ORIGINAL RESEARCH ARTICLE

# The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera L.* colonies in Europe

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## Summary

The survival and performance of 597 honey bee colonies, representing five subspecies and 16 different genotypes, were comparatively studied in 20 apiaries across Europe. Started in October 2009, 15.7% of the colonies survived without any therapeutic treatment against diseases until spring 2012. The survival duration was strongly affected by environmental factors (apiary effects) and, to a lesser degree, by the genotypes and origin of queens. Varroa was identified as a main cause of losses (38.4%), followed by queen problems (16.9%) and *Nosema* infection (7.3%). On average, colonies with queens from local origin survived 83 days longer compared to non-local origins (p < 0.001). This result demonstrates strong genotype by environment interactions. Consequently, the conservation of bee diversity and the support of local breeding activities must be prioritised in order to prevent colony losses, to optimize a sustainable productivity and to enable a continuous adaptation to environmental changes.

## La influencia del origen genético y su interacción con los efectos del medio ambiente en la supervivencia de las colonias de *Apis mellifera L.* en Europa

### Resumen

La supervivencia y el rendimiento de 597 colonias de abejas, representando cinco subespecies y 16 genotipos distintos, se estudiaron comparativamente en 20 apiarios en Europa. Iniciado en Octubre de 2009, el 15.7% de las colonia sobrevivieron sin ningún tratamiento

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# Introduction

Honey bees (*Apis mellifera* L.) represent an infrequent case of a single species naturally present across a wide range of latitudes. To successfully survive in the wide range of habitats where they naturally occur, as a result of the natural evolutionary process, honey bees have developed into many different geographical subspecies and into a wide variation of ecotypes (Ruttner, 1988; Whitfield *et al.*, 2006; De la Rúa *et al.*, 2009; Le Conte and Navajas, 2008; Meixner *et al.*, 2010). The honey bee sub-species are also described as 'geographic sub-species' since their distributions correspond to distinct geographic areas. Even within Europe, there are a wide range of climatic and vegetation zones which favoured differentiation, and at present about 10 subspecies of *A. mellifera* are recognized on the basis of morphometric and genetic markers (De la Rúa *et al.*, 2009).

The present distribution of the European honey bee subspecies has mainly been influenced by the last glaciation, when the mountain chains of the Pyrenees, the Alps and the Balkans acted as geographic barriers in maintaining isolation of the honey bee populations occurring on either side (Ruttner, 1988). Some of these subspecies have been found to be more attractive than others for beekeeping. This, as an economic and social activity plays a crucial role in the sustainable development of rural areas by providing important ecosystem services via pollination, thus contributing to the improvement of biodiversity of plants and agricultural crops (EU Parliament Report, 2011). However, in the last decade a decrease in the number of honey bee colonies has been reported in both the EU and other parts of the world (EFSA, 2008; van der Zee *et al.*, 2012, 2014; van Engelsdorp *et al.*, 2012, Spleen *et al.*, 2013; Steinhauer *et al.*, 2014). Recent research aiming to identify the factors involved in colony losses has mostly focused on diseases and parasites, most prominently the mite *Varroa destructor*, virus diseases, and the microsporidian *Nosema* spp. (Higes *et al.*, 2006; Cox-Foster *et al.*, 2007; Johnson *et al.*, 2009; de Miranda and Genersch, 2010; de Miranda *et al.*, 2010; Rosenkranz *et al.*, 2010). Another important factor shown to contribute to colony decline is the extended use of pesticides in agricultural systems (Desneux *et al.*,

2007; Frazier *et al.*, 2008; van Engelsdorp *et al.*, 2009; Chauzat *et al.*, 2009; van Engelsdorp and Meixner, 2010), and socio-political aspects such as trade or economics (van Engelsdorp and Meixner, 2010).

An understanding of the genetic variability of bee populations and their adaptation to regional environmental factors such as climate and vegetation, prevailing diseases and agricultural practices, is an important prerequisite for understanding problems in the health of honey bee colonies. It is widely accepted that extended and rapid changes in agricultural land use and climate have increased the adaptive pressure on local bee populations dramatically. Although honey bees, in contrast to other livestock, are only semi-domesticated, their populations in Europe have nonetheless been seriously affected by human activities (Moritz *et al.*, 2005). Introgressive hybridization modifies the genetic pool of local honey bee populations, leading to the loss of their genetic identity. Intensive queen breeding, use of imported queens and migratory beekeeping over long distances have also caused hybridization of local bee populations in many areas (De la Rúa *et al.*, 2009; Meixner *et al.*, 2010) and the extent to which these factors have affected the vitality of honey bee colonies is unknown.

We do know that distinct genotypes may vary in the degree to which their phenotypes are affected by specific environmental conditions. This phenomenon is known as "genotype-by-environment interactions" (GEI). Presence of the GEI indicates that the phenotypic expression of one genotype may be superior to another genotype in one environment but inferior in another environment (Falconer and Mackay, 1996). Genotype-environment interactions are known to occur in many organisms (plants and animals) and this concept has been applied to the study of different quantitative traits such as longevity (Vieira *et al.*, 2000), immunity and fecundity (Lazzaro *et al.*, 2008), and productivity (Hammami *et al.*, 2009). As a consequence, knowledge of the genotype-by-environment interactions is an increasing demand in livestock breeding programmes (Mulder and Bijma, 2012). In honey bees, it has been demonstrated that the colony development characteristics of different ecotypes may vary in regard to the floral availability of the environment (Louveaux *et al*, 1966).

A recent study indicated the presence of GEI in Italian honey bee

populations from different areas of origin on colony development and honey productivity (Costa *et al.*, 2012a).

Adaptation to local abiotic environment and GEI may also maintain genetic variation for resistance to infections. Observations about local strains of honey bees that are apparently less affected by losses and have better strategies to cope with varroa have been reported from different regions (Fries *et al.*, 2006; Le Conte *et al.*, 2007).

The aim of this study was to investigate the effects of GEI on the survival of honey bee colonies headed by queens originating from several areas in Europe and tested in a standardised way in various locations under differing environmental conditions. We refer to them as "genotypes" or "strains" as defined by the regional source of the queens and by the declared affiliation to a certain subspecies. A detailed analysis of the genetic constitution of the strains tested in the experiment is presented by Francis *et al.* (2014).

# Material and methods

## Field experiment

A total of 621 honey bee colonies, representing 16 different genotypes (Table 1), were set up in 21 apiaries across Europe in late summer of 2009. However, one apiary with 24 hives (Toulouse) could not be tracked until the end of the experiment and was therefore excluded from all evaluations. Most of the genotypes represent commercially successful strains selected for favourable traits such as high productivity and gentleness, while some genotypes were chosen to represent naturally selected or endangered populations. In each location, the local genotype was tested together with at least two non-local genotypes, with a minimum starting number of 10 colonies per origin (for details see also Fig. 1 in Francis *et al.* (2014)).

The colonies were started as package bees or nuclei and initially treated against *V. destructor*, ensuring uniform starting conditions in terms of strength and infestation level of all colonies within each location. Queens were produced either by the partner institutes or commercial partners and delivered by hand or express mail to the allocated partners. Queen introduction was completed on 1 October 2009 which was therefore defined as starting date for the survival test.

The colonies were managed by the partner institutes according to a standardised common protocol until 31 March 2012 and were not further treated with chemical substances for the control of *V. destructor* or other diseases. Colony and queen survival were recorded at least three times a year (in spring, summer and autumn) together with other traits and parameters. However, for most of the apiaries a much higher frequency of colony inspections was achieved. Full details of the experimental set up and data collection are presented in Costa *et al.* (2012b).

In some locations, colonies were removed from the experiment and considered collapsed when *V. destructor* infestation level in adult bees was higher than 10%, or when the number of adult bees in the colony was lower than 5000. Queenless colonies were also considered
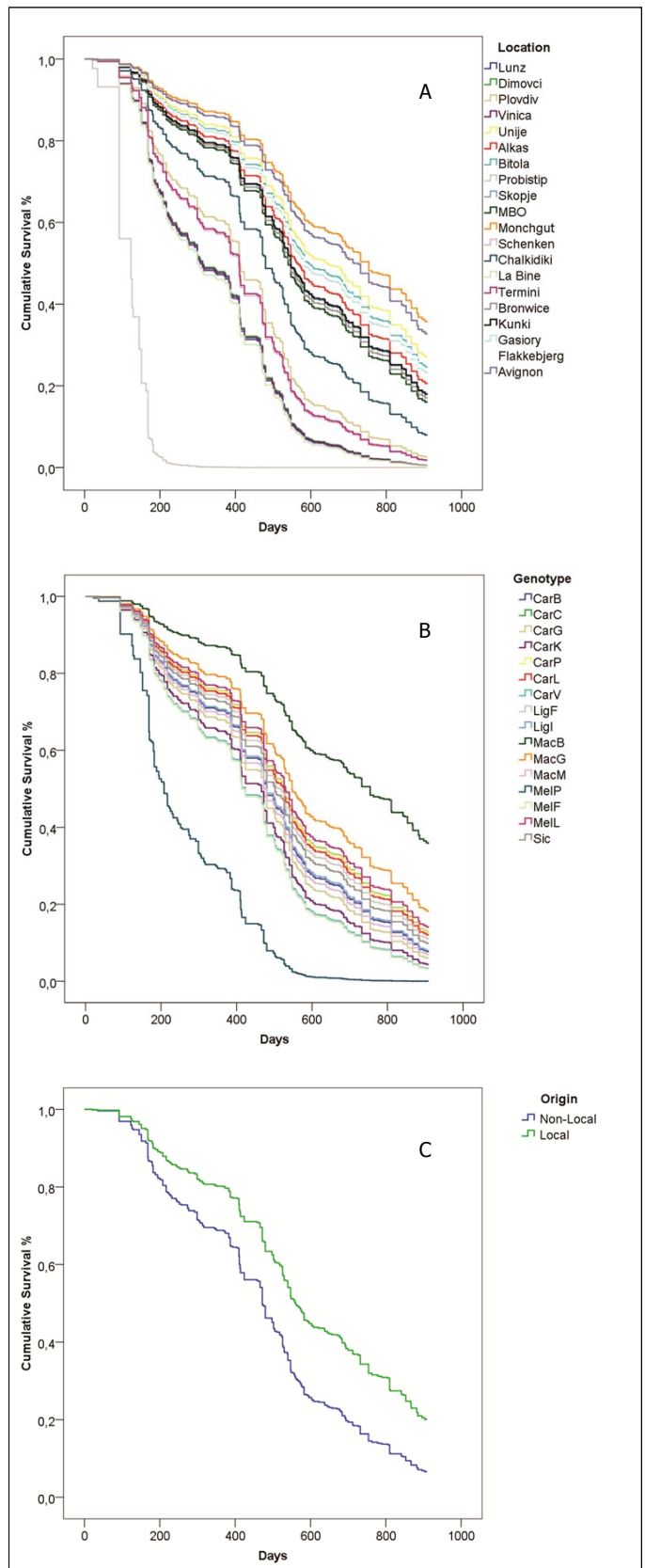


**Fig. 1.** Trajectories of colony survival for the different locations (**A**), genotypes (**B**) and the origin of queens (**C**) during the experiment.

collapsed if there was no natural supersedure. When colonies collapsed, the presumed cause of death was noted and classified based on analysis of previously collected samples and / or easily detectable symptoms (full data on *V. destructor*, *Nosema* spp. and virus infections are

**Table 1.** Details of the genetic origins used in the experiment.

| Subspecies | Strain | Country of origin | Abbreviation | Local strain in: |
|---|---|---|---|---|
| *A. m. carnica* | Bantin | Germany | CarB | Mönchgut |
| *A. m. carnica* | Croatia | Croatia | CarC | Unije |
| *A. m. carnica* | GR1 | Poland | CarG | Kunki |
| *A. m. carnica* | Kirchhain | Germany | CarK | Kirchhain |
| *A. m. carnica* | Kortowka | Poland | CarP | Bronowice; Gasiory |
| *A. m. carnica* | Lunz | Austria | CarL | Lunz |
| *A. m. carnica* | Veitshöchheim | Germany | CarV | Schenkenturm |
| *A. m. ligustica* | Finland | Finland | LigF | Äikäs |
| *A. m. ligustica* | Emilia-Romagna | Italy | LigI | Le Bine, Mantova |
| *A. m. macedonica* | Bulgaria | Bulgaria | MacB | Dimovci; Plovdiv; Vinica |
| *A. m. macedonica* | Greece | Greece | MacG | Chalkidiki |
| *A. m. macedonica* | Macedonia | Republic of Macedonia | MacM | Bitola; Probistip; Skopje |
| *A. m. mellifera* | Augustowska | Poland | MelP | Kunki |
| *A. m. mellifera* | Avignon | France | MelF | Avignon; Toulouse |
| *A. m. mellifera* | Læsø | Denmark | MelL | Flakkebjerg |
| *A. m. siciliana* | Sicily | Italy | Sic | Termini Imerese |

reported by Meixner *et al.*, 2014). The classes were: varroa, nosema, queen causes (queenlessness, drone laying queen, swarming without successful queen replacement, etc.), other (like American foulbrood, weakness, starvation, winter loss, robbing, apitechnical reasons), and unknown.

## Data analysis

Differences in colony survival were assessed by using the specific Survival Analyses function. For these analyses 1 October 2009 was used as a common starting date, identified as the earliest date where all colonies, at all locations, were established and populated by the experimental queens' progeny. For a general overview, the survival of the local genetic origin in each location was compared with the survival of non-local origins.

The survival analysis was performed with a Cox proportional hazard model (Cox, 1972). The hazard function or colony loss rate is the instantaneous probability of death for colonies still alive. The Cox model assumes that the individual hazard function depends on a common baseline hazard and the values of the covariates. Given two colonies with particular values for time-independent covariates, the ratio of the estimated hazards over time is supposed to be constant overtime.

The colony hazard functions are proportional to a common baseline hazard function. Based on pre-runs, the model used in analysis considered location of apiary (n = 20), genotype (n = 16) and origin of breed (local and non-local) as factors that significantly influenced the colony longevity.

In a second stage, General Linear Model (GLM) was used to examine statistical differences within each of the factors used in survival analysis. Differences among groups were assessed by applying post-hoc analysis using Tukey (HSD) test. All analyses were performed with SPSS software package, release 19.0.0.

**Table 2.** The effect of location (n = 20), genotype (n = 16) and origin of queen (local vs. non local) on longevity of colonies (n = 597) evaluated in survival analysis.

| Factors | Wald | df | p-value |
|---|---|---|---|
| Location | 165,1 | 19 | 0,000 |
| Genotype | 55,2 | 15 | 0,000 |
| Origin | 19,5 | 1 | 0,000 |

# Results

Of the 597 observed colonies, 94 (15.7%) survived until the end of the observation period (1 March 2012). The survival analysis showed highly significant effects of the test location (n = 20), the genotype (n = 16) and the origin of queen (local *vs.* non-local) on the longevity of colonies (Table 2). The final estimates of the survival curves are depicted in Fig. 1, where the differentiations within factors are presented.

The data were also analysed for the relevance of macro-effects and were thus grouped according to subspecies (n = 5) instead of genotype, region (Continental *vs.* Mediterranean) and weather clusters (n = 6; see Hatjina *et al.*, 2014) instead of location, and management factors (brood removal). However, none of these factors showed a significant influence on survival duration. The GLM analysis confirms

**Table 3.** GLM analysis of colony survival in days. a. R Squared = 0.829 (Adjusted R Squared = 0.818).

| Source | df | Mean Square | F | Sig. |
|---|---|---|---|---|
| Model | 36 | 4984582.7 | 75.5 | 0.000 |
| Location | 19 | 401200.9 | 6.1 | 0.000 |
| Genotype | 15 | 217437.6 | 3.3 | 0.000 |
| Origin | 1 | 826253.8 | 12.5 | 0.001 |
| Error | 561 | 66015.7 | | |
| Total | 597 | | | |

**Table 4.** Mean survival duration in days and standard error (SE) in different locations, adjusted for the effects of genotype and origin.

| Location | Mean ± SE |
|---|---|
| Lunz (A) | 342 ± 55 |
| Dimovci (BL) | 424 ± 70 |
| Plovdiv (BL) | 432 ± 82 |
| Vinica (BL) | 398 ± 76 |
| Unije (HR) | 619 ± 39 |
| Äikäs (FIN) | 619 ± 48 |
| Bitola (MK | 605 ± 86 |
| Probistip (MK) | 80 ± 86 |
| Skopje (MK) | 613 ± 86 |
| Kirchhain (D) | 597 ± 57 |
| Mönchgut (D) | 661 ± 58 |
| Schenkenturm (D) | 413 ± 58 |
| Chalkidiki (GR) | 536 ± 48 |
| Le Bine Mantova (I) | 314 ± 58 |
| Termini Imerese (I) | 434 ± 59 |
| Bronowice (PL) | 538 ± 48 |
| Kunki (PL) | 569 ± 50 |
| Gasiory (PL) | 646 ± 47 |
| Flakkebjerg (DK) | 688 ± 72 |
| Avignon (F) | 711 ± 62 |

**Table 5.** Mean survival duration in days and standard error (SE) in different genotypes, adjusted for the effects of origin and location.

| Genotype | Mean ± SE |
|---|---|
| CarB | 543 ± 58 |
| CarC | 555 ± 47 |
| CarG | 512 ± 57 |
| CarK | 460 ± 56 |
| CarP | 554 ± 37 |
| CarL | 635 ± 46 |
| CarV | 500 ± 56 |
| LigF | 513 ± 53 |
| LigI | 489 ± 59 |
| MacB | 659 ± 49 |
| MacG | 599 ± 45 |
| MacM | 560 ± 49 |
| MelP | 194 ± 68 |
| MelF | 481 ± 62 |
| MelL | 428 ± 80 |
| Sic | 509 ± 62 |

**Table 6.** Mean survival duration in days of local and non-local test colonies, adjusted for the effects of genotype and location.

| Origin | Mean ± SE |
|---|---|
| Local | 553 ± 21 |
| Non-local | 470 ± 16 |

the significant effect of the considered factors (Table 3). Adjusted means of the survival duration for the test locations, the genotypes and the origins are presented in tables 4, 5 and 6.

**Table 7.** Case example: mean survival duration of genotype MacG in the various locations in which it was tested, and the difference from each location mean.

| Location | Mean Mac G | Mean of Location | Difference |
|---|---|---|---|
| Dimovci | 399 | 424 | -25 |
| Plovdiv | 437 | 432 | 5 |
| Vinica | 287 | 398 | -111 |
| Äikäs | 588 | 619 | -31 |
| Bitola | 664 | 605 | 59 |
| Probistip | 128 | 80 | 48 |
| Skopje | 653 | 613 | 40 |
| Chalkidiki | 860 | 536 | 324 |

Much of the variability in the survival of colonies is connected to the test locations, actually representing differences in climate, vegetation, infestation pressure, and colony management. The average survival period ranged from 80 days for the test location "Probistip" in Macedonia, where all colonies were lost during the first winter period, to 711 days for the test location "Avignon", located in France. The differences between some of the locations were statistically significant.

The course of survival and the adjusted mean survival duration are quite similar for most of the genotypes, but a wide range of differences was observed between strains. The shortest observed average survival period was 194 days for the genotype MelP, while the longest was 659 days for the genotype MacB. A pair-wise comparison showed that the survival duration of the MelP genotype was significantly shorter than that of most other genotypes.

When survival of colonies with queens of the local strain were compared to colonies with queens of non-local origin throughout the whole test area, it was observed that colonies with local queens survived on average 83 ± 23 days (p < 0.001) longer than those with non-local queens. As a case example, we present the details of survival duration of the genotype MacG (*A. m. macedonica* from Greece) which was tested in its area of origin (Chalkidiki, Greece), but also in Bulgaria, in Macedonia and in Finland. The highest survival rate was observed in Greece, both in absolute terms (adjusted mean survival duration 860 days) and in relative terms, expressed as the difference between the mean survival of the MacG genotype at each of these locations and the mean survival of all genotypes at the same location (+ 324 days) (Table 7).

The main cause of loss reported for the 503 collapsed colonies was "varroa" (38.4% of losses), followed by "queen problems" (16.9%) and "nosema" (7.3%). "Other causes", including weakness, starvation, not further specified winter losses, robbing etc., were reported for 33.8% of losses, and 3.4% remained unknown. The survival duration depended on the cause of loss. Nosema losses mainly occurred in the early phase while losses due to varroa dominated in the late phase of the experiment (Meixner *et al.*, 2014).

## Discussion

Several reports from various regions of Europe document the management and survival of honey bee colonies in absence of control treatment against *V. destructor* (Berg *et al*. 2001; Büchler *et al.*, 2002; Kefuss *et al.*, 2004; Fries *et al.*, 2006; Le Conte *et al.*, 2007). For example, in non-treated populations in the South of France, mortality varied between 9.7% and 16.8% per year over a seven year period (Le Conte *et al.*, 2007) while in the non-treated population on the island of Gotland in Sweden, winter mortality rate was 76% in the third year (Fries *et al.*, 2006). In comparison to these studies, the number of surviving colonies in our two and a half year study (15%) appears slightly lower than expected.

We have to consider that the French project (Le Conte *et al.*, 2007) was started from colonies which had already been reported to have survived for some time without control treatment against *V. destructor*. This was not the case in our study, since we used mostly standard commercial stock. Also, in contrast to our study, the Gotland population (Fries *et al.*, 2006) was isolated, thus favouring the development of host-parasite balance and removing possible reinfestation with mites from neighbouring apiaries. Another factor to consider is the level of colony losses throughout Europe during the test period observed in colonies subject to normal management (i.e. treated against *V. destructor*): a questionnaire-based survey involving 19 European countries reported mean honey bee winter losses across Europe between 7 and 30% in the winter of 2009/2010, showing that high levels of colony losses may occur even under regular management (van der Zee *et al.*, 2012).

Although our colonies were not treated with acaricides of any kind, in some apiaries the biotechnical method of brood removal (Büchler and Meixner, 2008) was applied; furthermore, some of the colonies swarmed. Although neither of these factors was found to be statistically significant, both may have contributed to extending the duration of survival. Indeed, swarming has been shown to have an effect on mite infestation levels and, consequently, on colony survival (Fries *et al.*, 2003).

Our study highlighted a wide range of variability of survival duration in the different test locations. This is in good accordance with several reports from national monitoring programmes of colony losses (Mutinelli *et al.*, 2010; Van der Zee *et al.*, 2012, 2014; Spleen *et al.*, 2013; Steinhauer *et al.*, 2014; Van Engelsdorp *et al.*, 2012). Besides climatic effects, which directly influence the dynamics of colony development (Hatjina *et al.*, 2014) and are also a known factor in regard to parasites and diseases (de Guzman *et al.*, 1996; Meixner *et al.*, 2014), the availability of nectar and pollen resources and the pathogen abundance in the area can be regarded as major causes. Furthermore, most of these factors are directly or indirectly influenced by local beekeeping practices such as colony density, migration, disease treatment etc.

The observed effects of genotypes on mean survival duration underline the importance of genetic features and selection for the occurrence of colony losses. However, due to the unbalanced distribution of genotypes over locations in this large scale experiment, some genotypes may have been biased by unfavourable locations. In addition, not all of the stocks had a similar background of breeding conditions: some genotypes consisted of sister groups of queens from commercial selected lines with rigorous mating control, whilst others were from less intensely selected populations with a lower relatedness within the strain. Furthermore, breeding techniques and methods differ across Europe (Bouga *et al.*, 2011). We can thus assume that together with its genetic origin, each strain also represents the development of queen rearing methods specific to that stock / subspecies / country.

Another factor which should be taken into consideration is the size of the breeding populations available in different genotypes: small populations, such as those of the *A. m. mellifera* genotypes, may have been negatively influenced by low genetic variability, which is known to adversely affect colony fitness and its ability to cope with diseases and stress factors (Tarpy, 2003; Jones *et al.*, 2004; Mattila and Seeley, 2007).

Ecological studies have shown that genotype × environment interaction is an indicator for local adaptation and fitness. The most important form of this interaction is antagonistic pleiotropy, whereby different alleles have opposite effects on fitness in different habitats. This implies that no single genotype is superior in all environments, leading to a trade-off in adaptation to different habitats (Fry, 1993). Nonetheless, many fitness-related characters, likely to play a role in local adaptation, show polygenic variation, as is likely the case in the honey bee colony, where many individuals and many traits are involved in determining colony fitness. Bienefeld and Pirchner (1991) stated that most colony traits are composed of queen as well as worker effects, and that those effects are mostly negatively correlated. In the meantime, a model has been developed which considers both queen and worker effects on economically relevant traits of the honey bee as well as the genetic correlations between different traits in the genetic evaluation of colony performance (Bienefeld *et al.*, 2007). In contrast to single-locus models, the theory of polygenic traits under divergent selection is relatively unexplored (Gillespie and Turelli, 1989). Most of the theory relevant for local adaptation concentrates on the evolution of ecological specialization, assuming a trade-off in fitness across habitats mediated by a quantitative trait or traits (reviewed in Futuyma and Moreno, 1988; Jaenike and Holt, 1991; Fry, 1996). In simple words, local adaptation should result in improved fitness of each population in its own habitat (Kawecki and Ebert, 2004).

Due to the high number of genotypes and test locations, we could not realize a balanced distribution suitable for a complete analysis of genotype - environment interactions in our study. On the other hand, it must be noted that the "reciprocal transplant" (an experiment by which genotypes from the local and non-local populations are directly

compared under the same environmental conditions) which we attempted is known to be a method for demonstrating the pattern of local adaptation which is often impossible for practical, ethical or legal reasons (Kawecki and Ebert, 2004). Thus, we follow the indication of Kawecki and Ebert (2004) according to which the 'local *vs.* foreign' criterion should be regarded as diagnostic for the pattern of local adaptation. This criterion is directly relevant to the driving force of local adaptation, divergent natural selection, which acts on genetic differences in relative fitness within each habitat. In our study, we observed a significantly higher survivorship of the local genotypes compared to the non-local ones, clearly indicating a specific local adaptation of the honey bee populations considered in the experiment. The specific factors which are probably involved, and which were assessed in the course of the experiment (disease susceptibility, colony development, behavioural traits) are described by Meixner *et al.* (2014); Hatjina *et al*. (2014); Uzunov *et al*. (2014). One specific factor which may have contributed to the higher survival of the local stocks is the adaptation of the apiary and colony management to the local strains, which may have provided them with an advantage. However, we have no way of measuring this, and we can state that survival of the colony represents the sum of all the involved factors, and ultimately, it is this outcome which has both an ecological and a commercial importance.

The conservation of native European honey bees should be a pressing priority, given that the survival of honey bees in their natural habitats is ultimately determined by the unique behavioural and morphological traits exhibited by native subspecies which constitute important reservoirs of local adaptations (Randi, 2008). The predicted climatic changes together with the  growing demand for food and energy production on limited farmlands will most likely result in new challenges for European honey bee populations within the near future. Conservation of genetic diversity is therefore an essential precaution in order to preserve a high genetic adaptability of European honey bee populations. Furthermore, the results of our study show that it is not merely an ecological issue, but also a commercial one: the use of local honey bee populations provides a higher chance of colony survival, and the use of maladapted bees attributes to high colony losses, as recently observed in many regions. Thus, local breeding activities should be promoted and encouraged throughout the native range of *Apis mellifera*.

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