



Honey bee genotypes and the environment

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Although knowledge about honey bee geographic and genetic diversity has increased tremendously in recent decades (Meixner *et al.*, 2013), the adaptation of honey bees to their local environment has not been well studied. The current demand for high economic performance of bee colonies with desirable behavioural characteristics contributes to changing the natural diversity via mass importations and an increasing practice of queen trade and colony movement. At the same time, there is also a growing movement in opposition to this trend, aimed at conserving the natural heritage of local populations, with on-going projects in several countries (Strange *et al.*, 2008; Dall'Olio *et al.*, 2008, De la Rúa *et al.*, 2009).

Despite this, until now, no systematic comparisons of different strains of bees under standardised conditions in a range of environments have taken place. A bee that performs well in one region may not perform well in an area with different conditions, or indeed in another area with apparently similar conditions. And what do we mean by "performance" anyway, and how do we measure it?

Arguments between beekeepers and scientists may centre on the native ranges of subspecies (e.g. Carreck, 2008), whether native bees remain pure in areas where imports of bees from abroad have taken place (Bouga *et al.*, 2011), whether it is feasible to retain "pure" populations of threatened subspecies, or whether it is possible to 'protect' such populations. In addition, there are potential conflicts of interest between beekeepers keen to keep the most productive strains to maximise income from honey, and those keen to conserve and preserve diversity. But, are these factors really mutually exclusive? Are the commercial strains truly more "productive" than others when features apart from a large honey harvest are considered? Does the

improved honey production come at a cost? Might for instance commercially bred imported bees suffer more frequent winter losses, or be more prone to develop health issues from local pests and pathogens? Many of these questions are open and hot topics in other agricultural sectors: we are thus pleased to introduce this Special Issue of the *Journal of Apicultural Research*, which aims to address some of these questions through thirteen original research articles.

The honey bee research association COLOSS (Prevention of honey bee COLony LOSSes, (<http://www.coloss.org/>) was set up to study the possible causes of serious losses of honey bee colonies (Neumann and Carreck, 2010). Within this network, one Working Group (WG4, now the COLOSS Task Force on Sustainable Bee Breeding, (<http://www.beebreeding.net/>) concentrated on Genetic Diversity and Vitality. From the outset, it seemed that the interaction between genotype and environment and its impact on colony vitality might explain some of the variability in colony losses experienced in different regions. Thus, the main hypothesis was that the health of honey bee colonies cannot be understood without considering the genetic variability of honey bee populations and their adaptation to regional environmental factors, such as climate, vegetation and prevailing diseases (Meixner *et al.*, 2010). The objectives of the working group were: 1. to develop and test internationally recognised criteria for vitality; 2. to establish standardised methods to assess honey bee colonies based on these criteria, resulting in methodological and technical recommendations for breeders; 3. to investigate the role of interactions between genetic diversity and environment on honey bee colony vitality, and 4. to produce common standard protocols for characterising the subspecific variation of honey bees.

The above objectives resulted in a review of methods for discrimination of honey bee populations as applied to European beekeeping (Bouga *et al.*, 2011), two chapters of the COLOSS *BEEBOOK* (Meixner *et al.*, 2013; Büchler *et al.*, 2013) and in a review of methods assessing the quality of honey bee queens (Hatjina *et al.*, 2014).

Furthermore, an elaborate but comprehensive research plan to directly measure genotype-environment interactions in honey bees on a European scale was realised (Costa *et al.*, 2012). The results of this COLOSS Genotype-Environment Interactions experiment gave rise to six of the papers published in this issue. They reveal the existence of significant interactions between the genetic origin of the bees and the environment. A total of 621 colonies of 16 different genetic origins were set up in 21 apiaries in 11 different European countries and managed by 15 research partners. Each location housed a set of colonies of local origin, together with at least two sets of colonies of non-local origin, assumed to be less well adapted to the environmental conditions. The colonies were set up in the late summer of 2009 and were managed and evaluated according to a standard protocol used by all participants until March 2012. No chemical treatment against mites or diseases was performed to allow the expression of tolerance factors. Apart from parameters such as overwintering and colony build-up, close attention was paid to vitality parameters, such as mite infestation level, hygienic behaviour and the occurrence of other diseases.

The first of the papers describing the experiment details the genetic origin of the genotypes used in the experiment (Francis *et al.*, 2014a). Two morphometric methods, together with DNA microsatellite analysis and allozyme analysis were used, and confirmed that most of the bees used in the experiment belonged to the subspecies declared by the originating institution, but some were found to belong to a different subspecies, and a number were hybrids which could not be ascribed with confidence to any particular subspecies. Büchler *et al.* (2014) provide information on the survival of the colonies. In general, a strong interaction between genotype and environment was found, and the locally adapted bees survived better than introduced bees. Next, Meixner *et al.* (2014) describe the pests and pathogens found in the colonies: the results clearly demonstrated that apiary location had a significant and strong effect on their presence. Although in general no significant differences in disease incidence between local and non-local colonies were observed, a case study in one site in Greece (Francis *et al.*, 2014b) indicated that the level of pathogens in colonies of non-local origin was generally higher, which may be the result of poor adaptation to the local environment. Hatjina *et al.* (2014) describe the population dynamics of the colonies. It was found that both genotype and environment significantly affected colony development. Colonies in southern Europe tended to have lower adult bee populations compared to colonies in colder conditions, whilst the brood population tended to be smaller in the north, thus reflecting the shorter longevity of bees in warmer climates and the shorter brood rearing period in the north. A tendency towards specific adaptations in genotypes of local origin was observed,

especially in terms of adult bee population, honey production and overwintering ability. Uzunov *et al.* (2014a) describe the behavioural differences among the colonies. They found that overall variability among locations was higher than the variability among genotypes, but significant variability between the genotypes was also found, generally confirming the known characteristics of the subspecies they belonged to.

The conclusions from this comprehensive field experiment all tend to confirm the higher vitality of the local bees compared to the non-local ones, indicating that a more sustainable beekeeping is possible by using and breeding bees from the local populations, although the interactions are complex. This may seem logical and obvious to many bee scientists, but has not been proven on such a wide scale before. This conclusion may also come as surprise to some beekeepers who believe that queens purchased from sources outside their own region are in some way "better" than the bees they already have in their own hives. We hope that our results may provide them with additional information and entice the community to regard benefits other than the mere amount of honey produced in a season as important.

A group of five further papers then explores the genetic diversity and interactions with the environment found in different regions. Dražić *et al.* (2014) describe the results of a performance comparison of two strains of *A. m. carnica* transferred between Austria and Croatia in 1992 and 1993. Pinto *et al.* (2014) present results of a study using mitochondrial DNA and SNP techniques on populations of the Dark European honey bee *Apis mellifera mellifera* which either have been conserved by various legal or practical means, or remain without such protection in several north-western European locations. Keller *et al.* (2014) used morphometric techniques to evaluate *A. m. mellifera* populations and to predict areas where pure matings may be possible in the UK. Nedić *et al.* (2014) describe the genetic diversity of honey bees in the Serbia region using morphometry and DNA microsatellites. This region forms the border between the natural distribution of two subspecies, *A. m. carnica* and *A. m. macedonica*, but has long been used by migratory beekeepers who particularly favour *A. m. carnica*. In spite of this, in the south-east of the area bees of *A. m. macedonica* origin remain more frequent. Uzunov *et al.* (2014b) then explore the integrity of *A. m. macedonica* over its natural range within the Balkan Peninsula using DNA microsatellites. The study demonstrates that variation within the subspecies exists, but the bees appear relatively free from an influence of importations.

In contrast, Muñoz *et al.* (2014) used DNA microsatellites to study bees in the Macaronesian islands which lie off the Iberian Peninsula and North Africa. These islands historically had island-adapted populations of honey bees related to the African evolutionary lineage. However, in the past, beekeepers have imported bees to the islands of European origin (*A. m. carnica* and *A. m. ligustica*) over many years. The final paper in the issue by Zakour and Bienefeld (2014) is addressing practical matters, and sets out the criteria for establishing a honey bee breeding

programme using the example of the threatened Syrian honey bee (*Apis mellifera syriaca*).

There is now growing evidence of the adverse effects of the global trade in honey bees, which has led to the spread of novel pests and diseases such as the varroa mite and *Nosema ceranae* (Paxton, 2010; Mutinelli 2011, Fürst *et al.*, 2014). We hope that the evidence provided within the papers of this Special Issue will inspire beekeepers and scientists to explore and appreciate the value of locally bred bees, by developing and supporting breeding programmes. Damage from importations may arise from accompanying pests and pathogens, but it is also inevitable that introduced bees represent a burden to the genetic integrity of local populations. The spread of imported genes into the local population is likely, and the resulting increase in genetic diversity is not universally beneficial. Since maladapted genes will be selected against, this process may well in the short term contribute to colony losses, and in the long term, unsustainable.

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References

- BOUGA, M; ALAUX, C; BIENKOWSKA, M; BÜCHLER, R; CARRECK, N L; CAUIA, E; CHLEBO, R; DAHLE, B; DALL'OLIO, R; DE LA RÚA, P; GREGORC, A; IVANOVA, E; KENCE, A; KENCE, M; KEZIC, N; KIPRIJANOVSKA, H; KOZMUS, P; KRYGER, P; LE CONTE, Y; LODESANI, M; MURILHAS, A; SICEANU, A; SOLAND, G; UZUNOV, A; WILDE, J (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping. *Journal of Apicultural Research* 50(1): 51-84. <http://dx.doi.org/10.3896/IBRA.1.50.1.06>
- BÜCHLER, R; ANDONOV, S; BIENEFELD, K; COSTA, C; HATJINA, F; KEZIC, N; KRYGER, P; SPIVAK, M; UZUNOV, A; WILDE, J (2013) Standard methods for rearing and selection of *Apis mellifera* queens. In V Dietemann; J D Ellis; P Neumann (Eds) *The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research. Journal of Apicultural Research* 52(1): <http://dx.doi.org/10.3896/IBRA.1.52.1.07>
- 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. <http://dx.doi.org/10.3896/IBRA.1.53.2.03>
- CARRECK, N L (2008) Is the honey bee (*Apis mellifera* L.) native in the British Isles? *Journal of Apicultural Research* 47(4): 318-322. <http://dx.doi.org/10.3896/IBRA.1.47.4.15>
- COSTA, C; BERG, S; BIENKOWSKA, M; BOUGA, M; BUBALO, D; BÜCHLER, R; CHARISTOS, L; LE CONTE, Y; DRAZIC, M; DYRBA, W; FILLIPI, J; HATJINA, F; IVANOVA, E; KEZIC, N; KIPRIJANOVSKA, H; KOKINIS, M; KORPELA, S; KRYGER, P; LODESANI, M; MEIXNER, M; PANASIUK, B; PECHHACKER, H; PETROV, P; OLIVERI, E; RUOTTINEN, L; UZUNOV, A; VACCARI, G; WILDE, J (2012) A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality of honey bee colonies: methodology. *Journal of Apicultural Science* 56: 147-158. <http://dx.doi.org/10.2478/v10289-012-0015-9>
- DALL'OLIO, R; MUÑOZ, I; DE LA RÚA, P; LODESANI, M (2008) Estimating introgression in *Apis mellifera sicula* populations: are the conservation islands really effective? In *Proceedings of the Third European Conference of Apidology, Belfast UK, 8-11 September 2008*. pp 23-24.
- DE LA RÚA, P; JAFFÉ, R; DALL'OLIO, R; MUÑOZ, I; SERRANO, J (2009) Biodiversity, conservation and current threats to European honey bees. *Apidologie* 40(3): 263-284. <http://dx.doi.org/10.1051/apido/2009027>
- DRAŽIĆ, M M; FILIPI, J; PRĐUN, S; BUBALO, D; ŠPEHAR, M; CVITKOVIĆ, D; KEZIĆ, D; PECHHACKER, H; KEZIĆ, N (2014) Colony development of two Carniolan genotypes (*Apis mellifera carnica*) in relation to environment. *Journal of Apicultural Research* 53(2): 261-268. <http://dx.doi.org/10.3896/IBRA.1.53.2.07>
- 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 (2014a) Effect of genotype and environment on parasite and pathogen levels in one apiary - a case study. *Journal of Apicultural Research* 53(2): 230-232. <http://dx.doi.org/10.3896/IBRA.1.53.2.14>

- FRANCIS, R M; KRYGER, P; MEIXNER, M; BOUGA, M; IVANOVA, E; ANDONOV, S; BERG, S; BIENKOWSKA, M; BÜCHLER, R; CHARISTOS, L; COSTA, C; DYRBA, W; HATJINA, F; PANASIUK, B; PECHHACKER, H; KEZIĆ, N; KORPELA, S; LE CONTE, Y; UZUNOV, A; WILDE, J (2014b) The genetic origin of honey bee colonies used in the Genotype-Environment-Interactions experiment: a comparison of methods. *Journal of Apicultural Research* 53(2): 188-204. <http://dx.doi.org/10.3896/IBRA.1.53.2.02>
- FÜRST, M A; MCMAHON, D P; OSBORNE, J L; PAXTON, R J; BROWN, M J F (2014) Disease associations between honey bees and bumble bees as a threat to wild pollinators. *Nature* 506: 364-366. <http://dx.doi.org/10.1038/nature12977>
- 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. <http://dx.doi.org/10.3896/IBRA.1.53.2.05>
- HATJINA, F; BIENKOWSKA, M; CHARISTOS, L; CHLENO, R; COSTA, C; DRAŽIĆ, M M; FILIPI, J; GREGOREC, A; NESHOVA IVANOVA, E; KEZIC, N; KOPERNICKY, J; KRYGER, P; LODESANI, M; LOKAR, V; MLADENOVIC, M; PANASIUK, B; PAVLOV PETROV, P; RAŠIĆ, S; SMODIS SKERL, M I; VEJSNÆS, F; WILDE, J (2014) A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. *Journal of Apicultural Research* 53 (3): (in press).
- KELLER, E M; HARRIS, I; CROSS, P (2014) Identifying suitable queen rearing sites of *Apis mellifera mellifera* at a regional scale using morphometrics. *Journal of Apicultural Research* 53(2): 279-287. <http://dx.doi.org/10.3896/IBRA.1.53.2.09>
- MEIXNER, M D; COSTA, C; KRYGER, P; HATJINA, F; BOUGA, M; IVANOVA, E; BÜCHLER, R (2010) Conserving diversity and vitality for honey bee breeding. *Journal of Apicultural Research* 49(1): 85-92. <http://dx.doi.org/10.3896/IBRA.1.49.1.12>
- MEIXNER, M D; FRANCIS, R M; GAJDA, A; KRYGER, P; ANDONOV, S; UZUNOV, A; TOPOLSKA, G; COSTA, C; AMIRI, E; BERG, S; BIENKOWSKA, M; BOUGA, M; BÜCHLER, R; DYRBA, W; GURGULOVA, K; HATJINA, F; IVANOVA, E; JANES, M; KEZIC, N; KORPELA, S; LE CONTE, Y; PANASIUK, B; PECHHACKER, H; TSOKTOURIDIS, G; VACCARI, G; WILDE, J (2014) Occurrence of parasites and pathogens in honey bee colonies used in a European genotype - environment - interactions experiment. *Journal of Apicultural Research* 53(2): 215-229. <http://dx.doi.org/10.3896/IBRA.1.53.2.04>
- MEIXNER, M D; PINTO, M A; BOUGA, M; KRYGER, P; IVANOVA, E; FUCHS, S (2013) Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. In V Dietemann; J D Ellis; P Neumann (Eds) *The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research*. *Journal of Apicultural Research* 52(4): <http://dx.doi.org/10.3896/IBRA.1.52.4.05>
- MUÑOZ, I; PINTO, M I; DE LA RÚA, P (2014) Effects of queen importation on the genetic diversity of Macaronesian island honey bee populations (*Apis mellifera* Linnaeus 1758). *Journal of Apicultural Research* 53(2): 296-302. <http://dx.doi.org/10.3896/IBRA.1.53.2.11>
- MUTINELLI, F (2011) The spread of pathogens through trade in honey bees and their products (including queen bees and semen): overview and recent developments. *Scientific and Technical Review of Office International Epizooties* 30(1): 257-271.
- NEDIĆ, N; FRANCIS, R M; STANISAVLJEVIĆ, L; PIHLER, I; KEZIĆ, N; BENDIXEN, C; KRYGER, P (2014) Detecting population admixture in the honey bees of Serbia. *Journal of Apicultural Research* 53(2): 303-313. <http://dx.doi.org/10.3896/IBRA.1.53.2.12>
- NEUMANN, P; CARRECK, N L (2010) Honey bee colony losses. *Journal of Apicultural Research* 49(1): 1-6. <http://dx.doi.org/10.3896/IBRA.1.49.1.01>
- PAXTON, R J (2010) Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)? *Journal of Apicultural Research* 49(1): 80-84. <http://dx.doi.org/10.3896/IBRA.1.49.1.11>
- PINTO, M A; HENRIQUES, D; CHÁVEZ-GALARZA, J; KRYGER, P; GARNERY, L; VAN DER ZEE, R; DAHLE, B; SOLAND-RECKEWEG, G; DE LA RÚA, P; DALL' OLIO, R; CARRECK, N L; JOHNSTON, J S (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(2): 269-278. <http://dx.doi.org/10.3896/IBRA.1.53.2.08>
- STRANGE, J P; GARNERY, L; SHEPPARD, W S (2008) Morphological and molecular characterization of the Landes honey bee (*Apis mellifera* L.) ecotype for genetic conservation. *Journal of Insect Conservation* 12: 527-537.
- UZUNOV, A; COSTA, C; PANASIUK, B; MEIXNER, M D; KRYGER, P; HATJINA, F; BOUGA, M; ANDONOV, S; 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 (2014a) 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. <http://dx.doi.org/10.3896/IBRA.1.53.2.06>

- UZUNOV, A; MEIXNER, M D; KIPRIJANOVSKA, H; ANDONOV, S; GREGORC, A; IVANOVA, E; BOUGA, M; DOBI, P; BÜCHLER, R; FRANCIS, R M; KRYGER, P (2014b) Genetic structure of *Apis mellifera macedonica* in the Balkan Peninsula based on microsatellite DNA polymorphism. *Journal of Apicultural Research* 53(2): 288-295. <http://dx.doi.org/10.3896/IBRA.1.53.2.10>
- ZAKOUR, M K; BIENEFELD, K (2014) Basic considerations in the development of breeding plans for honey bee, illustrated by data on the native Syrian honey bee (*Apis mellifera syriaca*). *Journal of Apicultural Research* 53(2): 314-326. <http://dx.doi.org/10.3896/IBRA.1.53.2.13>