San Michele all'Adige Declaration

Appeal for Biodiversity Protection of Native Honeybee Subspecies of *Apis mellifera* Linnaeus, 1758 in Italy

Fondazione Edmund Mach,
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SUMMARY

This document, drawn up and signed by exponents of authoritative research institutions and by key figures in the beekeeping and environmental fields, wishes to make political administrations aware of the urgency of granting adequate protection to the honeybee[1] (*Apis mellifera* Linnaeus, 1758) and in particular to its indigenous subspecies. Despite being managed by beekeepers for many years, the honeybee cannot be considered as a domestic animal, and plays a key role in biodiversity conservation as a pollinator, with a high impact on agricultural production. The honeybee was originally distributed throughout most of Europe, Africa (including Madagascar), the Middle East, part of the Arabian Peninsula and some parts of Central Asia. From Europe, the honeybee was introduced to America, Asia and Oceania. Like all wild species, due to the evolutionary pathway and biology of this insect, adaptation to the environment is essential for the honeybee. This adaptation to a range of environmental conditions, together with geological and climatic changes in past eras, has resulted in subdivision of the *Apis mellifera* species into 31 subspecies. Due to the wide variety of environments, the Mediterranean area has the greatest intraspecific diversity. In the last 150 years, technological advances in beekeeping have caused a devastating genetic impoverishment, with an impact on honeybee production and pathologies, endangering conservation of the native subspecies of *Apis mellifera* in Europe. Evaluation of the impact of this phenomenon on the ecological equilibrium is still ongoing, while the negative effects that this problem is having on beekeeping are known and evident. This document sets forth the scientific arguments in support of this vision, on the basis of which we can proceed with concrete actions aimed to protect the honeybee, also as a biological entity, according to various operating methods. This document does not intend to oppose the actions of the beekeeping sector, but rather to contribute to a more global vision of the very serious problem of honeybee decline.

[1] In Italy the common name of *Apis mellifera* is "Ape mellifica", deriving from the Latin name subsequently proposed by Linneo, in 1761: *Apis mellifica*. This latter name is not accepted today by the International Commission on Zoological Nomenclature. The second name proposed by Linneo in 1761, Apis *mellifica*, means "honeybee producing honey" and would be more correct, while the first, *Apis mellifera*, literally means "honeybee carrying honey".

INTRODUCTION

The honeybee (*Apis mellifera* Linnaeus, 1758) is a species that has been used by humans for beekeeping for thousands of years. Since prehistoric times, wild colonies have been preyed on for honey, larvae and wax collection[2], as took place in the past and still happens today for all species of the genus *Api*s, but also for other *Apoidea apiformes*[3,4] in tropical areas. At all events, the bio-ethological characteristics of the honeybee allowed the development of beekeeping. There is a wealth of iconographic and documentary evidence regarding this noble human activity, based on archaeological finds dating back to at least 4,500 years ago. For example, among the many decorations found in the Shesepibre Temple in Egypt, built by Nyuserre Ini in around 2,500 BC, there is the oldest representation of a complex and advanced system for management of honeybees and honey, proving beyond doubt the development of beekeeping techniques beginning much earlier. It is indeed very likely that beekeeping of the honeybee developed around 10-12,000 years ago in the Fertile Crescent, during the era seeing the establishment of agriculture and the rearing and domestication of animals. Beekeeping has experienced an extraordinary development and diffusion over thousands of years, leading to a wide range of technical solutions, largely still preserved today in different areas of the Mediterranean basin and the Near East. The honeybee and other species of eusocial *Apoidea* living in complex and permanent societies (like some tropical bees from the genera *Trigona* and *Melipona*) have also inspired a series of symbols, beliefs, and myths, and therefore play an important role in the spiritual, cultural and political evolution of human society at global level.

Despite this very lengthy relationship between honeybees and humans, we can however declare with certainty that this extraordinary animal has never been domesticated.

Indeed, domestication is understood as the process by which an animal or plant species becomes domestic, namely dependent on cohabitation with man and on his control of feeding and reproduction conditions. Pliny the Elder (Gaius Plinius Secundus, 23-79 AD) had already expressed his opinion that honeybees managed by beekeepers had not been domesticated in the first paragraphs of the book dedicated to honeybees in his *Naturalis Historia*[5]. Likewise, in his work entitled "*Variation of Animals and Plants under Domestication*" Charles Robert Darwin (1809-1882) concluded that it was precisely the biological peculiarities of colonies of *Apis mellifera* that prevented this process of

[2] Crane E., 1999. The world history of beekeeping and honey hunting. Routledge Editore: 704 pp.

[3] Michener, C.D., 2000. The Bees of the World. The Johns Hopkins University Press, Baltimore: 913 pp.

[4] For convenience, they will subsequently be described as *Apoidea,* but refer to *Apoidea apiformes,* according to Michener, 2000.

[5] "*...cum sint neque mansueti generis neque feri…*" ovvero "*…pur non appartenendo né agli animali domestici né a quelli selvatici...*". Pliny the Elder, Naturalis Historia, Liber XI – 4.

domestication [6]. Furthermore, Eva Crane (1912-2007), the greatest 20th century expert on apiculture, also provided a clear definition of beekeeping, highlighting the distance from domesticated animals. Indeed, Eva Crane[7] defines beekeeping as "*the maintenance of strong healthy colonies of honeybees in hives designed for the convenience of the operator, and the removal from the hives (and subsequent processing) of the products for which the colonies are kept*" [8]. However, the comparison she proposed between beekeeping and the only other similar human activity to it is even more extraordinary: "*The use of bees as micromanipulators to harvest food from plants has its nearest parallel in the use of cormorants (on a neck-line which prevents swallowing) to catch fish. The beekeeper has an advantage over the fisherman in that the bees convert the nectar into honey, a very high energy food, before he takes his harvest*". Eva Crane refers to the traditional "ukai fishing" with cormorants, practiced in Japan.

It is precisely the wild nature of the honeybee and the fact that it is not a domestic animal that represents the starting point for this document.

Darwin observed that honeybees also behave like wild organisms when they are introduced to areas far from their original area of provenance. Today, when we talk about a wild species and its protection, it is important to establish whether it is an autochthonous or allochthonous organism. The honeybee is native to most of Europe, Africa, the Middle East, most of the Arabian Peninsula and some parts of Central Asia. It has colonised this extensive area, characterised by a variety of climates and vegetation, over thousands of years, diversifying through natural selection into well characterised populations that have been identified as subspecies, distinguishable firstly on a morphological and ethological basis, and more recently through molecular biology studies. In animal and plant biology, the subspecies is a taxonomic category consisting of one or more populations differentiated from others of the same species by a set of hereditary diagnostic characteristics and originated due to the selective action of various factors and geographical isolation. However, since there are no reproductive barriers between subspecies, if they come into contact, populations can crossbreed with each other, resulting in fertile offspring. For this reason, no different subspecies can be observed in the same area in nature [9]. It is important to note that when there is no insurmountable physical barrier between two subspecies, they will remain distinct, but in the contact area we can observe the presence of a more or less defined hybridisation zone. Most of the subspecies of *Apis mellifera* have areas in contact with one or more different subspecies, but there are also native subspecies of islands and therefore

[6] Darwin C. R., 1869. The Variation of Animals and Plants under Domestication. In two volumes. London: John Murray: VIII+411; VIII+486.

[7] Crane E., 1980. Apiculture. In: Perspectives in World Agriculture. *Farnham Royal, UK: Commonwealth Agricultural Bureaux*: 261-294.

[8] Indeed, Eva Crane uses the verb "*to keep" and not* "*to breed*", "*to raise*" or "*to rear*".

[9] O'Brien S. J & Mayr E., 1991. Bureaucratic Mischief: Recognizing Endangered Species and Subspecies. *Science, New Series*, Vol. 251, No. 4998. (Mar. 8, 1991), pp. 1187-1188.

not subject to hybridisation zones. If different subspecies are forced to live together in the same area, due to human activities, they are unavoidably destined to lose their respective unique genetic characteristics (e.g. *A. m. Siciliana* and *A. m. Ligustica*). To date, there are 31 subspecies of *Apis mellifera* officially accepted by the international scientific community[10, 11, 12, 13].

In Europe and the Caucasus region there are 15 subspecies:

A. m. mellifera Linnaeus, 1758 – Central and Northern Europe, up to Russia
A. m. ligustica Spinola, 1806 - Italy
A. m. remipes Gerstäcker, 1862 - Caucasus, Iran, Caspian Sea
A. m. adami Ruttner, 1975 - Crete
A. m. carnica Pollmann, 1879 - Slovenia, Eastern Alps and Northern Balkans
A. m. cypria Pollmann, 1879 - Cyprus
A. m. cecropia Kiesenwetter, 1860 – Southern Greece
A. m. caucasia Pollman, 1889 - Caucasus
A. m. siciliana Dalla Torre, 1896 - Sicily
A. m. taurica Alpatov, 1935 - Crimea
A. m. macedonica Ruttner, 1988 – Northern Greece
A. m. ruttneri Sheppard, Arias, Grech & Meixner, 1997- Malta
A. m. artemisia Engel, 1999 – Russian Steppes
A. m. iberiensis Engel, 1999 – Spain and Portugal
A. m. sossimai Engel, 1999 - Ukraine

In Africa there are a further 11 subspecies:

A. m. adansonii Latreille, 1804 - Nigeria, Burkina Faso
A. m. unicolor Latreille, 1804 - Madagascar
A. m. capensis Eschscholtz, 1822 - South Africa
A. m. scutellata Lepeletier, 1836 – Central and Western Africa
A. m. intermissa Buttel-Reepen, 1906 - Morocco, Libya and Tunisia
A. m. sahariensis Baldensperger, 1932 – desert oases in Morocco and North Africa
A. m. lamarckii Cockerell, 1906 – Nile Valley (Egypt and Sudan)
A. m. litorea Smith, 1961 - low altitude in East Africa

[10] Engel M.S., 1999. The taxonomy of recent and fossil Honey Bee (Hymenoptera: Apidae; *Apis*). *Journal of Hymenoptera Research,* 8 (2), 165-196.

[11] Sheppard W.S. & Meixner V.M., 2003. *Apis mellifera pomonella*, a new honey bee subspecies from Central Asia *Apidologie*, 34, 367–375.

[12] Meixner M.D., Leta M.A., N. Koeniger, Fuchs S., 2011. The honey bees of Ethiopia represent a new subspecies of *Apis mellifera-Apis mellifera simensis* n. ssp. *Apidologie,* 42:425–437.

[13] Chen C., Liu Z., Pan Q., Chen X., Wang H., Guo H., Shi W., 2016. Genomic Analyses Reveal Demographic History and Temperate Adaptation of the Newly Discovered Honey Bee Subspecies *Apis mellifera sinisxinyuan* n. ssp. *Molecular Biology and Evolution*, *33*(5): 1337–1348.

A. m. monticola Smith, 1961 - high altitude in East Africa
A. m. jemenitica Ruttner, 1976 - Somalia, Uganda, Sudan, Yemen
A. m. simensis Meixner *et al.*, 2011 - Ethiopia

There are a further 5 subspecies in the Middle East and Central Asia:

A. m. meda Skorikov, 1829 - Iraq
A. m. syriaca Skorikov, 1829 – Middle East and Israel
A. m. anatoliaca Maa, 1953 - Anatolia in Turkey and Iraq
A. m. pomonella Sheppard & Meixner, 2003 - Tien Shan mountains and Central Asia
A. m. sinisxinyuan Chen *et al.*, 2016 - Xinyuan (Central Asia)

In past centuries the honeybee was introduced to the Americas, Oceania and Asia, with the scope of extending beekeeping activities in these regions, activities which can be very profitable with this species, as has been stated. In recent years, the international scientific community has debated whether the massive introduction of honeybees to these regions has had or is having a negative impact on local populations of pollinating insects, especially the *Apoidea* species, although this seems unlikely according to numerous scientific investigations. However, this issue must be seen within the context of fundamental protection for native pollinator organisms.

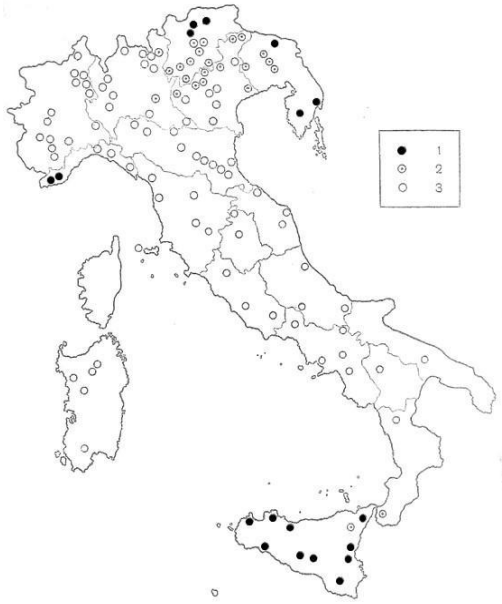
In Italy, which represents a unique case in Europe, there are natural populations attributable to 4 subspecies: *A. m. ligustica* and *A. m. siciliana* (endemic Italian subspecies), together with *A. m. mellifera* and *A. m. carnica* (the latter two probably only as populations crossbred to different degrees with *A. m. ligustica*).

Queens and worker bees of *A. m. mellifera, A. m. ligustica, A. m. carnica and A. m. siciliana*, the Italian subspecies

As regards the original distribution of honeybee subspecies in Italy, as well as Friedrich Ruttner's unsurpassed work[14], *Biogeography and Taxonomy of Honeybees*, first published in German and in English in 1988, we can refer to a previous Italian work published in 1927 by Anita Vecchi[15], entitled: "*Sulla distribuzione geografica dell'*Apis mellifica ligustica *Spin. in Italia*".

[14] Ruttner F., 1988. Biogeography and Taxonomy of Honeybees. Berlin: Springer-Verlag, 296 pp.

[15] Vecchi A., 1927. Sulla distribuzione geografica dell'*Apis mellifica ligustica* Spin. in Italia. *Boll. Zool. gen. agr. Portici*, 20: 150-168.



Anita Vecchi, 1927

In her work, Anita Vecchi analysed the chromatic patterns of numerous Italian populations, identifying honeybees with large clear bands in the first abdominal tergites in most of the peninsula, the presence of completely black honeybees in northern Italy and Sicily, and the presence of intermediate colours in certain areas. In the map presented by Anita Vecchi, the hollow circles represent places where there are only yellow honeybees (typical of *A. m. ligustica*), while the black circles correspond with locations marked exclusively by the presence of black honeybees, which could also represent populations of *A. m. mellifera*, *A. m. carnica* and *A. m. siciliana,* variously crossbred with *A. m. ligustica,* and circles with dots in central areas, where there are populations with intermediate colours. This distribution of *A. mellifera* subspecies in Italy, substantially confirmed by Ruttner's study, is well represented by the distribution map published in his text, cited above.

Friedrich Ruttner, 1987

A. m. mellifera, also called the black honeybee or German honeybee, was present in Italy in the Alps, along the borders with France and Switzerland and in a small but long area of the Liguria, Piedmont, Lombardy and Trentino Alto Adige Regions, mainly in hybridised form with *A. m. ligustica*. Today, the black honeybee is rare in these regions, but an awareness of the importance of protecting these populations has grown among beekeepers and institutions, first in France and more recently in Italy. *A. m. carnica* would appear to have been present on the border with Slovenia and Austria, but only in a small part of Friuli Venezia Giulia Region and perhaps in the northern part of the Veneto Region. Today *A. m. carnica*, or rather highly selected strains of this subspecies, and therefore far from having the characteristics of the original populations, are reared by a large number of beekeepers in the Friuli Venezia Giulia Region[16] and Veneto mountains, the Trentino Alto Adige Region, extensive areas of northern Italy and in scattered places throughout the Italian peninsula.

It is important to remember that the subspecies A. *m. mellifera* and A. *m. carnica* originally had marginal distribution in Italy, also in contact with *A. m. ligustica,* so the limited Italian areas of these two subspecies coincided largely with hybridisation zones [17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27.] The whole of the Italian peninsula and Sardinia (although with some particular characteristics, already highlighted by A. Vecchi and more recently in other studies by Floris

[16] In 1927, as is evident on the map in Anita Vecchi's publication, the Friuli Venezia Giulia Region included a large area now belonging to Croatia and Slovenia, where the *A. m. carnica* lives.

[17] Bolchi Serini G., Sommaruga A., Lapietra G., 1983. Studio biometrico di popolazioni alpine di *Apis mellifera* L. *Boll. Zool Agrar. Bachic.*, II, 17: 1-18.

[18] Comparini A, Biasiolo A., 1991. Genetic characterization of Italian bee *Apis mellifera ligustica* Spin, versus Carnolian bee, *Apis mellifera carnica* Poll, by allozyme variability analysis. *Biochem. Syst. Ecol.*,19: 189-194.

[19] Leporati M., Valli M., Cavicchi S., 1984. Étude biométrique de la variabilité géographique des populations d'*Apis mellifera* en Italie septentrionale. *Apidologie*, 15: 285-302.

[20] Marletto F., Manino A., Balboni G., 1984. Indagini biometriche su popolazioni di *Apis mellifera* L delle Alpi occidentali. *Apic. Mod.*, 75: 213-223.

[21] Nazzi F., 1992. Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy. *Apidologie*, 23: 89-96.

[22] Badino G., Celebrano G. and Manino A., 1982. Genetic variability of *Apis mellifera ligustica* Spin. in a marginal area of its geographical distribution. *Experientia*, 38: 540-541.

[23] Badino G., Celebrano G. and Manino A., 1983. Population structure and *Mdh-1* locus variation in *Apis mellifera ligustica*. *Journal of Heredity*, 74: 443-446.

[24] Badino G., Celebrano G. and Manino A., 1983. Identificazione di *Apis mellifera ligustica* Spinola sulla base di sistemi gene-enzima. *Boll. Mus. Reg. Sci. Nat.*, Torino 1 (2): 451-460.

[25] Marletto F., Manino A., Pedrini P. 1984. Integrazione fra sottospecie di *Apis mellifera* L. in Liguria. *L'apicoltore moderno*, 75: 159-163.

[26] Badino G., Celebrano G., Manino A., 1984. Population genetics of Italian honeybee and its relationships with neighbouring subspecies. *Boll. Mus. Reg. Sci. Nat.*, Torino 2(2): 571-584.

[27] Manino A., Marletto F., 1984. Il sistema enzimatico MDH in popolazioni di *Apis mellifera* L. della Valle d'Aosta. *L'apicoltore moderno*, 75: 89-94.

and Prota [28]) was populated by the Italian honeybee *A. m. ligustica*, while Sicily and the surrounding islands were populated only by *A. m. siciliana* [29,30], known as the black honeybee of Sicily.

The subspecies *A. m. ligustica* and *A. m. siciliana* are not only native but also endemic to Italy and their whole original distribution area is included within the Italian territory.

As regards *A. m. ligustica,* it should be emphasised that its distribution over such a vast territory, and above all different from the bioclimatic point of view, must originally have given rise to many local ecotypes [31, 32], each of these well-adapted to particular conditions, as can also be deduced from studies conducted in Sardinia.

A very important aspect of the different *A. mellifera* subspecies also concerns their origin. All the subspecies have been subdivided into four lines on a morphological basis: A (Africa), M (Western and Northern Europe), C (Eastern Europe and Asia Minor) and O (the Middle East and Central Asia). The autochthonous subspecies of European *A. mellifera* belong to three different lines (A, M and C) and were differentiated during the last great Ice Ages in remote areas in southern Europe (Spain, Italy and the Balkans) and in Africa, from where they recolonised central and northern European regions around 10,000 years ago.

As regards Italy, a study based on nuclear and mitochondrial markers showed that the two Italian endemic subspecies of A. *mellifera* (*A. m. ligustica* and *A. m. Siciliana*), originated from hybridisation between populations belonging to different evolutionary lines confined within the Italian peninsula and Sicily during the penultimate Ice Age (about 190,000 years ago). In *A. m. ligustica*, attributed to line C on a morphological and nuclear basis, there are also mitotypes of the M line, and in *A. m. Siciliana*, which only has mitotypes from the A-line, to which it also relates on a morphological basis, it is possible to observe some similarities with line C on a nuclear basis [33]. This fact, apparently unimportant in terms of conservation, is instead very important, because it highlights the complexity, and therefore the fragility, of the structure of European *A. mellifera* populations.

[28] Floris I., Prota R., 1994. Variazioni di alcune caratteristiche morfometriche nella popolazione di *Apis mellifera* L. della Sardegna nell'ultimo ventennio. *Apicoltura*, 9: 163-175.

[29] Manino A. & Longo S., 2010. The black Sicilian honey bee: a nomenclatural clarification. REDIA, XCIII, 2010: 103-105.

[30] Badino G., Celebrano G., Manino A., 1985. Enzyme polymorphism in the Sicilian honeybee. *Experientia*, 41: 752-754.

[31] An ecotype is a separate group of an animal, plant or organism that is closely connected with the environment in which it lives. As such, an ecotype has no taxonomic category.

[32] Costa C., Lodesani M., Bienefeld K., 2012. Differences in colony phenotypes across different origins and locations: evidence for genotype by environment interactions in the Italian honeybee (*Apis mellifera ligustica*). *Apidologie*, 43 (6): 634-642.

[33] Franck P., Garnery L., Celebrano G., Solignac M. & Cornuet J.-M., 2000. Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). *Molecular Ecology*, 9: 907–921.

The honeybee has some peculiarities that make it a key organism for conservation of biodiversity and therefore of the global ecological balance.

Honeybees obtain their nourishment from nectar and pollen (as well as honeydew), and by collecting these substances from flowers provide to the pollination and therefore reproduction of many plants that require the action of pollinating insects. The discovery of the role of insects in the reproduction of many plant species dates back to studies in the 18th century [34] and is therefore very recent. Darwin himself studied the benefits of the cross-fertilisation of plants and the relationship between certain plant species and the single or few insects capable of pollinating them. There are several thousand species of pollinators, most of which belonging to the superfamily *Apoidea*, a group of *Hymenoptera* differentiated precisely through a process of coevolution with *Magnoliophytes*, also called *Phanerogams* or flowering plants. The genus *Apis* derives from a long evolutionary pathway, and the complex and permanent societies into which the different species are organised play a fundamental role in the conservation of flora in their area of origin. When talking about pollination and pollinators, the tendency is often to consider only the important role that this mechanism has in agricultural production and therefore the direct consequences on human food (one can consider, for example, the fact that about a third of world agricultural production depends on animal pollination). In effect, since most cultivated plant species originate in areas where the main pollinator is the honeybee, this insect effectively plays an extraordinary role in food production worldwide. However, *A. mellifera* has an even greater role in the conservation of spontaneous flora [35], namely the plant world underlying almost all terrestrial ecosystems. Indeed, *A. mellifera* is able to pollinate more than 80% of *Magnoliophytes* species in its area of origin. Its environmental plasticity makes this species the main and fundamental pollinator in large parts of the world. One could therefore say that the flora of Europe, Africa, Middle East and Asia has been shaped by the relationship with local populations of this species. Native subspecies of *A. mellifera* are thus also fundamental for the conservation of native flora. In practice, honeybees are a typical example of an ecosystem service encouraging biodiversity, as is commonly stated today. In the last few years, numerous scientific studies have reported that *A. mellifera* could act as an invasive species with a major impact on biodiversity, especially in newly introduced areas (Oceania and the Americas)[36]. However, although the honeybee has become widespread in nature and has established wild populations in these new continents, the extent to which the honeybees introduced alter biodiversity remains controversial, and there is debate as to whether they have had an effect on the biodiversity of native pollinators, as the most likely

[34] Sprengel C. K., 1793. Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen. Berlin.

[35] As clearly stated in article 1 of Law no. 313 on beekeeping, issued by the Republic of Italy on 24 December 2004: "*This law recognises beekeeping as an activity of national interest useful for the conservation of the natural environment, the ecosystem and agriculture in general and aimed at guaranteeing natural pollination…*".

[36] Moritz R. F. A., Härtel S. & Neumann P., 2005. Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience*, 12(3): 289-301.

group of competing organisms[37,38]. However, the impact within the genus *Apis*, in terms of transporting new parasites or pathogens[39], the loss of genetic diversity and gene transfer between species has been proved.

In their areas of origin, *A. mellifera* and its autochthonous subspecies are wild *Apoidea*! In wildlife terms, protection of the honeybee should be considered in the context of conserving the natural equilibrium, as well as beekeeping.

Returning to the subspecies of *A. mellifera*, it is clear that being interfertile, these taxonomic entities are in a certain sense fluid and in many cases (neighbouring subspecies) need very precise and refined mechanisms for their survival, providing for continuing selective action in terms of climate and vegetation, but also a certain degree of gene exchange with neighbouring subspecies in the hybridisation zones. At the same time, as the different subspecies have evolved ethological mechanisms linked to eusociality that have made them more suitable for their habitats, in addition to adapting to the climate and local flora, it is clear that accidental remixing can destroy, or at least damage, these specific mechanisms for the adaptation of local subspecies to their respective environments. The contact areas between different subspecies allow natural and reciprocal gene exchange, albeit limited, helping to ensure greater potential adaptation to climate change within the subspecies and thus within the species as a whole.
It is therefore essential to repeat that in Italy and the areas of origin, *A. mellifera*, even when managed through beekeeping, has its own identity, representing a specific expression of biological information, and should therefore be protected as a component of the wildlife.

[37] Mallinger R. E., Gaines-Day H. R., Gratton C., 2017. Do managed bees have negative effects on wild bees?: A systematic review of the literature. *PLoS One*, 12(12): e0189268.

[38] Goulson D. & Sparrow K.R., 2009. Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. *Journal of Insect Conservation*, 13 (2): 177-181.

[39] Gordon R., Bresolin-Schott N. & East I.J. (2014). Nomadic beekeeper movements create the potential for widespread disease in the honeybee industry. *Australian Veterinary Journal*, 92 (8): 283-290.

CURRENT LEGISLATION

Within the framework of the European Union strategy for the protection of biodiversity, and which in point 31: "*calls on the Member States and the regions to use all means possible to protect local and regional honeybee species (strains of Apis Mellifera bees) from the undesirable spread of naturalised or invasive alien species having a direct or indirect impact on pollinators; supports the repopulation of hives lost through invasive alien species with bees of local native species; recommends Member States to create centres devoted to the breeding and safeguarding of native bee species; underlines in this regard the importance of developing breeding strategies to increase the frequencies of valuable traits in local honeybee populations; notes the possibilities provided for under Regulation (EU) No 1143/2014 on Invasive Alien Species, as well as potentially under the recently adopted Animal and Plant Health regulations (Regulations (EU) 2016/429 and (EU) 2016/2031 respectively*" [40]. Current national, regional and local legislation, of which a broad but not exhaustive review is provided, has a significant number of provisions that involve both a ban on introducing subspecies other than *Apis mellifera ligustica* and local ecotypes in large areas, and more generally, address the issue of protection and incentives for beekeeping. In the European context, an important precedent should be noted in the Republic of Slovenia's law on the breeding of animals, which defines *A. m. carnica* as a native subspecies and provides for special protection, according to which "*the breeding and commerce of reproductive material of other honeybee subspecies is not permitted*" in the whole of the national territory [41].

Laws prohibiting the introduction of subspecies other than the *Apis mellifera* ligustica and relative penalties

Of Italian legislative provisions, the law of 1925 states that: "*on the request of the Consortia or beekeepers concerned, or according to a provision of the Italian Ministry of Economic Affairs, Prefects may also prohibit the introduction or diffusion of species, subspecies and strains of honeybees other than A. m. ligustica in their respective provinces*" [42]. The Framework Law of 1991 prohibits: "*the introduction of alien species, plants or animals, that can alter the natural balance*" [43]. In 2015, an addition to the Criminal Law provided for imprisonment and financial penalties for crimes against the environment: "*Anyone who*

[40] Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (Habitats Directive); Communication from the Commission: Our life insurance, our natural capital: an EU Biodiversity Strategy to 2020 (COM (2011) 244); An Action Plan for nature, people and the economy {SWD (2017) 139 final}). The resolution is 2017/2115 (INI).

[41] Official Gazette of the Republic of Slovenia no. 18/2002, articles 68 and 70.

[42] Regio Decreto Legge of 23 October 1925, no. 2079, "*Provvedimenti per la difesa dell'apicoltura*", article 12.

[43] Italian law of 6 December 1991, no. 394 "*Legge quadro sulle aree protette*". Article 11 – Park regulation, paragraph 3.

unlawfully compromises or causes a significant and measurable deterioration to the following shall be punished with imprisonment from 2 months to 6 years and with a fine of from 10,000 to 100,000 Euro: 1. Water, air, and extensive or significant portions of the soil or subsoil; 2. An ecosystem, biodiversity of flora or fauna, also agricultural. When the pollution is caused in a protected natural area or area subject to landscape, environmental, historical, artistic, architectural or archaeological protection, or leads to damage to protected animal or plant species, the penalty shall be increased" [44]. A 2017 resolution adopted by the Chamber of Deputies included not only prohibitions but also protective actions: "*(omissis) commits the Government: to take initiatives to safeguard the subspecies A. m. ligustica, limiting or banning different subspecies, including hybrids (if not natural), in the Italian territory, through new agreements within the European Union, also implementing a strategy for the protection of biodiversity of this subspecies, providing for sufficiently extensive mating areas (at least 200 square kilometres) in areas where all natural or cultivated hives are inhabited by A. m. ligustica*" [45].

As regards regional laws, two regions provided for "*buffer zones*" in 1988 and 2009 respectively. The Regional Council of Emilia Romagna: "*after consulting the Regional Advisory Committee for Beekeeping, may set up buffer zones around farms, also on the request of a single breeder of queen bees included in the register of breeders stated in article 12, without prejudice to the application of the current regime of health checks to these. 2. From the moment the buffer zone is established, it is forbidden for third parties to introduce honeybees or increase the number of existing hives*" [46]. Likewise in Tuscany: "*Provinces and Mountain Communities (now integrated within the Region) may identify buffer zones around queen bee breeding centres on the basis of specific criteria issued by the Regional Government, concerning the characteristics of the buffer zones, the method for delimiting them and the period during which the ban on the entry of other beehives in the delimited buffer zone is applied, as well as identification of the parties authorised to make such a request*" [47]. In 1992 the Emilia Romagna Region provided for a total ban for the whole regional territory: "*it is forbidden to introduce and breed honeybees of strains other than A. m. ligustica, as well as interracial hybrids, within the regional territory*" [48]. There are also local bans, such as the 2015 order issued by the Mayor of the Municipality of Vetto

[44] Italian law of 22 May 2015, no. 68 "*Disposizioni in materia di delitti contro l'ambiente"*. Article 1: 1. After Section VI of the second volume of the criminal code the following is included: "Section VI-bis – Crimes against the environment. Article 452-bis. (Environmental pollution)."

[45] Republic of Italy. Chamber of Deputies, Resolution 7-01250 presented by Zaccagnini Adriano, 2 May 2017, no. 787.

[46] Emilia Romagna Region. Law of 25 August 1988, no. 35, "*Tutela e sviluppo dell'apicoltura*". Article 13, Buffer zone.

[47] Tuscany Region. Law of 27 April 2009, no. 21 - *Norme per l'esercizio, la tutela e la valorizzazione dell'apicoltura*" Article 11. Official Bulletin of the Tuscany Region no. 15 of 6/5/2009.

[48] Emilia Romagna Region. Decree no. 826 of 23 November 1992 of the President of the Emilia Romagna Region "*Divieto di introduzione e di allevamento sul territorio regionale di api di razza diversa dall'Apis mellifera ligustica*".

(Reggio Emilia) for a delimited area, which states: "*that in the territory of the Municipality of Vetto, within a radius of 3 km around Atticola, more clearly indicated on the map, honeybees other than those that are the object of the selection project (Ed. A. m. ligustica) may not be introduced or bred*" [49].

Laws for the protection of *A. m. ligustica*

An Italian law issued in 1992 protects *A. m. ligustica* as a form of wildlife: "*wildlife is a public asset of the State and is protected in the interest of the national and international community*". The fact that *Apis mellifera* is divided into various indigenous subspecies at local level means that the subspecies, especially if they are endemic, should also be protected as a genuine national heritage [50]. The 2004 Framework Law on Beekeeping: "*recognises beekeeping as an activity of national interest useful for the conservation of the natural environment, the ecosystem and agriculture in general, and is aimed at guaranteeing the natural pollination and biodiversity of honeybee species, with particular reference to the protection of Italian subspecies*", and more specifically at "*safeguarding and selection of the Italian honeybee (A. m. ligustica) and A. m. siciliana, promoting the use of Italian honeybee queens from genetic selection centres*" [51]. Finally, the 2009 ministerial provisions for the implementation of community regulations on organic production establish that: "*the choice of the strain to be used in apiculture must favour native subspecies according to their natural geographical distribution: A. m. ligustica, A. m. siciliana (limited to Sicily) and, limited to border areas, hybrids resulting from free crossing with subspecies from neighbouring countries*" [52].

Among regional regulations protecting *A. m. ligustica*, there is the 2015 measure by the Autonomous Region of Sardinia: "*the Region regulates, protects and promotes beekeeping and encourages the preservation of honeybee species, with particular reference to the Italian honeybee (A. m. ligustica) and populations of typical native bees*" [53].

The Umbria Region's regulations on beekeeping [54], establish in article 93 that: "*the Region can set up buffer zones around queen bee producers included in the national register of A. mellifera breeders and around mating station situated in the region. Nomadic beekeeping is also prohibited in these areas*".

[49] Provision no. 54 of 18 December 2015.

[50] Law of 11 February 1992, no. 157. Article 1.

[51] Law of 24 December 2004, no. 313 - Regulation of beekeeping. Article 1, paragraph 1; article 5, paragraph r.

[52] Ministerial Decree no. 18354 of 27 November 2009: "*Disposizioni per l'attuazione dei regolamenti (CE) n. 834/2007, n. 889/2008, n. 1235/2008 e successive modifiche riguardanti la produzione biologica e l'etichettatura dei prodotti biologici.*" Article 4, Animal production 1) Origin of biological animals in beekeeping – article 8 of Reg. (EC) 889/08.

[53] Regional Law of 24 July 2015, no. 19, *"Disposizioni in materia di apicoltura."* Article 1, paragraph 2. BUR Region of Sardinia no. 34 of 30 July 2015.

[54] Regional Law of 9 April 2015, no. 12.

HONEYBEES, THEIR SUBSPECIES AND CONSERVATION

The seriousness of the situation regarding the preservation of native populations of *A. mellifera* makes the issuing of clear ad hoc rules focusing exclusively on the problem urgent.

There is also a fundamental aspect to be clarified. For thousands of years, honeybees reared by beekeepers have coexisted with colonies of *A. mellifera* present naturally in different areas.
Although beekeepers have carried out intense selection activities, especially in the last 150 years, the way of mating of queen bees has always guaranteed extensive and beneficial genetic interaction between wild and managed honeybees. With the transfer of the parasitic mite *Varroa destructor* to *A. mellifera*, which will be discussed later, in the last 35 years wild colonies have almost completely disappeared in most of Europe, although there are recent data that could give new insight to this phenomenon[55].

This has meant that many debates today on the conservation of the honeybee from the wildlife point of view tend to distinguish colonies present naturally from those managed and selected by beekeepers, from whose swarms they often derive.

Since honeybees are not kept within a fenced and defined area, even when they are managed, **safeguarding of *A. mellifera* (and related subspecies) cannot consider the protection of colonies present naturally, by now extremely rare, separately from the protection of beehives maintained in the context of beekeeping, from which the wild colonies often derive.** Moreover, the protection of each subspecies must be extended to the whole of its original area, because all the local sub-populations (ecotypes) contribute to the conservation and continuous evolution of the subspecies, having adapted to the different habitats in this area. Protecting a subspecies means protecting its variability as extensively as possible. In this context, hybridisation areas with neighbouring subspecies are also fundamental. As regards the conservation of local ecotypes of different *Apis mellifera* subspecies, some studies have shown a certain stability of these populations[56], as Louveaux summarised for example, asserting that individuals not adapted for natural selection are condemned to die in a short time, meaning that local honeybees are a relatively stable ecotype everywhere[57]. A recent study involving many *Apis mellifera* populations at European level has shown that adaptation by local honeybees makes them able to survive longer in situations of environmental stress, that they tend to produce more honey and that they are

[55] Kohl P.L. & Rutschmann B., 2018. The neglected bee trees: European beech forests as a home for feral honey bee colonies. *Peer J*, 6: e4602 https://doi.org/10.7717/peerj.4602.

[56] Costa C., Lodesani M., Bienefeld K. (2012) Differences in colony phenotypes across different origins and locations: evidence for genotype by environment interactions in the Italian honeybee (*Apis mellifera ligustica*)? *Apidologie*, 43 (6): 634-642.

[57] Louveaux J., 1969. Importance of the notion ecotype in bees. *Apiacta*, 3.

more docile[58, 59, 60]. Unfortunately, the disappearance of naturally present colonies and increasing movement of bees outside their relative areas of origin, as well as the increasing use of commercial hybrids by beekeepers, makes the adoption of restrictive regulations urgently needed, given that if stabilisation is postponed the situation could soon be no longer recoverable.

An important aspect of biodiversity protection is the economic sustainability of the actions proposed to pursue the objective. In this context, the promotion and differentiation of products deriving from different subspecies of *A. mellifera* could represent an important feature, offering an economic return to the beekeeper with the sale of honey characterised by a specific genetic origin[61].

We cannot abandon conservation of European subspecies of *A. mellifera* with resignation just because they are declining rapidly today. This would mean surrendering, condemning to extinction not only these bees, but also the flora they have contributed to shaping. Sooner or later, extinction of European subspecies would also engulf beekeeping in vast areas of the Earth.

[58] Büchler R., Costa C., Hatjina F., Andonov S., Meixner M.D., Le Conte Y., Uzunov A., Berg S., Bienkowska M., Bouga M., Drazic M., Dyrba W., Kryger P., Panasiuk B., Pechhacker H., Petrov P., Kezic N., Korpela S., Wilde J., 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *Journal of Apicultural Research*, 53(2): 205- 214.

[59] Hatjina F., & Costa C., Büchler R., Uzunov A., Drazic M., Filipi J., Charistos L., Ruottinen L., Andonov S., Meixner M. D., Bienkowska M., Dariusz G., Panasiuk B., Le Conte Y., Wilde J., Berg S., Bouga M., Dyrba W., Kiprijanovska H., Korpela S., Kryger P., Lodesani M., Pechhacker M., Petrov P., Kezic N., 2014. Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research*, 53(2): 233-247.

[60] Uzunov A., Costa C., Panasiuk B., Meixner M., Kryger P., Hatjina F., Bouga M., Andonov A., Bienkowska M., Le Conte Y., Wilde J., Gerula D., Kiprijanovska H., Filipi J., Petrov P., Ruottinen L., Pechhacker H., Berg S., Dyrba W., Ivanova E., Büchler R., 2014. Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *Journal of Apicultural Research*, 53(2): 248-260.

[61] Utzeri V.J., Ribani A., Fontanesi L., 2018. Authentication of honey based on a DNA method to differentiate *Apis mellifera* subspecies: Application to Sicilian honey bee (*A. m. siciliana*) and Iberian honey bee (*A. m. iberiensis*) honeys. *Food Control*. Doi: 10.1016/j.foodcont.2018.04.010.

THE DECLINE OF BEES

Unfortunately, in Europe the conservation status of native subspecies of *A. mellifera* and their respective ecotypes has been seriously compromised.

The causes of this situation can be related to at least 6 factors.

1) The first, already known since ancient times, albeit to a lesser extent, is the **moving of subspecies from one region of Europe to another by beekeepers**. Several subspecies of *A. mellifera* have been involved in this movement. There is documentation at least from the 19th century of how certain colonies of subspecies known to be particularly docile or productive, or even because they are particularly "aesthetically pleasing", such as *A. m. cypria*, have been transferred from their area of origin to different regions of Europe. The most striking cases, however, concern *A. m. carnica* and *A. m. ligustica*. *A. m. carnica*, docile and productive was introduced in the last few centuries mainly in central Europe, where it was preferred by beekeepers to the local *A. m. mellifera*; in Italy this subspecies has also been very widespread in the last few decades, initially only in the southern Alps, but subsequently also in other parts of the country. *A. m. ligustica,* considered by many specialists in the beekeeping sector to be the best honeybee for honey production, has spread to many parts of Europe and also to Sicily (where it has almost completely replaced the local *A. m. Siciliana*) but also to many non-European countries, where the German black honeybee was initially introduced. In Malta, there has recently been some concern regarding the conservation of the local endemic subspecies *A. m. guttneri*, due to the introduction of *A. m. ligustica* and *A. m. Siciliana* [62].

2) The second phenomenon that has contributed to compromising the conservation of the native subspecies of *A. mellifera* is the result of techniques for **breeding queen bees**. With the larvae grafting technique above all it is possible to rear several thousand queen bees starting from the larvae of a single parent, believed to have positive characteristics for the beekeeper. In this context, selective pressure to reduce the swarming tendency or the production of drones is detrimental, because it contributes further to the loss of genetic diversity.
Breeding of queen bees on a large scale has on the one hand allowed the selection of highly productive bees for professional beekeeping, but on the other has facilitated the transfer of certain genetic traits of *A. mellifera* outside its own area of origin, dramatically increasing the effects of the first factor[63]. Furthermore, large-scale replication of the genetic heritage

[62] Zammit-Mangion M., Meixner M., Mifsud D., Sammut S. & Camilleri L., 2017. Thorough morphological and genetic evidence confirm the existence of the endemic honey bee of the Maltese Islands *Apis mellifera ruttneri*: recommendations for conservation. *Journal of Apicultural Research*, 56 (5): 514-522.

[63] Muñoz I., Pinto M. A. & De la Rúa P., 2014. Effects of queen importation on the genetic diversity of Macaronesian Island honey bee populations (*Apis mellifera* Linneaus 1758). *Journal of Apicultural Research*, 53:2, 296-302.

of a limited number of individuals today plays a negative role in conserving a large gene pool within the various indigenous sub-species. **It is indeed the same specific eusocial structure of honey bees that demands respect and safeguarding of its diversity**. The genus *Apis* is indeed characterised by the highest level of polyandry found among social *Hymenoptera*[64]. According to most experts, high polyandry, or the mating of queens with numerous males (a phenomenon that leads to high genotypic diversity in the offspring within honeybee colonies) is an evolutionary pathway that the genus *Apis* has pursued[65, 66] and which is essential to mitigate the effects of pests and pathogens on the colonies[67]. As a result of polyandry, the colony of honeybees is made up of a large number of worker bees called *step-sisters* (with the same mother but different fathers). However, within the colonies there are also a variable number of subgroups of *super-sisters* (with the same mother and father), equal to the number of drones with which the queen has mated. Because the drone is haploid (the spermatozoa produced by each drone are identical), the super-sister worker bees are individuals with a very high level of kinship, represented on average by 75% genetic similarity.

The presence of super-sister groups is at the basis of the honeybee's social structure, but a reduced presence of these groups can compromise the very survival of the colonies, by reducing the ability to respond to variables such as climate and food resources[68]. In an environment with poor genetic variability, a virgin queen on her mating flight will mainly find drones potentially related to each other and to her. Bees seek polyandry, but if during her only mating flight the queen encounters only males that are related to each other, following the large-scale reproduction of selected queens, it is as if she had mated with a small number of males and polyandry will not achieve the expected results[69].

3) The third negative aspect for the conservation of native subspecies of *A. mellifera* is the adoption of large-scale **nomadic beekeeping**. The diffusion of motorised vehicles in Europe and Italy in the 20th century made the transport of whole apiaries from one nectariferous area to another very simple and rapid, even for journeys covering several hundred kilometres. Thus many northern Italian beekeepers have moved their colonies to the south and vice-versa, mixing up genetically distant populations (ecotypes) of *A. m. ligustica,* but

[64] Strassmann J., 2001. The rarity of multiple mating by females of social Hymenoptera. *Insect Sociaux*, 48 (1): 1-13.

[65] Brown M. J. F. & Schmid-Hempel P., 2003. The evolution of female multiple mating in social hymenoptera. *Evolution*, 57(9): 2067-2081.

[66] Badino G., Celebrano G., Manino A., 2004. Allozyme evidence of recent genetic shift in honey bee populations of Italy. *Journal of Apicultural Research*, 43 (4):147-149.

[67] Tarpy D. R., 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270: 99-103.

[68] Mattila H.R., Rios D., Walker-Sperling V.E., Roeselers G., Newton I.L.G., 2012. Characterization of the Active Microbiotas Associated with Honey Bees Reveals Healthier and Broader Communities when Colonies are Genetically Diverse. *PLoS ONE*, 7(3): e32962.

[69] Tarpy D.R., and Page R E., 2002. Sex Determination and the Evolution of Polyandry in Honey Bees (*Apis Mellifera*). *Behavioral Ecology and Sociobiology*, 52 (2): 143–150.

also more recently, transferring *A. m. carnica* to southern Italy, or vice-versa bringing *A. m ligustica* to hybridisation areas in the Alps.
Even more radical movement takes place in the context of so-called pollination services. As nomadic beekeeping generally takes place during the season in which colonies have many drones and when the mating flights of virgin queen bees occur, the effect of nomadism is anything but theoretical or negligible.

4) However, the fatal blow to the conservation of native subspecies *of A. mellifera* has resulted from the transfer of the ectoparasitic mite ***Varroa destructor***, originally only linked to Asian species of the genus *Apis*, to the European honeybee. This mite, which like all parasites has co-evolved with the host species in such a way as not to cause irreparable damage to hives, once transferred to *A. mellifera* as a result of the introduction in Asia of this bee for production purposes, has become lethal for colonies, due to direct pathogenic effects and indirect effects related to the transmission and activation of viruses. Today, Varroa mites are one of the main problems for beekeeping in Europe and many other parts of the world, especially where there is very specialised beekeeping. Since the 1960s this parasite has spread rapidly throughout European honeybee colonies, both managed and wild. Until that time, in addition to managed colonies wild honeybees were present everywhere, inevitably crossbreeding with each other, but still subject to natural selection. The presence and abundance of these wild honeybees was essential to limit the negative effects of beekeeping on the conservation of native subspecies and local ecotypes. However, following the accidental arrival of *V. destructor*, there was an almost total disappearance of wild colonies of honeybees in Europe.
This fact, recently confirmed in a study on the conservation of European *Apoidea*[70] by the IUCN (International Union for Conservation of Nature), was a fatal blow for local populations of *A. mellifera*, to the extent that **today in a way, we are observing the paradox of a situation in which a species fundamental for the conservation of the natural equilibrium, as well as for human nutrition, is surviving in Europe almost only thanks to management by beekeepers**.

The presence of subspecies more or less tolerant to Varroa in various parts of the world and the discovery of colonies in Europe that can survive the parasite in the absence of chemical treatments[71], shows how, in principle, natural selection can lead to the development of colonies tolerating the parasite starting from local populations adapting to the environment of origin. Furthermore, recent studies comparing various honeybee strains in different European locations have shown that, in general, the colonies best tolerating the parasite tend to be local ones and that when these are moved away from their environment of origin

[70] Nieto A. *et al.*, 2014. European Red List of bees. Luxembourg: Publication Office of the European Union: 84 pp.

[71] Le Conte Y., De Vaublanc G., Crauser D., Jeanne F., Rousselle J.C. & Bécard J.M., 2007. Honey bee colonies that have survived *Varroa destructor*. *Apidologie*, 38: 566-572.

they lose this important characteristic [72, 73]. These data clearly indicate the usefulness of preserving local populations and also the possibility of obtaining colonies tolerant to Varroa mites from them, as already attempted in recent research projects at European level.

5) Another relatively recent phenomenon that is threatening the survival of native subspecies of *A. mellifera* is the diffusion of bees selected as **commercial hybrids** in many parts of Europe and within Italy. These hybrids derive from the extensive crossbreeding of different subspecies of *A. mellifera*, also from outside Europe. Distributed on a large scale and widespread among professional and non-professional beekeepers, these bees are further undermining the residual autochthonous heritage, and as they cannot be reproduced, except by a very few beekeepers and breeders, they represent both a source of genetic "pollution" and a reduction of the overall gene pool.
These hybrids are not stable and the supposed characteristics for which they are sold are related to heterosis (or hybrid vigour); in subsequent generations the characteristics segregate, with the formation of individuals completely different to each other and mostly with negative characteristics, which can however crossbreed with local populations, preventing beekeepers from implementing selection at local level.

The current lack of protection for native subspecies of *A. mellifera* in Europe also results partly from the fact that at European Community level, with a few exceptions, living organisms are only safeguarded at species level and therefore subspecies are practically ignored. This allows any European beekeeper to request, completely legally, to introduce any subspecies of honeybee coming from other European and non-European Countries, with the sole obligation to follow the veterinary policing obligations.

6) In addition to the previous serious problems contributing to the decline of autochthonous subspecies of *A. mellifera* in Europe, at least as regards their biological significance, namely as fundamental components of wildlife and key organisms for the conservation of local flora, and thus overall biodiversity, honeybees, like all the *Apoidea* family and other pollinating insects, are seriously threatened by other very serious **environmental factors of anthropic origin.** These are chemical pollution, especially due to massive and widespread use of agrochemicals [74], environmental changes, with a consequential reduction in nectariferous plants, and climate change. As regards agrochemicals, in recent years there has been an expansion in the use of chemicals active at very low doses and therefore more complicated to manage from an environmental point of view, whose most serious effects are often at

[72] Meixner M.D., Kryger P., Costa C., 2015. Effects of genotype, environment, and their interactions on honey bee health in Europe. *Current Opinion in Insect Science*, 10 (8): 177–184.

[73] Francis R M., Amiri E., Meixner M. D., Kryger P., Gajda A., Andonov S., Uzunov A., Topolska G., Charistos L., Costa C., Berg S., Bienkowska M., Bouga M., Büchler R., Dyrba W., Hatjina F., Ivanova E., Kezić N., Korpela S., Le Conte Y., Panasiuk B., Pechhacker H., Tsoktouridis G., Wilde J., 2014. Effect of genotype and environment on parasite and pathogen levels in one apiary - a case study. *Journal of Apicultural Research,* 53(2): 230-232.

[74] Tosi S., Costa C., Vesco U., Quaglia G., Guido G., 2018. A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. *Science of the Total Environment*, 61: 208–218.

sublethal doses. Extensive use of substances normally considered to be minimally toxic or non-toxic for bees, such as fungicides, has instead been shown to be a serious cause of decline for honeybees and *Apoidea* in general. This is also due to the negative effect on the gut microbiota of honeybees, namely the complex of micro-organisms on which honeybees partially base their glucose metabolism, and above all their protein diet. These micro-organisms are essential for the formation and conservation of bee bread[75, 76, 77]. All these factors, together with serious genetic deterioration, are placing the survival of local populations of *A. mellifera* and pollinators in general at risk, leading to serious problems for the conservation of flora and thus of habitats. The decline of bees and the impoverishment of flora endanger the survival of beekeeping, which is also of very high cultural significance in historical and social terms, in addition to producing extremely valuable substances for human food and health.

The problems are thus very complex, but it is necessary to take action immediately and on the basis of scientific data.

[75] Bee bread is the pollen stored by bees in the cells of the honeycomb, to which the bees' honey and digestive fluids are added, the latter containing a pool of beneficial microorganisms (microbiota) that acidify the mass, ensuring its conservation and contributing to its very high nutritional value.

[76] Loper G.M., Standifer L.N., Thompson M.J. & Gilliam M., 1980. Biochemistry and microbiology of bee-collected almond (*Prunus dulcis*) pollen and bee bread. I-Fatty Acids, Sterols, Vitamins and Minerals. *Apidologie*, 11 (1): 63-73.

[77] Vásquez A. & Olofsson T.C., 2009. The lactic acid bacteria involved in the production of bee pollen and bee bread. *Journal of Apicultural Research*, 48 (3): 189-195.

THE PROTECTION OF *APIS MELLIFERA*

Many organisations and institutions are working to protect honeybees, and many concrete actions to raise the awareness of political administrations at all levels have been carried out in Italy and Europe and are continuing. Most of these actions, however, are related to beekeeping and are thus based more on animal husbandry than a naturalistic approach. We are aware of the value of these measures to protect bees and raise awareness, but with this document we would like to stimulate public administrations at all levels to put into effect measures designed to protect honeybees and their indigenous subspecies, and in this way to guarantee concrete safeguarding of the environment and apiculture, as demonstrated by the extensive scientific literature.

Future protection strategies should prioritise: (1) creation of a national database on the heritage of *A. mellifera*, on a morphometric and genetic basis, to be linked to the National Honeybee Register, as a fundamental tool for regulating and managing the heritage, handling and trading of honeybees; (2) boosting of apicultural research to support adequate conservation strategies, encouraging studies aimed at identifying and enhancing local genetic lines and determining the impact of invasive species (plants, animals, parasites and pathogens), integrating this information to understand the potential impact of climate change on the current diversity of bees; (3) promotion of policies aimed at minimising habitat loss and making agricultural landscapes "bee-friendly".

We therefore wish to strongly urge all administrations and public institutions that can carry out administrative and legislative actions in this context to work urgently to prepare new and concrete measures for the protection of native subspecies of *A. mellifera*.
It is thus a question of safeguarding *A. mellifera* (with the indigenous subspecies and relative local ecotypes) as a species, not in opposition to the selection work carried out by beekeepers, but in harmony with this and according to established principles of conserving bee biodiversity and the related ecosystem services linked to it.

We make this appeal with the conviction that, as regards the 2 Italian endemic subspecies, protection of *A. m. ligustica* in the peninsula as a whole and Sardinia and protection of *A. m. Sicilian* in Sicily, as part of the wildlife and natural heritage, would be not an obstacle to Italian companies breading queen bees of these subspecies. It would rather make the work of the breeders even more fruitful, as by operating within a protected area they could focus their attention on lines of selection targeted at productivity and the health of the bees used by beekeepers.

San Michele all'Adige, 12 June 2018

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