

ORIGINAL RESEARCH ARTICLE



Population dynamics of European honey bee genotypes under different environmental conditions

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Summary

Adaptation of honey bees to their environment is expressed by the annual development pattern of the colony, the balance with food sources and the host - parasite balance, all of which interact among each other with changes in the environment. In the present study, we analyse the development patterns over a period of two years in colonies belonging to 16 different genotypes and placed in areas grouped within six environmental clusters across Europe. The colonies were maintained with no chemical treatment against varroa mites. The aim of the study was to investigate the presence of genotype - environment interactions and their effects on colony development, which we use in this study as a measure of their vitality. We found that colonies placed in Southern Europe tend to have lower adult bee populations compared to colonies placed in colder conditions, while the brood population tends 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. We found that both genotype and environment significantly affect colony development, and that specific adaptations exist, especially in terms of adult bee population and overwintering ability.

Dinámica poblacional de los genotipos de abejas europeas en diferentes condiciones ambientales

Resumen

La adaptación de las abejas melíferas a su entorno se expresa por el patrón anual de desarrollo de la colonia, el equilibrio con las fuentes de alimentos y el equilibrio parásito-hospedador, todos los cuales interactúan entre sí con los cambios en el medio ambiente. En el presente estudio, se analizan los patrones de desarrollo en un período de dos años en las colonias pertenecientes a 16 genotipos diferentes colocadas en áreas agrupadas en seis grupos ambientales por Europa. Las colonias se mantuvieron sin tratamiento químico contra el ácaro varroa. El objetivo del estudio fue investigar la presencia de interacciones genotipo - medio ambiente y sus efectos sobre el desarrollo de colonias, que fueron utilizadas en este estudio como una medida de su vitalidad. Encontramos que las colonias situadas en el sur de Europa tienden a tener poblaciones de abejas adultas menores en comparación con las colonias situadas en condiciones más frías, mientras que la población de cría tiende a ser menor en el Norte, lo que refleja la menor longevidad de las abejas en los climas más cálidos y el periodo más corto de cría en el Norte. Se encontró que tanto el genotipo como el ambiente afectan de manera significativa al desarrollo de la colonia, y que existen adaptaciones específicas sobre todo en términos de la población de abejas adultas y la capacidad de invernar.

Keywords: COLOSS, Genotype-Environment Interactions Experiment, *Apis mellifera* L., honey bee, population, development

Introduction

Honey bees (*Apis mellifera* L.) are increasingly in demand as pollinators for various key agricultural food crops, but globally their populations are in decline, and honey bee colony failure rates have increased (EFSA, 2008; van der Zee *et al.*, 2012, 2014; Spleen *et al.*, 2013; Steinhauer *et al.*, 2014; VanEngelsdorp *et al.*, 2012). There is now consensus among scientists that the causes for these colony losses are multi-factorial, with the major culprits being identified as diseases and parasites (Higes *et al.*, 2006; Cox-Foster *et al.*, 2007; de Miranda and Genersch, 2010; de Miranda *et al.*, 2010; Johnson *et al.*, 2009; Genersch *et al.*, 2010; Nazi *et al.*, 2013; Neumann and Carreck, 2010), the use of pesticides (Desneux *et al.*, 2007; Di Prisco *et al.*, 2013; Hatjina *et al.*, 2013; Nguyen *et al.*, 2009; Frazier *et al.*, 2008; vanEngelsdorp *et al.*, 2009; Chauzat *et al.*, 2009) and changes in land use (Foley *et al.*, 2005; Kremen *et al.*, 2007; Bartomeus *et al.*, 2013). Our working hypothesis was that loss of adaptation to local environment may also play a role in reducing colony survival by decreasing genetic variation for resistance to infections and other stressors (Meixner *et al.*, 2010).

The development of a honey bee colony is the result of a wide range of physiological and behavioural changes, which start from the individual bee level and then reflect on the whole colony. A single worker bee starts its existence as an egg, goes through a series of larval and pupal stages, and emerges as an adult 21 days later, with some variation (from a minimum of 16 days to a maximum of 24) (Winston, 1987) due to external factors, mainly temperature and nutrition, and to genotype (for example, bees of African descent have a shorter development time). The life span of the worker bee is mainly influenced by the season: the general pattern in temperate climates is that worker bees are short-lived in summer and long-lived in winter.

The longevity of summer bees ranges from 15 to 38 days, while the mean longevity for a winter bee is 140 days, with peaks of up to 320 days (Farrar, 1937; Sakagami and Fukuda, 1968; Winston, 1979).

Intermediate longevities have been observed for spring and autumn bees. The longevity of worker bees may also be strongly influenced by health status, as many pathogens (such as *Varroa destructor*, *Nosema* spp.) are known to shorten their lifespan (Malone and Gatehouse, 1998; Downey and Winston, 2001). The longevity of individual bees is one of the factors that affect the size of a colony throughout the season: the number of adult bees in a colony plays a role on the amount of brood that can be reared, in turn, the adult bee population and the brood interact via pheromones with a feedback system which regulates colony functions according to its need (reviewed by Bortolotti and Costa, 2014).

The size of the colony population (the amount of brood and the number of adult bees) and its interaction with the environment around it, determine the amount of food (nectar and pollen), which is collected, and the amount of food which is consumed. In other terms, the ability of a colony to make the most of the available floral resources, defined in apicultural terms as the productivity of a colony (honey yield and annual food balance), is related to the adult bee population force and to the annual cycle of the colony.

On the other hand, food availability and diseases may impose limits on colony development. Adaptation of honey bees to their environment is expressed by the annual development pattern, the balance with food sources and the host – parasite balance, all of which interact among each other and with changes in the environment. The honey bee colony shows a wide range of developmental patterns, which correspond to the wide range of *Apis mellifera's* geographical distribution. Availability of food sources and the length of the active flying season are probably some of the most important environmental

factors affecting the dynamics of population growth. It is known that honey bees are not active when the outside temperature is below 10°C and when it rains, and that activity decreases with high temperatures (above 30°C) (Heinrich, 1996).

Apiculturists long ago realized that knowledge of the colony's population dynamics could be an important tool for understanding its functions, and to make choices in beekeeping. A booklet from the end of the 19th century described "the basic law of brood and colony development", under the assumption that such information was essential for anyone wishing to keep bees (Gerstung, 1890). Since then, many bee scientists have recognized the truth of this, and many studies have investigated how colony population size affects colony growth, behaviour, and survivorship. To perform these studies, different ways of assessing colony population size have been used and are extensively reviewed in Imdorf *et al.* (2011) and Delaplane *et al.* (2013). Models have also been constructed for estimating the population and brood size of a colony based on actual data (Harris, 1985). The size or "strength" of a colony is greatly influenced by geographical factors (such as latitude and altitude), by the quality and amount of pollen and nectar producing flora, and by its genotype, and has been reported to vary from a maximum population of 60,000 thousand bees (Farrar, 1937) to just a few thousand bees in an overwintering colony (Harbo, 1986). In a temperate climate, the population is typically at its lowest during the winter and then grows rapidly in the spring leading to a peak in size at the beginning of the summer, followed by a gradual reduction through the rest of summer and autumn into the winter. This annual development pattern is determined to a greater extent by the environment, but several studies have shown that the genetic makeup of the colony also has an influence on the dynamic of its development (Louveaux, 1966; Costa *et al.*, 2012a; Uzunov, 2013). For example, African colonies respond more rapidly with increased brood rearing when foraging conditions become favourable (Rinderer and Hellmich, 1991) when compared to honey bees from temperate climates.

Population growth is the best predictor of a colony's ability to survive over the winter and to reproduce by swarming (Michener, 1964; Winston, 1979, 1980; Winston *et al.*, 1981; Seeley and Visscher, 1985; Lee and Winston, 1985, 1987; Harris, 2010). The ability to store honey, which is the basis of the survival of the honey bee colony during winter, shows natural variation among and within honey bee populations, and has also represented the main selection trait even in the simplest breeding programmes (Bar-Cohen *et al.*, 1978; Guzman-Novoa and Page, 1999). The environmental conditions that allow a honey bee colony to be active are of great importance when we consider the colony productivity in terms of population, as well as of collected food.

Thus, long-term adaptations express suitable population dynamics of the bee colony, which enable the colony to make the most of the available resources and to successfully resist threats like unfavourable seasonal living conditions (Parker *et al.*, 2010), disease and parasite

pressure (Fries *et al.*, 2006; Le Conte *et al.*, 2007). Adaptations can be recognised by genotype – environment interactions (GEI), in which distinct genotypes vary in the degree to which their phenotypes are affected by environmental conditions (Falconer and Mackay, 1996). GEI 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.*, 2008). To the plant or animal breeder, GEI have in the past represented a problem, for they limit the application of results from varietal or performance tests, as one genotype may perform better than another in a first environment but worse in a second (Burdon, 1977). In honey bees, a few studies have found GEI at the colony level: Louveaux *et al.* (1966), showed that different ecotypes of honey bee colonies maintain the adaptation to the annual cycle of floral availability of their native environment when moved out of it; Recently, similar findings were reported by Uzunov (2013) for two genotypes of *A. m. macedonica*, by Charistos (2013) for three genotypes of *A. m. macedonica* and a genotype of *A. m. cecropia* which maintained their annual colony developmental trajectories in non-local conditions and by Rasic (2013) for 4 genotypes of *A.m. carnica*. Costa *et al.* (2012a) suggest the presence of GEI in Italian honey bee populations when considering their spring development and honey production.

Following these reports, the aim of this study was to comprehensively investigate the effects of genotype, of the environment, and the interaction of the two factors, on the colony development of different European honey bee genotypes, thereby gaining further insight into the complex process of adaptation. We included 16 different genotypes coming from different backgrounds (some from breeding programmes with strong focus on specific traits, others from conservation programmes with little selection) in the experiment and tested their development and performance in different environments, represented by 21 locations in 11 countries across Europe.

Material and method

Honey bee genotypes and locations

The experiment was set up in the late summer of 2009 and ran until March 2012. It included 597 colonies from 16 different genetic origins belonging to five *Apis mellifera* subspecies (*carnica*, *ligustica*, *macedonica*, *mellifera*, *siciliana*), located in 20 apiaries/ locations, distributed in 11 European countries, ranging from Scandinavia to the Mediterranean, across Central Europe and the Balkans (see Table 1 in Büchler *et al.*, 2014). A detailed map showing the distribution of genotypes at the experimental locations across Europe is shown in Francis *et al.* (2014). At each location the local strain of bees was tested together with at least two "foreign" origins. No chemical treatments against varroa or other pathogens were applied during the experiment. A detailed

description of the distribution of the strains across the locations can be found in Costa *et al.* (2012b) and is graphically depicted in Francis *et al.* (2014).

Environmental conditions

In the experimental set up, each location represented not just a geographic area, but a sum of characteristics, related to local environmental conditions, management practice, management techniques, influence from neighbouring apiaries, flowering plants etc. Given the above, areas with similar environmental conditions suitable for bee activity might have the same impact on colony development and production. Therefore, in this study we considered the different locations with similar environmental conditions, as clusters of similar climatic conditions, as we assume that food availability can influence both the number of brood successfully reared to adulthood, therefore population and the time of the year the population will reach its maximum.

Meteorological data (mean, minimum and maximum daily temperature, rain fall and humidity) were collected for the experimental locations from local meteorological stations. The weather parameters for the year 2010 were used for statistical analysis (for this year we had complete data for all locations). Daily temperatures (average, minimal, maximal) and days with rain were used to obtain for each location the number of days with minimum temperature below 0°C, maximum temperature above 30°C, average temperature below and above 10°C, and number of days with rainfall for each location. These data together with average annual temperatures and latitude positions of the locations were used as dataset in order to group the locations with similar environmental conditions. Locations were clustered by Ward's minimum variance method using *proc* CLUSTER, and a dendrogram was produced by *proc* TREE (SAS, 2009). Following cluster-analysis, the 20 different locations were grouped into six distinct clusters (Fig. 1) and the average values for each location as well as for each cluster are given in Table 1. The six environmental clusters were named according to length of the active season (defined on the basis of average number of days with temperatures above 10°C) and were used for analysis instead of the 20 locations, for ease of interpretation.

Assessment of colony development traits

Colony development was assessed by considering several parameters, based on the assumption that a honey bee colony is 'productive' not only for its honey yield but also as a whole (bees, brood and food stores): a) population (=number of adult bees); b) amount of brood (=number of brood cells); c) overwintering index, estimated as the ratio between number of adult bees in spring to number of adult bees in the previous autumn; d) pollen storage; e) honey yield. A complete census for each experimental colony was performed in autumn, spring and summer from autumn 2009 until summer 2011 (two complete years). Colony size was determined by estimating the adult bee

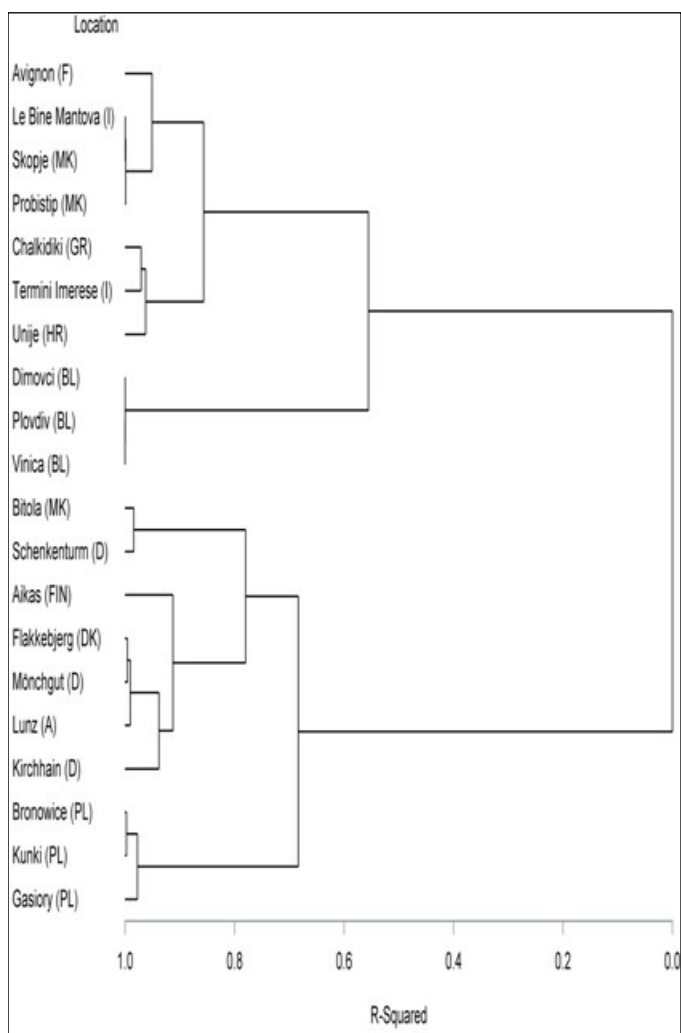


Fig. 1. Clustering of the 20 locations according to environmental conditions shown in Table 1.

population and the amount of brood present in the colony in accordance with the "Liebefeld method" (Imdorf *et al.*, 1987; Delaplane *et al.*, 2013). The amount of pollen in the colony was evaluated by assigning a score, based on the amount of pollen in relation to the amount of brood. Harvested honey was weighed and any supplementary feeding or placement/removal of honey combs was also noted. The testers were trained to assure uniform measuring (for more details on colony assessment methods see Costa *et al.*, 2012b).

Statistical analysis

A General Linear Model (GLM) was used to examine statistical difference among the considered factors; genotype ($n = 16$), origin of breed (local vs. non-local), environmental cluster ($n = 6$), season (spring, summer and autumn) and year (2010 and 2011) were used as fixed effect factors and pollen storage was used as a covariate. Differences among factors were assessed by applying post-hoc analysis using a Bonferroni test. Pearson's correlation coefficients (r) were calculated using the SPSS software package, release 19.0, as for all the above analysis.

Table 1. Average values of the meteorological data for each location and cluster. All parameters were used in the cluster analysis apart from 'Days with average T > 10° C' which is reported to illustrate length of the active flight season.

Cluster name	Location	GPS N	Average T°C	Days with average T > 10°C	Days with average T < 10°C	Days with MinT < 0°C	Days with Max T > 30°C	Days with rain
Long season	Chalkidiki	40°22'0"	16.95	293	72	16	64	109
	Termini Imerese	37°58'3.42"	16.65	313	52	0	25	118
	Unije	44°38'58.3	15.40	272	93	2	36	145
	Average		16.15	293	72	6	42	124
Medium-long season	Avignon	43°56'58"	13.84	246	119	50	54	89
	Le Bine Mantova	45° 8'18.85"	13.27	235	130	55	58	142
	Skopje	41°59'06.8"	13.00	243	122	61	60	144
	Probstip	41°59'40.0"	13.00	243	122	61	60	130
	Average		13.28	242	123	57	58	126
Medium season	Dimovci	42°66'07"	13.02	234	131	83	67	43
	Plovdiv	42°13'54"	13.02	234	131	83	67	43
	Vinica	42°9'67"	13.02	234	131	83	67	43
	Average		13.02	234	131	83	67	43
Medium-short season	Bitola	41°02'20.64"	11.15	215	150	45	14	180
	Schenkenturm	49°48'53.31	8.65	176	189	52	0	199
	Average		9.90	195	170	49	7	190
Short season	Bronowice	51°25'	9.37	182	183	120	40	99
	Kunki	50°26'	8.78	186	179	119	21	107
	Gasiory	53°40'40.8	7.98	172	193	119	16	139
	Average		8.71	180	185	119	26	115
Very short season	Äikäs	60°49.732'	3.68	124	241	174	5	229
	Flakkebjerg	55°19'32"	7.08	145	220	113	1	164
	Monchgut	54°19'34,6"	7.77	159	206	108	6	182
	Lunz	47°50'981"	7.12	150	215	137	6	196
	Kirchhain	50°44'089"	8.02	160	205	114	16	231
	Average		6.73	148	217	129	7	200

Results

Adult bee population

The size of the adult honey bee population was significantly affected by all considered factors: the genotype, origin (local or non-local), the environmental cluster, the year and the season (Table 2). In general, the number of adult bees was lower in spring than in autumn and much higher in the summer (Fig. 2). Environmental conditions significantly affected the development of the honey bee populations with the colonies in the countries near the Mediterranean region having the lowest overall numbers of adult bees, along with the highest numbers of days with T > 10°C (Table 3). The two full years of data collection were different to each other, as most of the colonies were alive and strong in the first year while in the second year many of them had collapsed or were collapsing (average number of adult honey bees were 15,055 ± 389 for the first year and 11,351 ± 386 for the second year of

assessment; see also Fig. 1 in Büchler *et al.*, 2014). Colonies of local origin had significantly higher numbers of bees than colonies placed outside their area of origin (14,734 ± 651 and 11,672 ± 378 honey bees respectively).

Number of brood cells

The number of brood cells was significantly influenced by most considered factors (Table 4). The general trend was that brood production was lower in autumn than in spring, opposite to what happens with the adult bee population, and higher in the summer, as for the number of adult bees (Fig. 3). The environmental conditions significantly affected the brood development of the honey bee colonies, but differently from the adult bee population we found that the lowest overall numbers of brood cells were in the colonies placed in the colder locations or in the clusters with very short active period (and low numbers of days with T > 10°C respectively) (Table 5). We did not

find a significant difference in number of brood cells according to the origin (local or non-local) while similar to the adult bee population we found that the year of the test significantly affected brood production with average brood cells reaching $15,138 \pm 482$ in the first year and $10,566 \pm 477$ in the second year.

Table 2. GLM analysis of adult bee population using 'genotype', 'cluster', 'season', 'origin' and 'year' as fixed effect factors and 'pollen' as a covariate. a. R Squared = 0.858 (Adjusted R Squared = 0.849).

Source	df	Mean Square	F	Sig.
Model	84	4.351E9	97.148	0.000
Cluster	5	8.563E8	19.120	0.000
Genotype	15	1.906E8	4.256	0.000
Origin	1	5.689E8	12.704	0.000
Year	1	3.018E9	67.394	0.000
Season	2	6.854E9	153.035	0.000
Pollen	1	3.324E9	74.212	0.000
Cluster * Genotype	28	4.551E8	10.162	0.000
Genotype * Season	30	1.891E8	4.222	0.000
Error	1351	44784117.605		
Total	1435			

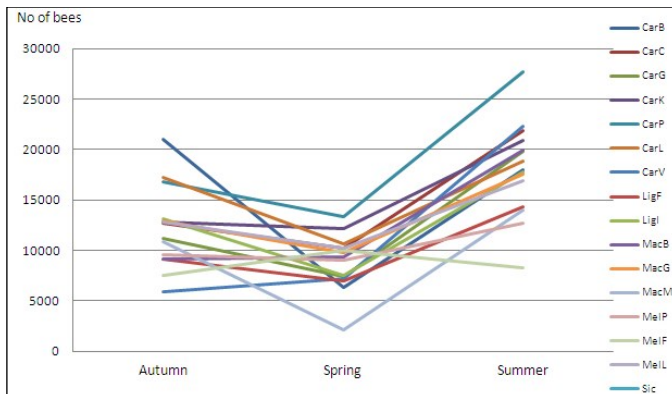


Fig. 2. Number of adult bees of each genotype in the three seasonal censuses. Data are reported as LS means of the two years considered, adjusted for the effects of year, origin and environmental cluster and their interactions.

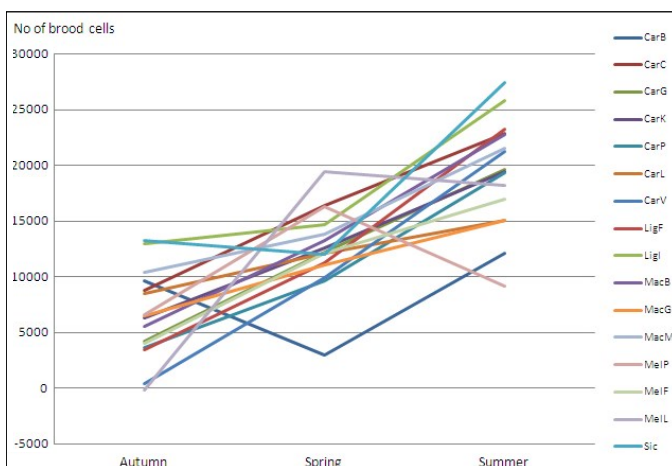


Fig. 3. Number of brood cells of each genotype in the three seasonal censuses. Data are reported as LS means of the two years considered, adjusted for the effects of year, origin and environmental cluster.

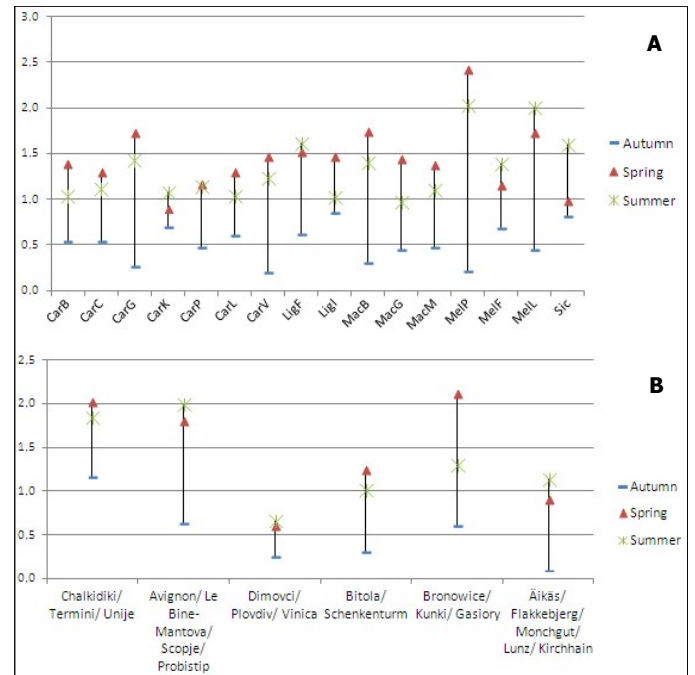


Fig. 4. Brood to adult bee ratio: values across the seasons (mean of both years); **A.** in the different genotypes; **B.** in the different environmental clusters.

Relation between developmental stages

The ratio of brood to adult bees was higher in spring compared to autumn for all genotypes, and higher than 1 in most cases (indicating a higher level of brood compared to adult bees), with values ranging from 0.91 in CarK to 2.42 in MelP (Fig. 4A). In autumn the ratio was always lower than 1 (indicating a higher level of adult bees compared to brood) and ranged from 0.21 in MelP to 0.86 in LigL. Values in the summer were mostly intermediate and closer to 1, ranging from 0.97 in MacG to 2.03 in MelP, showing the greater balance between adult bees and brood in the summer (Fig. 4A). When considering the ratios according to the environmental conditions, it is notable that the warmest regions had higher autumn and summer ratios, confirming the higher production of brood in the summer and showing how brood production continues longer into the autumn, compared to the colder regions. As can be observed in Fig. 4B, the autumn ratio ranged from 0.10 in Äikäs Flakkebjerg / Monchgut / Lunz / Kirchhain to 1.17 in Chalkidiki / Termini / Unije. In spring the ratio was higher than 1 in all clusters apart from the coldest region and Bulgaria, ranging from 0.60 in Dimovci / Plovdiv / Vinica to 2.10 in Bronowice / Kunki / Gasiory. In the summer the ratio was higher than 1 in all clusters apart from Bulgaria, ranging from 0.66 in Dimovci / Plovdiv / Vinica to 2.0 in Avignon / Le Bine Mantova / Scopje / Probstip (Fig. 4B).

Overwintering ability

The environmental conditions, but not the year, significantly affected the overwintering ability of the various genotypes (Table 6). Also, the genotypes had a different overwintering ability depending on whether they were in their local environment or not, as highlighted by the significant interaction between genotype and origin in the GLM analysis

Table 3. Adult bee population of each honey bee genotype (expressed as LS Mean \pm S.E) in the different environmental clusters adjusted for the effect of origin and year (significant differences among clusters after Bonferroni post hoc analysis are indicated under the clusters, Mean values with * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$).

Location	Chalkidiki/ Termini Imerese/ Unijie	Avignon/ Le Bine Mantova/ Skopje/ Probistip	Dimovci/ Plovdiv/Vinica	Bitola/ Schenkenturm	Bronowice/ Kunki/Gasiory	Äikäs/ Flakkebjerg/ Monchgut/Lunz/ Kirchhain
Cluster name	Long season	Medium-long season	Medium	Medium-short	Short	Very short
No. of days with average T > 10°C	293	242	234	196	180	148
CarB	.			11,635 \pm 1,636	.	18,550 \pm 996
CarC	10,619 \pm 1,438	13,401 \pm 1,984		18,600 \pm 1,912	17,135 \pm 945	
CarG			17,904 \pm 1,903	11,196 \pm 1,415	14,416 \pm 842	
CarK	11,698 \pm 1,467				16,849 \pm 1,189	17,318 \pm 1,236
CarP			19,560 \pm 1,943		16,071 \pm 651	23,196 \pm 1,143
CarL	12,994 \pm 1,230				16,162 \pm 1,109	17,611 \pm 947
CarV	7,205 \pm 1,666			7,091 \pm 1,515	21,040 \pm 1,109	
LigF	13,640 \pm 1,334	13,598 \pm 1,923				3,284 \pm 1,571
LigI	11,217 \pm 1,044	14,344 \pm 1,279				
MacB	11,766 \pm 1,335	1,344 \pm 2,594	23,376 \pm 1,289		14,652 \pm 1,325	
MacG	12,867 \pm 1,198	10,168 \pm 1,788	21,523 \pm 2,423	17,415 \pm 1,787		7,121 \pm 1,610
MacM	13,149 \pm 1,176	9,153 \pm 1,998		14,395 \pm 2,273		17,064 \pm 1,290
MelP	.	105 \pm 4,246			11,218 \pm 2,372	15,839 \pm 5,634
MelF	11,861 \pm 1,496	423 \pm 3,044				19,103 \pm 1,270
MelL	10,555 \pm 1,878					6,665 \pm 2,377
Sic	11,573 \pm 1,117	14,768 \pm 2,624				13,630 \pm 1,993
MEAN	11,595 \pm 438	8,565 \pm 840	20,661 \pm 954	13,388 \pm 719	15,942 \pm 495	14,489 \pm 676
	*	*	***	*	*	**

Table 4. GLM analysis of number of brood cells using 'genotype', 'cluster', 'season', 'origin' and 'year' as fixed effect factors and 'pollen' as a covariate. a. R Squared = 0.828 (Adjusted R Squared = 0.817).

Source	df	Mean Square	F	Sig.
Model	84	5.325E9	77.341	0.000
Cluster	5	1.833E9	26.625	0.000
Genotype	15	2.184E8	3.173	0.000
Origin	1	2.585E8	3.754	0.053
Year	1	4.631E9	67.258	0.000
Season	2	7.506E9	109.013	0.000
Pollen	1	1.014E10	147.254	0.000
Cluster * Genotype	28	4.163E8	6.046	0.000
Genotype * Season	30	2.236E8	3.247	0.000
Error	1353	68854106.769		
Total	1437			

(Table 6). Illustration of the differences between the genotypes in local vs non local areas are shown in Fig. 5A. Significant differences were also observed between the environmental clusters, which are shown in Fig. 5B, where numbers of spring bees were plotted against numbers of autumn bees. When data is above the diagonal line of the graph, thus the overwintering ability is >1 the number of spring bees is higher

than autumn bees, while when the data is below the diagonal line the number of spring bees is lower than autumn bees, indicating a poor development or large loss of bees in the winter. Interestingly, the clusters with shorter active season tend to have an overwintering index < 1.

Honey yield

The collected data showed great differences in honey yield among the considered factors (Table 7). The overall average honey yield in our experiment was 23.4 kg. Genotypes belonging to the commercially used subspecies *A. m. ligustica* and *A. m. carnica* tended to have higher honey yields compared to the genotypes belonging to *A. m. mellifera* and *A. m. macedonica* (ranging from 40 kg in CarK and LigI to 15.2 in Mell) (Fig. 6A), although care must be placed in interpretation of these data, as significant differences among environmental clusters were also observed, with the most Southern locations (longer active season) having the highest honey yields (Fig. 6B). Overall, local genotypes collected higher amounts of honey than non-local ones (with 24.5 and 22.7 kg of honey respectively); although this difference was not significant, the interaction between genotype and origin was. The strong influence of environmental conditions is evident also by the

Table 5. Number of brood cells of each honey bee genotype (expressed as LS Mean \pm S.E) in the different environmental clusters adjusted, for the effect of origin and year (significant differences among clusters after Bonferroni post hoc analysis are indicated under the clusters, Mean values with * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$).

Location	Chalkidiki/ Termini Imerese/ Unije	Avignon/ Le Bine Mantova/ Skopje/Probistip	Dimovci/ Plovdiv/Vinica	Bitola/ Schenkenturm	Bronowice/ Kunki/ Gasiory	Äikäs/ Flakkebjerg/ Monchgut/Lunz/ Kirchhain
Cluster name	Long season	Medium-long season	Medium	Medium-short	Short	Very short
No. of days with average T < 10°C	293	242	234	196	180	148
CarB				6,573 \pm 2,029		9,887 \pm 1,234
CarC	22,341 \pm 1,752	10,982 \pm 2,461		16,130 \pm 2,371	14,620 \pm 1,172	
CarG			5,085 \pm 2,355	6,138 \pm 1,755	17,908 \pm 1,044	
CarK	15,430 \pm 1,819				12,621 \pm 1,474	10,253 \pm 1,533
CarP			10,192 \pm 2,404		17,031 \pm 809	8,291 \pm 1,418
CarL	17,833 \pm 1,525				11,819 \pm 1,376	6,035 \pm 1,175
CarV	5,940 \pm 2,066			4,547 \pm 1,879	20,977 \pm 1,376	
LigF	19,143 \pm 1,654	15,245 \pm 2,384				3,552 \pm 1,948
LigI	14,939 \pm 1,294	20,727 \pm 1,586				
MacB	10,390 \pm 1,656	14,919 \pm 3,216	13,580 \pm 1,599		16,452 \pm 1,643	
MacG	17,696 \pm 1,472	7,943 \pm 2,217	12,135 \pm 3,003	12,524 \pm 2,216		4,211 \pm 1,995
MacM	17,564 \pm 1,458	14,941 \pm 2,477		18,333 \pm 2,818		10,191 \pm 1,599
MelP		17,217 \pm 5,264			13,032 \pm 2,941	1,757 \pm 6,986
MelF	18,352 \pm 1,855	9,548 \pm 3,775				5,098 \pm 1,575
MelL	18,271 \pm 2,372					6,762 \pm 2,971
Sic	24,119 \pm 1,385	15,734 \pm 3,253				12,807 \pm 2,354
MEAN	16,834 \pm 544 ***	14,139 \pm 1042 **	10,981 \pm 1,249 *	10,707 \pm 892 *	15,557 \pm 613 ***	7,167 \pm 837 *

Table 6. GLM analysis of overwintering index (ratio of spring bees to autumn bees) using 'genotype', 'cluster', 'origin' and 'year' as fixed effect factors. a. R Squared = 0.478 (Adjusted R Squared = 0.431).

Source	df	Mean Square	F	Sig.
Model	51	28.931	10.268	0.000
Genotype	15	4.352	1.545	0.085
Cluster	5	32.629	11.581	0.000
Origin	1	0.666	0.236	0.627
Year	1	4.227	1.500	0.221
Genotype * origin	13	6.786	2.408	0.004
Genotype * Year	15	10.290	3.652	0.000
Error	572	2.818		
Total	623			

Table 7. GLM analysis of honey yield from the colonies using 'genotype', 'cluster', 'origin', and 'year' as fixed effect factors. a. R Squared = 0.765 (Adjusted R Squared = 0.747).

Source	df	Mean Square	F	Sig.
Model	33	11524.604	42.611	0.000
Genotype	14	1657.718	6.129	0.000
Cluster	4	3833.318	14.173	0.000
Origin	1	39.755	0.147	0.700
Year	1	1491.137	5.513	0.019
Genotype * Origin	12	1852.690	6.850	0.000
Error	431	270.459		
Total	464			

difference between the two years. Although colonies were weaker in the second year, having lower amounts of bees and brood, the honey harvested was higher than in the first year (25.5 kg and 21.5 kg respectively).

Relations between development parameters, varroa infestation and colony survival

The complete data set gave us the possibility of investigating relations between parameters: we thus found that:

- the number of adult bees in the autumn of both 2010 and 2011 was negatively correlated to varroa infestation level in July, August, and September of the same year ($r = -0.218$, $P < 0.005$; $r = -0.247$, $P < 0.005$; $r = -0.516$, $P < 0.001$, respectively for 2010; and $r = -0.348$, $P < 0.001$; $r = -0.445$, $P < 0.001$; $r = -0.675$, $P < 0.001$, respectively for 2011);
- the number of bees in spring 2010 was negatively correlated with varroa infestation levels in the previous October ($r = -0.405$; $P < 0.005$) (for varroa infestation levels see: Meixner *et al.*, 2014);
- varroa infestation levels during June and July were positively correlated with the number of adult bees and number of brood cells during the previous spring ($r = 0.209$, $P < 0.005$; $r = 0.409$, $P < 0.001$ for the number of bees and $r = 0.325$, $P < 0.001$; $r = 0.135$, $P < 0.05$ for the brood cells);

- varroa infestation levels during September were also found to be positively correlated with number of bees and number of brood cells during summer ($r = 0.139, P < 0.05$; $r = 0.151, P < 0.05$, for number of bees and number of brood cells respectively);
- the survival duration of the honey bee colonies was positively correlated to the number of bees and brood cells in summer and to the number of bees in autumn of the first year ($r = 0.299, P < 0.001$; $r = 0.340, P < 0.001$; $r = 0.428, P < 0.001$, respectively for summer bees, summer brood and autumn bees of 2010) (for details on duration of survival see Büchler *et al.*, 2014);
- the overwintering ability of the colonies was positively correlated to honey yield in the next season ($r = 0.368, P < 0.000$);
- survival days were not correlated to overwintering index ($r = 0.084; P > 0.05$).

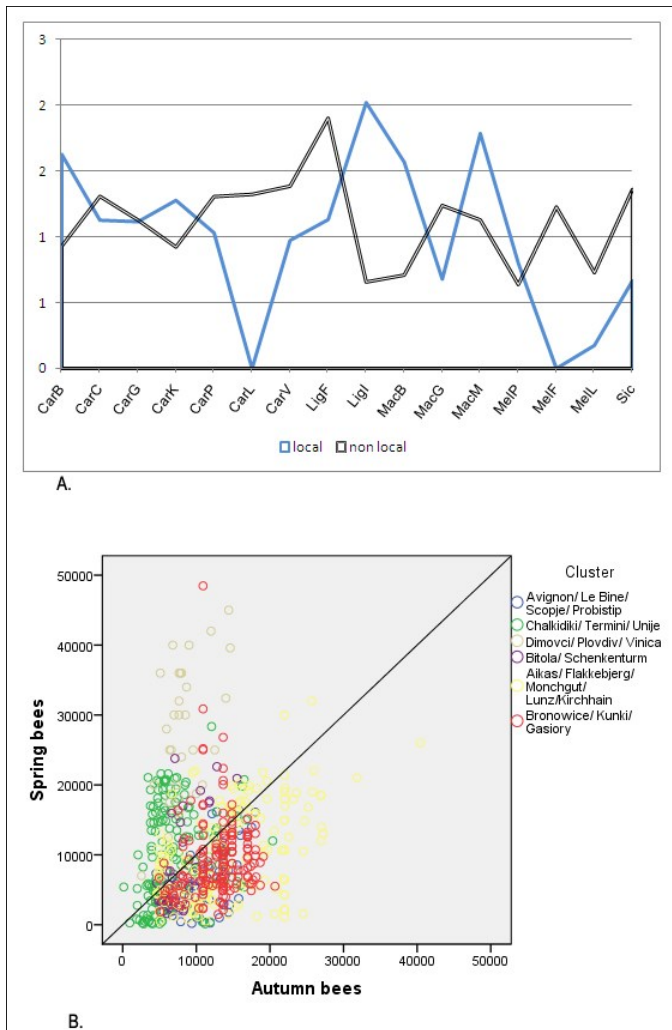


Fig. 5. Illustration of differences in overwintering index as an average for both years of assessment: **A.** between genotypes tested in local and non local areas. The Y axes of the figure represents the ratio between spring to autumn bees; **B.** between the environmental clusters.

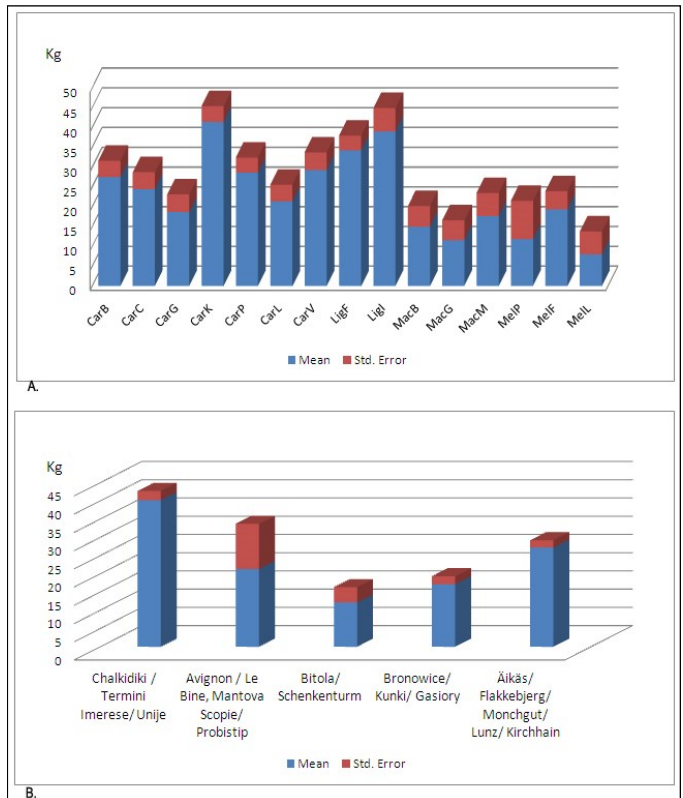


Fig. 6. Graphical illustrations of the honey (kg) harvested from: **A.** each specific genotypes; **B.** in each environmental cluster.

Discussion

Along the distribution range of honey bees, the ecosystem circumstances can vary from extremely hot deserts in southern regions to long and hard winters with temperatures of -45°C in northern European forests. Accordingly, the annual active flight and forage season can be the whole year round or be restricted to just a few months (in Northern Europe the bees are forced to stay in winter cluster for almost seven months). The annual cycle of colony development of European honey bees has been described in many independent studies from America (Avitabile, 1978; Harris, 2008; 2009; 2010) to Europe (Wille and Gerig, 1976; Liebig, 1996) and Asia (Gong, 1980). Honey bees display a great range of behavioural and morphological differences resulting from adaptation to such diverse environments.

During our experiment the average annual temperature ranged from 3.68°C in Äikäs, Finland to 16.95°C in Chalkidiki, Greece, with the lowest number of days $< 10^{\circ}\text{C}$ in Termini Imerese, Italy; Unije, Croatia and Chalkidiki, Greece (52, 53 and 72 respectively) and highest number of days $< 10^{\circ}\text{C}$ in Äikäs, Finland followed by Flakkebjerg, Denmark (241 and 220 days, respectively). The opposite trend was found for the days with $T > 10^{\circ}\text{C}$ and with $T > 30^{\circ}\text{C}$ (Table 1). These parameters clearly demonstrate the high differences in the climatic conditions and the consequence in terms of possible bee-active days among the European locations considered in our experiment.

The most striking effect of the different environmental conditions on colony development was the lower number of adult bees in southern Europe (longer active season) compared to northern Europe (shorter active season). This value, which refers to the whole two years of the experiment, could reflect the tendency of the colonies placed in cold climates to keep high numbers of bees to increase probability of survival during the long inactive season. The fact that local genotypes had higher adult bee populations in their area of origin than outside, could indicate specific adaptations to environmental conditions that allow individual bees to survive longer and thus to generate a larger colony population. This hypothesis finds confirmation in the fact that the same differences were not observed in the brood population, which was actually highest in the southern-most cluster: thus the number of bees is lower and the number of brood cells is higher in locations with longer active season. The above difference and its relationship with the number of days with $T > 10^{\circ} \text{C}$ indicates one of the following two factors: 1. shorter life-span of bees in areas of longer active season; 2. a higher proportion of foraging bees (not considered in the estimation). It has indeed been shown that reductions in colony population are associated with shorter worker life spans, younger worker foraging ages, and increased rates of comb building, brood rearing, and population growth (Winston and Fergusson, 1985; Winston *et al.*, 1985). The second hypothesis could be due to a more precocious onset of foraging, which can be the result of pathological conditions - it is well known that bees infected by *Nosema* spp. start foraging earlier (Wang and Moller, 1970a, b; Tofilski, 2009) or simply to a higher number of bee-active days and general better foraging conditions - the active flight season and the honey yield were indeed highest in the most southern cluster. An indication of large differences in adult bee life-span come from the ratio between the two developmental stages: if the ratio is multiplied by the length of development in days we find that the estimation of life-span ranges from 12 to 42 days, showing a strong influence of the cluster (environment) with the tendency of a higher average life expectancy in the colder regions.

It is also important to state that the number of bees developed in a specific location is also the result of parameters such as management techniques and measuring accuracy, parameters that we tried to keep as constant as possible (e.g. self-evaluation of measuring accuracy; see Costa *et al.*, 2012b), but still subject to error and variation. However, we feel that these estimates are more accurate than estimates made by measuring brood area and then making assumptions on adult bee life. For example, a study by Hauser and Lensky (1994) reports for a Mediterranean location an average population of *A. m. ligustica* adult bees of 66,000, a value much greater than the ones found in our study at any location. It is true that in our study we applied no chemical treatment to control varroa mites, and thus our colonies were smaller in the second year than what they probably would have been with treatment, thus lowering the overall mean value. However, it must also be noted that Hauser and Lensky did not use the 'Liebefeld method'

or a similarly accurate one for estimating the actual number of adult bees; rather, they calculated the number of adult bees based on the brood area and on an assumption of the duration of adult bee life-span.

As mentioned above, the colonies were found to be weaker during the second year of the experiment, in terms of both adult bee population and number of brood cells, probably as a result of increased varroa infestation during the second year, and maybe the increased age of the queens (Woyke, 1984; Genç, 1992; Kostarelou-Damianidou *et al.*, 1995; Akyol *et al.*, 2008). Indeed we found significant negative correlations between mite infestation levels and the number of adult bees in the following months, and after the winter. Previous studies have shown that colony losses are linked to varroa infestation levels, but also to the age of the queen and the size of the colony in the autumn (Genersch *et al.*, 2010). An increased number of brood cells and adult bees in the summer also results in an increased number of varroa mites, which in turn may result in a higher virus titers of the bees (see also Genersch *et al.*, 2010; Meixner *et al.*, 2014). It is therefore interesting to know or even to predict the survival of a colony according to varroa levels and population in summer and autumn. Our strategy of not treating the colonies allowed us to observe the natural interactions between genotype and environment and their effects on colony vitality. The same can also be measured by the ability of the colony to overwinter with a high number of adult bees, which probably will result in a more successful spring development, as has been shown by Harris (2008, 2010). Locations with long active season tend to have higher numbers of spring bees compared to autumn bees (thus higher overwintering index) but lower numbers of bees as an overall. We also found that the various genotypes performed differently in local or in non-local environments, thus demonstrating the adaptation of the local populations to their specific area of origin. Similar effects have been demonstrated with dairy cattle (Hammami *et al.*, 2008).

The performance of a honey bee colony can also be described by its ability to collect honey and be productive. Although the management of the colonies during this experiment did not allow normal practice for honey production, and it was not specifically orientated to honey production, we collected data in order to see the effect of the GEI on this important apicultural characteristic. Under the restrictions of the limited data available, the local genotypes showed a trend to collect more honey than the non-local ones, which shows their ability to develop higher adult bee populations (as we found in the present study) and better ability to forage on the local flora. This adaptation and their longer survivorship (see Büchler *et al.*, 2014) could also explain the fact that the survived colonies (most of them of local genotypes) had higher honey production during the second year. GEI which resulted in higher honey production, linked to higher spring development, have also been shown by Costa *et al.* (2012a) on different Italian honey bee populations. Although our experiment did not show any relationship between survival duration and overwintering ability, is very possible that colonies which survive have also higher number of

bees in spring compared to autumn and they can probably develop quicker and stronger in order to produce higher amounts of honey. And indeed this was found in our experiment: colonies with higher overwintering ability produce more honey.

However, we should always keep in mind that in our experimental conditions colony assessments, both in spring and autumn, were performed when it was permitted by the environmental conditions. Furthermore, each genotype was tested in different environmental conditions/locations, and it is possible that some genotypes were tested in more favourable conditions than others, especially in terms of honey production. This might explain the overall not significant effect of the factor 'origin' but the significant effect of the interaction between 'genotype' and 'origin'.

Based also on the correlations performed between the survival period or varroa levels and the colonies' population or brood we can state that high numbers of bees in spring leads to high number of bees in summer, which leads to high levels of varroa in summer and autumn and eventually in low number of bees in autumn and probably low survival for next spring.

Pollen storage levels may also have a direct effect on colony fitness as they are related to immediate colony growth rates via brood production (Brodtschneider and Crailsheim, 2010; Odoux *et al.*, 2014). However, pollen storage in this study was recorded in a way to show shortage or levels of abundance only. Therefore, although it showed significant influence on all characters determined, this was not enough to be analysed further for its specific differentiated effects on colony growth.

Intensive breeding activities during the last decades are limiting the number of subspecies or ecotypes as they favour specific breeds or commercial lines. However, it is well documented that high diversity of honey bee populations still exists in Europe (De la Rúa *et al.*, 2009; Bouga *et al.*, 2011; Ivanova *et al.*, 2012). Therefore, the questions to be answered are: why does this high diversity exist? Do we need to preserve it for specific reasons? The results from the colony development in the European GEI experiment show that there are good reasons to believe that the diversity is the result of natural selection favouring specific phenotypes with important local adaptations, resulting in improved fitness of each population. Furthermore, the data highlights the significance of using local populations in breeding programmes.

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