; 2008; Fries *et al.*, 2007; Le Conte *et al.*, 2007; Seeley, 2007). Resistance of honey bees against parasitic mites has also been demonstrated in selection programmes that focused on behavioural strategies enabling the bees to limit the growth of the mite population (Spivak, 1996; Harbo and Harris, 2000; Spivak and Reuter, 2001; Ibrahim and Spivak., 2006; Harris, 2007).

While criteria like resistance against pathogens and parasites are of little importance in traditional breeding protocols, recent projects increasingly bring the development of traits related to colony vitality into focus (Garrido *et al.*, 2005; Büchler *et al.*, 2007; 2008). While maintaining the traditional traits described by Ruttner (1972) and continuing comparative performance testing for these traits, these projects introduce and evaluate additional traits related to colony vitality, such as hygienic behaviour, mite infestation development, and overwintering ability, thus basing the final selection decision on all traits that were compared. The aim of these projects is to improve the mite resistance of the selected stock without sacrificing traits that are of importance for beekeepers. In addition, the concept of tolerance mating stations has been developed, where drones of the selected population are reared under high infestation pressure in colonies that remain untreated for a long time (Büchler *et al.*, 2009). Since mating success of these colonies will depend on differences in the individual colony's ability to cope with parasite pressure (Büchler *et al.*, 2006), the fitness of drones can thus be integrated in the selection process. The basic idea is to introduce elements of natural selection into the breeding protocols, rather than preventing it from taking place.

Aims and perspectives of the Coloss Working Group "Diversity and Vitality"

The existing subspecies and ecotypes of honey bees in Europe represent an important resource for breeding of disease and stress resistant strains. Anecdotal evidence suggests that locally adapted strains of bees suffer less from elevated losses than non native bees. The ultimate goal of our group is to reduce the currently prevailing dependence on medication and to improve overall bee health by facilitating and propagating comprehensive breeding efforts that include colony vitality in addition to the traditional traits. To achieve this, our proximate objectives are: 1. to develop and test internationally recognized criteria for vitality; 2. to establish standardized methods to assess honey bee colonies based on these criteria, resulting in methodological and technical recommendations for breeders and; 3. to investigate the role of interactions between genetic diversity and environment on honey bee colony vitality.

Our experimental approach to these aims consists of a common experiment to reveal interactions between the genetic origin of the bees and the environment. To this end, a total of 670 colonies from 17 different genetic origins have been set up in 16 locations across Europe, where each location contains the local strain of bees together with two "foreign" origins. The colonies have uniformly been set up in the summer of 2009 and will be managed and evaluated according to a standard protocol that is used by all participants. Chemical treatment against mites is precluded, except when a colony is in immediate danger of collapsing. At that moment it can be treated to prevent mite invasion into neighbouring colonies, but with the exclusion of that colony from the experiment. Apart from parameters such as overwintering and colony buildup, close attention will be paid to vitality parameters, such as mite infestation level, hygienic behaviour and the occurrence of other diseases.

From the results of the experiment, which is designed to run until 2012, we expect insights into the interactions between factors such as genetic origin and local adaptation, and contributions to our understanding of interactions within the bee-mite-environment system. In addition, the participants will have established and evaluated a standardized test methodology to estimate the vitality of bee colonies under varying environmental conditions across Europe.

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