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Analysis of Iberian honey bee (*Apis mellifera iberiensis*) colony phenotypes across two different origins in Portugal: searching for evidence of genotype by environment interactions

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Fainna maAAa alAAusri yusra. Inna maAAa alAAusri yusra.

« Derriere chaque difficulté, il y'a une facilité. Derriere chaque difficulté, il y'a certe une facilité »

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Abstract

*Apis mellifera iberiens*is is a honey bee subspecies native to the Iberian Peninsula. Its adaptive potential allows the development of several phenotypes depending on the environment. The aim of this study is to understand to what extent there is local adaptation in this subspecies of Iberian Peninsula, which has proved to be highly diverse and complex at the genetic level.

To this end, we established two experimental apiaries at the latitudinal extremes of Portugal (Gimonde, in the Northeast, and Zavial, in the Southwestern). Three (Bragança, Algarve and Basque Country) and two (Algarve, Bragança) different genetic origins of the Iberian honey bee, represented by 18 colonies each, were deployed in the apiary of Gimonde and Zavial, respectively.

The data from this study was collected between October of 2015 and May of 2017. During this period, the colonies were evaluated for the following parameters: varroa infestation level (measured by the sugar test), hygienic behavior (measured by the pin test), colony strength (measured by the weight and the Lieberfeld method for adult bees, brood, and food resources), honey yield and survivorship.

The results of the varroa infestation level suggest that (1) the apiary location has a main role on the varroa's infestation level; (2) there is no difference in varroa incidence between local and non-local colonies

The results of the hygienic behavior indicate that (1) the variation in the hygienic behavior in each evaluation may result from different proportions of workers with hygienic behavior (between 15-17 days of age) in the colony; (2) there are origins with better hygienic behavior than others. The latter supports the results of the varroa infestation level, although further research is needed to confirm this trend.

The colony strenght suggests that (1) within the Iberian honey bee variation, local honey bees are not better adapted than the non local. However these results may have been influenced by the high level of varroa infestation that some origins showed in a given period of time. Nevertheless, a higher survivorship of the local origin compared to the non-local ones was detected suggesting local adaptation.

These finding improve our knowledge on the adaptive process of the Iberian honey bee. They suggest also the preservation of local honey bees which is more sustainable (higher survivorship) for beekeeping and promote the elaboration of appropriate management plans.

Keywords: *Apis mellifera iberiens*is, local adaptation, survival

Resumo

A *Apis mellifera iberiens*is é uma subespécie de abelhas do mel nativa da Península Ibérica. O seu potencial adaptativo permite o desenvolvimento de vários fenótipos dependendo do ambiente. O objetivo deste estudo é compreender em que extensão há adaptação local nesta subespécie na Península Ibérica que tem demonstrado ser altamente diversa e complexa a nível genético.

Para este fim, nós estabelecemos dois apiários experimentais nos extremos latitudinais de Portugal (Gimonde, no Nordeste e Zavial, no Sudoeste). No apiário de Gimonde foram colocadas três origens genéticas diferentes da abelha ibérica (Bragança, Algarve e País Basco) enquanto no apiário do Zavial apenas duas (Algarve, Bragança), representadas por 18 colônias cada. Os dados deste estudo foram coletados entre outubro de 2015 e maio de 2017. Durante este período, as colónias foram avaliadas para os seguintes parâmetros: nível de infestação de varroa (medido pelo teste de açúcar), comportamento higiénico (medido pelo teste de pin), força da colónia (medida pelo peso e pelo método de Lieberfeld para abelhas adultas, criação e reservas alimentares), produção de mel e sobrevivência.

Os resultados do nível de infestação de varroa sugerem que (1) a localização do apiário tem um papel importante ao nível da infestação de varroa; (2) não há diferença na incidência varroa entre colónias locais e não locais.

Os resultados do comportamento higiénico indicam que (1) a variação do comportamento higiénico em cada avaliação pode resultar de diferentes proporções de obreiras com comportamento higiénico (entre 15-17 dias) na colónia; (2) existem origens com um comportamento higiénico melhor do que outras. Este último suporta os resultados do nível de infestação de varroa, embora sejam necessários mais estudos para confirmar essa tendência.

A força da colónia sugere que (1) dentro da variação da abelha ibérica, as abelhas locais estão tão adaptadas como as não-locais. No entanto, esses resultados podem ter sido influenciados pelo alto nível de infestação de varroa que algumas origens mostraram em um determinado período de tempo. Não obstante, detetou-se uma maior sobrevivência da origem local em comparação com as não-locais, sugerindo adaptação local.

Essas descobertas melhoram nosso conhecimento sobre o processo adaptativo da abelha ibérica. Eles sugerem também a preservação da abelha local que é mais sustentável para a apicultura (maior sobrevivência) e promove a elaboração de planos de maneio adequados.

Palavras chave: *Apis mellifera iberiens*is, adaptação local, sobrevivência

Résumé

Apis mellifera iberiensis est une sous-espèce d'abeilles originaire de la péninsule ibérienne. Son potentiel d'adaptation lui permet de développer plusieurs phénotypes différents en fonction de son environnement. Le but de cet étude est de comprendre les circonstances de l'adaptation locale chez l'abeille ibérienne, qui s'est révélée être extrêmement diverse et complexe au niveau génétique.

Pour ce faire, nous avons choisi deux sites expérimentaux situés de part et d'autre du Portugal (Gimonde, au Nord-Est et Zavial au Sud-ouest). Trois (Bragança, Algarve et Basque Country) et deux (Algarve, Bragança) différentes origines de l'abeille ibérienne, représentées par des colonies de 18 chacune, sont aussi déployées dans les ruches de Gimonde et Zavial respectivement. Au cours de cette étude, les données sont collectées entre Octobre 2015 et Mai 2017. Durant cette période, les colonies sont évaluées pour les paramètres suivant : le niveau d'infestation en varroa (déterminé grâce au sugar test), le comportement hygiénique (déterminé grace au pin test), la vigueur de la colonie (défini par la mesure du poids des colonies et de la production de miel, le potentiel de survie et l'estimation grâce a la méthode de lieberfeld de la population d'abeilles adultes, de la quantité d'abeille en cours de maturation et de la quantité de nourriture et de pollen disponible).

Les résultats suggèrent que l'infestation en varroa des colonies est principalement déterminée par (1) la position géographique du rucher. (2) L'appartenance de certaines colonies à l'environnement local n'a par contre pas un effet majeur sur le niveau d'infestation.

Les résultats indiquent par ailleurs que le comportement hygiénique pourrait être du aux (1) différences de proportion en abeilles hygiéniques (âgées de 15 a 17 jours) entre les colonies. (2) Par ailleurs, il existe des origines d'abeilles avec un meilleur comportement hygiénique que d'autres. Ces résultats expliquent le niveau d'infestation obtenu dans certaines colonies même si des recherches supplémentaires seraient nécessaires afin de confirmer cette thèse.

L'étude de la vigueur des colonies suggère qu'avec (1) les variations qui existent au sein de l'abeille ibérienne, l'abeille locale n'est pas toujours plus productive que la non locale. Cependant, ces résultats peuvent être influencés par l'importante infestation de varroa qu'a connues, pendant une période certaines colonies. D'autres parts, les abeilles locales présentent un fort potentiel de survie comparé aux abeilles non locales qui sont quant à elles, plus sensibles. Ceci pourrait s'expliquer par une adaptation locale des abeilles autochtones.

Les résultats obtenus à l'issu de ces études permettent d'améliorer nos connaissances par rapport au potentiel d'adaptation de l'abeille ibérienne. Ils suggèrent aussi la préservation

des abeilles locales, qui permettent une exploitation plus durable pour les apiculteurs (fort potentiel de survie). Sur cette base, des plans de gestion rentables et appropriés peuvent être conçus afin d'améliorer l'activité apicole.

Mots clés: *Apis mellifera iberiensis*, adaptation local, potentiel de survie

Index

List of Figures

List of Tables

List of abreviation

% -Percentage

(i) -Top left

(ii) -Bottom right

Ac -Area of a cells

$A = \pi r^2$ -Area of circle

cm -Centimeter

cm² -Square centimeter

g - Gramme

GEI -Genetique by environment interaction

Int -Interval

Int -Interval

kg -Kilogramme

N -Number of colonies

N(A) -Number of colonies from Algarve

N(B) -Number of colonies from Braganca

N(BC) -Number of colonies from Basque Country

px -Pixels

QTL -Quantitative trait loci

rc -Radius of a cell

SNP-Chip -Single-nucleotide polymorphism- Chip

VSH -Varroa Sensitive Hygiene

X -Position on the x-axis

Y -Position on the y-axis

1. Introduction

Insect pollinators are essential for the equilibrium of ecosystems and for maintaining biodiversity. Among the different insect taxa, the honey bee (*Apis mellifera* L.) is one of the most important pollinator species of both wild plants and crops.

In this work, I will focus on a specific honey bee species native to the Iberian Peninsula, the *Apis mellifera Iberiensis*. Taxonomically, the genus Apis belongs to the Apidae family, order Hymenoptera, class Insecta, and phylum Arthropoda. Most phylogeographic studies suggest that the origin of *Apis* is in Asia because eight of the nine extant *Apis* species occur in Asia. *Apis mellifera* is the only *Apis* species with a distributional range outside of Asia; it occurs in African, Middle East and Europe (Ruttner, 1988).

The Iberian honey bee, *A. m. iberiensis*, is a species that is naturally present in the Iberian Peninsula, and was probably introduced into the Balearic Islands and in Macaronesia (reviewed by Chávez-Galarza et al., 2016). The adaptive potential and evolutionary process of the Iberian honey bee allow it to live in a wide variety of environments and climates.

One genotype can develop different phenotypes depending on the environment. This phenomenon is named genotype-by-environment interaction (GEI). It is an adaptation of the genotype against the environment. So one genotype can have different phenotype expressions and one phenotype can be more adapted to an environment and less to another. The honey bees have differentiated into numerous subspecies, also known as geographic races. This differentiation is caused by adaptation to diverse climates and vegetation types (Ruttner, 1988; Whitfield et al., 2006; De la Rúa et al., 2009; Le Conte and Navajas, 2008; Meixner et al., 2010).

The current distribution of honey bee subspecies in Europe is explained by the last glaciation when the mountain chain of Pyrenees, Alps and Balkans constituted into barriers which isolated honey bee populations in glacial refuges (Ruttner, 1988). Specifically, *A. m. iberiensis* had an important refuge in the south of Europe during the quaternary glaciation (reviewed by Chávez-Galarza et al., 2016).

In the last decades, the number of honey bees colonies have experienced a sharp decrease and numerous studies were performed to explain this phenomenon. Many scientists have suggested that the main factors of colony losses are diseases and parasites. They focused mostly on the effect of *Varroa destructor* mites, virus diseases and the microsporidian *Nosema ssp*. (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). Scientists have also identified human

pressure as an important factor underlying colony losses. This pressure has been materialized through reduction of natural areas, increase of agricultural land, use of dangerous pesticides or just inappropriate apicultural practices (Desneux et al.,2007 ; Frazier et al., 2008 ; van Engelsdorp et al., 2009 ; Chauzat et al., 2009; van Engelsdorp and Meixner, 2010). Indeed, some beekeeping practices like intensive queen breeding and trading of commercial stock has caused hybridization of native honey bee population.

Introgressive hybridization modifies the gene pool of local honey bees leading to losses of their genetic identity. These practices widely affect the vitality of honey bees (Buchler et al., 2014) and are supported by the high economic values of honey bees which incite the beekeepers to choose preferentially some commercial strains. However, the imported bees are more sensitive to winter or diseases because they are less adapted to climate change than the local honey bees (Meixner et al., 2014).

To further understand the problem of honey bee health, at first we need to focus on genetic variability and adaptation of honey bees to their environment. The environmental factors, which act directly on honey bees, are mostly climate, vegetation, prevailing diseases and human activities. These factors have increased dramatically the adaptive pressure on local honey bee populations (Moritz et al., 2005). However, the GEI phenomenon maintains the genetic variability and improves the resistance and colonies' vitality. Genetics and the environment affect the development of a colony (Buchler et al., 2014).

In southern Europe, the colonies have usually less adult bees but more brood population than in northern Europe, which is characterized by colder climate. That can be explained by the short longevity of bees in warmer climates and a short brood period in colder climate (Hatjina et al., 2014). Furthermore, some studies found that the introduced bees are indirectly more sensitive to pathogens, although the disease incidence is similar to local bees (Meixner et al., 2014). The difference is in the infestation level, which is more important in the introduced bees, can be explained by the poor adaptability (Francis et al., 2014). Despite the fact that introduced commercial bees can be more productive (especially if they are fed with artificial food), it is more sustainable for beekeepers to use the local bees (Meixner et al., 2014).

Going back to my thesis, a reciprocal translocation experiment was performed to assess whether local genotypes performed better than introduced genotypes. To that end colonies of two different origins were exchanged between two different geographic contexts: one in Gimonde, Bragança, and another in Zavial, Vila do Bispo.

Bragança is located in the northeast extreme of Portugal. Located at the heart of a mountainous zone, the apiary is placed in a civil parish named Gimonde. This region has a

continental climate. The thermal amplitudes are very large. Zavial, the second experimental location, is in the southwest of Portugal. In general, the region of Algarve, where Zavial is located, is characterized by a moderate Mediterranean climate and is punctuated with low annual pluviometry. Even in winter, temperatures range between 11 °C and 15°C, which makes Zavial a good environment for beekeeping. Both of these regions, Gimonde and Zavial, differ by their climates but also by their vegetation and prevailing diseases.

The biological material deployed in the two apiaries has different origins. In the apiary of Gimonde, there are three colony origins: one from the Basque Country, one from Algarve, and one from Bragança. In the apiary of Zavial there are colonies of two distinct origins: one from Bragança and another from Algarve.

This experimental setting allowed us to follow the temporal evolution of colonies and to assess adaptation of colonies to their environment by measuring parameters such as longevity of queen, colony strength, honey productivity, and hygienic behavior, among others.

The results may serve as a basis to find solutions for improving colony health, for developing profitable and adapted strategies of breeding, which will promote honey bee vitality and preserve local populations. Additionally, the results may deepen our understanding on the interactions between genetic diversity and environment and their influence on honey bees vitality.

2. Materials and methods

This experiment was divided into three main parts: (1) installation of two apiaries, in Gimonde (Northest of Portugal) and another in Zavial (Southwest of Portugal) with a total of 54 and 36 colonies, representing three and two genetic origins, respectively, each with 18 colonies (Figure 1). (2) Periodical evaluation of colonies in both apiaries of the following parameters: queen status (present or replaced); surface area covered by bees, brood, pollen and honey estimated by the Lieberfeld method; weight; honey production; hygienic behavior; and percentage of *Varroa destructor* infestation. (3) Analysis of the parameters evaluated in each apiary for each origin.

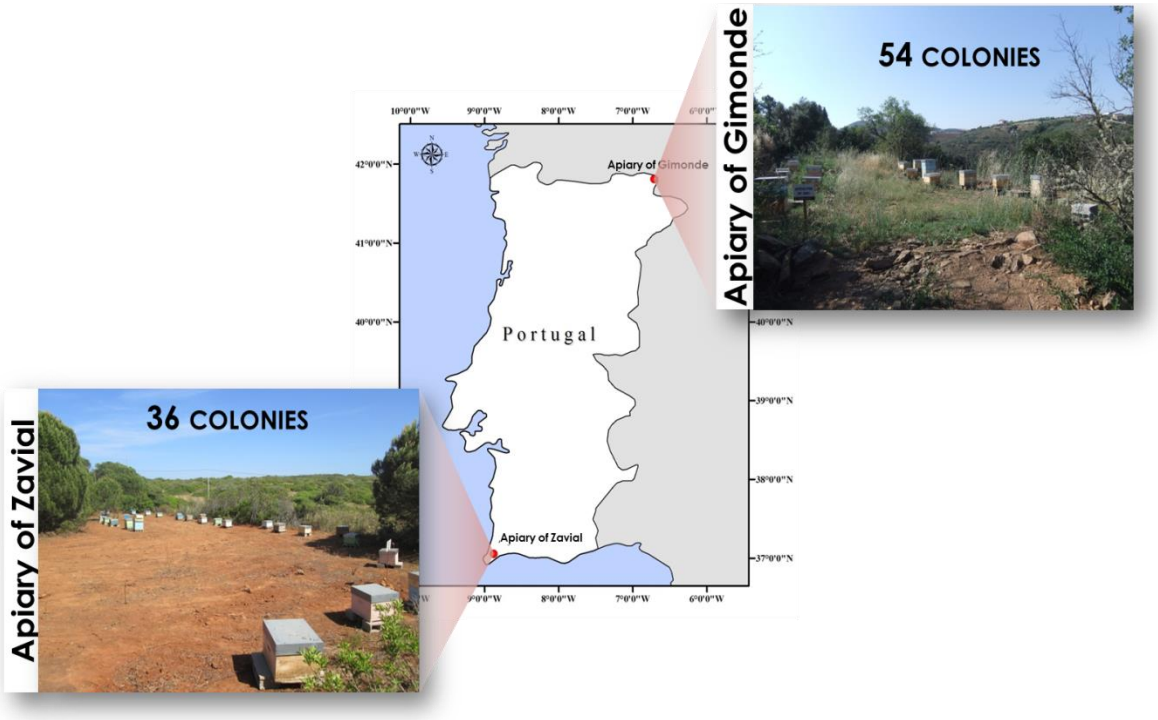

Figure 1. Location of the two Apiaries in the latitudinal extremes of Portugal.

2.1. Installation of the test apiaries located in two latitudinal extremes of Portugal

Colonies of *A. m. iberiensis* of different origins were uniformly set up in Zavial (Algarve and Bragança origins) and in Gimonde (Bragança, Algarve, and Basque Country origins), in September and October of 2015, respectively. We prepared colonies for each of the three origins with approximately the same number of bees, brood, honey and pollen in Langstroth hives with hygienic bottom boards. The queens of each colony were marked with a blue (color attributed to queens born in 2015) dot on the thorax, and a unique ID was assigned to each colony. The homogenization process took place between May and September-October, when the experiment

began. All colonies were treated against *V. destructor* with Apivar in September (Gimonde) and October (Zavial). The 36 colonies (18 of each of the two origins) in Zavial and the 54 colonies (18 of each of the three origins) in Gimonde were placed randomly in each apiary.

2.2. Evaluation of the vitality and performance of the colonies

Queen status (presence/absence) was checked in all colonies at each evaluation. When a queen of 2015 swarmed, superseded or if an emergency queen was produced, the new colony stopped being assessed with standard protocols. The mechanism that led to queen replacement was assessed, whenever possible, by the queen cell type.

In both apiaries, colonies with a queen of 2015 were assessed similarly by the same team. Smoke was always used during evaluation. The colonies were only opened with dry and calm weather conditions.

Foundations were provided to each colony whenever needed. Supers were added when needed during the honey season. The honey harvest took place in Zavial in 11/07/2016 and in Gimonde in 12/09/2016. The treatment against *V. destructor* was performed with Apivar at the beginning of the Spring and after the honey harvest, specifically at the following dates: Zavial in 17/02/2016 and 11/07/2016 and Gimonde in 01/03/2016 and 12/09/2016. Colonies have been managed following the traditional methods and were fed once only in the first Winter.

2.2.1 Estimating the number of bees, number of cells with brood, honey and pollen

Following the Lieberfeld method, we overlapped a grid, divided in 8 sections for nest frames and 4 for super frames to visually estimate the number of sections occupied by bees, brood, honey and pollen in each of the 10 and 8 frames of nest and supers, respectively (Figure 2).

Figure 2. Evaluation of colony strength by the Lieberfeld method.

The area of each section of the grid for nest frames was approximately 106 cm^2 and 121 cm^2 for super frames. To infer the number of large cells (drones) and small cells (workers, honey and pollen) per grid section for nest and supper frames we considered that:

- The radius of the small cells is 36 pixels (px) and that of the large cells is 41 px for *A. m. iberiensis*, using the method of Rodrigues *et al.* (2016).
- Coordinates of the internal vertices of a Langstroth nest frame are: (i) top left (236 px, 447 px) and (ii) bottom right (5838 px, 924 px) using the method of Rodrigues *et al.* (2016).
- The internal length of the Langstroth nest/super frame is 42,7 cm.

The subtraction of xii and xi resulted in an internal length for a Langstroth frame of 5602 px, as shown with the following equation:

$$\text{Int. length of the Langstroth frame (px)} = \text{xii (px)} - \text{xi (px)}$$

Table 1: Calculator table of the Interval length of the Langstroth frame

Internal vertices of a Langstroth nest frame	Coordinates	
	x	y
top left (i)	236 px	447 px
bottom right (ii)	5838 px	924 px

Int. length of the Langstroth frame	5602 px

The internal length of the Langstroth frame in pixels and in centimeters was used to convert the radius of the small cells and the radius of the large cells from pixels to centimeters, following the equation:

$$\text{rc (cm)} = \frac{\text{rc (px)} \times \text{Int. length of the Langstroth frame (cm)}}{\text{Int. length of the Langstroth frame (px)}}$$

Table 2: Conversion table of the radius of the small cells and the radius of the large cells from pixels to centimeters

Radius of a cell (rc)	small	36 px
	large	41 px
Int. length of the Langstroth nest frame		5602 px
		42,700 cm

Radius of a cell (rc)	small	0,274 cm
	large	0,313 cm

Using the area of a circle ($A = \pi r^2$), the area of the small cell was 0,237cm^2 and the area of a large cell was 0,307cm^2. Finally, taking into account those areas, the number of small and large cells was estimated for grid sections of the nest and the super frames, using the following equation.

$$\text{Number of cells in a part of the grid} = \frac{\text{Area of a part of the grid (cm}^2\text{) x 1 cell}}{\text{Ac (cm}^2\text{)}}$$

Table 3: Estimation of the number of small and large cells in a grid section of the nest and the super frames

Area of a cell (Ac)	small	0,237 cm^2
	large	0,307 cm^2
Area of a section of the grid	nest frames	105,750 cm^2
	super frames	120,800 cm^2

		Conversion factor
Number of small cells in a section of the grid	nest frames	447,050
	super frames	510,683
Number of large cells in a section of the grid	nest frames	344,662
	super frames	393,721

The number of honey bees per grid section of both frame types was estimated by using the following equation and considering that in the Langstroth frames there are 1,25 bees per 1 cm^2 (Imdorf et al, 1987):

$$\text{Number of honey bees in a section of the grid} = \frac{\text{Area of a part of the grid (cm}^2\text{) x 1,25 Honey bees}}{1(\text{cm}^2)}$$

Table 4: Estimation of the number of honey bees per grid section of the nest and the super frames

Area of a section of the grid	nest frames	105,750 cm^2
	super frames	120,800 cm^2
Number of honey bees in a cm^2		1,25

		Conversion factor
Number of honey bees in a section of the grid	nest frames	132,188
	super frames	151,003

After calculating the conversion factors, the bee, brood, honey, and pollen data obtained in the apiary by visual estimation were converted into number of bees and number of cells with brood, honey and pollen by colony using Microsoft OfficeTM Access.

2.2.2 Hygienic behavior

The hygienic behavior of the colonies was assessed four times a year using the pin test (Buchler et al., 2013). This test (Figure 3) consisted in selecting a comb region with sealed worker brood at the stage of young white or reddish-eyed pupae, where a pattern (10 x10 cells wide) was placed. Then, 50 cells were pierced with an entomological pin (size nº 2)

Figure 3. Evaluation of hygienic behavior with the pin test method

Each pin was placed into absolute ethanol after use to avoid contamination between colonies. We marked the cell 51 and the frame on the top bar with a pen to identify the area with the pupae that we killed. The removal of dead pupae by adults' honey bees was calculated 24 hours later by counting and recording the number of unopened cells and number of cells with pupal remains. For calculating the removal rate of each colony, and therefore evaluate the hygienic behavior, we used the following equation:

$$\text{Removal rate (\%)} = [50 - (\text{Number of unopened cells} + \text{Number of cells with remains})] \times 2$$

2.2.3 Infestation rate of *V. destructor*

The infestation rate of the mite *V. destructor* of each colony was assessed with the sugar test (Dietemann et al., 2013) six times. To that end, we first weighted 36 g of icing sugar and we measured an initial weight of a jar of shaking with fixed metal mesh (size 2,8mm). Then we collected about 50 g of bees (approximately 500 worker bees) from the outer frame of each colony by shaking it for a jar through a funnel.

Figure 4. Evaluation of varroa infestation with the sugar test method

Next, we measured and recorded the final weight of the jar containing the honey bees and added the icing sugar. We carefully shook the jar every 3 minutes to distribute the sugar homogeneously in the honey bees. We inverted and shook the jar for about 1 minute so that the sugar and the mites passed through the mesh. We counted and recorded the number of mites per colony. Finally, knowing that 10 g of honey bees correspond roughly to 100 honey bees, we estimated the infestation rate of *V. destructor* by using the following formulas:

$$\text{Bees net weight} = \text{Final weight of the jar of shaking with honey bees} - \text{Initial weight of the jar of shaking}$$

$$\text{Varroa infestation level (\%)} = \frac{\text{Number of varroas x 10 (g)}}{\text{Bees net weight (g)}}$$

2.2.4 Colony weight and honey yield

The weight collected continuously can be a non-invasive way to monitor colony strength (Meikle et al. 2016). We weighted all the colonies in both apiaries monthly during the active season. In total, colonies in Zavial were weighted 13 times and in Gimonde 12 times.

In July and September of 2016 the colonies were weighted before and after the honey harvest in Zavial and in Gimonde, respectively, to assess the honey yield by subtracting the initial and the final weight. The weight was obtained by using a U-shape metal device with pulleys to hang a dynamometer and to lift the colonies, which were secured with straps. Pre-weighted supers and nest were subtracted from the total weight of the colony (Kg).

Figure 5. The weight of colony

2.3. Analysis of the parameters evaluated in each apiary for each origin

We calculated the average and confidence intervals of the number of bees, number of cells with brood, honey and pollen, hygienic behavior (%), infestation rate of *V. destructor* (%), and weight for each origin in each apiary. Our study happened between October/November of 2015 to April/Mai of 2017.

3. Results and discussion

In our work, the number of colonies under evaluation changed/reduced across time, for several reasons: (1) when a queen of 2015 has swarmed, superseded or if an emergency queen was produced, the new colony stopped being ceased to be assessed with standard protocols, (2) colony mortality and (3) it was not possible to perform the pin test, or the sugar test, due to lack of brood, or the small number of adult bees, respectively.

3.1. Infestation level of *Varroa destructor*

The life cycle of the mite *V. destructor* occurs inside capped brood cells. It all starts when an adult varroa female enters the opened brood cells, just before they are sealed for pupation, and hide in the larval liquid. Inside the sealed cell, varroa feeds on the brood and lays the first egg, which will develop into a male, just 60 to 70 hours after entering. Subsequently, 3 to 6 eggs are laid by the female. The number of female eggs depends on the type of cells (drone or worker brood). The development is completed in the closed cells and that is why the production of honey bee brood is essential for varroa. Its life cycle is correlated with the life cycle of honey bee host (Vidal-Naquet, 2015). In a field study conducted in Greece, Hatjina et al. (2014) demonstrated that the level of varroa infestation is correlated with the amount of brood in the Spring and the amount of adult honey bees in the Summer.

In a pan-European field experiment, Büchler et al. (2014) reported that 38.8% of the losses were caused by varroa, 16.9% by queen problems, and 7.3% by Nosema sp.

In this study, Varroa's infestation level was measured by the sugar test in every colony deployed in the apiaries of Gimonde and Zavial. The infestation levels across time are shown for both apiaries in Figure 6 and 7.

In the apiary of Gimonde, the colonies of different origins showed a low average infestation level in the first two and the last two evaluation periods (22-10-2015, 29-04-2016, 08-03-2017 and 02-05-2017). In contrast, a high average infestation level in all origins was observed in the two intermediate evaluations (12-09-2016 and 27-10-2016). In the first evaluation of this study (22-10-2015), the average varroa infestation level of the 25 colonies from Bragança was 0.3% ± 0.2 and of the 18 colonies from Basque Country was 0.1% ± 0.1. The colonies of the Algarve origin were not evaluated on this date since they were not yet in the apiary of Gimonde. The low level of infestation in the first evaluation resulted of the treatment against *V. destructor*, applied in September.

On the following evaluation period, which took place in the Spring (29-04-2016), the average varroa infestation level of the remaining 16 colonies from Bragança was 0.3% ± 0.4; of the 10 colonies from Algarve was 0.4% ± 0.3, and of the 14 colonies from Basque Country was 0.3% ± 0.2. The average infestation level was low because the treatment against varroa with Apivar was performed a few weeks before (in 01/03/2016).

In the following evaluation period (12-09-2016), the average infestation level increased considerably in the three origins: the 13 colonies from Bragança showed an average infestation level of 7.3% ± 5.3, the 7 colonies from Algarve was 8.7% ± 10.4, and the 12 colonies from Basque Country was 2.4% ± 1.4. The average infestation level on 27-10-2016 decreased in all origins, except for the Basque Country, an expected result given that the treatment against varroa was performed on 12-09-2016. At this date, the average infestation level of the 12 colonies of the Bragança origin was 4% ± 2.8; of 7 colonies from Algarve was 5.6% ± 3.1 and of 12 colonies from Basque Country was 3.8% ± 1.8. In the following Spring (08-03-2017), the average infestation level of the 12 colonies from Bragança was 0.4% ± 0.5; of 5 colonies from Algarve were 0% and of 10 colonies from Basque Country were 0.5% ± 0.6.

In the last evaluation, on 02-05-2017, the average infestation level of 10 colonies from Bragança was 0.02% ± 0.03, of 2 colonies from Algarve was 0%, and of 7 colonies from Basque Country was 0.03% ± 0.04 (Figure 6.).

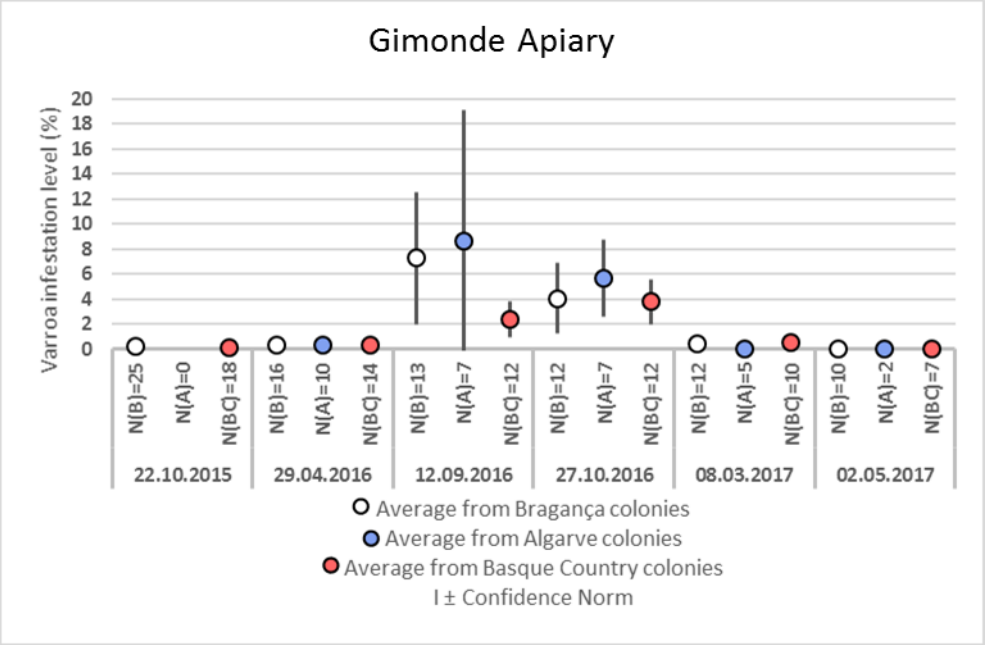

Figure 6. Temporal pattern of varroa infestation levels expressed by colonies of different origins in the apiary of Gimonde. N represents the number of colonies. The colonies from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, the colonies of each origin presented a low average infestation level in the first two and the last three evaluations (08-11-2015, 16-04-2016, 05-11-2016, 13-02-2017 and 08-04-2017 ; Figure 7)

A high average infestation level was observed on 11-07-2016 in both origins deployed in Zavial. In the first assessment, on 08-11-2015, the average infestation level of 36 colonies from Algarve (before 18 were moved to Gimonde) was 0.1% ± 0.1. This low level of infestation in the first evaluation resulted of the treatment against *V. destructor*, applied in October.

The average infestation level on 16-04-2016 increased slightly, although we had performed the treatment against *V. destructor* in 17/02/2016. The average infestation level of 17 colonies from Algarve was 0.9% ± 0.4 while that of 15 colonies from Bragança was 0.5% ± 0.6. Both origins showed a high average infestation on 11-07-2016: 10 colonies from Algarve had an average infestation of 17% ± 8.4 while 11 colonies from Bragança had 8.1% ± 2.2. On 05-11-2016, the average infestation level decreased as a result of the treatment against *V. destructor* performed in 11/07/2016. In this evaluation date, 5 colonies from Algarve had 0.2% ± 0.1 and 7 colonies from Bragança had 0.3% ± 0.3. On 13-02-2017, the average infestation level of 5 colonies from Algarve was 0.1% ± 0.1 and that of 7 colonies from Bragança was 0.5% ± 0.7. The average infestation level of both origins (2 colonies from Algarve and 6 colonies from Bragança) was 0% on 08-04-2017.

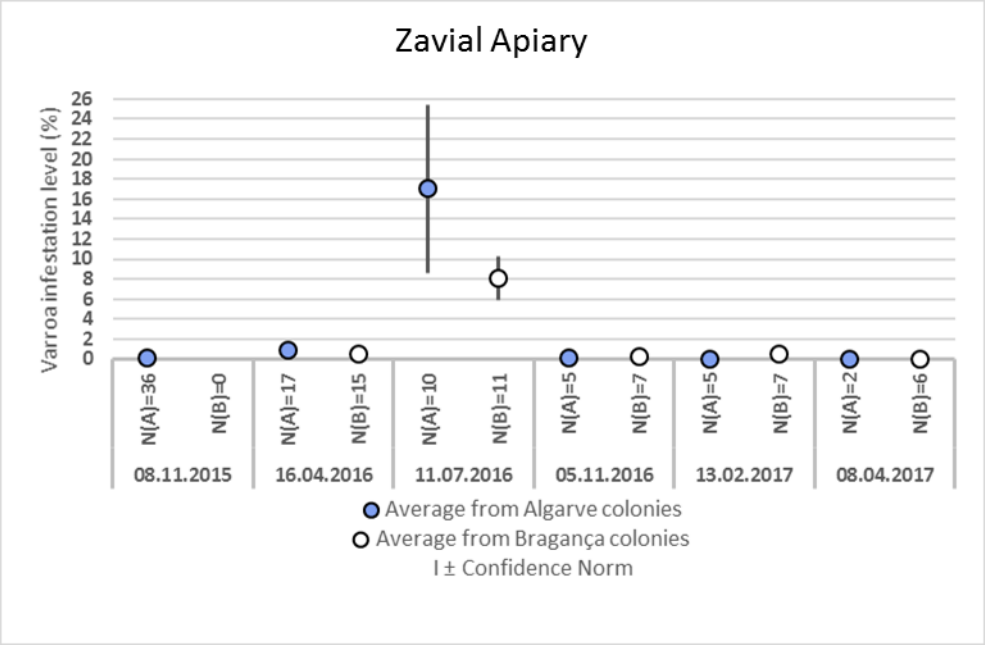

Figure 7. Temporal pattern of varroa infestation levels expressed by colonies of different origins in the apiary of Zavial. N represents the number of colonies. The colonies from Algarve and Bragança are indicated by A and B, respectively.

In the apiary of Gimonde, the colonies from Basque Country, showed the lowest average infestation level, except in the last two evaluation dates, in which it was the origin with the highest infestation levels. However, the levels were so low that it was not relevant. Bragança was the second origin with the lowest average infestation levels across all evaluations of Gimonde. Algarve was the origin with the highest average of infestation in Gimonde, except in the last two evaluation dates. In the evaluations of Zavial for the period from 16-04-2016 to 11-07-2016, the colonies from Algarve showed a higher average infestation level than the colonies from Bragança. In contrast, in the period from 05-11-2016 to 13-02-2017, the colonies from Bragança showed slightly higher infestation levels than the colonies from Algarve. Colonies from Bragança and Algarve did not have varroa in the last evaluation. It should be noted, however, that the number of colonies from Algarve in the last three evaluations was so low (5, 5 and 2), due to colony mortality, swarming or queen replacement, that the average infestation level cannot be representative of the population studied.

Our results suggest that (1) the apiary location has a great effect on the presence of varroa because in Zavial there was more varroa than in Gimonde; (2) there is no difference in varroa incidence between local and non-local colonies because the colonies from Algarve showed more varroa in both apiaries, and the colonies from the Basque Country, showed lower infestation levels than local colonies in most evaluations of Gimonde. Similar results were obtained by Meixner et al. (2014), who monitored the occurrence and levels of parasites and pathogens of 621colonies of 16 different genetic origins, set up in 21 apiaries across 11 European countries. In each location and country, there was a set of colonies of local origin and at least two sets of colonies of non-local origin and no chemical treatment against mites or diseases was done. The authors showed that apiary location had a significant and strong effect on the presence of pathogens, although in general they did not find significant differences in disease incidence between local and non-local colonies. However, in another study in Greece, the level of pathogens in colonies of non-local origin was usually higher than in colonies of local origin (Francis et al., 2014b). This result suggested a poor adaptation of non-local colonies to the environmental conditions of the apiary.

Although this study is unable to determine whether the Iberian honey bee harbors genetic variation underlying tolerance or resistance to varroa (untreated colonies would be needed for addressing that question), it is possible that in Iberia there are genetic origins more resistant or tolerant than others and this question deserves to be investigated. Honey bees may exhibit resistance/tolerance mechanisms against varroa at both individual and social level (Figure 8). Different mechanisms of varroa resistance at the individual level have independently

evolved by natural selection and are based on inhibition of varroa's reproduction after it enters the brood cell (Le Conte et al., 2007; Locke and Fries, 2011; Locke et al., 2012). At the social level, the defense mechanisms include swarming (Fries et al., 2003), absconding of all adult individuals of the colony, leaving infested brood behind (Hepburn and Radloff, 1998), grooming behavior, ability of adult honey bees to remove phoretic mites from themselves or other bees (Le Conte et al., 2015), and Varroa sensitive hygiene, which will be discussed in more detail later.

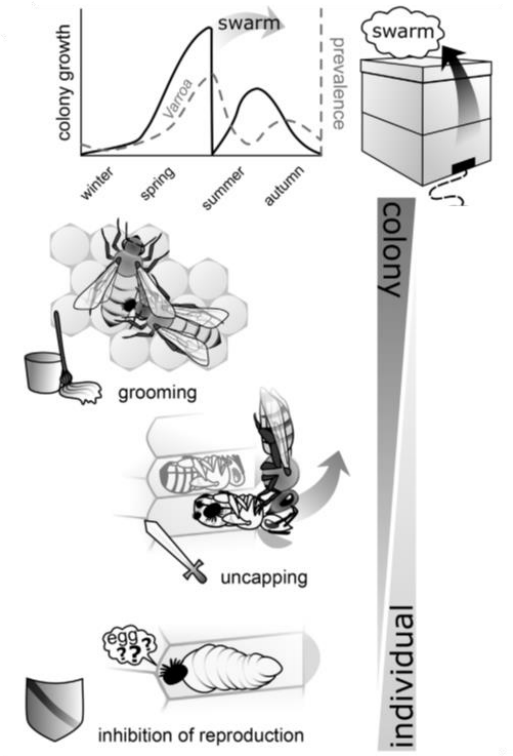

Figure 8. Defense mechanisms against varroa, ranging from the colony level to the individual level. Adapted from Kurze et al. (2016).

3.2. Hygienic behavior

The hygienic behavior consists of detecting, uncapping and removal of dead, diseased or parasitized brood by about 18% middle-aged worker bees (between 15-17 days of age) that have not yet begun foraging (Arathi et al. 2000). The hygienic behavior plays a major role in the resistance of the colony to pathogens, like *Ascosphaera apis* (which causes chalkbrood disease) (Gilliam et al. 1983), Paenibacillus larvae (which causes American Foulbrood) (Spivak et al. 1998) and varroa (Danka et al. 2008; Harbo et al. 2009), which acts as a reservoir, vector and incubator of several honey bee viruses (Gisder et al. 2009). The removal of larvae and pupae infected occurs before the disease-causing organism reachs the infectious stage, thereby limiting the spread of infection. The behavior of removal of varroa-parasitized larvae or pupae,

also called varroa sensitive hygiene (VSH), interrupts the reproductive cycle of this mite (Boecking et al. 2000; Harbo et al.2005).

The hygienic behavior observed in colonies of different origins in the apiaries of Gimonde and Zavial is shown in Figure 9 and Figure 10 respectively

In the apiary of Gimonde, in periods with intensive nectar flow and pollen collection, 30-05-2016 and 02-05-2017, all colonies of the different origins showed a good and similar average hygienic behavior. In the first evaluation of Gimonde, on 30-05-2016, the average hygienic behavior of the 14 colonies from Bragança was 91.9% ± 5; of 9 the colonies from Algarve was 96% ± 4.3 and of the 11 colonies from the Basque Country was 94.7% ± 4.9. In the last evaluation (02-05-2017), the average hygienic behavior of the 10 colonies from Bragança was 94,8% ± 5; of the 2 colonies from Algarve was 91% ± 12.5 and of the 7 colonies from the Basque Country was 98.9% ± 0.7. In the intermediate evaluations, periods without (12-09-2016) or with little (08-03-2017) nectar flow and pollen availability, colonies of different origins showed distinct hygienic behaviors. On 12-09-2016 all origins showed on average lower rates of hygienic behavior, namely: 10 colonies from Bragança with 61.6% ± 16; 7 colonies from Algarve with 53.4% ± 25.5 and 12 colonies from Basque Country with 59.2% ± 14.2. On 08-03-2017, the average hygienic behavior of the different origins started to improve: 11 colonies from Bragança with 71.5% ± 13.2 ; 5 colonies from Algarve with 68% ± 21.2 and 10 colonies from the Basque Country with 80.4% ± 13.8

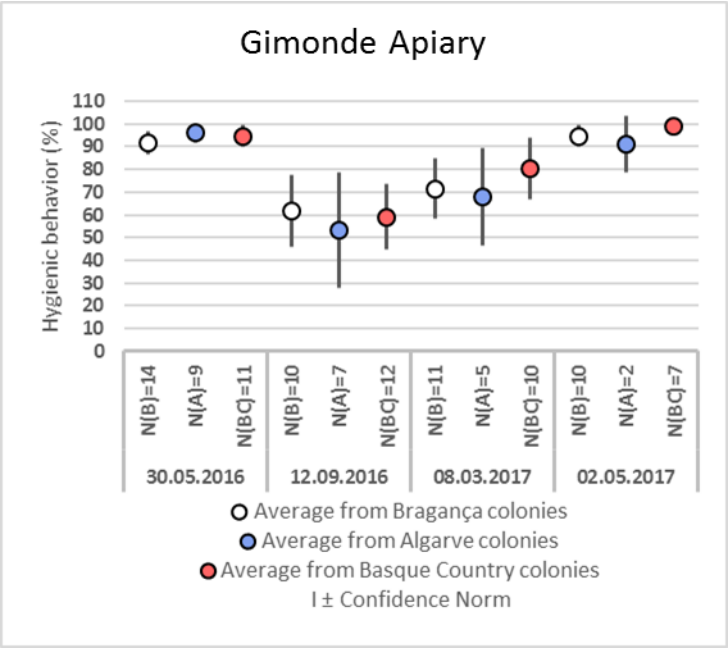

Figure 9. Hygienic behavior of colonies of different origins in the apiary of Gimonde. N represents the number of colonies from one origin. The colonies from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, in periods with intensive nectar flow and pollen collection, 16-04-2016, 13-02-2017 and 08-04-2017 the colonies of different origin showed a good but identical hygienic behavior. In the first assessment of Zavial, on 16-04-2016, the average hygienic behavior of 17 colonies from Algarve was 92.6 % ± 3.7 and of 15 colonies from Bragança was 84.5 % ± 9.6. On 13-02-2017, the average hygienic behavior of 5 colonies from Algarve was 84 % ± 20.2 and of 6 colonies from Bragança was 90.7% ± 8.6. In the last evaluation, 08-04-2017, the average hygienic behavior of the 2 remaining colonies from Algarve was 86 % ± 16.6 and of the 6 colonies from Bragança was 89 % ± 6.7. On 11-07-2016, a period with little or no nectar and pollen sources available around the Zavial apiary, the colonies of the different origins showed a distinct average hygienic behaviors: 9 colonies from Algarve with 65.1% ± 26.3 and 11 colonies from Bragança with % 91.3 ± 12.4.

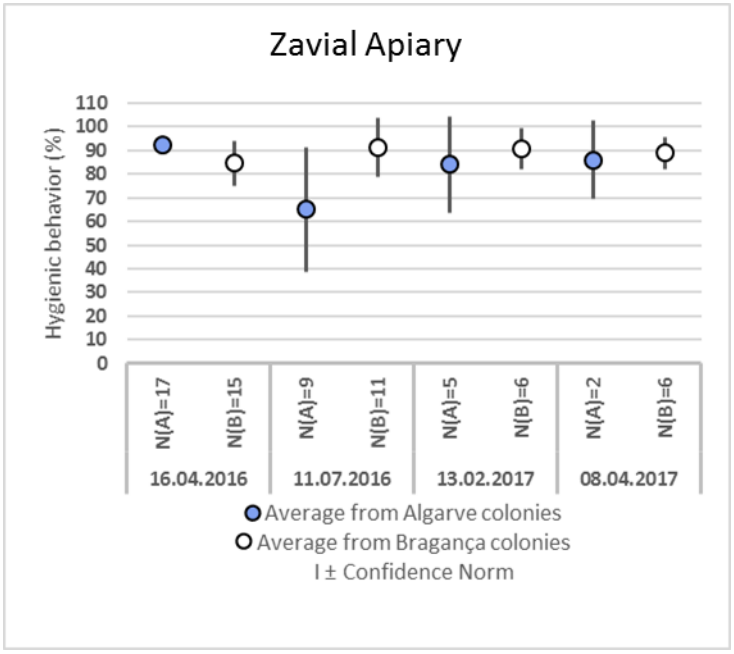

Figure 10. Hygienic behavior of colonies of different origins in the apiary of Zavial. N represents the number of colonies from one origin. The colonies from Algarve and Bragança are indicated by A and B, respectively.

In the apiary of Gimonde, the colonies from Algarve showed the worst average hygienic behavior in all evaluations, except in the first one, which showed the best performance. The colonies from Bragança had the worst average hygienic behavior in the first evaluation, the best in the period with no nectar and pollen availability and the second best in the remaining evaluations. The colonies from Basque Country showed the second best average hygienic behavior in the first two evaluations and the best performance in the last two.

In the apiary of Zavial, the colonies from Algarve showed a worse average hygienic behavior than those of Bragança in all evaluations, except in the first one.

As a follow up study, it would be interesting to test whether there is any correlation between hygienic behavior and flowering since in both apiaries the colonies of different origins showed a good and similar hygienic behavior at this moment. Other studies have shown that the hygienic behavior is influenced by environmental conditions, like the abundance of nectar (Trump et al., 1967; Momot and Rothenbühler, 1971), and seasonal factors remain unclear (Mondragón et al., 2005) and can change every year. However, Bigio et al. (2013) reported that the availability of nectar, simulated by feeding or no feeding with sucrose syrup, does not affect hygienic behavior and that hygienic behavior is not considerably affected by environmental factors. The authors detected only a significant reduction of the hygienic behavior in colonies with added brood that was not fed sucrose syrup in spring.

The hygienic behavior has also been associated with genetic factors. Rothenbuhler (1964) proposed that the uncapping of brood cells with dead, diseased or parasitized larvae or pupae is controlled by one locus, while the removal of the cell contents is controlled by another one. Moritz (1988) re-evaluated this model and suggested that three or more loci control the removal. Lapidge et al. (2002) suggested that this trait is polygenic involving 7 loci with quantitative inheritance. Oxley et al. (2010) identified 6 quantitative trait loci (QTL), which cannot be compared with the two previously mentioned studies due to the use of different genetic maps. Tsuruda et al. (2012) reported one major QTL for VSH behavior on chromosome 9 with a small-scale SNP-Chip (1340 informative SNPs). The influence of colony genotypic composition on the expression of hygienic behavior, studied by Arathi et al., (2001) by creating colonies with normal age structures but with different proportions of bees belonging to the hygienic and non-hygienic lines showed that (1) colonies with only 25% workers with hygienic behavior, hygienic bees performed the task well beyond middle age (between15-39 days) and were more persistent, (2) colonies with 100% workers with hygienic behavior, despite a lack of persistence, was more efficient, and (3) colonies with 50-100% workers with hygienic behavior, partitioned the hygienic behavior into subtasks (some bees uncapped cells at higher frequencies than removed cell contents). In a recent two-year study with *Apis mellifera macedonica,* Xonis et al. (2015) found a great variation in hygienic behavior each month in 74% of the colonies and a little variation of hygienic behavior in 26% of colonies. The low variation in hygienic behavior of the 26% of the colonies was likely due to the higher proportion of workers with hygienic behavior, which resulted with the cleaning of cells. So, variation in the hygienic behavior in each evaluation detected in our experiment may result from different proportions of workers (between 15-17 days of age) in the colony that displayed hygienic behavior (Arathi et al. 2000). Finally, our results suggest that there are origins with better hygienic behavior than others and supports the results of the varroa infestation level (Kurze et al. 2016), although further research is needed to confirm this trend.

3.3. Colony strength

3.3.1. Relationship between weight and quantity of bees, brood, honey and pollen

Changes in colony weight is a good indicator of foraging activity, colony food consumption (Meikle et al. 2008), swarming and colony health (Meikle et al. 2015). The monitoring of colony strength of the different origins in the apiaries of Gimonde and Zavial took into account the weight (Figure11 and 12), number of adult bees (Figure 13 and 14), number of brood cells

(Figure 15 and 16), number of cells with honey (Figure 17 and 18), and number of cells with pollen (Figure 21 and 22) respectively.

In the apiary of Gimonde, the colonies of different origin, showed that before the winter period, on 22-10-2015, the weight of 36 colonies from Braganca was 11.2 Kg ± 0.7; and of 18 colonies from Basque Country were 10.9 Kg ± 1.1 **(**Table 5). No colony from Algarve was evaluated on this date since they were not yet in the apiary of Gimonde. All colonies had more than 5500 adult bees, little brood and pollen cells, and some honey cells **(**Table 6.). Although the colony strength was similar between the two origins, Bragança showed slightly greater values that those of the Basque Country.

The average weight of the colonies in the following Spring (23-03-2016) was similarly low across origins **(**Table 5). This shows that the honey and pollen was consumed. In contrast, the number of honey bees and the number of brood cells increased (Table 6).

In the following month (29-04-2016), the average weight of all origins continued to drop slightly (Table 5). At this date, although the number of pollen and brood cells had increased, the number of honey cells and the number of honey bees decreased (Table 6).

In May (30-05-2016), when the blooming season was at its maximum, the average weight of all origins started to increase and continued to increase in June and July (Table 5).

In August (28-08-2016), the average weight of the colonies from Bragança and Basque Country continued to increase but the colonies from Algarve started to decrease (Table 5). The weight gained, probably resulted in an increase in reserves, brood and honey bees. The weight loss observed in the colonies from Algarve could be the result of other factors, like the varroa. As shown on 12-09-2016 (Figure 6), colonies of the Algarve origin exhibited higher infestation levels.

In the evaluation of September (12-09-2016), the average weight of all origins started to decrease (Table 5.), indicating the end of the flowering season, together with the continuous food consumption. In this month, all colonies had about 16500 honey bees, a low number of brood cells, a low number of pollen cells and a high number of honey cells (Table 6).

The colony weight measurements carried out after honey harvesting (18-09-2016) produced the following values: 17.3Kg ± 2 (Bragança), 16.4Kg ± 2.5 (Algarve), and 16.2Kg ± 1.7 (Basque Country).

One month later (27-10-2016), the average weight of all origins decreased (Table 5.), concurrently with a decrease in the number of adult bees and brood cells **(**Table 6.), when we compared with the evaluation of 12-09-2016. However, in comparison with the evaluation of 12-09-2016 the number of pollen cells increased to colonies of the Bragança and Algarve but

decreased to the Basque Country, and the number of honey cells was higher than 36000 in all origins (Table 6).

Until the Spring of the following year (08-03-2017), the average weight of each origin continued to decline (Table 5.), with the honey and pollen consumption and the reduction in the number of adult bees, despite the increase in the number of brood cells (Table 6.).

In May (02-05-2017), at the maximum of the flowering season, the average weight of all origins started to increase (Table 5). In this assessment, the number of adult bees, the number of brood and pollen cells increased in all origins. The number of honey cells decreased in the colonies of Bragança and Algarve origins, but it increased in the Basque Country origin (Table 6).

Table 5. Colony weight in the apiary of Gimonde. N represents the number of colonies of each origin.

	N		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	36	0	18
23-03-2016	18	18	15
29-04-2016	17	13	14
30-05-2016	15	9	13
28-06-2016	14	9	12
28-07-2016	14	9	12
28-08-2016	14	9	12
12-09-2016	13	8	12
18-09-2016	13	8	12
27-10-2016	13	7	12
08-03-2017	12	5	11
02-05-2017	10	2	8

	Average weight ± Confidence Norm (Kg)		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	11.2 ± 0.7	N/A	10.8 ± 1.1
23-03-2016	4.3 ± 0.7	4.2 ± 0.3	3,6 ± 0.6
29-04-2016	3.4 ± 0.6	3.4 ± 0.6	3.0 ± 0.6
30-05-2016	10.3 ± 2.9	10.1 ± 2.3	8.1 ± 2.6
28-06-2016	17.7 ± 4.6	15.1 ± 4.0	14.7 ± 4.2
28-07-2016	29.8 ± 5.7	23.7 ± 7.1	28.3 ± 6.3
28-08-2016	30.7 ± 5.9	21.9 ± 6.3	30.5 ± 5.5
12-09-2016	29.5 ± 6.2	23.5 ± 5.5	29.4 ± 5.4
18-09-2016	17.3 ± 2.0	16.4 ± 2.5	16.2 ± 1.7
27-10-2016	15.2 ± 1.9	15.2 ± 2.1	14.6 ± 1.8
08-03-2017	10.4 ± 1.9	11.1 ± 1.8	10.0 ± 1.4
02-05-2017	12.8 ± 3.3	13.3 ± 4.4	16.6 ± 5.9

Table 6. Number of adult bees, number of brood cells, number of honey cells and number of pollen cells by colony origin in the apiary of Gimonde. N represents the number of colonies of each origin.

	N		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	36	0	18
23-03-2016	18	18	15
29-04-2016	17	13	14
12-09-2016	13	8	12
27-10-2016	13	7	12
08-03-2017	12	5	11
02-05-2017	10	2	8

	Average number of adult bees ± Confidence Norm		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	5737 ± 448	N/A	5508 ± 453
23-03-2016	6376 ± 1346	8758 ± 816	6568 ± 998
29-04-2016	4971 ± 1550	3828 ± 1229	4018 ± 953
12-09-2016	14705 ± 2934	14873 ± 5061	20051 ± 2877
27-10-2016	6518 ± 1614	7516 ± 2493	7920 ± 1630
08-03-2017	4632 ± 1792	6081 ± 2916	6898 ± 2140
02-05-2017	15175 ± 3711	12954 ± 3481	16994 ± 5656

	Average number of brood cells ± Confidence Norm		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	2921 ± 478	N/A	2912 ± 583
23-03-2016	6150 ± 1906	5591 ± 1433	5306 ± 1526
29-04-2016	11676 ± 4321	7256 ± 3497	9963 ± 3777
12-09-2016	5433 ± 1428	5672 ± 1829	9360 ± 2211
27-10-2016	1582 ± 819	1373 ± 438	1658 ± 791
08-03-2017	10598 ± 4399	13312 ± 7636	15642 ± 4924
02-05-2017	29073 ± 6993	33736 ± 6935	36780 ± 11119

	Average number of honey cells ± Confidence Norm		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	25637 ± 1841	N/A	24861 ± 2466
23-03-2016	3576 ± 1610	1995 ± 895	2096 ± 891
29-04-2016	750 ± 323	696 ± 332	894 ± 289
12-09-2016	64734 ± 11950	51024 ± 14154	65255 ± 10227
27-10-2016	36263 ± 5479	37393 ± 5102	39117 ± 5411
08-03-2017	19913 ± 5517	17480 ± 5863	16836 ± 3110
02-05-2017	16540 ± 5543	15200 ± 5266	23625 ± 12125

	Average number of pollen cells ± Confidence Norm		
Date	**Bragança**	**Algarve**	**Basque Country**
22-10-2015	1316 ± 274	N/A	931 ± 293
23-03-2016	562 ± 256	596 ± 238	734 ± 464
29-04-2016	1723 ± 520	2657 ± 950	2491 ± 578
12-09-2016	6577 ± 1596	7279 ± 1716	5579 ± 2257
27-10-2016	7626 ± 2622	8861 ± 2933	5178 ± 2168
08-03-2017	6063 ± 2244	4269 ± 2668	5334 ± 1088
02-05-2017	9680 ± 2456	10282 ± 5266	7666 ± 2802

In the apiary of Zavial, the colonies of each origin, showed that before the winter period, on 08-11-2015, the weight of 36 colonies from Algarve was 9.1 Kg ± 0.5 (Table 7.). The colonies from Algarve started with N(A)=36: 10998.9 ± 745.3 adult bees, little brood and pollen cells, and some honey cells. In this date, no colony from Bragança was in the apiary of the Zavial (Table 8).

At the end of the Winter (17-02-2016), the average weight of the colonies of both origins was the low (Table 7). The colonies from Algarve consumed some honey, slightly reduced the number of adult bees, but they increased the number of pollen cells and brood. The colonies of the Bragança origin had a greater number of honey cells and a lower number of adult bees and brood cells than those of the Algarve origin (Table 8).

In the following month (16-03-2013) the average weight of the two origins increased slightly (Table 7), indicated the beginning of the flowering season.

One month later (16-04-2016), the average colony weight of both origins continued to increase (Table 7) due to the raise in the number of adult bees and number of brood, honey, and pollen cells (Table 8). The average colony weight of both origins continued to increase in May, June and July (Table 7).

In evaluation of July (11-07-2016), before the honey harvest was carried out, the average weight of the colonies of both origins increased (Table 7**)**. In this date, the quantity of adult bees and brood cells decreased and the quantity of honey and pollen cells increased (Table 8). The second weighting of July (14-07-2016) showed the average weight of the two origins after the honey harvest (Table 7).

In the following month (17-08-2016), the average colony weight of the Algarve origin decreased, compared to the previous evaluation, while that the weight of the Bragança origin increased (Table 7). The weight reduction observed in the Algarve origin probably resulted from the higher loads of varroa in the previous month (Figure 7), right before the treatment with Apivar was performed.

In September (20-09-2016), the average weight of the colonies from Algarve increased whereas that of the colonies from Bragança decreased slightly, when compared to the previous month (Table 7.).

However, about one month later (05-11-2016), the average weight increased in the two origins (Table 7). In this evaluation date, it was observed an increase in the number of adult bees in the Algarve origin in comparison to July (Table 8). The number of adult bees and the number of brood, honey and pollen cells (Table 8) unfortunately cannot be compared with last month (uncollected data) to know what increased the weight. One possibility is the existence

of some flowering in this period, which sustained colony growth probably in population (brood and/or adults) an in the amount of food reserves.

At the end of the Winter (13-02-2017), the average weight of both origins decreased (Table 7). This resulted from a consumption of honey and pollen by both origins, which was greater than the gain of brood. Furthermore, the colonies of the Algarve origin showed an increase in the number of adult bees while those of Bragança origin showed a decrease, when compared to the previous evaluation (Table 8).

Finally, in the last evaluation date (08-04-2017), the average weight of both origins started to increase (Table 7), in response to the beginning of flowering season. This increment resulted from the increase in the number of adult bees and of brood, honey and pollen cells (Table 8).

Table 7. Colony weight in the apiary of Zavial. N represents the number of colonies of each origin.

	N	
Date	**Algarve**	**Bragança**
08.11.2015	36	0
17.02.2016	18	18
16.03.2016	18	17
16.04.2016	17	15
14.05.2016	17	15
15.06.2016	17	15
11.07.2016	10	11
14.07.2016	10	11
17.08.2016	9	10
20.09.2016	6	8
05.11.2016	5	7
13.02.2017	5	7
08.04.2017	2	6

	Average weight ± Confidence Norm (Kg)	
Date	**Algarve**	**Bragança**
08.11.2015	9.1 ± 0.5	N/A
17.02.2016	7.0 ± 0.8	6.7 ± 1.0
16.03.2016	8.2 ± 1.0	7.1 ± 1.0
16.04.2016	14.5 ± 2.1	11.8 ± 1.8
14.05.2016	23.5 ± 3.7	19.5 ± 3.0
15.06.2016	26.0 ± 4.1	21.1 ± 4.1
11.07.2016	30.5 ± 5.1	21.8 ± 3.6
14.07.2016	11.4 ± 1.3	11.8 ± 1.7
17.08.2016	11.3 ± 2.3	13.8 ± 2.4
20.09.2016	13.1 ± 5.9	13.2 ± 2.8
05.11.2016	15.2 ± 2.5	15.2 ± 3.7
13.02.2017	13.1 ± 2.4	11.0 ± 3.4
08.04.2017	16.0 ± 3.1	18.6 ± 5.9

Table 8. Number of adult bees, number of brood cells, number of honey cells and number of pollen cells by colony origin in the apiary of Zavial. N represents the number of colonies of each origin.

	N	
Date	**Algarve**	**Bragança**
08.11.2015	36	0
17.02.2016	18	17
16.04.2016	17	15
11.07.2016	10	11
05.11.2016	5	7
13.02.2017	5	7
08.04.2017	2	6

	Average number of adult bees ± Confidence Norm	
Date	**Algarve**	**Bragança**
08.11.2015	10999 ± 745	N/A
17.02.2016	10773 ± 1097	6913 ± 1060
16.04.2016	24095 ± 3377	17282 ± 2456
11.07.2016	11376 ± 5310	15405 ± 3701
05.11.2016	13866 ± 2269	9451 ± 2700
13.02.2017	14921 ± 4121	7261 ± 2442
08.04.2017	20836 ± 562	18555 ± 3995

	Average number of brood cells ± Confidence Norm	
Date	**Algarve**	**Bragança**
08.11.2015	3133 ± 933	N/A
17.02.2016	14691 ± 2387	7718 ± 1108
16.04.2016	41564 ± 4536	38540 ± 3829
11.07.2016	10923 ± 4269	11933 ± 2281
05.11.2016	10528 ± 2926	7313 ± 2164
13.02.2017	27444 ± 8989	10914 ± 3820
08.04.2017	31035 ± 787	40415 ± 7157

	Average number of honey cells ± Confidence Norm	
Date	**Algarve**	**Bragança**
08.11.2015	16033 ± 1432	N/A
17.02.2016	6588 ± 2460	10368 ± 2583
16.04.2016	16648 ± 4072	11641 ± 3251
11.07.2016	45782 ± 9366	37629 ± 6108
05.11.2016	27766 ± 8289	32545 ± 12343
13.02.2017	17044 ± 7191	16885 ± 9071
08.04.2017	18148 ± 5239	25974 ± 13931

	Average number of pollen cells ± Confidence Norm	
Date	**Algarve**	**Bragança**
08.11.2015	2775 ± 486	N/A
17.02.2016	2962 ± 822	1801 ± 506
16.04.2016	12590 ± 1653	6237 ± 1590
11.07.2016	34100 ± 5095	17288 ± 1511
05.11.2016	13500 ± 3542	8503 ± 1222
13.02.2017	7900 ± 1898	4201 ± 1546
08.04.2017	17578 ± 5154	9292 ± 1266

3.3.2. Colony weights per origin

In the apiary of Gimonde, the colonies from Bragança always had the greatest weight, except in 27-10-2016, 08-03-2017, and 02-05-2017. The colonies from Algarve had the second greatest weight between 23-03-216 and 28-06-2016. The smaller weight of the Algarve origin was between 28-07-2016 and 12-09-2016, probably due to the high rate of varroa infestation (Figure 11). On 18-09-2016 and 02-05-2017, Algarve had again the second greatest weight, and in 27-10-2016 and 08-03-2017 this origin showed even greater weight. However, in the last evaluations, the number of assessed colonies of the Algarve origin was very low (N=2), as compared with the other two origins. In the first four evaluations (22-10-2015 to 28-06-2016), the colonies of the Basque Country origin had the lowest weight. Between 28-07-2016 and 12-09-2016, this origin exhibited the second greatest weight, but from 18-09-2016 to 08-03-2017 they had again the worst weight. In the last assessment, the colonies from the Basque Country had the best weight.

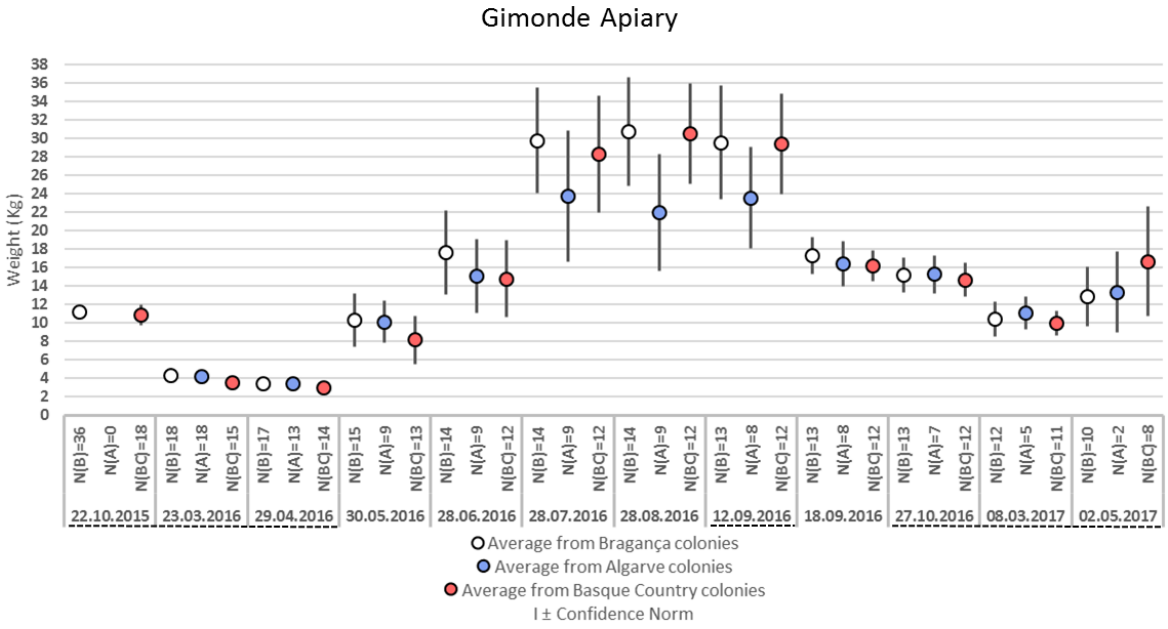

Figure 11. Colony weight in the apiary of Gimonde. N represents the number of colonies of each origin. Colony origin of Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, the colonies of the Algarve origin had the greatest weight from 17-02-2016 to 11-07-2016. From 14-07-2016 to 05-11-2016 the colonies from Bragança showed a higher weight, probably because even though the colonies from Algarve produced

more honey than Bragança in 14/07/2016 (Figure 12), they were weakened by the excess of varroa.

Colonies from Algarve had once again the greater weight in 13-02-2017, but in 08-04-2017 the colonies from Bragança exceed the colonies from Algarve. In both apiaries, local colonies showed some times more weight than non-local colonies. However, the result is not always constant, probably due to high levels of varroa infestation observed on the evaluation of 12-09-2016 in Gimonde and of 11-07-2016 in Zavial.

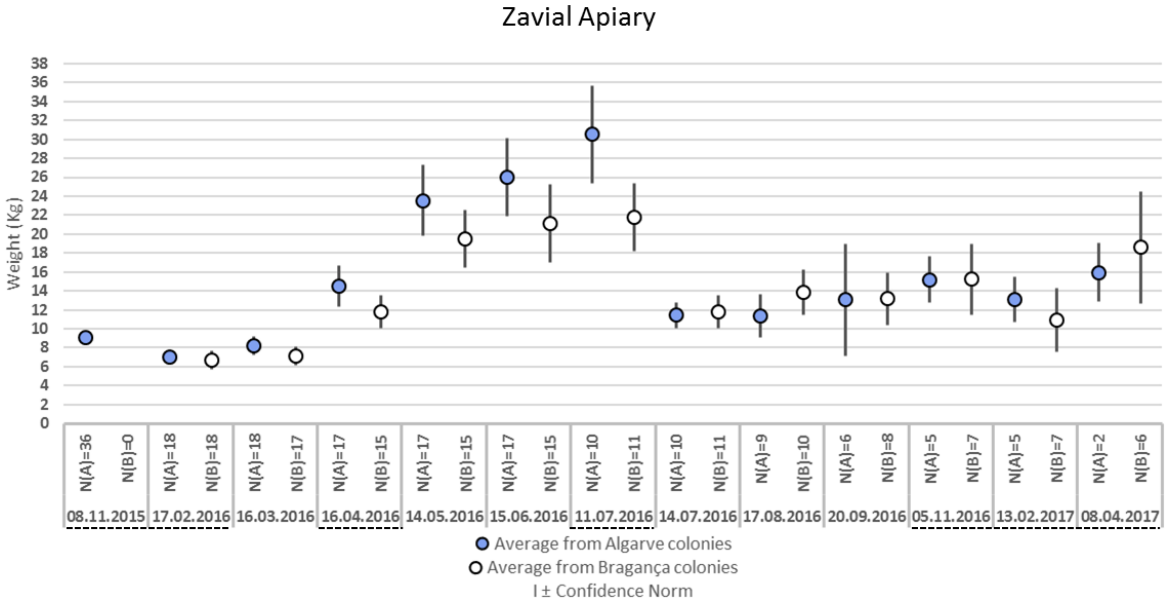

Figure 12. Colony weight in the apiary of Zavial. N represents the number of colonies of each origin. Colony origin of Algarve and Bragança are indicated by A and B, respectively.

3.3.3. Number of adult bees per origin

In the apiary of Gimonde, the colonies of the Bragança origin showed the smallest number of adult bees in four evaluation dates (23-03-2016, 12-09-2016, 27-10-2016 and 08-03-2017), the largest in two dates (22-10-2015 and 29-04-2016), and in one date it was the second origin (02-05-2017). The colonies of the Algarve origin were the second regarding the number of adult bees in three evaluations (12-09-2016; 27-10-2016 and 08-03-2017). This origin had the smallest number of adult bees on 29-04-2016 and 02-05-2017. Although, in the last evaluations, the number of assessed colonies of the Algarve origin was very low (N=2), as compared with the other two origins. This origin had the largest number of adult bees on 23-03-2016. The colonies of the Basque Country origin had the largest number of adult bees in the last four evaluations (12-09-2016, 27-10-2016, 08-03-2017 and 02-05-2017) and the second largest in the first three (22-10-2015, 23-03-2016 and 29-04-2016).

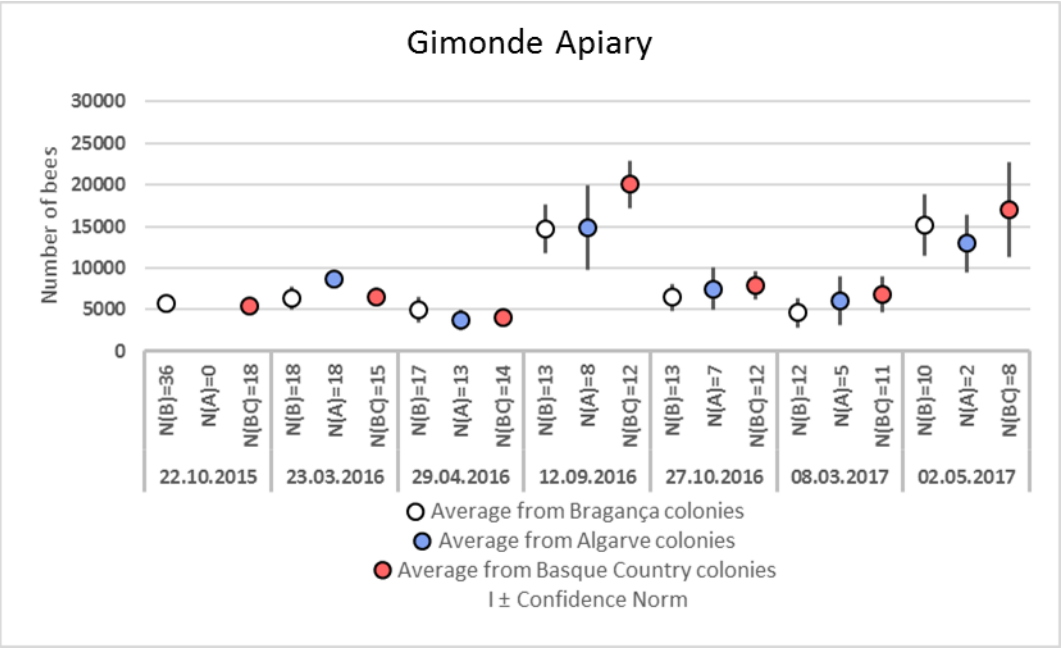

Figure 13. Number of adult bees by colony origin in the apiary of Gimonde. N represents the number of colonies of each origin. Colony origin from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, the colonies of the Algarve origin had the largest number of honey bees in all evaluations, except in one (11-07-2016; Figure 14).

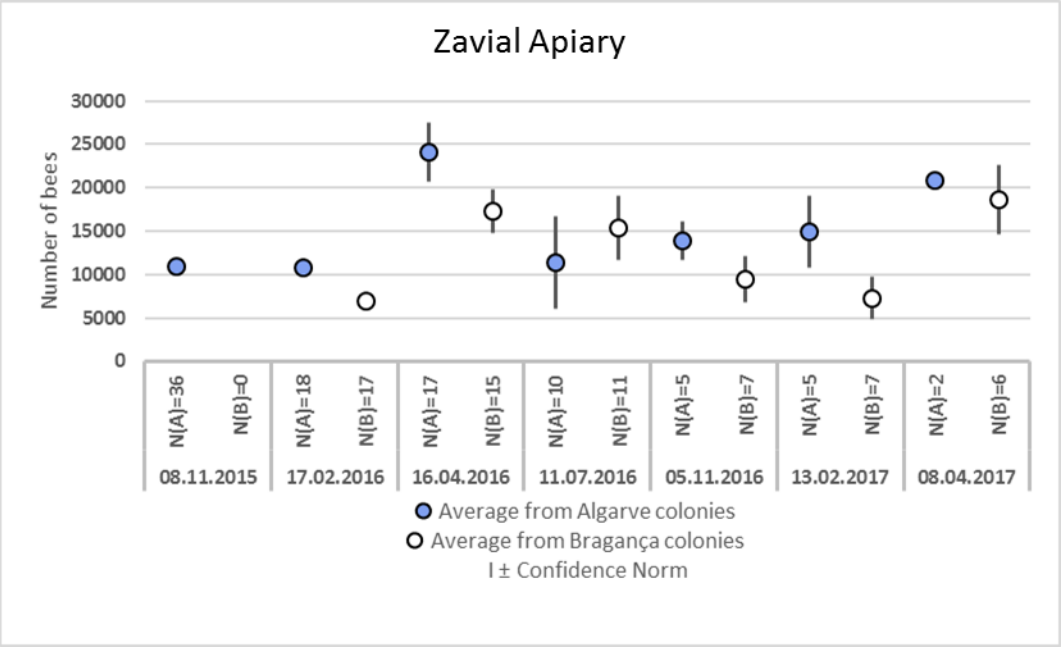

Figure 14. Number of adult bees by colony origin in the apiary of Zavial. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

In the apiary of Gimonde, colonies of the Basque Country origin generally had more adult bees, followed by the Algarve origin (Figure 13). The colonies of the Algarve origin generally had in both apiaries an adult bee population larger than the Bragança origin. This suggests that queens of the Algarve origin seem to invest more in colony development than the other origins, regardless the environmental conditions. Nonetheless, the differences are minor and likely not statistically significant. Furthermore, the sample size of the Algarve origin in the last evaluations is small (N<5) and therefore the results should be interpreted with caution. Although our results are not conclusive and further experiments should be implemented, it seems that, within the Iberian honey bee variation, local honey bees are not better adapted than the non-local. In a similar reciprocal translocation study, but testing variation within and between different honey bee subspecies, Hatjina et al. (2014) analyzed 597 colonies from 16 different genetic origins representing five subspecies (*A. m. carnica*, *A. m. ligustica*, *A. m. macedonica*, *A. m. mellifera*, *A. m. siciliana*), located in 20 apiaries, in 11 European countries. At each apiary, the local origin was tested with at least two "foreign" origins without no chemical treatments against varroa. Unlike in this study, the authors detected a tendency towards specific adaptations in genotypes of local origin, especially in terms of adult bee population, honey production and overwintering ability, with local origins performing generally better than non-local. Furthermore, they found that colonies placed in Mediterranean countries (hot weather) tended to have lower adult bee populations and higher brood population compared to colonies in colder climates, thus reflecting the shorter longevity of bees in warmer climates and the shorter brood rearing period in the north. In contrast, in our study, the Apis mellifera showed a greater number of adult bees in the southern apiary than in the northern apiary.

3.3.4. Number of brood cells per origin

In the apiary of Gimonde, the colonies of the Bragança origin had the largest number of brood cells in the first three assessments, the second largest number on 27-10-2016 and the smallest number on 12-09-2016, 08-03-2017 and 02-05-2017. The colonies of the Algarve origin had the second largest number of brood in four evaluations (23-03-2016, 12-09-2016, 08-03-2017 and 02-05-2017) and the smallest in two evaluations (29-04-2016 and 27-10-2016). The colonies of the Basque Country origin had the largest number of brood cells in the last four assessments, the second largest in two (22-10-2015 and 29-04-2016) and the smallest in one (23-03-2016).

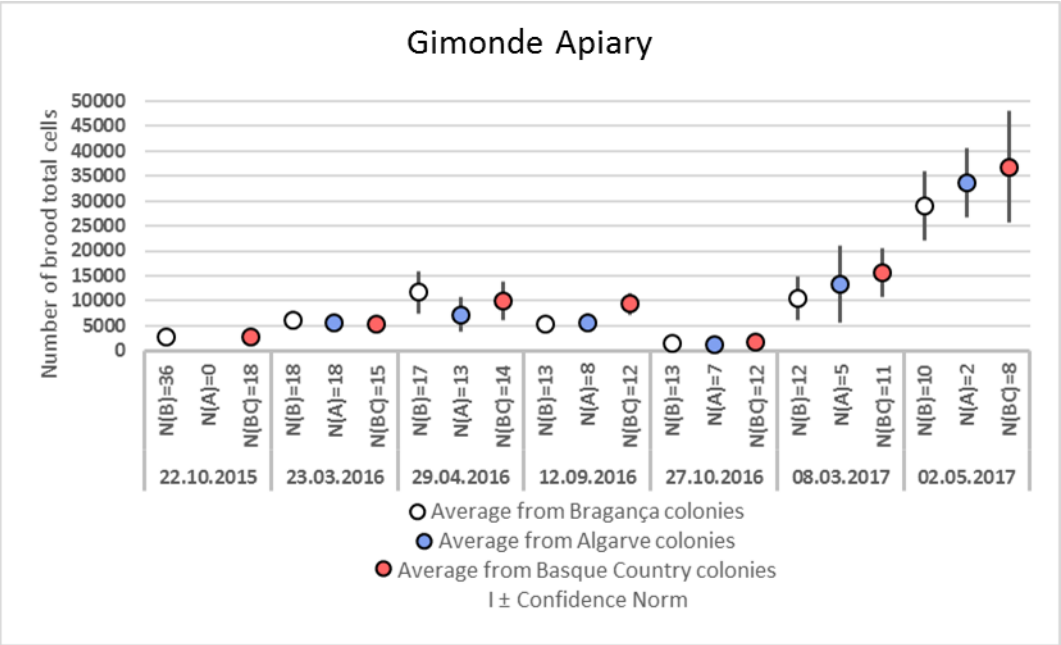

Figure 15. Number of brood cells by colony origin in the apiary of Gimonde. N represents the number of colonies of each origin. Colony origin from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, the Algarve origin had in most evaluations the largest number of brood cells, while the Bragança origin just showed more brood in two evaluations (11-07-2016 and 08-04-2017). In contrast, in the apiary of Gimonde, colonies of the Algarve origin never were the origin with more brood. This suggests that colonies of the Algarve origin are more adapted to their local environment. In the apiary of Gimonde, the number of brood cells and the number of adult bees was higher for the Basque Country origin, suggesting that this non-local origin is adapted to the local environment.

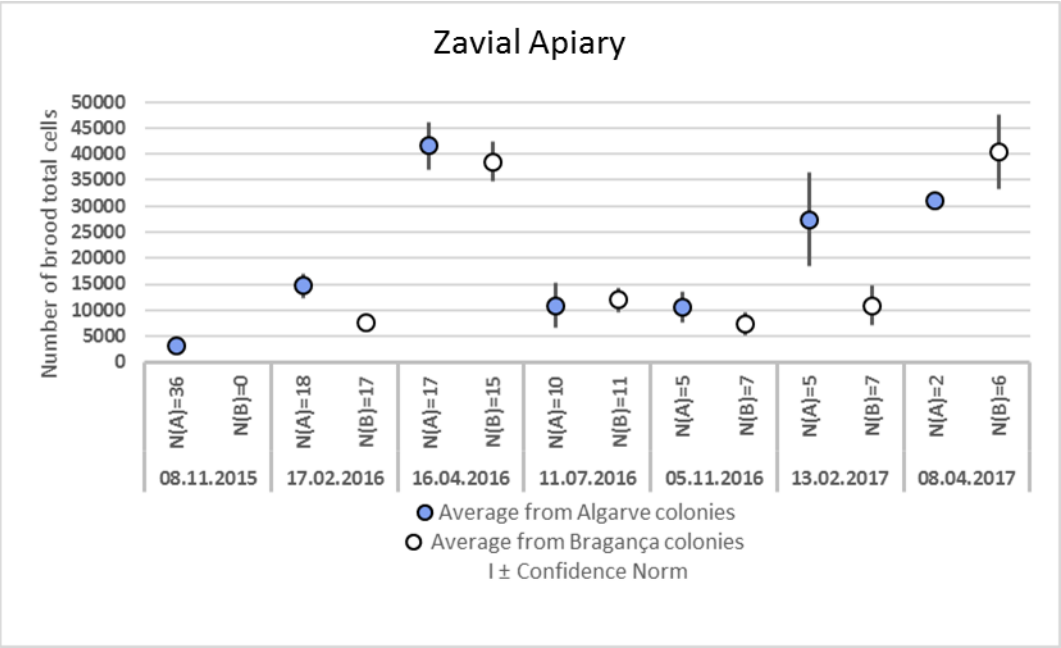

Figure 16. Number of brood cells by colony origin in the apiary of Zavial. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

3.3.5. Number of honey cells and honey yield per origin

Regarding the number of honey cells, in the apiary of Gimonde, the colonies of the Bragança origin had the largest number of honey cells in the first two and penultimate assessments, the second largest in three assessments (29-04-2016, 12-09-2016 and 02-05-2017) and the smallest on 27-10-2016 (Figure 17). The colonies from Algarve had the smallest number of honey cells in all evaluations, except on 27-10-2016 and 08-03-2017 that had the second largest number. Most of the time the colonies of Basque Country origin had the largest number of honey cells (29-04-2016, 12-09-2016, 27-20-2016 and 02-05-2016). The Basque Country origin only had twice the second largest number of honey cells (22-10-2015 and 23-03-2016) and once the smallest (08-03-2017).

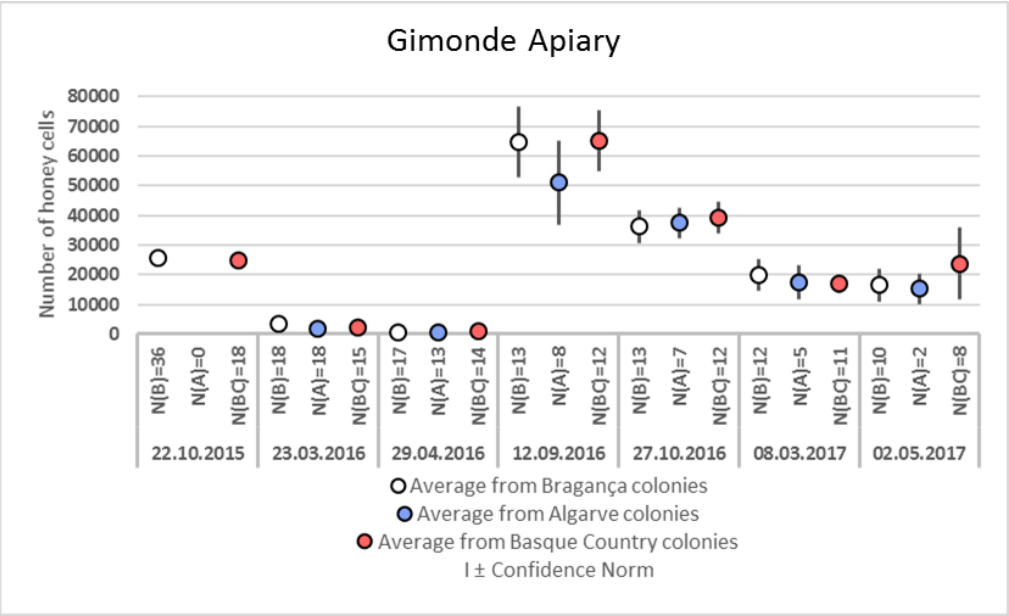

Figure 17. Number of honey cells by colony origin in the apiary of Gimonde. N represents the number of colonies of each origin. Colony origin from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In the apiary of Zavial, the Algarve origin had more honey cells in 16-04-2016, 11-07-2016 and 13-02-2017, while the Bragança origin had more honey cell in 17-02-2016, 05-11-2016 and 08-04-2017.

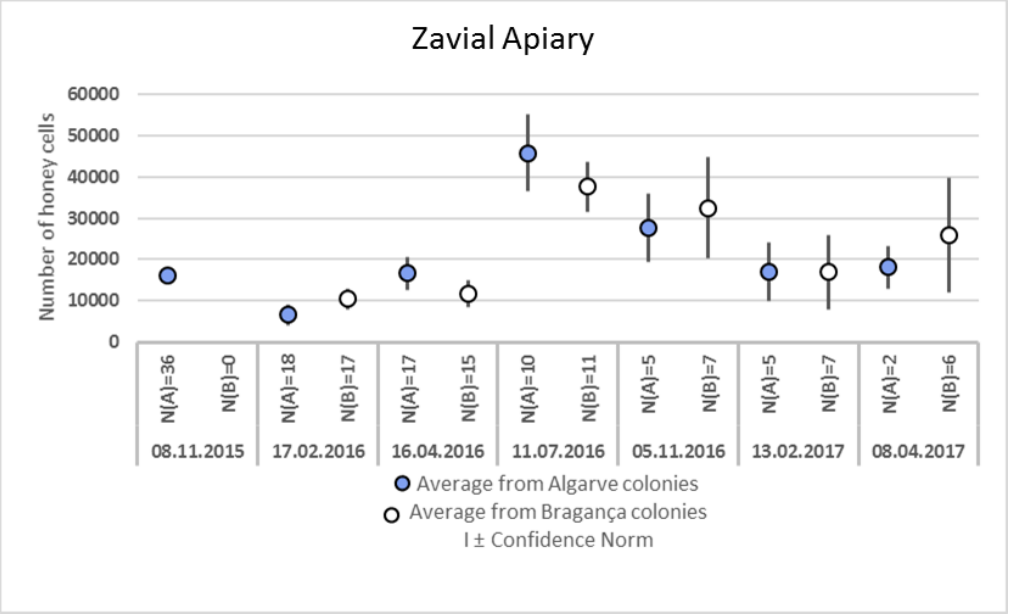

Figure 18. Number of honey cells by colony origin in the apiary of Zavial. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

The origin that showed highest honey yield in the apiary of Gimonde in 2016 was the Basque Country with an average of 13.2Kg ± 5.3 per colony, followed by Bragança with 12.3Kg ± 5.8, and the Algarve with 7,1Kg ± 4.6.

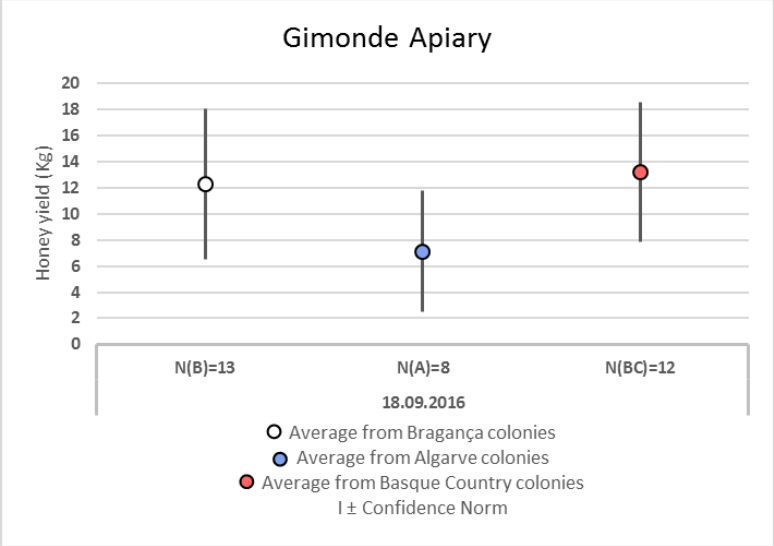

Figure 19. Honey yield per origin in Gimonde. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

The origin that showed highest honey yield in the apiary of Zavial was the Algarve origin with 19.1Kg ± 4.4 followed by Bragança with 10kg ± 3.9.

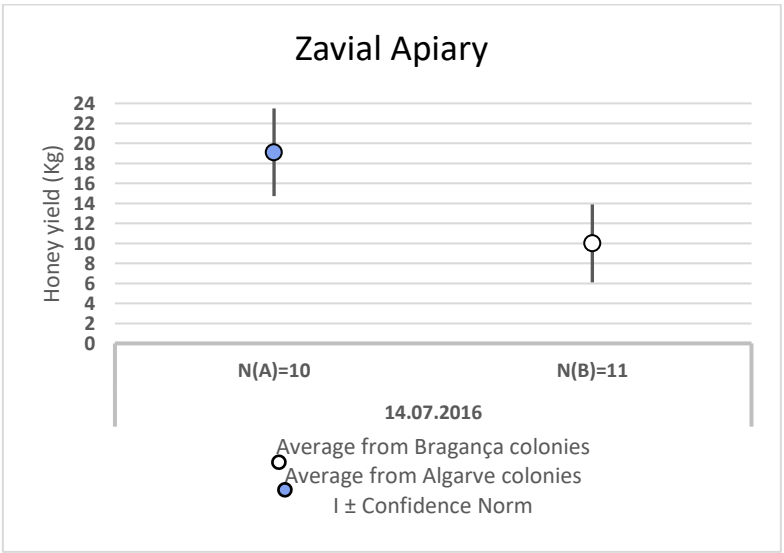

Figure 20. Honey yield per origin in Zavial. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

Comparing the honey yield produced by Bragança and Algarve origins in a local and non-local environment, we found that colonies of the local origin are more productive than the non-local, suggesting the existence of local adaptation, as measured by honey production. This is supported by the study of Hatjina et al. (2014) previously mentioned. However, in the apiary of Gimonde it was a non-local origin (Basque Country) that produced more honey, suggesting that this origin is pre-adapted to the Gimonde environment.

3.3.6. Number of pollen cells per origin

In the apiary of Gimonde, the Bragança origin had the largest number of pollen cells in two evaluations (22-10-2015 and 08-03-2017), the second largest in three (12-09-2016, 27-10-2016 and 02-05-2017), and the smallest in two (23-03-2016 and 29-04-2016). The Algarve origin had the largest number of pollen cells in all assessments, except in 23-03-2016 and 08-03-2017, that had the second largest and the smallest, respectively. Finally, the Basque Country origin had the largest number of pollen cells in one evaluation (23-03-2016), and three times the second largest (22-10-2015, 29-04-2016 and 08-03-2017) and the smallest (12-09-2016, 27-10-2016 and 02-05-2017).

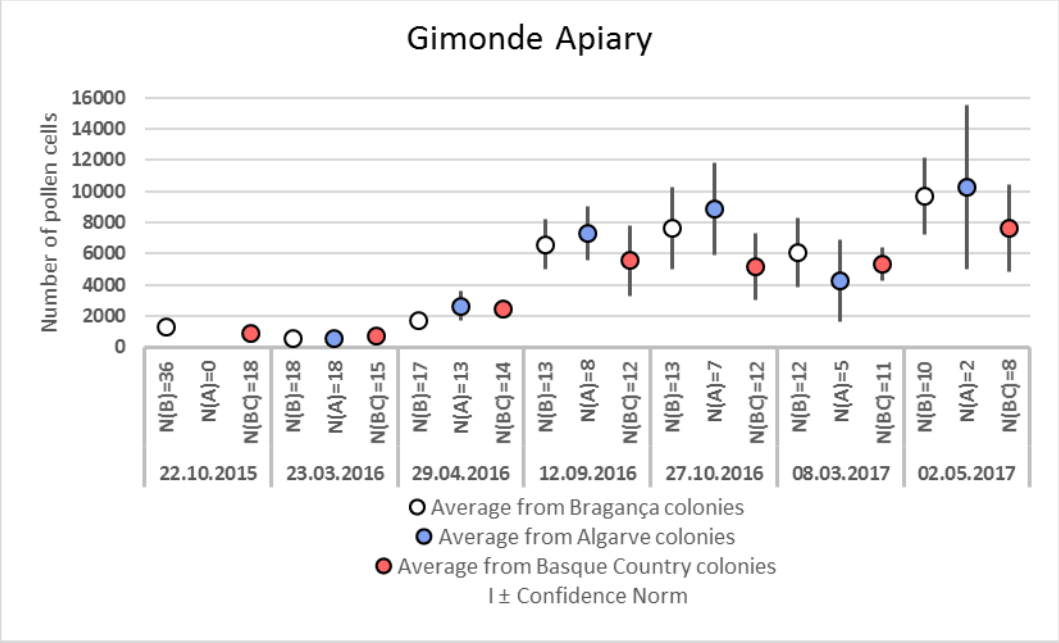

Figure 21. Number of pollen cells by colony origin in the apiary of Gimonde. N represents the number of colonies of each origin. Colony origin from Bragança, Algarve and Basque Country are indicated by B, A and BC, respectively.

In Zavial, the colonies of the Algarve origin always had the largest number of pollen cells and the same trend was shown in Gimonde.

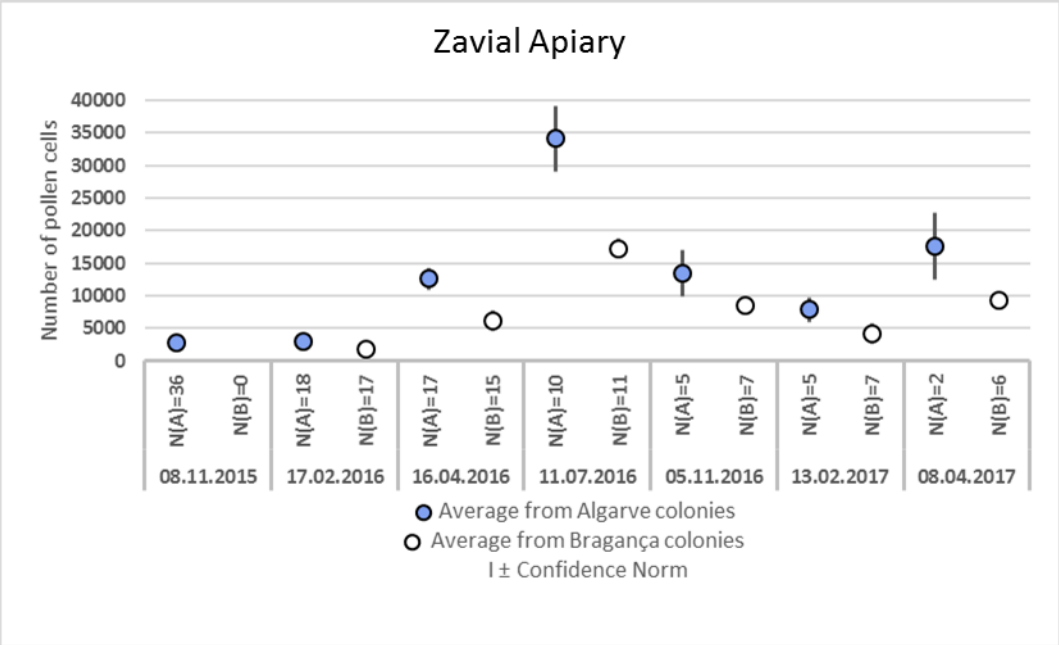

Figure 22. Number of pollen cells by colony origin in the apiary of Zavial. N represents the number of colonies of each origin. Colony origin from Algarve and Bragança are indicated by A and B, respectively.

4. Colony longevity per origin

In the apiary of Gimonde, 559 days after we started the experiment with 54 colonies (18 colonies x 3 origins), we only had 38.9% (21) of the initial colonies, including 11.1% (2) of colonies of the Algarve origin, 50% (9) of colonies of the Basque Country origin and 55.6% (10) colonies of the Bragança origin **(**Figure 22).

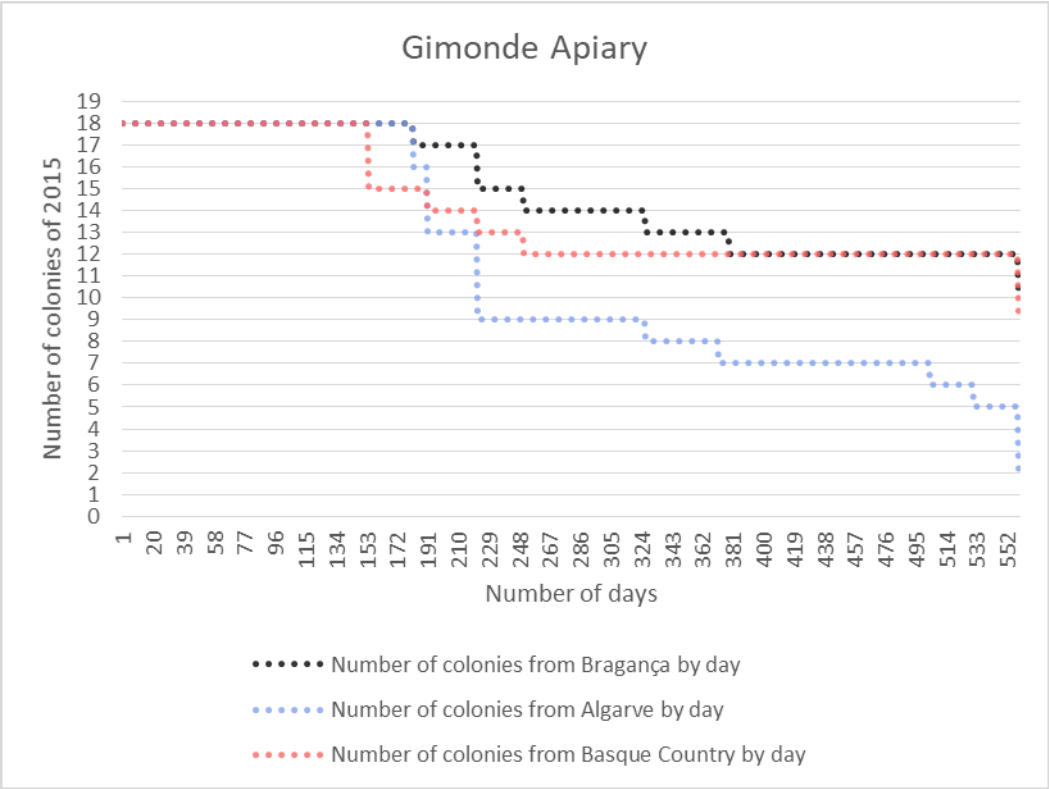

Figure 23. Longevity of colonies of 2015 with different origins in the apiary of Bragança.

Furthermore, in the apiary of Gimonde, of the 44.4% (8) of colonies of the Bragança origin that did not survive, 27.8% (5) died without to show any signs of the most common diseases with observable symptoms such as American foulbrood, chalkbrood, deformed wing virus, 5.6% (1) replaced the queen, and 11.1% (2) swarmed **(**Figure 23).

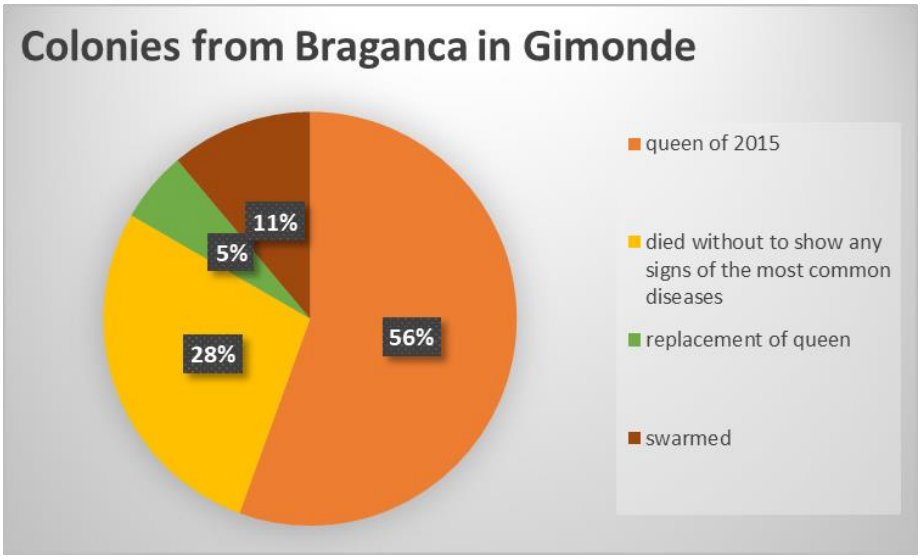

Figure 24. Causes of bee colony mortality or replacement of the Bragança queen of 2015 in Gimonde.

Regarding the Algarve origin, of the 88.9% (16) of the colonies that did not survive, 50% (9) died without to show any signs of the most common diseases, 22.2% (4) superseded the queen, and 16.7% (3) swarmed (Figure 24).

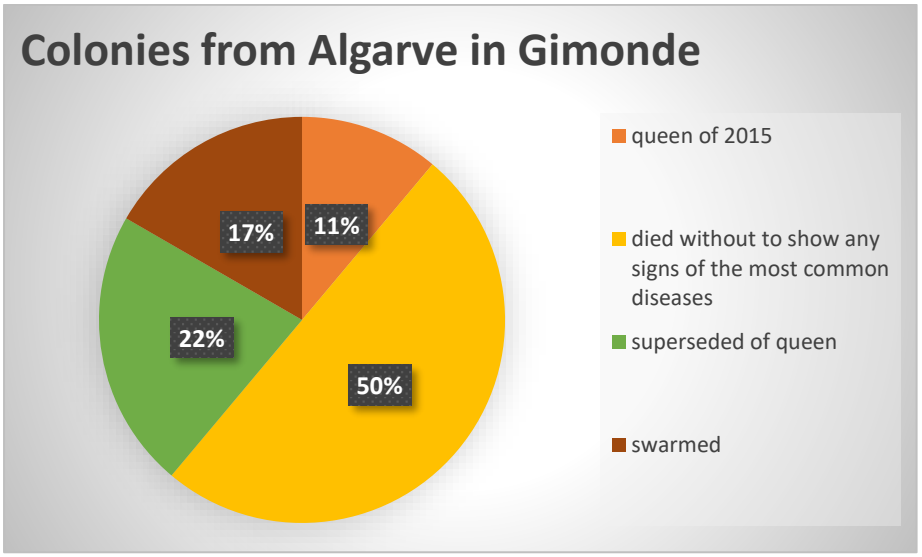

Figure 25. Causes of bee colony mortality or replacement of the Algarve queen of 2015 in Gimonde.

Regarding the Basque Country origin, of the 50% (9) of the colonies that did not survive, 11.1% (2) died without to show any signs of the most common diseases, 5.6% (1) died from diseases, 11.1% (2) superseded the queen, and 22.2% (4) swarmed (Figure 25).

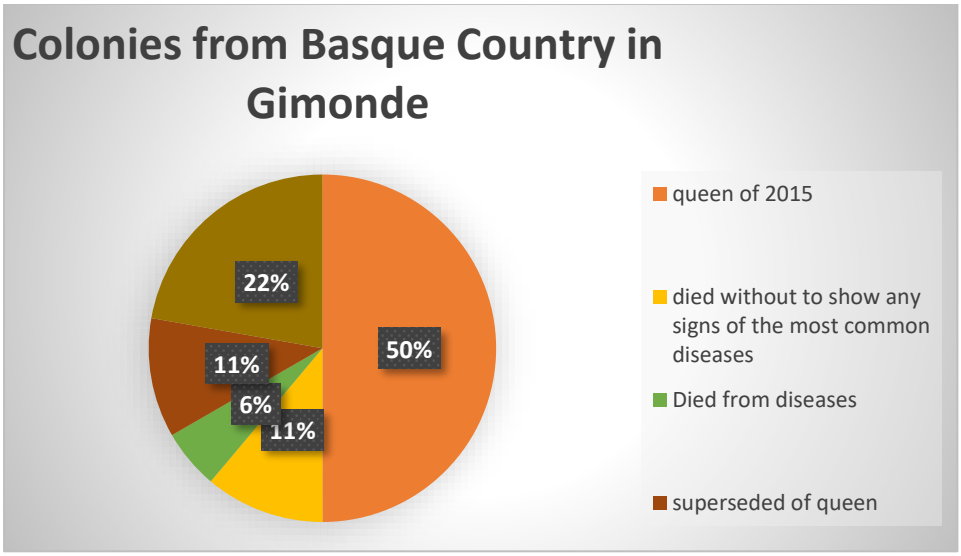

Figure 26. Causes of bee colony mortality or replacement of the Basque Country queen of 2015 in Gimonde.

In the apiary of Zavial (Figure 26), 518 days after we started the experiment with 36 colonies (18 colonies x 2 origins), we had 25% (9) of the initial colonies, including 16.7% (3) of colonies from Algarve and 33.3% (6) of colonies from Bragança.

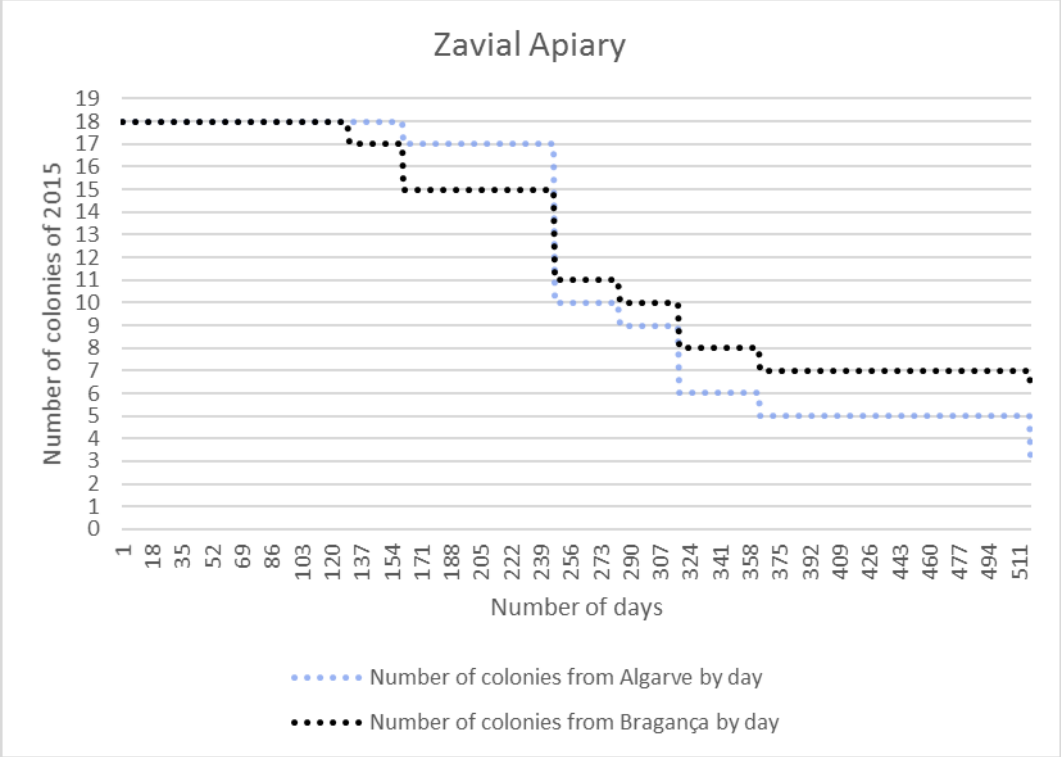

Figure 27. Longevity of colonies of 2015 with different origins in the apiary of Zavial.

In the apiary of Zavial, of the 83.3% (15) of colonies of the Algarve origin that did not survive, 5.6% (1) died without to show any signs of the most common diseases, 22.2% (4) died by disease, 5.6% (1) superseded the queen, and 38.9% (7) swarmed, and 11.1% (2) queen replacement (Figure 27).

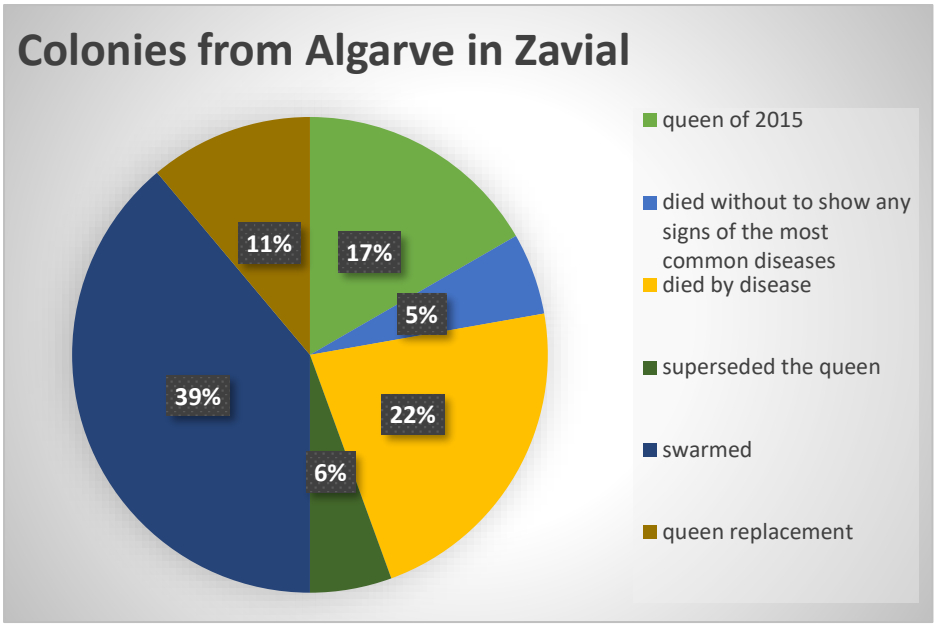

Figure 28. Causes of bee colony mortality or replacement of the Algarve queen of 2015 in Zavial.

Regarding the Bragança origin, of the 66.7% (12) colonies that did not survive, 22.2% (4) died without to show any signs of the most common diseases, 5.6% (1) died by disease, 5.6% (1) died due to management, 5. 6% (1) superseded the queen, 22.2% (4) swarmed and 5.6% (1) queen replacement (Figure 28).

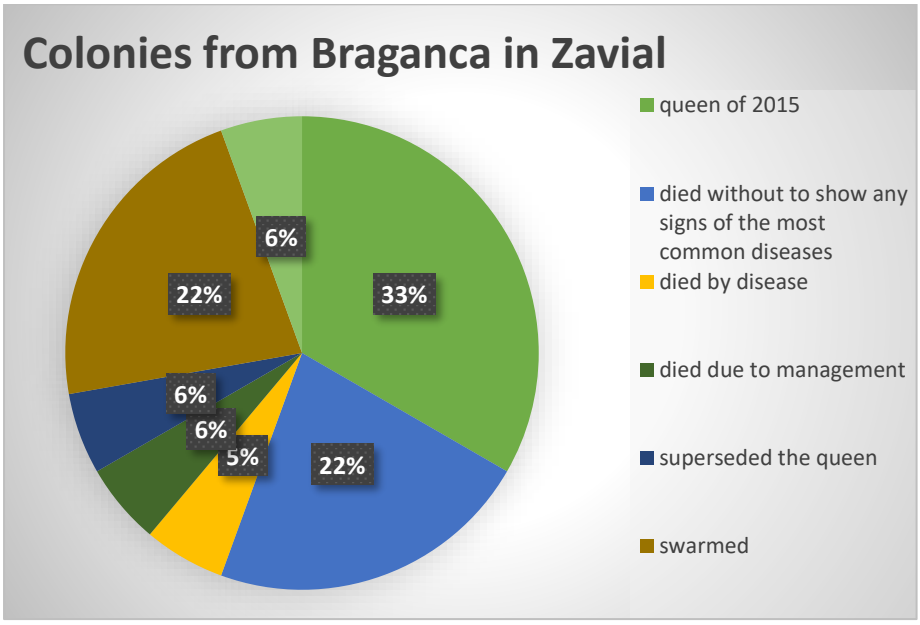

Figure 29. Causes of bee colony mortality or replacement of the Bragança queen of 2015 in Zavial.

A higher survivorship of the local origin compared to the non-local ones was observed suggesting local adaptation. A similar result was reported by Büchler et al. (2014), who analyzed 597 colonies representing five subspecies (*A. m. carnica, A. m. ligustica, A. m. macedonica, A. m. mellifera, A. m. siciliana*) and 16 genotypes deployed in 20 European apiaries. In this study, 15,7% of the colonies survived between the Fall of 2009 and the Spring of 2012 without any therapeutic treatment against varroa diseases (Büchler et al. 2014). The authors showed a significantly higher survivorship of the local genotypes compared to the non-local ones, indicating a specific local adaptation of the honey bee populations.

5. Conclusion

Our research was focus on the Iberian honey bee, *Apis mellifera iberiensis* which has proved to be highly diverse and complex at the genetic level. The adaptive potential allows it to live in a large range of environments.

The major aim of this study was to understand the local adaptive process of honey bees under different environmental conditions.

The evaluation of the vitality and performance of the colonies were performed in two apiaries, one in Gimonde (Northest of Portugal) and another in Zavial (Southwest of Portugal) with three and two genetic origins, respectively, between October of 2015 and May of 2017. The evaluations suggest that:

(1) the apiary location has a main role on the varroa's infestation level;

(2) there is no difference in varroa incidence between local and non-local colonies;

(3) the variation in the hygienic behavior in each evaluation may result from different proportions of workers with hygienic behavior (between 15-17 days of age) in the colony;

(4) there are origins with better hygienic behavior than others;

(5) Regarding the colony strenght (weight and adult bees, brood, and food resources), local honey bees are not better adapted than the non local. However these results may have been influenced by the high level of varroa infestation that some origins showed in a given period of time;

(6) the local origin has a higher survivorship when compared to the non-local ones, suggesting local adaptation.

Finally, this research contributes to the improvement of knowledge about the adaptive potential of honey bees, bringing emphasis on the importance of preserving the local honey bees and suggests a pragmatic approach to combine profit with sustainability. Therefore, ours results would be a good help for beekeepers who wish to elaborate sustainable management plans that are more adapted to their honey bee colonies.

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