Restructuring of the Honey Bee Chain and Varroa Resistance Breeding & Selection Programme

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Content

Abbreviations and definitions

CB	Commercial beekeeper
CS	Case study
HYG	Hygienic behaviour (pin test)
OW	Overwintering index
PT	Performance tester
REC	Recapping behaviour
SMR	Suppressed mite reproduction
VSH	Varroa sensitive hygiene

1. Abstract

The EurBeST study explores possibilities for increasing the varroa resistance of commercially available honey bees by selective breeding and analyses ways to improve beekeepers' access to resistant material.

Analysis of the EU market for reproductive material, including queen production and trade, shows high diversity of organisation, but also a weak development of breeding structures in some countries. EU beekeepers are satisfied with the quality of breeding material, except for varroa resistance traits. Despite growing demand, no established market for varroa-resistant stock in Europe exists, and supply of queens is limited.

To provide reliable data regarding the performance of resistant stocks, specific lines were compared under commercial conditions in five case studies, including traditional traits and varroa resistance (VSH, SMR, REC, hygienic behaviour). Strong genotype-environment interactions affected many traits, highlighting the importance of local adaptation. Local lines produced more honey, but the test lines had fewer mites at the end of the study. Some lines from long-term selection programs combined good productivity and improved varroa resistance.

A cost-benefit analysis provides insights into the economic aspects of queen production, colony evaluation, and selection for varroa resistance.

Based on the results, recommendations are developed for beekeepers involved in breeding and policy makers.

2. Assignment and mission of the study

Although the apicultural sector in Europe is small compared to other agricultural sectors, it has a major impact on the efficiency of European food production and the sustainability of ecosystems due to its pollination services. However, intensive agricultural production systems, climatic and environmental changes can strongly affect the well-being of honey bees and other pollinators. On top of these challenges, the near-ubiquitous prevalence of the invasive parasitic varroa mite, together with associated virus infections, continues to burden the productivity, health and survival of honey bee colonies in Europe.

It is important to realise that apiculture differs from other animal production sectors in some crucial aspects:

- although managed by man, the main food source of a honey bee colony is the direct environment of the hive where they forage for nectar and pollen and therefore depend on the floral resources of the bees' natural habitat; in addition, the colony self-regulates and stabilises its nest climate according to the conditions outside the hive; both of these factors constitute an extremely high level of interaction between colonies and their natural environment;
- high diversity exists in the motivation, education and experience of the active beekeeping community across countries, with a high proportion of apiculturists practising beekeeping as a hobby or a sideline source of income;
- the economic and marketing structures for beekeeping products (including pollination service) as well as those for honey bee reproductive material are in part not very well developed.

As a consequence of the intensive interactions with the individual environment and climate, honey bees have developed a huge geographic diversity during their evolutionary history. This is reflected in the presence of about 13 separate honey bee subspecies that occur naturally in Europe and that can in part be further differentiated into ecotypes with specific regional adaptations. Recent research has demonstrated the importance of the significant interactions between honey bee genotypes and environmental factors for colony survival, health and productivity parameters.

Together with the complex mating biology, characterised by multiple matings of the queen and resulting in high genetic diversity of the worker bee population of a colony, this enables honey bees to adapt to variable environmental conditions and their changes over time. The independent natural development of varroa resistance in several local European honey bee populations confirms this general capability.

However, honey bee colonies from populations that are the result of natural selection for varroa resistance sometimes do not perform well in regard to traits that are considered crucial by commercial beekeepers, such as honey production, gentleness, or swarming behaviour. Nonetheless, breeding and disseminating varroa resistant bees to European beekeepers could significantly improve honey bee colony health and honey production in Europe, provided these honey bees are suitable for commercial beekeeping. This could be achieved by implementing selection strategies that combine mite resistance traits together with the already established economically relevant traits. However, the relevance of various specific resistance traits, their interactions with other characters of economic interest and the efficacy of selection strategies are still under discussion.

Although impressive examples for successful selective breeding in honey bees exist, many European regions suffer from structural deficiencies and a lack of implementation of suitable methods. This might partly be explained by an insufficient educational and economic development of the sector, but specific technical challenges due to the complex biology of honey bees may also play a role.

To reduce high colony losses due to varroa infestation regular applications of therapeutics are common in many European countries, causing extra costs and labour for the beekeepers. Furthermore, the quality and reputation of bee products can be impaired by residues from miticide treatments, and treatments are not always effective. If the selection and spread of varroa resistant bees could make a contribution to significantly lowering the loss rate of the commercially used stock and also to reduce the average demand for medication, positive effects, not only on the profitability of the beekeeping sector but also on the pollination-dependent plant growing sector and on the stability of European ecosystems, can be expected.

To achieve a sustainable improvement of the varroa situation, beekeepers across the EU need to get access to queens resistant to varroa that also possess good production qualities. Information on the availability, types and quality of the reproductive material placed on the EU market needs to be disseminated, and practical knowledge on how to produce and use such reproductive material must be shared. Beekeepers have to be made aware of the availability and quality of this reproductive material, and be trained to use it.

To explore the current and future possibilities for increasing the varroa resistance of commercially available honey bees by selective breeding, and to improve the beekeepers' access to such selected material, the EU commission launched the EurBeST study in December 2017. To achieve this goal, the EurBeST team first analysed the EU market for reproductive material of European honey bees, including data on production and trade of all member states. The team then reviewed the state of play for the production and keeping of European honey bees resistant to varroa. To explore the expectations and experience of customers of honey bee reproductive material and their views on the potential of varroa resistant honey bees to arrive at a long-term sustainable solution, an online survey was developed and distributed among beekeepers in various countries.

To assess the potential of honey bee selection for improving bee health and production for commercial breeding and beekeeping in Europe, an expert team of 131 queen breeders, performance testers and commercial beekeepers from seven countries was established. With support from this network, the core team successfully organised case studies in France, Germany (also involving some beekeepers from Austria and Croatia), Greece, Italy and Poland. To ensure that the results of the case studies can be taken as representative for the honey bee reproductive market, the countries were selected among those in which production and structure of the breeding market is more significant, but also diversified, to reflect the variability identified by the previously conducted overview of the reproductive market and state of play of varroa resistance in commercially available stock.

Together with the European expert team, a methodology was established to develop commercial production of varroa resistant honey bees by breeders and to promote the dissemination of such bees to commercial beekeepers. A testing scheme for validation of the methodology within the case studies was developed, involving the assessment of the commercial qualities and varroa resistance traits of 23 honey bee selection lines over one full apicultural season, and followed by an extended statistical analysis of the study data.

The full data set is presented with this report and provides a representative description of the status of European breeding stock in terms of varroa resistance and commercial qualities. The report identifies key elements that will enable the further development of commercial production of reproductive material from varroa resistant European honey bee lines and improvement of their availability on the EU market for use by commercial beekeepers.

3. Analysis of the European market for honey bee breeding stock

3.1. Introduction

Commercial production of queens started in the late 19th century, together with the spread of "rational beekeeping". By the end of the century, commercial production of queens was well developed in both Europe and the USA, including their shipment within and across countries by mail. Before that, whole colonies or swarms were the object of trade.

Trade of reproductive material is driven by the commercial choice to regularly replace queens (so that the operation will on average have young queens, thereby reducing risk of swarming and natural replacement) or desire to have stock with more favourable apicultural traits compared to the own stock.

Unlike for the numbers of honey bee colonies, there is no official reporting system for honey bee queen production in the EU, or for the production of other kinds of reproductive material. The only data available so far come from two scientific studies which report on numbers of queens produced in Europe, both based on collection of data via questionnaires (Lodesani & Costa, 2003[1]; Chauzat et al, 2013[2]).

We here report an overview of the market of apicultural reproductive material for all 28 EU Member States. The revised version of the report "Overview of the

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[1] Lodesani, M, Costa, C (2003) Bee breeding and genetics in Europe. Bee World, 84(2): 69-85. https://doi.org/10.1080/0005772X.2003.11099579

[2] Chauzat, M-P, Cauquil, L, Roy, L, Franco, S, Hendrikx P, et al. (2013) Demographics of the European apicultural industry. PLoS ONE, 8(11): e79018. https://doi.org/10.1371/journal.pone.0079018

EU market for reproductive material of European honey bees", produced in May 2018, is contained in the Annex.

The overview was obtained by using various kinds of data sources: EU trade statistics[3], TRACES System, questionnaires to experts (EurBeST team survey) and Member States' national apicultural programmes (referring to 2018 data).

3.2. Results

In honey bees, the fundamental reproductive unit is the queen bee. Queen bees are traded with or without the colonies they are heading. Thus, the following main categories of honey bee reproductive material are recognised:

- Queen bees (typically a young egg-laying queen, recently mated, held in a small cage together with about 10 worker bees; however, exceptions apply in some countries, see below)
- Small colonies, also termed "swarms", "nuclei", or "nukes" (usually on 4-5 frames including brood and honey, with an egg-laying queen)
- "Package bees", (generally consisting of 1.5 - 2 kg of adult bees without frames) that, however, sometimes are sold without a queen present.

Full-sized colonies are also traded, but to a much smaller extent, and insufficient data was collected to give any results. Trade of honey bee semen within Europe appears to be marginal.

The main findings of the overview are summarised in Table 3.1, where parameters relating to production and international trade are given as total for EU28 (with some exceptions, see Annex), and prices of honey bee reproductive material are given as average per EU country.

Table 3.1. Overall values of the EU honey bee reproductive market, based on the EurBeST team survey.

Production				International trade*		Prices (€)			
No. bree-ders	No. queens	No. swarms	No. package bees	No. queens exported	No. queens imported	Queens		Swarm	Package bees
						Open mated queen **	Breeder queen**		
2 739	1 772 975	603 000	108 400	54 604	212 105	21	38	105	83

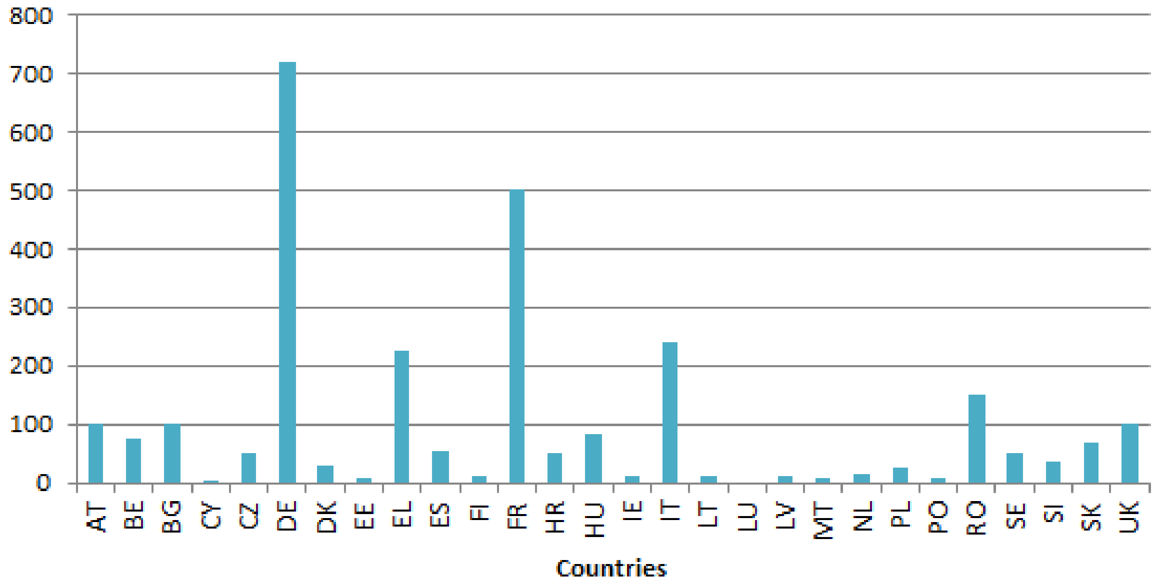
* within and extra EU; ** see 3.2.2. for further explanation

[3] https://trade.ec.europa.eu/access-to-markets/en/statistics?includeUK=true.

3.2.1. Total and average numbers of breeders, queens, swarms and package bees

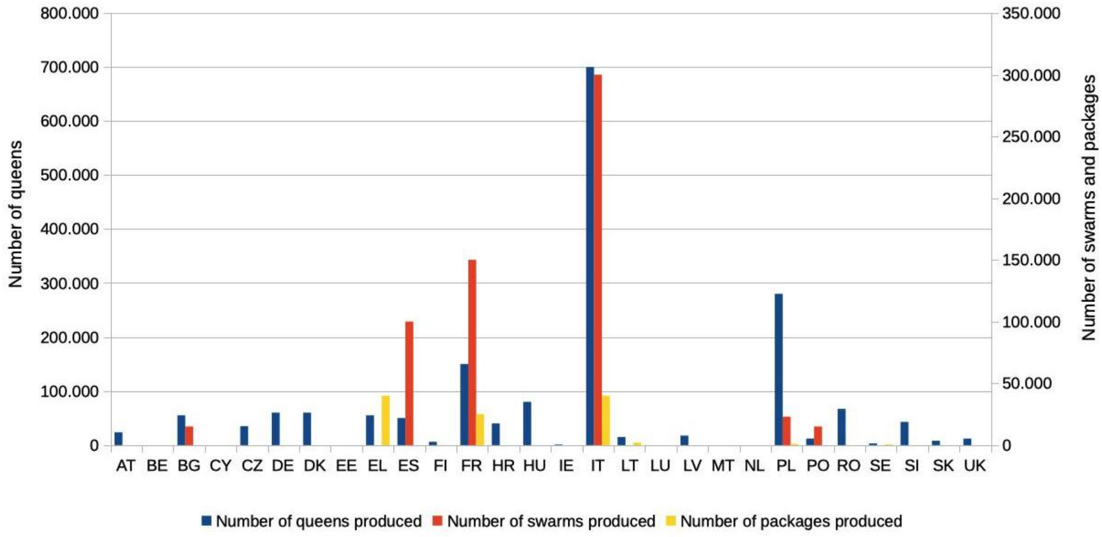
The estimated total number of queen breeders in the EU is 2 739 (excluding Luxembourg). Queen breeders are defined as beekeepers who specialise in queen production and who obtain part of their income from trade with queens, swarms or package bees. The highest numbers of breeders are in Germany and France (equal or over 500), followed by Italy and Greece (over 200) and Romania (over 100) (Fig 3.1). The total number of breeders corresponds to an average 0.4% of the total number of EU beekeepers (as given by MS for the National Apiculture Programmes).

Figure 3.1. Estimated number of breeders in EU countries



The total number of queens produced annually is 1 772 975 (no data from Belgium, Cyprus, Estonia, Luxembourg, Malta and the Netherlands). More than one third of these queens are produced in Italy (700 000), followed by Poland with 280 000 and France with 150 000. Production of an annual total of 603 000 commercial swarms was reported from six countries (Bulgaria, France, Italy, Poland, Portugal and Spain), while 108 200 package bees are estimated as being produced each year in France, Greece, Italy, Lithuania, Poland and Sweden (to a much lesser extent in the latter three countries) (Fig. 3.2).

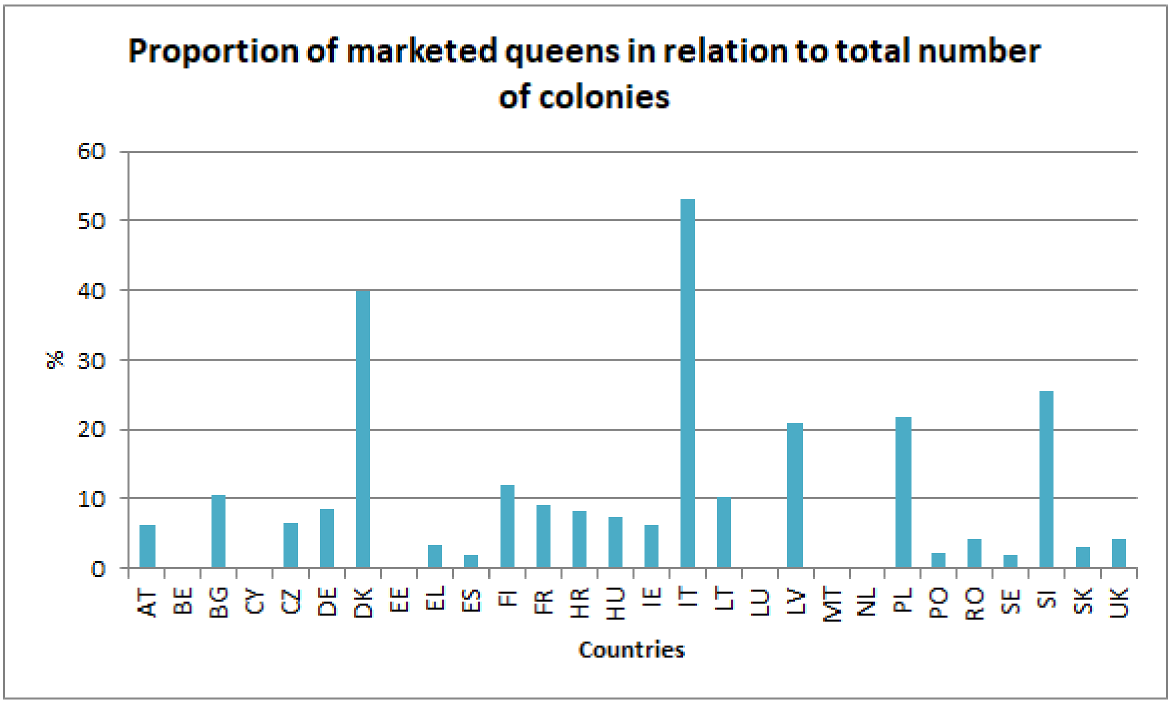
Figure 3.2. Annual numbers of queens (left axis), swarms and package bees (right axis) produced in EU countries



If we relate the number of produced queens to the number of breeders, we find that the average number of queens produced per breeder per year is 1 2531, ranging from over 10 000 per breeder in Poland (a few breeders are producing very high numbers), followed by Italy (about 3 000 queens per breeder) and Denmark (3 000 queens per breeder), to very small scale producers in Germany (83 queens per breeder) and Sweden (60 queens per breeder). These results reflect the different beekeeping traditions in different countries. For example, in Poland many of the traded queens are unmated or even merely queen cells, which require a much lower production effort.

The estimated number of queens produced annually represents 11% of the total number of EU honey bee colonies, the swarms 4% and the package bees 1%. These proportions vary greatly across MS. Fig. 3.3 shows the differences between countries for the proportion of queens compared to colonies (we show only queens as they are produced in almost all countries, while the swarm and package bee market is restricted to few specialised countries). The values range from 53% in Italy to 2% in Sweden, reflecting the importance of the breeding sector in the different countries.

Figure 3.3. Proportion of marketed queens on total number of colonies per country



3.2.2. Average prices and estimation of value of the honey bee reproductive market

Within the category "queens" various levels of quality can be identified. The main distinction is between:

- "breeder" queens = queens which have undergone strict selection and are used for multiplying genetic material; mating has been controlled either through instrumental insemination or by strictly isolated mating stations;

- "open mated" queens = queens produced for direct use in colonies; mating is not usually controlled or occurs in non-strictly controlled mating stations;

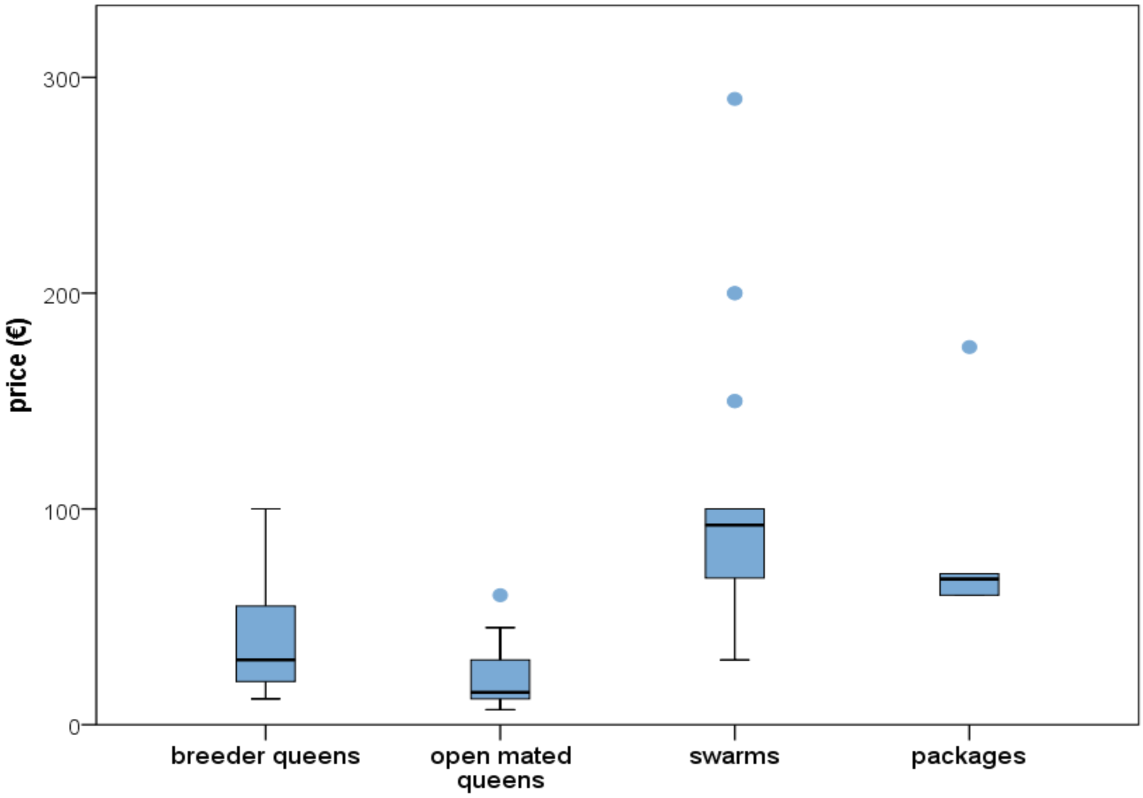
- unmated queens and queen cells (larvae) = in these cases only the maternal contribution is sold; the buyer has to care himself for mating the queen or for raising the queens from larvae.

However, this distinction does not exist in all countries, and the trade of unmated queens or queen cells is large scale only in Poland, and was thus included in the "open mated " queens.

Figure 3.4 shows the variability in prices of reproductive material across the EU. We found that the average price of a breeder queen is 38 €, ranging from a maximum of 100 € in Slovenia to 12 € in Croatia. The average price of an open mated queen is 21 €, ranging from a maximum of 60 € in Denmark (although this value probably is the country average, including breeder queens), to a minimum 4 € in Poland (the unmated queens). Other countries with open mated queen price under or equal to 10 € are Croatia, the Czech Republic, Italy and Romania. The average price for swarms is 105 €, and 83 € for package bees.

For swarms, there was a great variation in the reported prices (maximum value of 290 € in Finland and minimum 30 € in Lithuania), which could be due to the different interpretation of "swarms" (as described above, these are generally intended as small colonies on 4-5 frames including brood and honey, with a laying queen, but there are many variants, such as more or fewer frames, with a young queen or with an older queen, sold in spring or at the end of the season). Package bees instead are more standard and this uniformity is reflected in the price range, between 70 € and 60 €, apart from Sweden where the price is much higher (175 €).

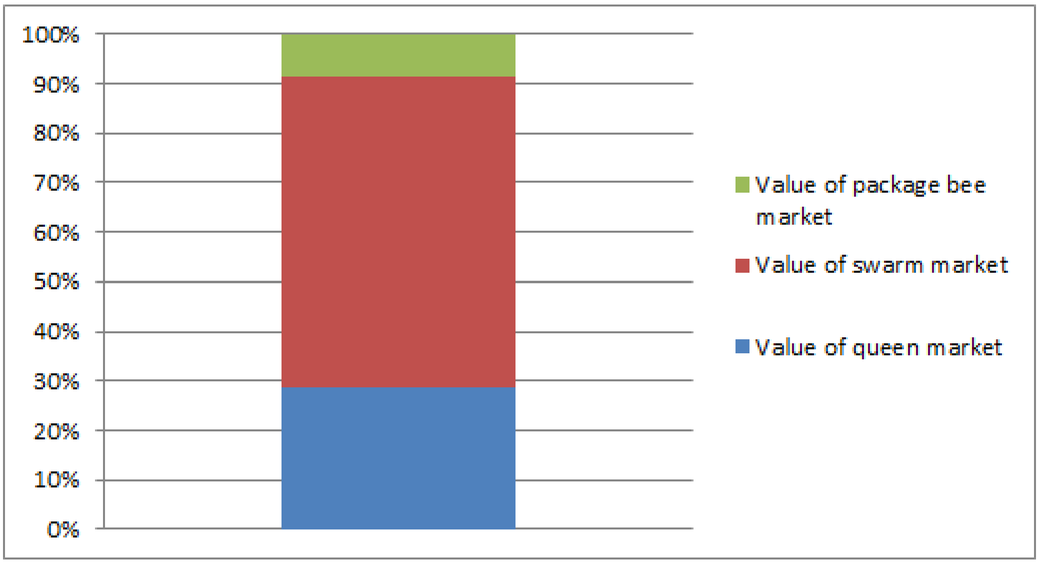
Figure 3.4. Distribution of prices of reproductive material in the EU (minimum, first quartile, median, third quartile and maximum; circles are outliers)



Based on the above described data, we calculated the total annual value of the honey bee reproductive market (including queens, swarms and package bees) to be around 86 M € (probably underestimated, as income from queens was assessed conservatively). This value corresponds to about 5% of the value of the honey market (as given from Member States National Apiculture Programmes). Almost half of the value of the honey bee reproductive market is attributed to Italy, with 40 M €; next are France (18 M €) and Spain (7.5 M €), while the remaining countries are all under 5 M €.

The contributions of the different kinds of reproductive material are represented in Figure 3.5. Contribution of the queen market may be underestimated as the lowest reported queen price was used in the calculation. As expected, the country with the highest value of the queen market is Italy (about 7 M €) followed by Denmark (3.6 M €), France (3 M €) and Poland (2.8 M €). Italy is also the country with the highest market value of swarms with about 30 M €, followed by France (13.5 M €) and Spain (7 M €); the situation for package bees is more equally distributed among the countries involved, mainly Italy (2.8 M €), Greece (2.4 M €) and France (1.7 M €). Details are included in the Annex.

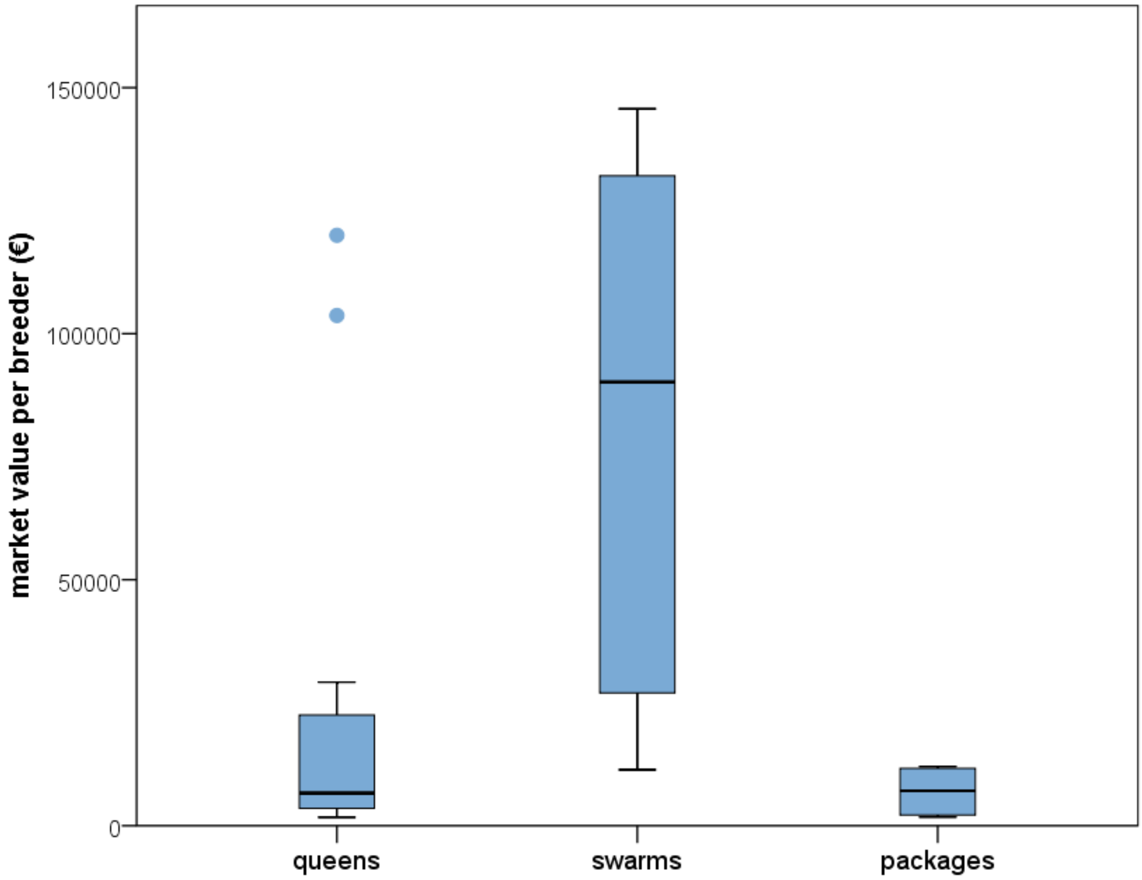
Figure 3.5. Share of queens, swarms and package bees in the market of reproductive material



3.2.3. Breeders' incomes and relative cost of queens for beekeepers

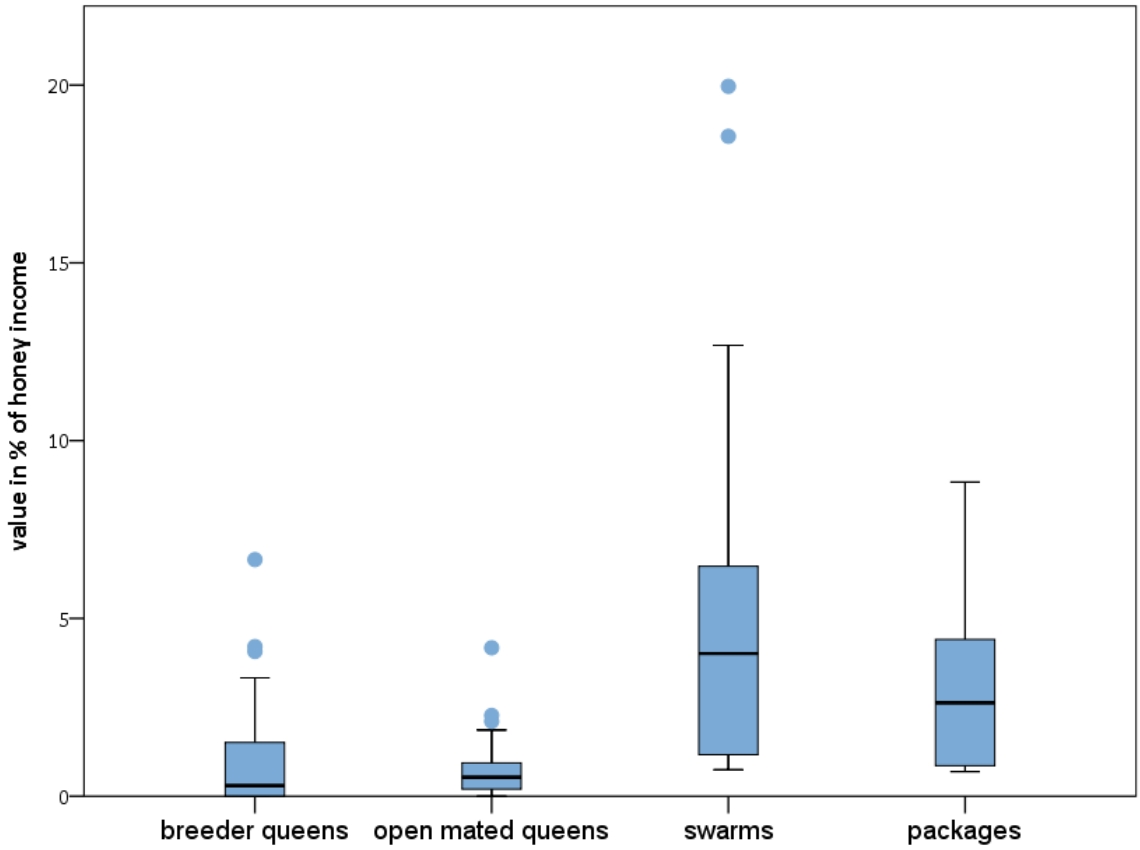
The average value of the queen market for each European breeder is 19 746 €, calculated from data referring to 22 EU countries (excluding the six countries for which we could not obtain estimates for the total number of queens produced annually). Again, this value is probably underestimated because we used the value for open mated queens, considering that these are the majority of queens present on the market. Breeders in Denmark and Poland are those who obtain the largest income from queen production (due to a high price or the large number of queens produced per breeder, respectively). The average value of the swarm market per breeder is much higher: 82 754 €, while the value of the package bee market is lower: 6 958 €. Distribution of these values across EU countries is summarised in Figure 3.6.

Figure 3.6. Distribution of value of market of reproductive material per breeder across Europe (minimum, first quartile, median, third quartile and maximum; circles are outliers)



The variation in the price of reproductive material in terms of the proportion of average annual income from honey production gives indications on various factors which affect the market of reproductive material: environmental (availability during the season, number and length of honey flows), socio-economic (price of honey, cost of labour) and cultural (breeding traditions). On average, and in relation to the country of production, one breeder queen represents 1.2% of a beekeeper's annual honey income, one open mated queen 0.8%, one swarm 5.5% and one bee package 3.3%. There is great variation among countries: breeder queens represent the highest proportion of a beekeeper's income in Slovenia (6.6%), Germany (4.2%) and Poland (4.1%), while the lowest proportions are found in Greece and Croatia (0.2%); open mated queens represent the highest proportion of a beekeeper's income in Ireland (4.2%), Sweden (2.3%) and Germany (2.1%), while the lowest proportions are again in Greece and Croatia (0.1%), closely followed by Portugal and Hungary (0.2%). An even greater variation is seen for swarms, as mentioned above probably attributable to the many different kinds of swarms available on the EU market. The price of one swarm represents the highest proportion of a beekeeper's annual honey income in the Czech Republic (20%), followed by Ireland (18%) and Estonia (13%). The extent of the above described differences are summarised in Figure 3.7.

Figure 3.7. Distribution of value of reproductive material across Europe in % of the honey income (minimum, first quartile, median, third quartile and maximum; circles are outliers)



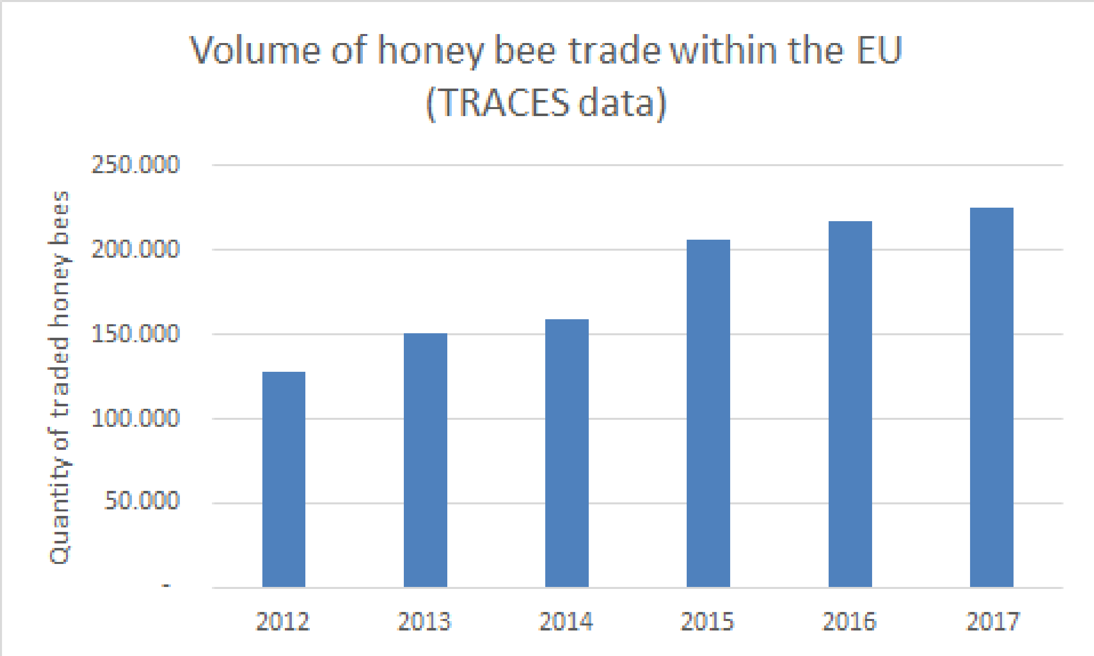
3.2.4. Trade of reproductive material within and outside the EU

The extent of import / export within the EU was assessed by expert knowledge, by the TRACES system and by official EU trade data.

From the expert knowledge, we estimate that around 55 00 queens are exported from their countries of origin each year (about 3.5% of the total queen production). The highest numbers of exported queens originate from Italy and Slovenia (both around 15 000), but the proportion compared to the total number of queens produced is much higher for Slovenia, which exports 36% of its total queens. Imports of queens are in much greater numbers; the estimated total number of imported queens is around 210 000, of which 150 000 are by France from South America and other European countries. For swarms and package bees we did not obtain this information.

TRACES data, which report numbers of traded units (queens, swarms, package bees or colonies) within the EU, show a steady increase from 2012 to 2017 in the volume of trade of live honey bees (Fig. 3.8). As our expert knowledge did not consider trade of swarms and package bees, the values found in our survey and reported by TRACES seem to coincide.

Figure 3.8. Number of traded honey bees (queens, colonies, swarms, package bees) within the EU (plus AD, LI, NO, CH), registered by the TRACES system



Although not explicitly stated, we deduced that the official EU trade statistics, giving the value of the import / export market within and outside the EU (Access2Markets, DG Trade) include the bumble bee market for pollination. Thus, three countries with extremely high values of live bee trade that are known for a flourishing bumble bee market were excluded from further calculations (Belgium, The Netherlands and Slovakia). The adjusted annual values of the import and export market within the EU, considering data from 2012 to 2017, were thus found to be 2 403 712 € and 2 570 349 €, respectively. Compared to the total estimated value of the reproductive market, the within EU export of reproductive material seems to correspond to about 3%, similar to the proportion of exported queens based on the expert knowledge survey. However, as shown in Figure 3.9 with the contributions of the different MS to the EU live bee export market, the major role played by Italy and Slovenia is not highlighted. This could be due to the fact that the bumble bee market is included in these data.

Fig. 3.9. Average export of live bees within EU countries for the time period 2012-2017, excluding Belgium, The Netherlands and Slovakia (source: EC helpdesk)

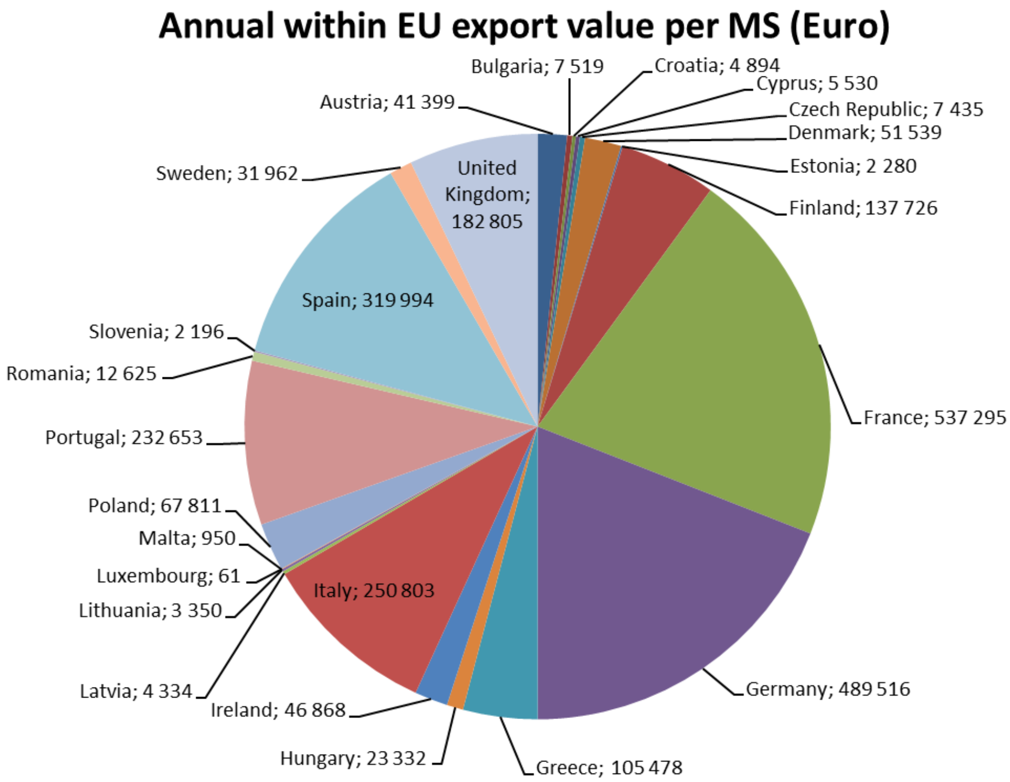
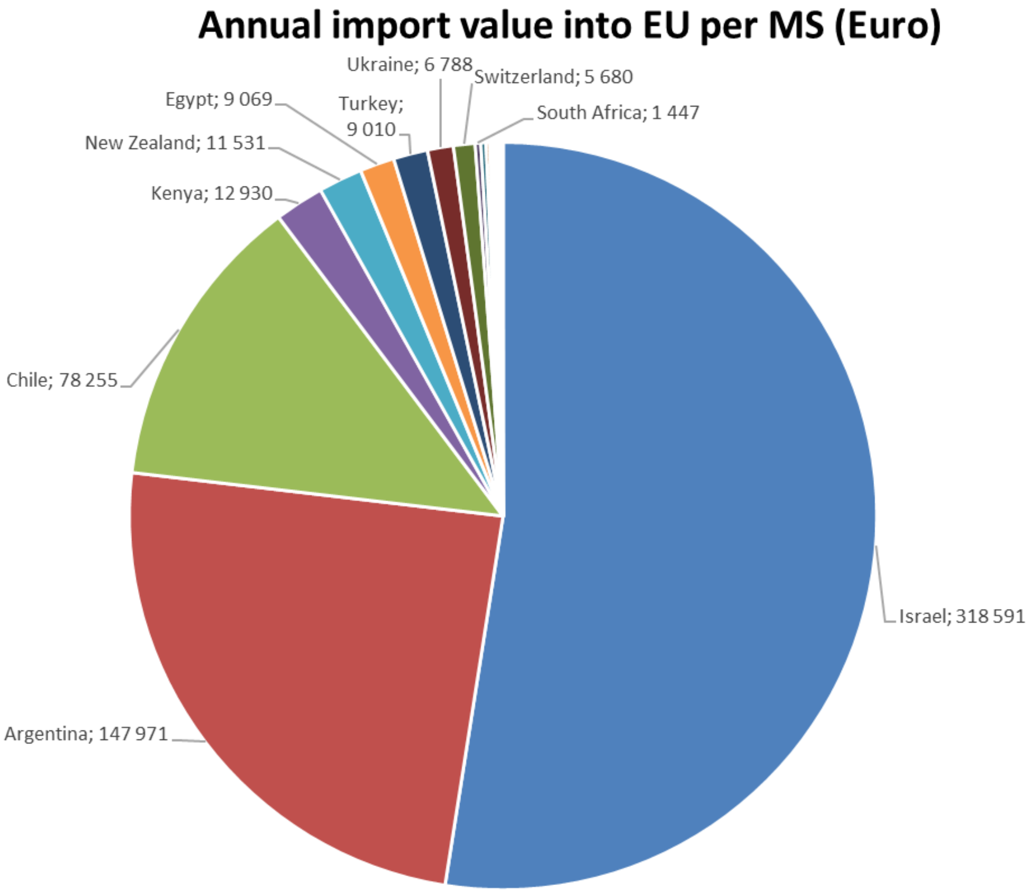


Figure 3.10 shows the contribution of Third Countries to the average annual imports to the EU, considering the years 2012-2017. The total average volume of imports for this period is about 600 000 €. For the imports, in our opinion the share of market attributed to Israel concerns bumble bees, as our questionnaires did not highlight any imports of honey bee reproductive material from Israel. Instead, the imports from Argentina, Chile, New Zealand and Turkey were noticed also in our survey and are thus likely to represent honey bee queens. The total average volume of exports for the 2012-2017 time period is about 1.3 M €, with main destination countries Switzerland, Morocco, Korea, Tunisia, China. The exports to Switzerland may, at least in part, represent honey bee reproductive material, while we have no information concerning export of honey bee reproductive material to the other main countries of destination, and thus assume that this figure is more likely to reflect the bumble bee trade.

Fig. 3.10. Average import of live bees into the EU from Third Countries for the time period 2012-2017; import values lower than 1 000 € are not depicted (source: EC helpdesk)



3.3. Conclusions

Bee breeders in the EU represent less than 1% of the beekeepers, and the reproductive material they produce for the market adds up to 16% of the EU colonies, with a total annual value of 86 000 000 €. The countries with the highest numbers of breeders are Germany, France, Italy, Greece and Romania; countries with the highest queen/swarm/package bee production are Italy, France, Poland, Spain, Greece.

International trade seems to affect the honey bee reproductive market to a small extent, as the evidence we found shows that within EU trade represents about 3% of the total market value, and extra EU trade an even smaller proportion. However, according to TRACES data, the market of reproductive honey bee material within the EU has been in constant increase in recent years.

The collected data shows that the sector is very variable, with big differences even between closely neighbouring countries: in some countries the production of reproductive material is a profession in itself, and there are large commercial operations which produce thousands of queens per year; in others, production of reproductive material is in the hands of many small scale beekeepers who also specialise in queen production. The production of queens varies in size of operations, but appears to be present in the majority of EU MS, while the production of swarm and package bees is restricted to a few countries, mostly located in southern Europe, where the climatic conditions allow for an earlier breeding season.

4. Spread and selection of European honey bees resistant to varroa

4.1. Introduction

Developing resistance to the varroa mite in honey bees is a major goal for apicultural science and practice. Here we present a literature review and survey of resistant populations and selection programs in the EU and elsewhere, including expert interviews. We illustrate the practical experiences of scientists, beekeepers, and breeders in search of resistant bees. We describe numerous resistant populations surviving without acaricide treatments, most of which developed under natural infestation pressure. Their common characteristics: reduced brood activity, limited mite population growth, and low mite reproduction, may cause conflict with the interests of commercial beekeeping. Since environmental factors affect resistance, particular strains must be evaluated under different local conditions and colony management. The resistance traits of grooming, hygienic behaviour and mite reproduction, together with simple testing of mite population development and colony survival are significant in recent selection programs. Advanced breeding techniques and genetic and physiological selection tools will be essential in the future. Despite huge demand, there is no well-established market for resistant stock in Europe to date. Moreover, reliable experience or experimental evidence regarding the resistance of stocks under different environmental and management conditions is still lacking.

4.2. Review of scientific research

Why should we select bees resistant to the mite?

The parasitic varroa mite (*Varroa destructor*) spread throughout Europe in the second half of the last century and now represents the greatest problem for the Western honey bee, *Apis mellifera*. Since then, the beekeeping industry and hobby beekeepers have had to face a major challenge. The regular use of chemical treatments to control the mite has several disadvantages such as high costs and labour, residues in bee products, and the rapid emergence of mite populations resistant to acaricides. Consequently, there is an urgent need to use alternative control methods for the mite. Using mite resistant honey bees is generally agreed to be the most sustainable way to proceed. There are several different approaches to obtaining varroa resistant bees.

One approach is to consider that some honey bee populations living with the mite for many generations without varroa control will naturally develop resistance that will contribute to an equilibrium between the parasite and its host. This has been demonstrated with the original host of *Varroa* spp., the Eastern hive bee *Apis cerana*, and with *A. m. scutellata* in southern Africa or imported to South America as the Africanized bee, which has a high degree of resistance to the mite. In Europe and the USA, a few small populations of European strains have been found to be naturally resistant to the mite.

Another approach can be mass selection, using a large group of varroa infested honey bee colonies, which are allowed to live with the mite without any treatment and either die or survive. This has been called the "Bond test" ("Live and let die!"), and was developed in Sweden and in France and then in few other European countries.

Finally, a more academic approach is to develop a genetic selection based on chosen phenotypic characters and quantitative genetic tools. This approach has been utilised more or less successfully since the 1980s by several different research teams around the world.

4.2.1. Naturally selected populations and their known mechanisms

Apart from *Apis cerana*, which is the original host, there are few cases of naturally varroa resistant populations in *Apis mellifera* subspecies.

a. Apis cerana

The Eastern hive bee *Apis cerana*, the original host of *Varroa* spp., tolerates infestation without suffering serious damage. Several mechanisms enable it to do this, all of which operate to limit mite population development. Firstly, and most importantly, the mites reproduce successfully mostly in the drone brood of *A. cerana*. This means that mite reproduction can only take place during the relatively short period of the year when drone brood is present. Furthermore, in cases where multiple infestation of a drone cell occurs, and the pupa dies, perhaps due to viral infection, the worker bees do not uncap the cell, thus entombing the dead pupa together with the mites, which thereby cannot complete their cycle, and also sealing off infectious agents. Should the mites enter worker cells, adult bees can detect them and uncap the cells before the mites can reproduce. The bees open the cell, remove the mite and reseal it, allowing the pupa to develop normally.

In *A. cerana* colonies, a high proportion of infested worker larvae show abnormal development, thereby triggering a behaviour of the adult workers analogous to the Varroa Sensitive Hygienic behaviour (VSH) described in *A. mellifera* and preventing mite population growth from reproduction in worker brood.

Workers of *A. cerana* also show several forms of grooming behaviour, whereby mites are removed from other workers and damaged, often by the removal of legs. The quantitative contribution of grooming to mite resistance in *A. cerana* is still not clear and may be very limited.

b. Africanized honey bees

In the 1950s, *A. m. scutellata* queens from South Africa were experimentally introduced to Brazil. Some swarms accidentally escaped, and the bees indeed proved to be well adapted, rapidly spreading throughout the continent, into Central America and soon thereafter reaching the southern USA. Although producing high honey yields, the aggressive behaviour of these "Africanized" bees resulted in a fearsome reputation. Varroa mites arrived in South America already in 1971, but are not viewed as a serious problem by beekeepers using Africanized bees (AHB) in Brazil. Few colonies are treated, yet mite populations remain small.

There are several different variants or haplotypes of *V. destructor*, notably the Korean haplotype, which seems very virulent, and the Japan / Thailand haplotype, which seems less virulent. Due to different invasion events, the Japan / Thailand haplotype became established in South America, whilst the Korean haplotype spread throughout Europe and USA. The reduced fertility of the Japan / Thailand haplotype was initially believed to be the main reason why Africanized bees (AHB) were resistant to varroa.

However, during the past 20 years in Brazil the original Japan / Thailand haplotype has been replaced by the more virulent Korean haplotype, and there has been a corresponding increase in both the mite fertility (from 35 % to 72 %) and the number of mites producing at least one viable offspring in worker brood (from 56 % to 80 %). Despite this dramatic increase in the mite's reproductive ability, however, infestation levels remain low, and AHB remain varroa tolerant with high hygienic behaviour. This also supports findings from Mexico, where AHB have long been known to be varroa tolerant despite the presence of the Korean haplotype. This suggests that several resistance mechanisms are at work in the Africanized bees. Recent findings suggest that the resistance of Africanized bees to *V. destructor* in Mexico is related to adult bee mechanisms such as, for example, hygienic and grooming behaviour.

The wax brood cells made by *A. m. scutellata* are slightly smaller than those made by European strains, and this could lead to a reduction in the infestation rate.

Bees of *A. m. scutellata* have a post-capping stage duration (PSD) significantly shorter than that of European strains in Germany, which may explain the lower rate of infestation. Conversely, more extensive studies in Mexico found no significant differences in the PSD of experimental colonies of *A. m. scutellata* and European strains. The observed differences therefore may represent the large natural variability in post capping times at different times of the year and under different climatic conditions.

Worker brood from European strains has also been found to be twice as attractive to mites than that of *A. m. scutellata*.

Grooming behaviour has been suggested as a possible resistance mechanism in *A. m. scutellata*, as reported by various studies. But it seems that grooming is unlikely to be a significant factor in limiting mite population growth. Several studies have also noted removal of infested brood in *A. m. scutellata* colonies, but mites are not removed from the colony and have subsequently been observed to enter brood cells and breed again. However, the reproductive potential of these removed mites is reduced.

In most studies, the ability of *A. m. scutellata* to tolerate mites seems to be associated with the degree to which the mites fail to lay eggs or to produce viable offspring. Only 43% of female mites produced offspring when infesting *A. m. scutellata* in Brazil, as compared to 76% in European bees in Germany.

c. African honey bees

Varroa destructor presence has been confirmed since about 1990 in North Africa; it was found in South Africa in 1997, and since then has been documented in various countries of west and east Africa. To date, the mite is known to occur in 31 African countries, but there are still several regions with no data available, so this is likely an underestimation.

Beekeeping in Africa relies on constant influx from the huge reservoir of wild colonies. Beekeeping operations, even large commercial ones, acquire their colonies mostly by trapping swarms, and deliberate breeding is nearly absent.

However, infestation with varroa mites does not seem to lead to important colony losses, nor to greatly negatively affect bee colonies. It has been reported that in South Africa resistance to varroa developed within six to seven years after the first invasion. Various characters have been described that contribute to the resistance against mites, for instance: the short post-capping stage, especially in *A. m. capensis*, enhanced grooming behaviour, and the removal of mites through hygienic behaviour and recapping behaviour. In *A. m. scutellata* colonies, survival has been attributed to reduced varroa population growth and the low prevalence of viruses. In addition, absconding, a specific type of swarming behaviour, typical for tropical bees, may contribute to significantly reducing mite infestation levels. In the case of high mite infestation, an absconding colony will thus get rid of all reproducing mites trapped in the brood nest. In Ethiopia, the demonstrated resistance of *A. m. simensis* is partly explained by low brood infestation levels, low capability of producing reproductive progeny, as well as high failure to produce adult male progeny.

d. A. m. capensis

Workers of *A. m. capensis*, the Cape honey bee found in the extreme south west of South Africa, have a development time in the sealed cell (PSD) nearly one day shorter than that of European subspecies. This is sufficient to ensure that the third mite offspring (*i.e.*, the second daughter mite) does not reach the adult stage before the worker bee emerges. Together with the proportion of mites that do not produce fertile offspring, which appears to be greater than in European strains, this contributes to a low mite population increase in the worker brood. Researchers found that hybrid bees between *A. m. capensis* and *A. m. carnica* showed the same reduced development time. *A. m. capensis* is, however, unsuitable for beekeeping in Europe for several reasons. For instance, when introduced into colonies of other *A. mellifera* subspecies, such as in a hybrid zone with *A. m. scutellata* in South Africa, it becomes a social parasite, producing "pseudoqueens" which take over the colony. *A. m. capensis* is slightly larger than other African bees, and the amount of room in the brood cell can affect the reproductive success of the mite in some extreme cases. When the oversized parasitic *A. m. capensis* are reared in *A. m. scutellata* colonies, they fill the entire cell, thus preventing the male varroa mite from reaching the feeding site on the abdomen of the pupa, so all the female offspring cannot mate and remain infertile. Despite this discovery, however, reducing the cell size of the comb has so far failed as a varroa control mechanism, because it is not the size *per se*, but the amount of space within the cell that is important.

e. Survivors from France

Varroa mites invaded France in the 1980s, and most wild and untreated colonies were killed by the mites within two years. A first observation of naturally occurring varroa surviving bee colonies (VSB) was made in 1994 in the west of France, near Le Mans, where wild and untreated colonies seemed to survive the mite infestation for a few years. In 1999, ten out of twelve such untreated colonies were still surviving. Then, 82 colonies that were untreated for at least two years were collected in two apiaries, one in the North of France (Le Mans) and one in the South (Avignon) to characterise their survival without varroa control. These colonies were managed only for their survival. They were allowed to swarm and to naturally replace their queens. On average, the survival of those colonies was 7.88 ± 0.3 years, with a maximum of 15 years.

Varroa populations were estimated by counting natural mite mortality using a screened bottom board to collect the mites. The number of mites collected in the VSB was three times lower than in varroa susceptible control colonies all year round, suggesting that VSB have developed resistance mechanisms to inhibit the growth of varroa populations.

The VSB have a better ability to recognise the mites compared to control bees and are also able to detect and remove mite-infested pupae from their cells. The VSB from Avignon and Le Mans had low fertility and they show suppressed mite reproduction (SMR). Interestingly, gene expression analysis of the VSB shows over-expression of a set of genes related to responsiveness to olfactory stimuli compared with varroa susceptible bee colonies.

Differential virulence of the mite was also hypothesised to explain the survival of VSB. A less virulent parasite, which would not kill the host, would thus have an increased individual fitness. The hypothesis of sub-populations of mites with different levels of virulence was tested using mitochondrial and nuclear microsatellite markers. Recent findings could show significant changes in the genetic structure of the mite populations in different honey bee populations.

Acute bee paralysis virus (ABPV) and deformed wing virus (DWV) are resident in honey bee colonies and become more harmful when associated with varroa, which can transmit them between adult bees and brood and *vice versa*. Therefore, survival of VSB could be due to a higher tolerance of the bees to those viruses. This hypothesis was tested, and data have shown that the VSB had less ABPV and CPV (chronic paralysis virus) compared to control bees. However, the VSB did not survive longer compared to control bees when injected with the two viruses. This suggests that the VSB have fewer viruses because they have fewer mites to transmit viruses in the bee population. Nevertheless, it is reasonable to suggest that honey bee resistance, varroa virulence and virus prevalence are constantly under selection pressure and that natural selection favours a co-evolution that secures the survival of both the host and the parasite.

The effect of the environment and apicultural methods contributing to the survival of VSB cannot be excluded. Those areas where the experiments were carried out are outside France's major agricultural zone and are very favourable to the development of honey bee colonies. The colonies were manipulated only if necessary and were not moved or managed, as professional beekeeping would recommend.

f. Survivors from Norway

A managed population of local honey bees which had survived for more than nineteen years without varroa treatment in the Østlandet region of Norway recently was the subject of scientific study. Colonies from the population, which were of mixed ("Buckfast") origin, were monitored for mite population levels and mite reproductive success, and two possible resistance mechanisms, grooming behaviour and varroa sensitive hygiene (VSH) were evaluated. Mite infestation levels were found to be significantly lower in the survivor colonies compared to control colonies. The authors concluded, however, that whilst reduced mite reproductive success seemed to be a key factor in survival, neither grooming or VSH appeared to be important to explain the differences in survival. More recent investigations have shown that a shorter postcapping period may also contribute to natural colony survival of this population, while it is not the case for cell size. Moreover, recapping behaviour has been shown to be an important factor in the survival of this bee population.

g. Survivors from the USA

Tom Seeley studied a unique honey bee population of feral colonies nesting in trees in the Arnot Forest, south of Ithaca, NY, USA. In 2002, 15 years after the arrival of varroa, he observed the survival of the colonies. Inspection of the colonies showed that the population as a whole remained stable over three years despite mite infestation, and a comparison with susceptible control colonies did not show differences in mite infestation growth rate.

It was found that the smaller nest cavities and more frequent swarming of feral colonies contributed to their persistence without mite treatments. He found that young one-year-old colonies survived less well compared to already established colonies. Moreover, established colonies had a mean lifespan of 5-6 years and a queen turnover (swarming) each summer. Using a population model, he demonstrated that these life-history traits can produce a stable population of colonies. Interestingly, the feral colonies in the 1970s and the 2010s have essentially identical sets of life-history traits before and after the arrival of varroa, which suggests that the feral colonies possess defenses against the mite that are not costly. Because feral colonies in the 2010s have to invest in defenses against the mite, Seeley suggests that small colony size and frequent swarming endow them with good defenses against varroa, so they didn't have to evolve costly new defenses against the mites. However, he does not exclude the possibility that the feral colonies have needed to evolve some new defenses against *V. destructor*, including hygienic behaviour and grooming behaviour, but that these new defenses should not be costly.

h. Survivors from Russia

The general rule that in time parasites become less virulent and that their hosts become more resistant, led Tom Rinderer and colleagues at the USDA lab at Baton Rouge, Louisiana, USA, to examine bees from the far east of Russia, where varroa had first been reported to be a problem in the 1950s. Preliminary field studies in the early 1990s led to importations of bees to the USA from the Primorsky region, near Vladivostok, in 1997. After evaluation, these bees were released to commercial breeders in 2000, and studies have shown that these commercially available stocks are indeed more varroa resistant than other commercial strains, and that careful crossing has avoided inbreeding, given the limited original gene pool. The precise mechanisms for the varroa survival of the honey bee colonies remain somewhat unclear, as does the degree to which these bees will survive without varroa treatment. But it is clear that a number of factors are involved, in particular a reduced number of viable female offspring, an increased hygienic response, the removal of infested brood preventing successful mite reproduction, and the removal of phoretic mites through grooming.

i. The case of wild honey bees in Europe

Wild bees are an important issue within the framework of varroa resistance of honey bees. Whilst colonies kept by beekeepers have a limited chance to evolve varroa resistance because they are systematically treated against the mite, this is not the case for wild colonies, which can be a reservoir for naturally selecting varroa resistance genes.

In Europe, the spread of varroa and viruses led to the belief that wild colonies had disappeared. Recently, however, there was a first assessment of the occurrence and density of wild colonies in natural beech (*Fagus sylvatica* L.) forests in two German woodland areas. It remains unclear, however, whether these colonies indeed constitute a sustainable varroa resistant wild population, or whether they represent recent swarms escaped from nearby surrounding managed apiaries. Based on those findings, we can extrapolate that there could exist several thousand wild honey bee colonies in German woodlands. Indeed, the role of forests as a reservoir for the occurrence of sustainable naturally varroa resistant colonies should be taken into account when assessing their role in providing ecoservices to the surrounding area. It has been demonstrated in the USA that feral colonies have lower varroa population growth compared to managed colonies. It would be interesting to know whether wild colonies are similarly spread in natural forests at the European level.

4.2.2. Mass selection

The principle is simple: put together as many varroa infested honey bee colonies as possible in the same place and environment and study their survival when allowed to develop without any treatment for mites, in order to select for varroa resistance. The next year and the following generations, the selection is done on the best surviving colonies. This approach was called the "Bond test" ("Live and let die!") and has been used successfully in France, Sweden and in the Netherlands.

a. Gotland bees

For the original "Bond test", 150 colonies were established to study survival rates of untreated colonies and the development of the parasite population under Scandinavian climatic conditions. It was done on a small peninsula on the Swedish island of Gotland. Shortly after set up, the colonies were provided with an artificial mite infestation. No varroa treatments were performed, colony management was reduced to a minimum, and the colonies were allowed to swarm freely. Swarms were collected and set up in colonies in the experimental apiary. After three years, the annual colony mortality rate had increased to 80%, after which time it decreased and reached significantly lower levels below 20% after six years.

Whilst the frequent swarming of the colonies was not found to have a significant effect on the buildup of detrimental mite levels, the resistant colonies appeared to have developed adaptive characteristics that allowed them to limit mite population growth, such as a significantly smaller broodnest than non-resistant colonies that were regularly treated. In addition, infertile mites and mites with dead offspring were observed significantly more frequently in resistant colonies compared to control colonies. Mites also showed signs of delayed egg-laying, which has been suggested to result from potential inhibition of egg-laying, maybe through pupal volatiles. In addition, a recent study suggests that virus tolerance, rather than reduced susceptibility or virus resistance, is an important component of the natural survival of the Gotland bees.

b. Kefuss bees

John Kefuss and colleagues initiated their first Bond test in 1993 on 12 *A. m. intermissa* colonies known to be resistant to varroa in Tunisia. These bees were imported from Tunisia to France, near Toulouse. The resistance of these bees was compared with 12 varroa susceptible *A. m. carnica* colonies after exposure to heavy varroa infestations. Only the *A. m. intermissa* colonies survived. These bees hybridised with the local bee populations, and most of the hybrids survived mite infestation, indicating a genetic control of the resistance.

In 1999, a survival field test was done on 268 original European honey bee colonies. After losses of over two-thirds of the colonies, new colonies were made from the survivors. In 2002, genetic material from these survivors was bred into an independent group of 60 colonies. In 2013, 519 non-treated colonies from both groups were being used for commercial beekeeping, and mite populations were very low. Since 1999, no treatments against varroa have been used by Kefuss et al. in their professional beekeeping enterprise. From this naturally surviving stock, they subsequently select their breeder colonies for economic traits. The best colonies are then tested for hygienic behaviour (using a freeze-killed brood assay) and for varroa infestation. Apart from one year, their colony losses are comparable to other beekeepers in the region who still treat their hives with acaricides. The adult bee infestation usually remains below 5% and, according to their report, does not economically justify the use of chemicals. The underlying mechanisms are unknown, but a recent study identified an ecdysone-induced gene significantly linked to resistance; ecdysone initiates metamorphosis in bees and reproduction in varroa. This indicates that under commercial beekeeping conditions, simple methods can be used to select for reduced mite populations.

c. Blacquière bees

Blacquière et al. started selecting for surviving colonies in 2007 and 2008, in two isolated locations in the Netherlands. The population of Tiengemeten partly descends maternally from the Gotland (Sweden) population. The population of Amsterdamse Waterleidingduinen is a population of "hybrid" Dutch colonies, established with 70 colonies in 2008, of which 20 were used as controls and 50 as the starting group to select for resistance. No varroa control has been performed since 2007 in Tiengemeten and since 2008 in Amsterdamse Waterleidingduinen.

The main traits of selection were the ability of the colonies to grow, to survive winter despite the presence of varroa, and then to again develop well in spring. Only those colonies were kept and allowed to produce the following generation that survived the winter, increased in size and produced drones in spring. The different groups of colonies were kept in remote areas during mating. After significant losses during the first few years, the size of the untreated populations became stable, and the colonies now have consistently low levels of mite infestation, varying between 5% and 13% of phoretic mites in broodless conditions. The mechanisms behind mite resistance in these populations are still unclear. There is no difference in grooming behaviour between the two selected populations and the control population. VSH had increased strongly in one of the selected populations, where up to 40% of the infested cells with mites and pupae were removed. However, it had decreased in the Tiengemeten population, compared to the control colonies. The different VSH responses between the two selected resistant honey bee populations lead to the conclusion that more than one mechanism of resistance may have evolved.

After 10 years of this successful program, Blacquière et al published their selection scheme that they have called "Darwinian black box" selection for resistance, so that it can be used by other scientists or beekeepers and promoted the use of honey bees' natural resilience in beekeeping.

4.2.3. Genetic selection on chosen characters

The development of genetic resistance can also result from the successful implementation of deliberate breeding programs that use suitable resistance characters in the selection process of honey bees. The selection for varroa resistance in treated populations has to rely on indirect selection characters, because the direct trait of survivability cannot be studied while the colonies are influenced by veterinary treatments. Much research in European institutes focused on the identification of suitable selection characters based on comparative studies with varroa surviving or resistant colonies to understand the mechanism of varroa resistance in honey bees. In addition to the biological relevance, the heritability and the practicability of testing under field conditions were considered to be of major importance in the implementation of such characters in breeding programs.

The characters that have been used in breeding programs for increased mite resistance are the following, and the interactions of resistance traits with colony and environmental parameters are illustrated in figure 4.1.

a. Hygienic behaviour is the act by which worker honey bees detect and remove diseased or infested brood.

b. Suppressed Mite Reproduction (SMR). The non-reproduction of mites (SMR) was found to be correlated with mite population development and would be caused by two heritable traits.

c. Varroa Sensitive Hygiene (VSH) is the hygienic removal of pupae infested with mites.

d. Uncapping - recapping of varroa infested brood cells is another mechanism, which is more likely to be favored by natural selection, as it reduces mortality of the bee pupae and increases colony competitiveness.

e. Mite non-reproduction (MNR) is the sum of the effects of VSH and Recapping by adult bees and SMR induced by the brood . This definition may be important to use in the future to precisely describe which phenotype we are selecting.

f. Grooming behaviour (GRO) refers to an act that honey bees perform in physically dislodging mites from their bodies by using their mouthparts or legs. Adult bees can remove mites from their own bodies (auto-grooming) or they can be helped by their nestmates (allo-grooming).

g. Attractiveness of the brood. Varroa mites predominantly rely on olfactory triggers to identify and enter brood cells suitable for reproduction, and it has been demonstrated that brood from different sources can have different attractiveness to the mite.

h. Mite population dynamics. Regular monitoring of mite populations in order to calculate mite population development is interesting, and some breeding programs have been based on simply selecting those colonies with the lowest mite population development without understanding the underlying mechanisms.

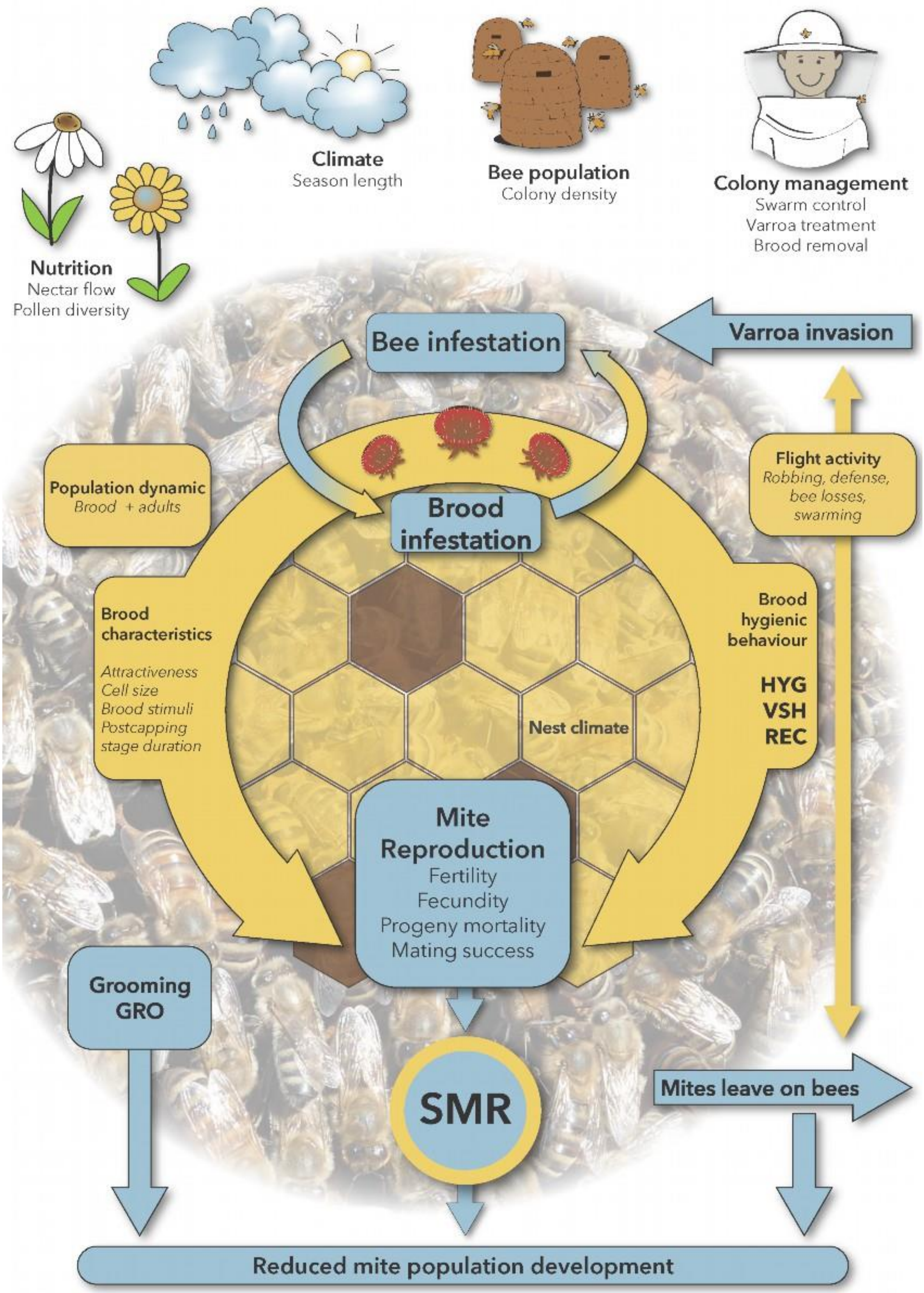
i. The postcapping stage duration (PSD) can be calculated as the time between the capping of a cell containing a last-stage bee larva by the nurse workers and the time of the emergence of an adult bee from the cell. This duration is directly correlated with the number of offspring a mother mite can produce.

j. Brood cell size is based on the principle that less available space in the brood cell would inhibit varroa reproduction.

k. Varroa versus virus selection? Varroa mites are known to be closely associated with other pathogens, especially viruses. Therefore, we can also think about a potential virus resistance associated to the varroa survival of the bees.

l. Genomic analysis of varroa resistant bees is developing, as using genetic markers is for sure a potential future tool for genomic assisted selection on varroa resistance.

Figure 4.1. The interactions of resistance traits with colony and environmental parameters on mite population development.



4.2.4. Conclusion from the literature

Considerable time, effort and finance has been devoted to understanding the mechanisms underlying varroa resistance and to breeding bees resistant to the mite. However, progress has often been slow, and some desirable traits, demonstrable in experimental colonies, show low heritability or, alternatively, show benefits that are too small to render them practicable in breeding programs. Bee populations apparently resistant to varroa in one location sometimes cease to remain resistant when moved elsewhere and exposed to different environmental conditions. Nonetheless, significant progress has been made in organised breeding programs and, alternatively, in identifying "survivor" stocks and in understanding the underlying mechanisms of resistance that may support a more rapid progress in the future.

4.3. Survey on the presence of naturally selected resistant honey bee populations and the state of selection programs on varroa resistance across the EU

In the following section, we present the results of a survey on the presence of naturally selected resistant honey bee populations and the state of selection programs on varroa resistance across the EU and some associated countries. We describe the practical experiences of those searching for varroa resistant bees, whether they are bee research institutes, universities, or beekeepers, including those running large commercial operations, enthusiastic breeding groups, and individuals.

For collecting the data for an overview of the EU market for reproductive material of European honey bees, we designed a questionnaire and circulated it among contact persons for each country from our scientific networks: COLOSS (www.coloss.org), RNSBB (www.beebreeding.net) and SMARTBEES (http://www.smartbees.eu/). This questionnaire (see "T3_Description of the state of play in varroa resistance_2018-05-18.pdf" report file in the Annex) contained questions on the presence of naturally selected honey bee populations and the state of selection programs on varroa resistance in each country. A summary of the 45 answers is presented in Table S2 of the earlier mentioned report. Only seven countries have one naturally selected population present, but for most of them there are no proven examples or anecdotal reports of survivors. But most of the countries have selection programs for varroa resistance.

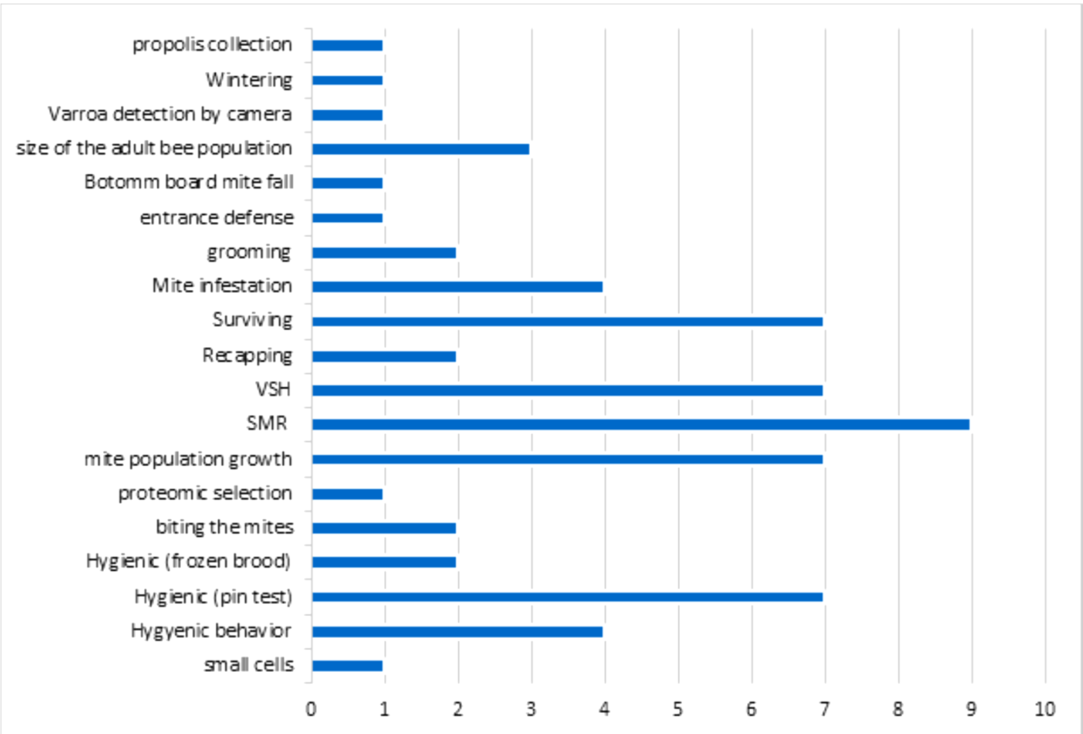
4.3.1. Interviews with experts in the field (beekeepers, breeders, researchers) to obtain information on practical experience with selection for varroa resistant bees

Interviews were carried out with scientists and beekeepers known to be involved in breeding varroa resistant honey bees and based on our own knowledge, and from the relevant networks. We focused on the various varroa resistant honey bee populations, which are known throughout Europe, including both naturally selected populations, and those which have been deliberately genetically selected as part of bee breeding programs. We also included a few international experts from outside Europe.

Data were collected from the returns of the questionnaires (see Annex in the EurBeST report) and interviews. We distributed questionnaires and then followed up by mail with the different contacts. Forty-eight interviews were completed using the questionnaire from 19 different countries: 41 from Europe and seven from North America. The questionnaire and the original data are presented as supplementary material (see "T3_Description of the state of play in varroa resistance_2018-05-18.pdf" in the Annex). Twenty-one breeders are using naturally selected populations, whilst twenty-nine genetically select their bees as part of bee breeding programs. Four are using both approaches.

The breeders using naturally selected bee populations are mostly interested in one main trait: the survival of the colonies. The breeders selecting their bees for varroa resistance use 19 different selection characters, with a maximum of five characters per breeder (see Figure 4.2. and Table in "T3_Description of the state of play in varroa resistance_2018-05-18.pdf" in the Annex).

Figure 4.2. Frequency of different characters used for varroa resistance selection by honey bee breeders. Data are presented as the number of breeders using each specific character.



The characters most frequently used are the three linked (SMR, VSH and recapping) characters, mite infestation and population growth, colony survival, and hygienic behaviour. Figure 4.2. lists the underlying mechanisms that beekeepers consider responsible for varroa resistance.

Looking at the extent and mechanisms of varroa resistance, the answers suggested that these mechanisms were diverse in different populations and focused on the characters within the studied population or between populations. When the breeder considered the within the population extent, for most of their answers, SMR and VSH seem to be common worldwide.

The breeders deliberately selecting their bees for varroa resistance are using 16 different criteria with a maximum of six criteria per breeder (see Table S1 in Annex1 of the EurBeST report). The criteria most frequently used are mite infestation; VSH/SMR/Recapping; survival of the colony; and hygienic behaviour.

The selection strategies are very diverse. Whilst breeders of naturally surviving populations allow their bees to carry out natural selection by themselves, breeders deliberately selecting their bees for varroa resistance usually include one to four characters related to varroa resistance to their already established selection program on, for example, productivity, gentleness, and swarming behaviour.

Breeders of naturally surviving populations have generally no mating control, except when they are using an isolated area like an island. Breeders selecting their bees for varroa resistance sometimes have no mating control, but more generally use drone saturated areas for their queen rearing, and / or artificial insemination.

Eleven of the 21 breeders of naturally surviving populations carry out assessment of queen quality, as do 15 of the 28 breeders selecting their bees for varroa resistance. Breeders of naturally surviving populations use colony survival as a trait for selection, so bad quality queens will not survive.

Nine of the 21 breeders of naturally surviving populations, and 15 of the 28 breeders selecting their bees for varroa resistance have local or regional beekeeper collaborations or networks.

4.3.2. Availability of stocks for beekeepers

Queens from breeders of naturally surviving populations:

In Europe, four beekeepers reproducing naturally surviving populations make their stock commercially available to other beekeepers.

Three of them are from Greece: one rears queens and sells about 20 000 queens per year at 15 € each; another one allows colonies to produce their own queens and rears about 3 000 queens per year to sell at 15 € to 60 € per queen; the third one supplies fewer than 300 queens each year for local beekeepers. According to the producers, no varroa treatment is needed.

In Norway, less than 500 queens are sold locally for 50 € to 70 € per queen. This stock is bred and reared as a commercially viable stock for the southern regions of Norway and for some commercial honey production. It is classified as "Buckfast", and no varroa treatment is needed according to the producer.

In Puerto Rico, queens are distributed to participating beekeepers from the only breeding center, but no stock is available for sale. According to the producer, no varroa treatment is needed.

Queens from breeders selecting their bees for varroa resistance

Fifteen of the 28 breeders make their stock available to beekeepers. Most of the breeders include at least one trait for varroa selection in their selection program. Only four breeders produce queens that are actually claimed to be varroa resistant without the need of control measures: one in Finland, one in France and two from the USA. In Finland, one beekeeping operation sells varroa resistant queens at 500 € per queen. In France, one beekeeper supplies queens at about 10 € per virgin queen. In the USA, scientists supply breeding material to queen producers at a price of $ 200 to $ 350 per queen. Whether these queens are actually 100% varroa resistant under all conditions is untested and needs to be confirmed.

In Germany, one beekeeper supplies SMR selected queens, according to him no varroa treatment is needed. Members of one breeding association offer *A. m. carnica* queens, and those of another one offer "Buckfast" queens, which are supposed to have improved resistance traits, especially for hygienic behaviour and SMR, combined with excellent commercial traits. Some of them cooperate, especially in the selection for SMR in well established breeding lines. There is a high demand for such queens at the national and international level. To date, such stock can usually be managed with a reduced chemical treatment regime, but not without any kind of treatment.

Only a few positive answers were provided on location and numbers of breeding and training centers, etc.. One breeder in France provides training. Five groups provide training within the framework of varroa resistance selection programs. Only four breeding training centers were identified: in Kirchhain/Germany, in Olsztyn/Poland, in Mugla, Turkey, and one in Puerto Rico.

The basic attraction of varroa resistance stock to the beekeepers is to reduce colony losses, while avoiding the need to chemically treat the colonies. Breeders of naturally surviving populations tend to sell their queens for use in a similar environment. A lack of selection for commercial traits in some of those populations could limit the commercial attractiveness of those queens. However, in Norway, the commercial attractiveness is important as the stock ("Buckfast") is bred and reared as a commercially viable stock for the southern regions of Norway, which gives the proof of concept. Breeders selecting their bees for varroa resistance combined with other commercially attractive traits experience different levels of attention for their breeding products. An increasing demand for queens is observed by many German breeders, but most of them are cautious to advertise their stock with the label "resistant" as there are only a very few examples of highly varroa resistant bees produced. However, in the USA there is a clear demand, greater than the supply, for such bees.

Productivity is of secondary importance for breeders of naturally surviving populations. This is not the case for breeders selecting their bees for varroa resistance among other commercially attractive traits such as production, gentleness and swarming. No breeding values were recorded, with exception of a group of German carnica breeders (Arbeitsgemeinschaft Toleranzzucht – AGT), who use the www.beebreed.eu database system.

In Greece, five beekeepers were identified who select their colonies on survival ability. They seem to be successful, as three of them sell thousands of daughter queens from those selected breeder colonies. In Italy, three beekeeping operations have used naturally selected colonies for five to 15 years and plan to develop them in protected areas. In the Netherlands, one operation has maintained a surviving population on Texel Island for 15 years, but the availability of the material is limited. There is also one survival program started in the Netherlands, which seems to be successful, but there will be no stock available for some time. In Norway, one varroa survival population has been maintained since 1998 and seems successful. Queens are sold locally and abroad for approximately 50 € per mated queen. This operation produces an average of 400 queens per season from a single geographical breeding center. In France, two populations of naturally resistant bees are maintained since 1999, but no material is available for sale.

Outside Europe, in Puerto Rico, gentle Africanized bees which are naturally varroa resistant are available for beekeepers. About 1 000 queens per year are produced in one breeding and training center. The Arnot Forest bee population identified by Tom Seeley in the USA is not available to beekeepers.

In France, one beekeeper has for many years sold virgin queens from his "Bond test" bees at a low price of 10 € per queen. In Finland one group have not treated their colonies since 2008 and produce queens. In Germany a beekeeper produces varroa resistant queens only for beekeepers interested in varroa resistance breeding. Several breeders from Germany and neighbouring countries offer queens from their lines selected on SMR and further resistance traits, but most of them avoid advertising them as varroa resistant as this is not yet proven under different environmental and management conditions.

In Sweden, the Gotland bee population is not available for beekeepers.

Outside Europe, the USDA Baton Rouge team have been leaders in this field for many years and developed the VSH and SMR methods that are now used by many beekeeping operations and scientists throughout the world. They have conclusively demonstrated that selection using these characters can be efficient.

Prices for resistant breeder queens seem to be very variable in the range of 10 – 500 €.

Overall conclusions about varroa resistant honey bees in Europe

If we focus on the various varroa resistant honey bee populations - those for which there is no need for varroa treatment - in the EU, we identified six countries which have naturally selected varroa resistant populations (France, Italy, Ireland, Lithuania, the Netherlandsand Sweden), and several, where bee breeding programs focusing on varroa resistance are being conducted, albeit often on a small scale. Supplies of queens are, however, very limited in most areas; alternatively, breeders participating in selection programs are often very cautious about advertising their stock as "resistant". However, several countries have recently initiated new selection and breeding programs, so it is clear that there is an increasing interest in developing these aspects, either by using naturally varroa resistant bees, by adding suitable selection characters to existing selection schemes, or by devising entirely new programs. A recent survey made in Switzerland demonstrated that many beekeepers are interested in developing a breeding strategy for resistant stock even though their bees would produce less honey, swarm more often or be less gentle, showing a clear desirability for resistance traits. There may also be many naturally resistant populations, which have yet to be identified. It is necessary to strengthen cooperation among beekeepers and breeders and to develop sustainable and effective infrastructures for the promotion of varroa resistant and commercially attractive honey bee stocks in the EU.

For references and more details please use the original Task 3 output "Description of the state of play for the production and keeping of European honey bees resistant to Varroa" in the Annex 1 of the EurBeST report.

5. Customer survey on expectations and experience with common honey bee breeding stock

For each case study, the experience and expectations of the breeders' customers was assessed using a tailor-made questionnaire. The aim of the survey was to gain insight on the degree of satisfaction that beekeepers have toward the queens they are buying, what characteristics they are seeking for when they buy reproductive material, and, more specifically, what is their level of interest and hope towards varroa resistant stock. The questionnaire consisted of six questions with multiple answering alternatives. The online questionnaire was translated into six languages (DE, EL, FR, HR, IT and PL). In total, 28 breeders from all case studies were asked to share the translated online questionnaire with at least ten of their clients. Due to precautions taken by the project team to achieve anonymity, the queen producers were asked to share/spread the online questionnaire by themselves. The online survey was launched on 7th March 2020, and total responses were collected on 17th October 2020.

5.1. Survey response rate metrics

In the early phase of data collection, due to COVID-19 pandemic restrictions, in most of the CSs the beekeepers were struggling to perform their regular field activities, resulting in a low response rate during the active beekeeping season. In addition, some of the queen producers failed or hesitated to communicate the links with their customers due to time limitations, insufficient IT skills, as well as lack of experience or awareness of the market perception of their products (queens and swarms). However, the response rate significantly increased during the early autumn, once the main beekeeping field activities were reduced.

The total number of responding customers surpassed the expectations of the project team. The case study coordinators devoted a significant amount of effort to obtain 83% of the targeted responses (Table 5.1).

Table 5.1 Descriptive metrics of the customers' response rates and overall performance of the survey

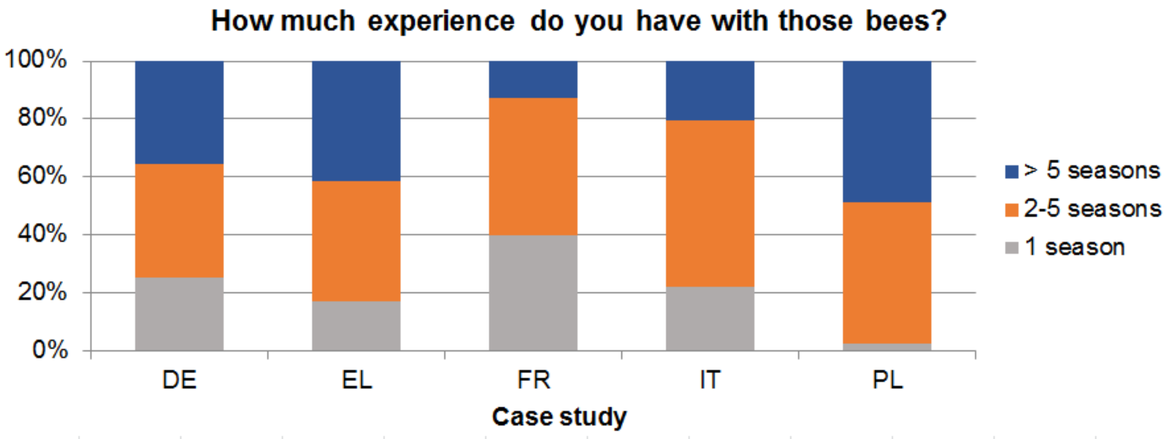
Case study	Number of surveyed QP	Number of QP with their customers responses	Response percentage	Total number of responses from the customers	Average number of responses per QP
DE	8	7	87.5	102	14.6
EL	4	4	100.0	29	7.3
FR	3	2	66.7	85	42.5
IT	10	6	60.0	68	11.3
PL	3	3	100.0	112	37.3
	28	**22**	**82.8**	**396**	**22.6**

The overall response rate and the high number of almost 400 answered questionnaires provide solid data about the customers' (beekeepers) expectations, as well as their appreciation for the queens purchased from the queen producers. The results are even more relevant due the fact that on average the queen producers were appraised by around 23 of their customers.

5.2. Survey outcome

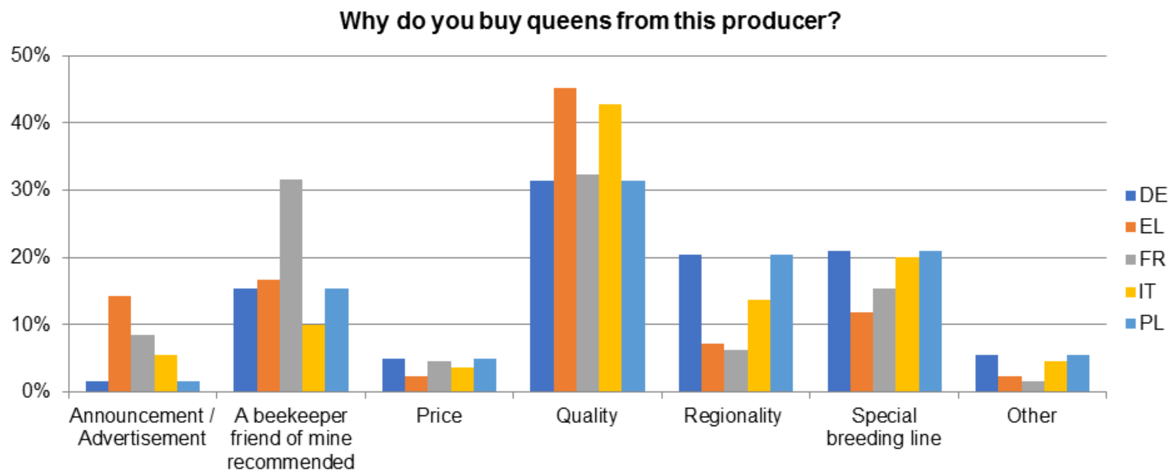
The relevance of the responses to the survey is highlighted by the fact that almost 80% of the customers had a working experience of more than two seasons with colonies headed by the queens purchased from the assessed queen producers (Fig. 5.1). Moreover, 1/3 of the customers had more than 5 years of beekeeping experience with the purchased stock.

Figure 5.1 Percentage and ratios of customers' beekeeping experience with the colonies headed by queens purchased from the assessed queen producers



Queen quality, not only in a sense of the queens' physiological status, but also in regard to the overall performance of the colonies headed by them, is the most prominent reason (>36% of the customers) for buying queens from a certain queen producer (Fig. 5.2). In addition, the high appreciation of the quality parameters is also confirmed by the customers' preference for queens from special breeding lines, which indicates consideration of particular selection traits relevant for the practice. The higher appreciation of this factor in DE, IT, PL could be interpreted as result of extended systematic breeding efforts or pure race breeding in these countries compared to EL and FR, where systematic breeding was established only recently, or less attention is given to subspecies origin.

Figure 5.2 Customers' view on the factors relevant for choosing a queen producer

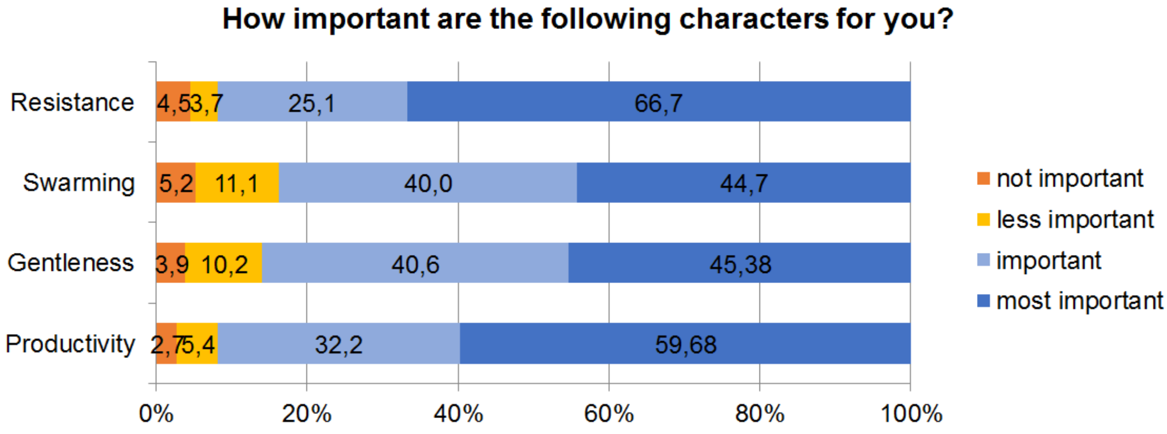


Regionality (proximity to the queen producer) and recommendations from other beekeepers are factors of overall secondary importance to the beekeepers, with exception of FR where the customers' decision is significantly influenced by recommendations provided from their colleagues.

Surprisingly, purely commercial aspects, such as price and marketing (advertisement) turned out to be less relevant for the beekeepers' decision from where to purchase queens, leading to the conclusion that there is readiness for higher investment in quality.

Beekeepers across the assessed CSs share the perceptions of which parameters are the most important ones for their beekeeping operations. More than 2/3 of all customers identified disease and parasite resistance as the most important trait, followed by productivity (Figure 5.3). With the exception of EL, where productivity is recognised as most important, resistance is the leading trait among the remaining CSs (see "Survey details" in the Annex). Thus, beekeepers prefer working with productive colonies that do not require extra efforts and investments in disease control. Interestingly, behavioural traits such as gentleness and swarming, are of overall secondary importance for the customers.

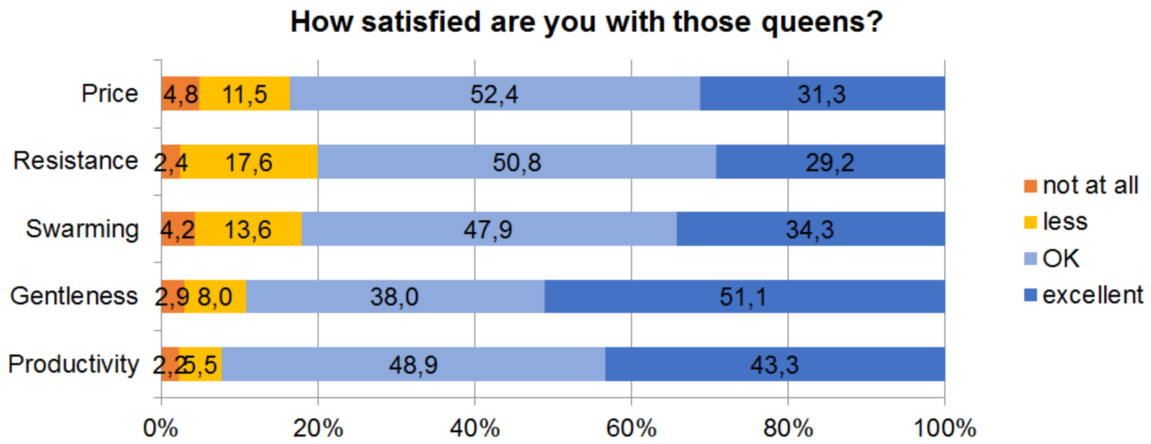
Figure 5.3 The overall customers' responses on the relevance of the traits



When assessing beekeepers' satisfaction with the used stock (queens), the results show that the experience is opposite to the expectations. Indeed, overall, the beekeepers are most satisfied with the traits gentleness, productivity and swarming (Figure 5.4). In contrast, they are less satisfied with the price paid for the queens and even less satisfied with the resistance traits. This result highlights that beekeepers have high expectations and develop a growing demand for high quality queens originating from populations with improved resistance. However, it must be noted that overall, the participating customers tended to be satisfied with all traits and aspects, including disease and parasite resistance, for which more than 80% of the beekeepers expressed their positive perception (scores excellent and OK).

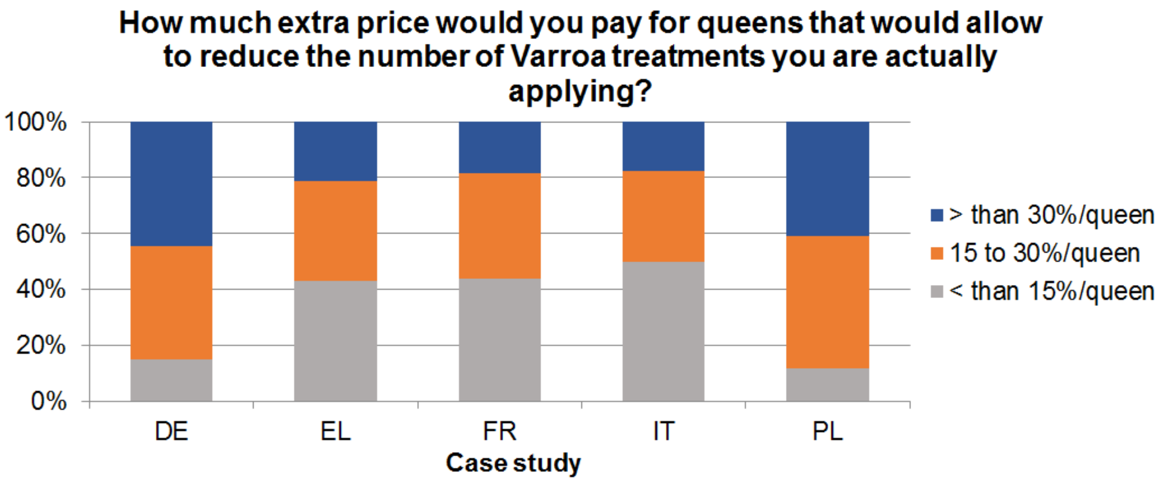
Still, there are variations among the CSs, with the most evident difference noted in the DE case study, the region with the longest systematic breeding for resistance traits. Here, a total of 93% of the beekeepers replied that they are satisfied with the resistance trait of the queens (54% excellent + 39% ok; see DE figure in "Survey details" in the Annex). In contrast, the beekeepers in FR and PL show the lowest appreciation of the resistance trait, with around 20% and 25% of the beekeepers scoring as less satisfied or not satisfied at all.

Figure 5.4 The overall customers' responses on the relevance of the traits



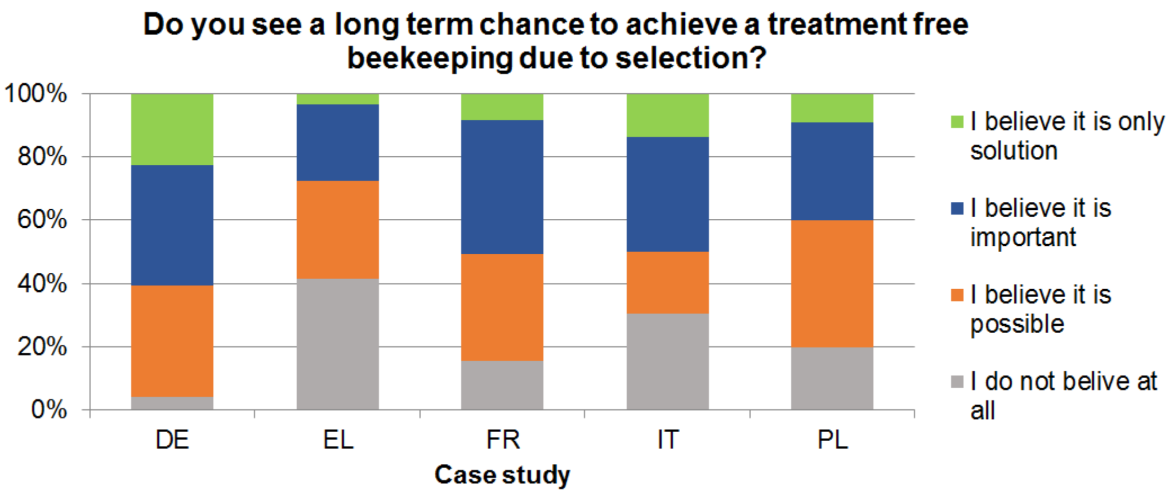
More than 2/3 of all surveyed customers (67.3%) are ready to pay an extra of more than 15% in addition to the current price for stock (queens) that would require no or only reduced treatments against the varroa mite (Figure 5.5). Interestingly, 28.6% of them are ready to pay more than 30% in addition to the current price per queen, confirming the previous result that quality and expression of resistance are considered more important than the price. However, we observed variations among the CSs, where the customers from Central Europe (DE and PL) are more affirmative concerning the investment in queens, compared to the more southern regions (EL, FR and IT), where it seems that beekeepers are more sceptical and less inclined to invest more. This outcome could be a result of the fact that selection initiatives for varroa resistance are less frequent in the South of Europe and, compared to DE and PL, the selection results have not yet reached comparable levels.

Figure 5.5 Customers' readiness to invest in stock which will require no or limited treatment against the varroa mite



When asked about perspectives for the future in terms of achieving varroa resistance (Fig. 5.6), the expectations mirrored the outcome from the previous question. The customers who are not ready to pay more are also the ones who are most sceptical about the chance to achieve treatment-free beekeeping resulting from selective breeding. From that group of CSs, only the beekeepers from FR were expectant and positive about the possibility of achieving treatment-free beekeeping. Overall, almost 50% of the total surveyed customers are optimistic (it is important and it is only solution) that this goal is attainable. For 10% of them (from 23% in DE to 3% in EL), selective breeding is the only solution towards treatment free beekeeping.

Figure 5.6 Customers' expectations concerning the achievement of treatment-free beekeeping due to selective breeding



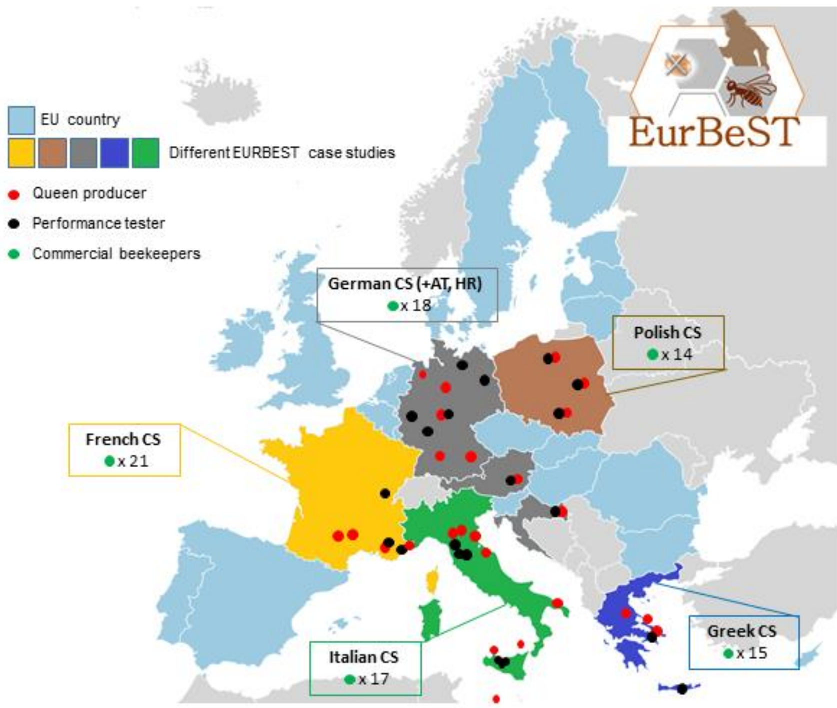
6. Breeding and distributing commercially viable varroa resistant bees: a case study approach

The most central part of the EurBeST study was the implementation of five large-scale case studies to validate the methodology of honey bee selection for varroa resistance and the qualities of representative genotypes. To achieve this task, an expert team of 131 queen breeders, commercial beekeepers and performance testers was established. They cared for the production and dissemination of test queens and implemented a methodology for testing and further selection for varroa resistance and other commercially important traits.

The selection of case study countries reflects the variability in terms of market structures and breeding infrastructure identified by the analysis of the European market for honey bee breeding stock and the given infrastructure for honey bee selection. In consultation with the steering group of the EU-commission, Germany (including also some beekeepers from Austria and Croatia), Greece, France, Italy and Poland were finally chosen to run the large-scale field-testing of preselected stock (Figure 6.1).

The genotypes included in the case studies were either coming from selective breeding programs with a variable degree of selection for varroa resistance or from naturally selected populations with increased varroa resistance potential. Commercial beekeepers compared such lines to their common stock in order to check for differences in productivity, varroa infestation levels and colony losses. At the same time, a more comprehensive testing of the selected lines was carried out by specialised performance testers to better describe biological differences between the genotypes and to estimate the relevance of genotype – environment interactions.

Figure 6.1: Locations of queen producers and performance testers and the number of commercial beekeepers involved in the EurBeST study



6.1. Expert team and case study methodology

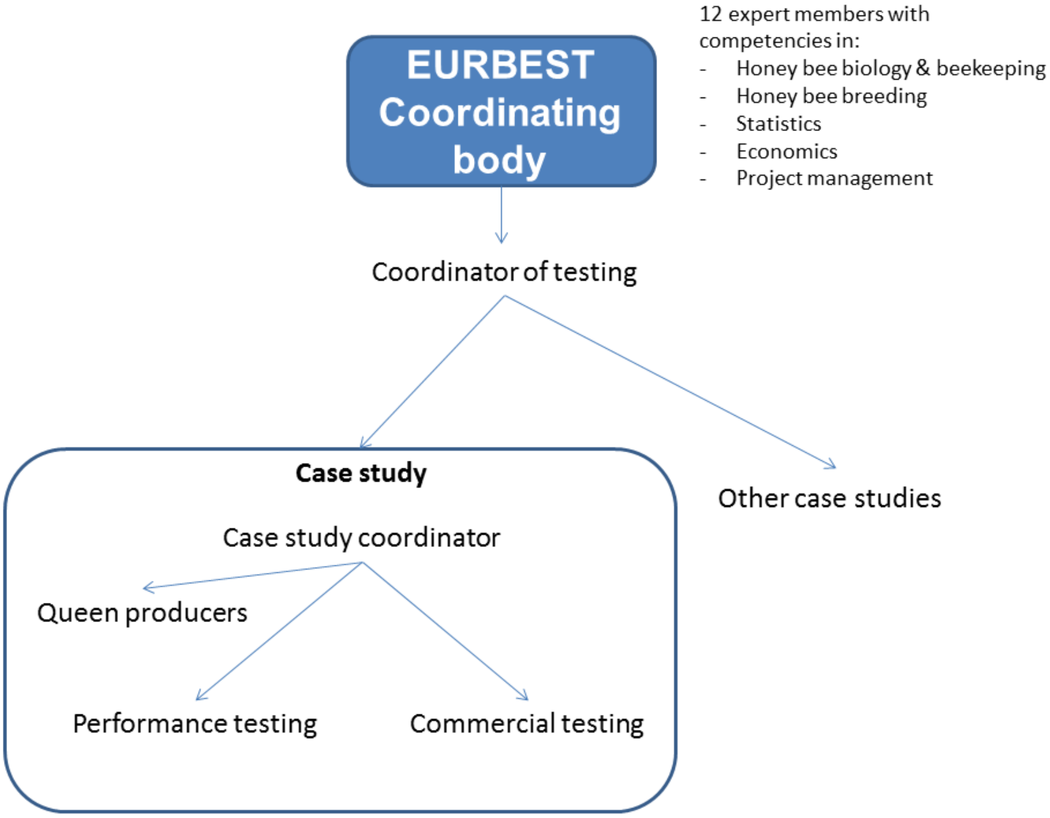
6.1.1. Coordination and management of the expert team

A detailed description of the composition of the EurBeST expert team is presented in deliverable "Task4, output 1" in the Annex. The hierarchical structure of the network and task distribution among the partners is shown in Figure 6.2.

The network was managed by 12 persons who formed the "coordinating body". The coordinating body was responsible for development and design of the study setup, the working protocols and project coordination. One of these persons was in charge of developing the testing manuals, collated in the "Book of Methods" (see English versions for PTs and for CBs in Annex) and for the task assignment and monitoring of the field-testing in each case study.

Each regional case study coordinator was responsible for supporting the activities within the case study, including adaptation and translation of the manuals, training of the involved partners and coordination and monitoring of the tasks by the participating queen producers, performance testers and commercial beekeepers.

Figure 6.2. Structure of the expert team and task distribution



The field comparison of the selected lines was conducted by 106 beekeepers (Table 6.1.), either running comprehensive performance testing or comparative testing under common commercial conditions.

Table 6.1. Number of queen producers (QP), performance testers (PT) and commercial beekeepers (CB) participating in the study

Case study	Number of QP	Number of PT	Number of CB
DE	7	7	18
EL	3	2	15
FR	4	3	21
IT	8	6	17
PL	3	3	14
Total	**25**	**21**	**85**

Performance testing (PT; n = 21) took place in qualified breeding centres where all selected lines of the regional CS were compared by comprehensive and thorough performance testing in regard to the traditionally most important traits, but also with special attention to the parameters related to varroa resistance. Each line was represented by a half-sister group of queens with known pedigree. Many of those queens were mated on isolated mating stations or by artificial insemination to identify also the paternal heritage. In each PT, two or three lines, with 8-10 colonies each, were compared. At the same time, each line was tested in at least three different PT apiaries. The colonies in PT were initially built from artificial swarms. To standardise the varroa infestation within and between different apiaries, an initial treatment was applied; thereafter no varroa treatment was applied during the full testing period. At the end of the study, the queens were caged to stop brood production and to estimate the final mite infestation. The performance testers were recording the values for all selected biological parameters for varroa resistance, colony development and performance (Table 6.4.). Additional testing on the specific resistance characters SMR, REC and VSH was performed in some selected test apiaries in close cooperation with local laboratories. Finally, data on work load and costs of testing and apiary management were collected for a detailed economic analysis (see chapter 7.2).

Comparative testing *by commercial beekeepers* (CB; n = 85) was run by the commercial beekeepers who compared about 20, usually open mated, queens of one line to the commercial stock they normally use. Most selected lines were tested in three to five commercial apiaries. Colony management and varroa treatment were following the common practice and apiary standards of the respective beekeeper. Data were recorded for honey yield, manageability, varroa infestation and colony losses as traits of major apicultural interest (Table 6.3.), and specific relevance was given to the assessment of economic aspects of using stock selected for resistance vs. non-selected stock (see chapter 7.3.).

6.1.2. Description of the breeding stock

Most of the 23 pre-selected lines used in the case studies originated from breeding programs or were included based on preliminary evidence of elevated resistance by the experts/beekeepers. Besides the origin, which is the home country of the breeder, we recorded the subspecies assignment and the duration of selective breeding for specific traits according to information provided by the breeders (Table 6.2.).

Table 6.2. Description of the selected lines used in the EurBeST study

Geno-type	Origin	Subsp./ Race	Selection criteria*	Case study of testing	No. of PTs	No. of colonies	No. of records
A	EL	Unspecified	SUR	EL	2	20	521
B	DE	Carnica	HYG, MPD, REC, SMR	DE, PL	4	40	1 744
C	FR	Hybrid	SUR	IT, FR, DE	4	40	2 366
D	AT	Carnica	HYG, MPD, SMR	DE, PL	4	36	2 010
E	EL	Unspecified	MPD	EL, IT	2	20	766
F	IT	Buckfast	SUR	IT, DE, FR	7	69	4 960
G	PL	Carnica	HYG	PL	3	31	582
I	IT	Siciliana	SUR	IT	1	10	630
J	FR	Hybrid	SMR, VSH	FR	4	51	1 262
K	FR	Hybrid	SMR, HYG	FR	4	58	1 814
L	PL	Carnica	HYG	PL, DE	4	43	1 149
M	IT	Siciliana	HYG, MPD	IT	1	10	212
N	IT	Ligustica	SUR	IT	3	30	2 113
O	DE	Buckfast	MPD, SMR	DE, IT	3	30	1 183
Q	EL	Macedonica	MPD	EL	2	16	360
R	PL	Carnica	HYG	PL, DE	5	49	912
S	IT	Ligustica	HYG	IT, EL	3	30	1 776
T	DE	Buckfast	MPD	D, FR	4	30	1 331
U	HR	Carnica	HYG, REC, SMR	DE, PL	4	41	4 342
V	DE	Buckfast	MPD, SMR	DE	3	29	4 728
X	IT	Siciliana	SUR	IT	1	10	400
Y	DE	Carnica	HYG, MPD, SMR	DE	3	27	4 055
Z	FR	Hybrid	SMR	FR	4	52	1 326
				Total		772	40 542

*) HYG = general hygiene behaviour, MPD = reduced mite population development, REC = Recapping of infested cells, SMR = suppressed mite reproduction, SUR = survival of untreated colonies, VSH = Varroa sensitive hygiene

The production and the challenging exchange (shipment) of the queens, within and between the CSs (Figure 6.1.), was well coordinated and completed without major problems. The total number of selected queens distributed for testing was

2 562, out of which on average more than 86% were accepted in the PT and CB colonies. Another 1 347 queens were contributed by the commercial beekeepers for their own control colonies.

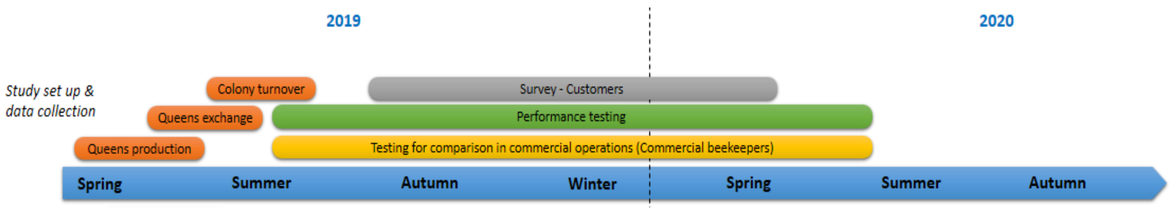
Table 6.3. Number and rate of received and accepted queens in PT (performance test) and CB (commercial beekeeper) apiaries and total number of queens accepted

	Performance testing			Commercial bee-keepers				
Case Study	Received	Accepted	% of acceptance	Received	Accepted	% of acceptance	Accepted own queens	Total accepted
DE	222	190	85.59	519	477	91.91	264	931
EL	100	80	80.00	233	163	69.96	161	404
FR	120	101	84.17	362	300	82.87	421	822
IT	159	136	85.53	301	261	86.71	282	679
PL	120	110	91.67	426	400	93.90	219	729
Total in test	721	617	85.58	1 841	1 601	86.96	1 347	3 565

6.1.3. Colony management and timeline

The production, mating and exchange of the queens and their establishment in colonies all took place during a short period of two months in the first half of 2019 (Figure 6.3). To minimise possible risks (queen losses, low acceptance rate, frequent queen supersedure incidents etc.), about 20% more queens than required were produced as backup.

Figure 6.3. Timeline of the activities related to colony management and data collection



The testing period started in mid-summer 2019, after a period of 6 weeks required for the turnover of the genetic composition of the colonies after the introduction of the new queen, and was completed in all locations until September 2020.

6.1.4. Data management and evaluation

For proper data management and security, a central database was developed for electronic record keeping of the data from the PT and the CB. The database was partitioned for PT and CB. Full access to all files was only given to the project management team. Data entry, validity and verification was in the responsibility of the beekeepers and respective local coordinators. However, in order to avoid errors by typing or misinterpretation, logical controls were predefined for each entry cell. Stable and secure data collection was ensured by regular backups. The database was closed for any further changes on 15th of October 2020.

6.1.5. Statistical analyses

Most of the traits measured on the colony level in the case studies were analysed by Generalized Linear Model (GLM), where, according to the traits' specifics (distribution, number of cases), different factors were chosen to be tested in the models. The analysed factors were:

- genetic line to which the colony belonged (Table 6.2);
- apiary in which the colony was tested (belonging to a PT or a CB);
- origin: in the PT apiaries lines were classified as local (belonging to the autochthonous subspecies and reared and selected in the same region as testing for several generations), intermediately local (autochthonous subspecies but selected under different environmental conditions; or non-autochthonous subspecies / race, but selected under the same environmental conditions), or non-local (non-autochthonous subspecies / race, and selected under different environmental conditions as testing);
- subspecies / race to which the line belonged (Table 6.2);
- selection efforts: lines were classified based on whether and how much selection effort has been invested for a specific trait (no effort, 1, 2, 3 or more generations).

For the aim of achieving a more fair comparison, all the traits are presented as adjusted means, in which specific location variances are separated. The differences between adjusted means were tested for significance using the Bonferroni test. Only the factors with significant differences are reported.

Colony losses in PT and CB apiaries, gentleness and swarming in CB apiaries (details in 6.2) were measured as categorical traits and thus are presented as frequencies.

6.2. Testing parameters

The methods used to measure and assess the biological parameters in the EurBeST project were already validated in previous studies and most of them are currently used within breeding initiatives across Europe. For the EurBeST study, the colonies were assessed for 11 traits that finally enabled analysis and comparison of 17 parameters (Table 6.4.).

Standardisation and harmonisation of the data collection between and within the CSs was ensured by the development of handbooks entitled “Book of

Methods for performance testers” and “Book of Methods for commercial beekeepers)”, respectively (see copies of the English versions in the Annex). Each folder, available in electronic and hard-copy formats, included detailed step by step description of the methods and the record-keeping cards for data collection.

In addition, before the beginning of field testing, one to three meetings and training sessions were held in each CS to demonstrate and practice the methods.

Table 6.4. List of traits and derived parameters registered in the EurBeST study

Trait/parameters	Level of application	Data collection frequency
Colony strength (adult bees)	PT & CB	LT Regular
Colony strength (brood area)	PT	LT Regular
Honey yield (net weight)	PT & CB	One time
Swarming tendency (scoring)	PT & CB	ST Regular
Gentleness (scoring)	PT & CB	LT Regular
Adult bee infestation (powdered sugar)	PT & CB	LT Regular
Brood infestation (brood inspection)	PT	ST Regular
Natural mite mortality (fallen mites)	PT (optional)	ST Regular
Hygienic behaviour (pin test)	PT	ST Regular
SMR & REC (brood inspection)	Selected PTs with lab support	One time
VSH (brood inspection)	Selected PTs with lab support	One time
Queen and colony losses	Derived parameters	
Overwintering index		
Absolute strength at first inspection in 2020		
Absolute strength in summer 2020 (Average for the season)		

CB - commercial beekeeping, QP - queen production, PT - performance testing, ST - short term, LT - long term, lab – laboratory

Queen and colony losses

Losses of queens and entire colonies are one of the most obvious indicators concerning the health status and the fate of honey bees. These indicators are widely recognised as a barometer of the population health status and have great economic impact. For the EurBeST study, the time of queen or colony losses was recorded for all PT and CB test colonies together with the causes for such events, based on the beekeepers' observations.

Overwintering index

The winter, or the non-active season, due to unfavourable weather and general absence of forage, is the most delicate period for honey bee colonies. Moreover, during this period, the beekeeper also has very limited options to intervene to support the colony, if problems occur. The fate of the colony during the non-active season is of major importance for the colony performance and productivity in the forthcoming active season. The parameter can also be used as a good indicator of the health and quality of the winter bee population in consequence of the colony condition in the previous autumn.

To describe the wintering success of colonies, the overwintering index is calculated as the ratio of bees in early spring (number of combs occupied with bees in early spring) to the number of combs occupied with bees before the preceding winter (usually mid to late autumn). In the EurBeST study, we estimated the overwintering index for all the colonies surviving the winter season 2019/2020.

Colony strength and development

The seasonal changes in bee population and brood activity, together constituting colony strength, are important to describe the adaptation, wintering ability and productive potential of the colonies. Colony strength was assessed by visually assessing the number of combs and spaces occupied by bees and the number of combs with brood. In order to directly compare the strength of colonies between apiaries using different comb types, the relative sizes of the combs compared to modified Dadant frames (448*285 mm) were used to correct the measured number of combs. Consequently, all results in this report refer to the Dadant frame size.

Colony strength and development was thus assessed in the following way:

- Absolute strength at first inspection in 2020

From an apicultural point of view, the most determining parameter for the colony´s potential to exploit resources in the forthcoming season is the strength of the colony in early spring. Usually, the most prolific colonies, concerning the production of honey and other bee products, are those with the highest number of worker bees and brood in early spring. That is particularly true for regions with an early nectar flow. Thus, in our study, we took this parameter as an indicator for the colony´s adaptation to the environment and of its potential for production in the season 2020.

- Average strength during the season 2020

Repeated records for the number of combs occupied with bees and for the number of brood combs during the season 2020 were taken to calculate average strength parameters. In the CB apiaries, one measure in summer was taken. These records are used as additional indicators for the colony´s potential and health status.

Traditional apicultural traits

The behavioural traits (gentleness and swarming) were assessed during most of the inspections of the colonies by the performance testers and by the commercial beekeepers. However, in the performance testing the traits were evaluated and recorded as scores from 1 to 4, where 1 is the most negative and 4 most positive observation, while the commercial beekeepers evaluated the traits by registering them as better (+), no difference (0), or worse (-) compared to their expectations based on the stock they are used to managing.

- Gentleness

The gentleness of colonies directly affects all apicultural manipulations, but also, and in particular, the reputation of beekeeping by other persons. Therefore, it has traditionally been one of the most highly regarded selection traits in almost every breeding program. Gentleness was assessed by classifying the defensive behaviour and response of the bees during handling according to a 4 point scale (1 most defensive = aggressive to 4 = less defensive = gentle). At the end of the season, the average of all registered scores throughout the season was assigned to each colony.

- Swarming

Beekeepers want to prevent swarming to achieve a continuous seasonal development of their colonies and to maximise honey production. Colonies with low swarming tendency are easier to handle and do not require any special management efforts for the control and prevention of swarming. This trait is therefore particularly relevant for the commercial beekeeping operations. For the EurBeST study, swarming tendency was evaluated according to a 4 point scale (1 swarming could not be prevented except by the most severe manipulations and 4 the colony did not show any intention to swarm), and at the end of the season the lowest score registered for each colony was assigned as ultimate evaluation of the colony.

- Honey yield

The honey production is surely one of the most important traits for commercial beekeeping and dominantly affects the economic prosperity of beekeeping. For the EurBeST study, honey production was assessed by weighing the honey supers before and after extraction. The production of each honey harvest was registered and summed to calculate the total honey production per colony for the entire season.

Varroa destructor infestation

Differences in varroa infestation between colonies can directly relate to varying degrees of resistance. The parameter is therefore commonly used as a selection parameter for mite resistance. Furthermore, infestation levels can help the beekeeper decide on the need for treatments against varroa and closely correlate with the risk of colony losses. There are several methods available for the estimation of varroa infestation. Natural mite mortality and estimation of the adult bee infestation are the most commonly used ones.

- Natural mite mortality

Evaluation of mite mortality rates gives an indirect measurement of varroa infestation levels in colonies. This method is easily accessible to beekeepers and does not require opening the colonies, which is of particular advantage during the winter and early spring periods. In the EurBeST study, the method was optionally recommended for the performance testers.

- Adult bee infestation

Sampling of adult bees allows evaluating the level of infestation by mites that are present only on the adult bees. There are two simple and reliable methods (powdered sugar and washing bees) available to separate the mites from the bees and to calculate the infestation levels as the number of mites per 10 g of bees (roughly equals the % of bee infestation). The testing for adult bee infestation was obligatory for all beekeepers (PT and CB) during the pre-wintering period in 2019 and at the end of the testing period in late summer 2020.

Specific traits of varroa resistance

Several traits are recognised as resistance mechanisms against the varroa mite. Amongst them, hygienic removal of brood infested with mites (Varroa Sensitive Hygiene – VSH) and opening and closing the capped brood (recapping – REC) cause lower reproduction success of varroa (Suppressed Mite Reproduction – SMR). These traits have been shown to be heritable and possible to select for. However, implementation of selection for these traits in breeding operations tends to be difficult as they are time consuming (both in terms of implementation and selection progress), demand specialised education and additional equipment, which significantly increases the cost of queen breeding. Consequently, breeding and selection for resistance using these traits is not widely developed and mainly limited to research institutions.

The EurBeST project offered the opportunity to evaluate the progress of selection on different resistance traits and put a value on breeding efforts that have to be conducted.

- Hygienic behaviour (Pin test)

Hygienic behaviour of bees is defined as the ability of worker bees to recognise, open and remove diseased or dead brood. In all PT apiaries, hygienic behaviour was evaluated using the "pin-test" method. On one frame per colony, 50 sealed brood cells at the developmental stage of young pupae with white to reddish eyes were pierced with an entomological pin. Six hours later, the number of sealed (untouched) cells was counted and recorded. The hygienic score was calculated as proportion of cells opened by the bees in regard to all pierced cells. Testing was performed at least twice during the active season, and the average hygienic score was calculated for each colony.

- VSH

Worker bees with highly expressed Varroa Sensitive Hygiene are able to recognise and open brood cells infested with varroa mites. In this study, VSH was tested by checking the removal of brood artificially infested with varroa mites. Briefly, 30 brood cells close to capping were marked on a transparent sheet and infested with varroa mites 6-8 hours later, once they were capped. Using a scalpel, selected brood cells were carefully opened, a live adult varroa was introduced with a fine brush, and the cell was immediately closed again. Mites for infestation were collected from highly infested colonies using the sugar shake method. In addition to infested cells, an equal number of brood cells was opened and closed again without infesting them, serving as controls. Eight days after the infestation (before the bees started to emerge), the manipulated brood was checked for hygienic removal. For control cells, the same procedure was performed. In calculation of VSH (removal of artificially infested cells), the rate of removal of control cells was taken into account to correct the VSH score (proportion of artificially infested cells that were removed). The following formula was used:

$$VSH = (RI / (INF / 100) - RC / (CON / 100)) / (100 - RC / (CON / 100)) * 100$$

where: RI – number of removed infested brood cells, RC – number of removed control brood cells, INF – number of infested brood cells, CON – number of control brood cells.

The VSH testing was realised in some selected PT apiaries with support of institute laboratories.

- SMR and REC

The investigation for SMR and REC was combined on the same brood samples. Capped brood samples containing pupae in late developmental stages (7-12 days post capping) were collected and stored in a freezer (-18°C) until examination. Brood cells of each sample were opened and examined under a stereomicroscope until 35 cells infested with a single foundress varroa mite were found. When it was not possible to find 35 infested cells (due to low infestation rate or a small brood sample size), a lower number was used, but never less than 10 single infested cells. The capping of each cell was carefully opened using fine forceps or a scalpel, and the structure of its inner side was investigated to determine if it had previously been recapped (opened and closed again) by the bees. When a brood cell containing varroa was found, the developmental stage of the bee pupa and that of the varroa offspring was determined. A varroa foundress was considered non-reproductive if there was no offspring, if the offspring was too young to mature before the bee would emerge, or if the male offspring was missing. Similar to the VSH testing, the estimation of SMR and REC behaviour was realised only in some selected PT apiaries with support of institute laboratories.

6.3. Case study results

Data from most of the traits considered in the case studies were analysed statistically. Adjusted mean values for the lines in performance testers' (PT) apiaries, mean values for their own stock and for the EurBeST lines in commercial beekeepers' (CB) apiaries as well as statistical parameters are presented for all measured traits that are linked to colony development and production, to colony behaviour and to varroa resistance in the Annex (see file: "PT and CB case study data and statistical parameters.pdf").

6.3.1. Colony strength and development

The factor that mostly affected colony strength and development was "apiary", confirming the strong dependency of the honey bee colony on the environment in which it is placed. Also, the factor "apiary" includes the beekeepers' management style, thus its influence reflects both the environmental influence, but also the beekeeping practices enacted. The apiary effect was significant for all traits, both at the PT and CB level. The genetic type of the bees, in terms of specific lines, had a significant effect on number of adult bees in the productive season (summer), at both PT and CB level. Instead, colony strength assessed as number of brood combs was not affected by "line", highlighting the effect of environment on the adult bees / brood ratio. The interaction between line and apiary was significant for all traits connected to development in CB apiaries. Furthermore, when colonies were classified according to their origin (local or non-local in each apiary), we found that the interaction between the apiary and the origin was highly significant, suggesting an important role of adaptation to the local environment, as local colonies in general displayed higher values of colony strength and of production. The importance of environment and of environmental adaptation on colony development, observed in other studies, is confirmed by the EurBeST case studies.

6.3.1.1. Queen and colony losses

At the end of one productive season, after one year without treatment against varroa, 57% of the colonies in the PT apiaries survived (Fig. 6.4). On average, colonies in the German case study had the highest survival rate, while in the Polish and Italian case study the survival rate was lowest.

In the CB apiaries, the overall survival rate of colonies was higher (78%) than in the PT apiaries. However, it must be noted that colonies in PT apiaries were not treated against varroa after the initial starting treatment, while in the CB apiaries most of the beekeepers performed a winter treatment to reduce the mite loads. Some of the EurBeST lines suffered higher losses than the beekeepers' own stock (Fig. 6.5), indicating better adaptation of the local stock to the environmental conditions and local beekeeping practices.

Figure 6.4. Status of colonies (lost or survived) at the end of the study in PT apiaries. Each bar represents the proportion per line of lost and survived colonies, per case study (CS).

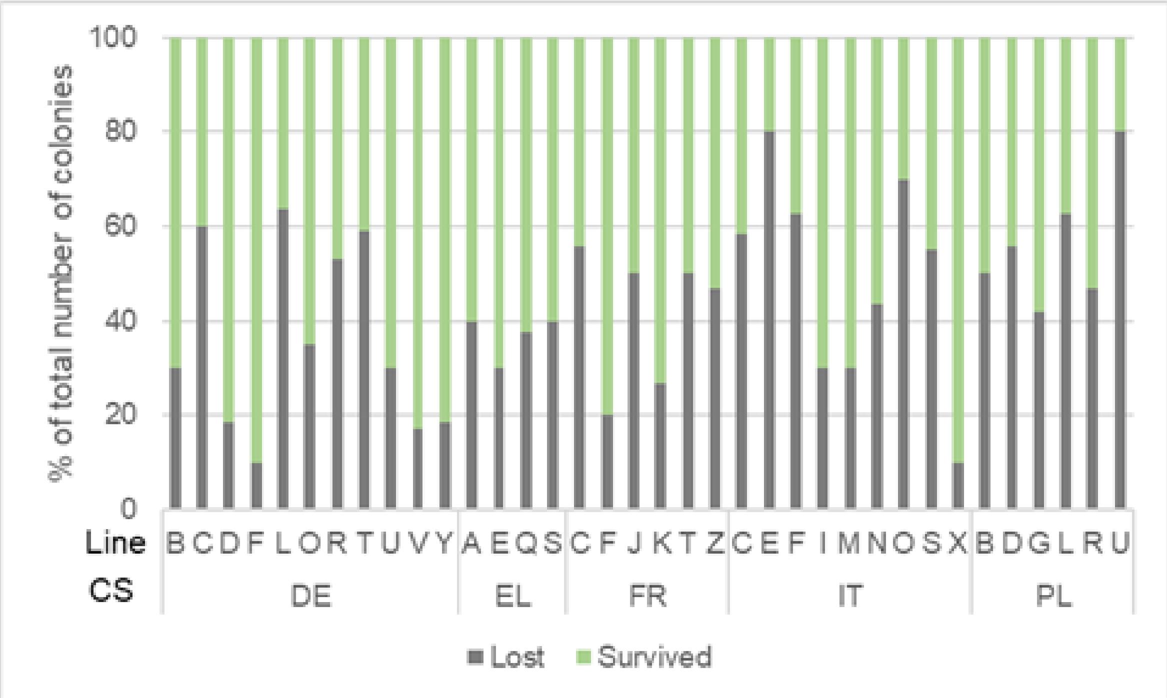
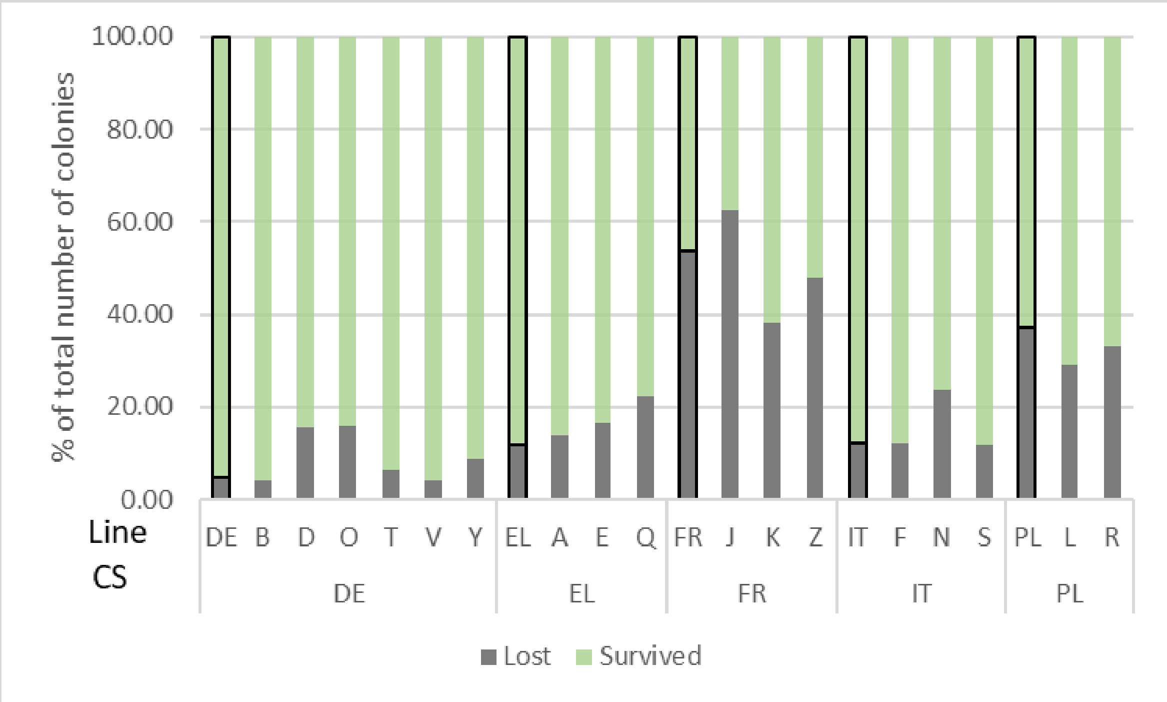
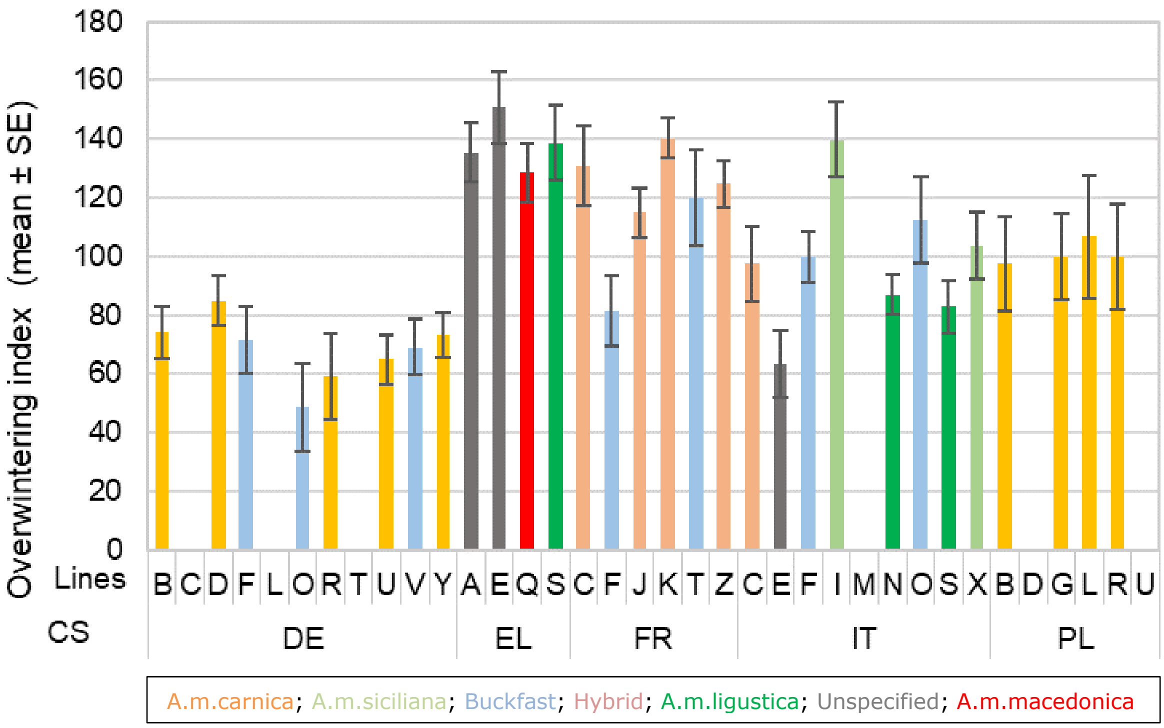


Figure 6.5. Status of colonies (lost or survived) at the end of the study in CB apiaries. Each bar represents the proportion per line of lost and survived colonies. For each case study (CS), the average rate of survival / loss of CB stock is given.



6.3.1.2. Overwintering index

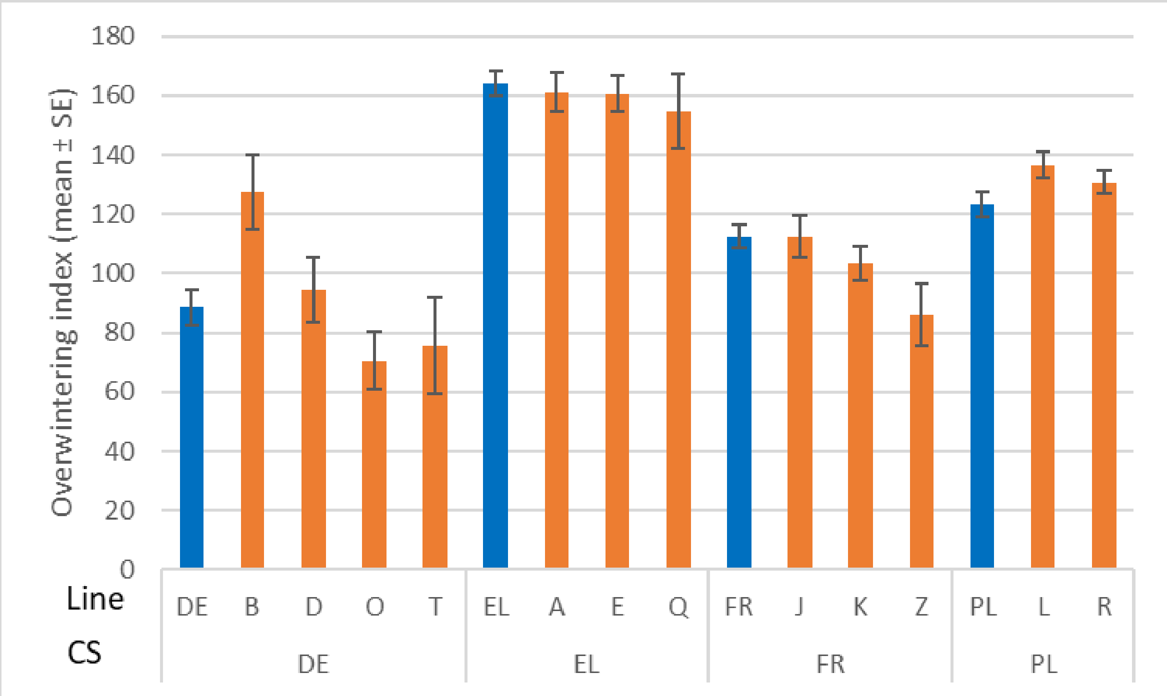
Figure 6.6. Overwintering index (%) per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.



The overwintering (OW) index is the ratio in % between number of bees at the end of the winter and number of bees at the beginning of the winter. A ratio of 100% means that the colony is as strong in spring as it was in the previous autumn, thus that fewer bees died in the colony during the winter and/or that the colony managed to better compensate for the lost bees. In the PT apiaries, there were significant differences in the OW index based on apiary ($F = 16.04$, $p < 0.001$), line ($F = 2.63$, $p < 0.001$), and their interaction ($F = 2.93$, $p < 0.001$). In the Greek case study, all lines had an OW index over 100%, while in the German case study all lines were under 100% (Figure 6.6), highlighting the strong effect of environment (which includes beekeeping management) on this trait, for the considered lines. Indeed, no statistically significant effect of selection effort towards colony development ($F = 0.96$, $p = 0.41$) or origin of the queens (local, intermediately local, or not local) ($F = 1.12$, $p < 0.33$) was found by our analyses.

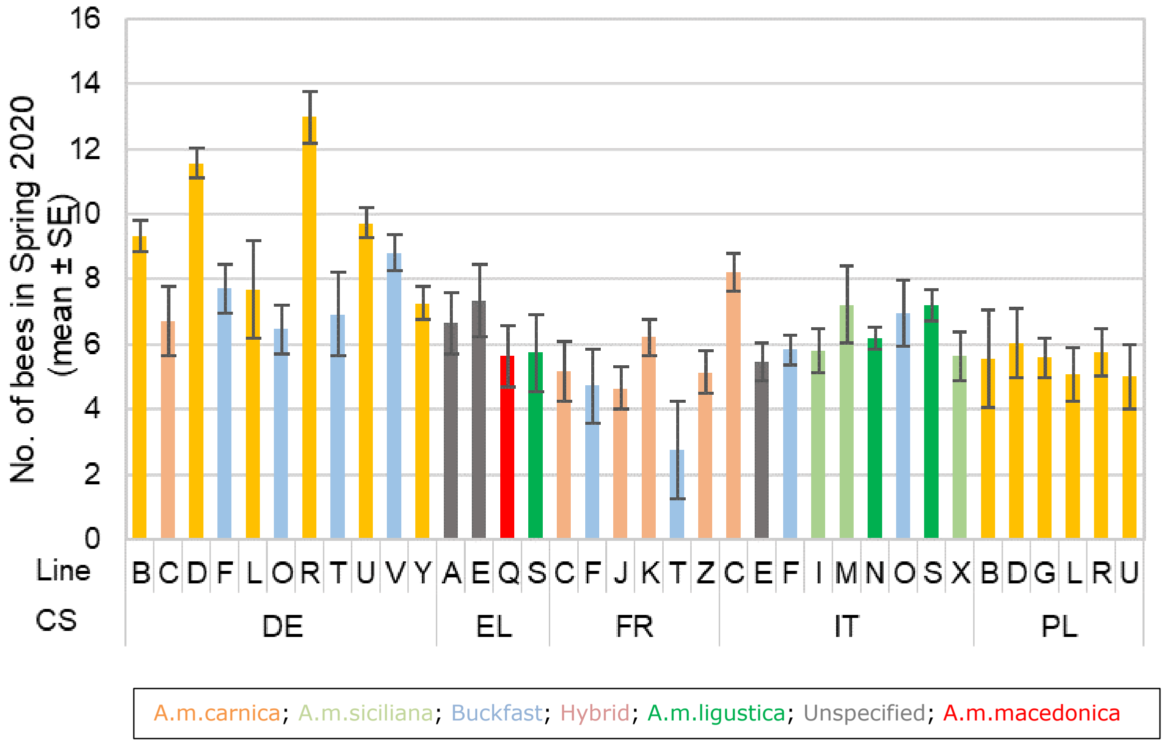
The two lines tested in the Polish case study by commercial beekeepers and two lines in the German case study were found to have a higher OW index compared to the beekeepers' own stock (Figure 6.7). Instead, in the French and Greek case study the EurBeST lines were found to have a lower OW index compared to beekeepers' lines. For some lines it wasn't possible to calculate the index due to missing data. Statistical analyses show that the main factor influencing this trait was the apiary ($F = 87.87$, $p < 0.001$), while line and origin (own stock / EurBeST stock) were not significant. However, the interactions between apiary and line (F = 1.85, p < 0.001) and between apiary and origin (F = 2.11, p < 0.001) were significant, showing that genotype - environment interactions play a role in the expression of the trait.

Figure 6.7. Overwintering index (%) in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).



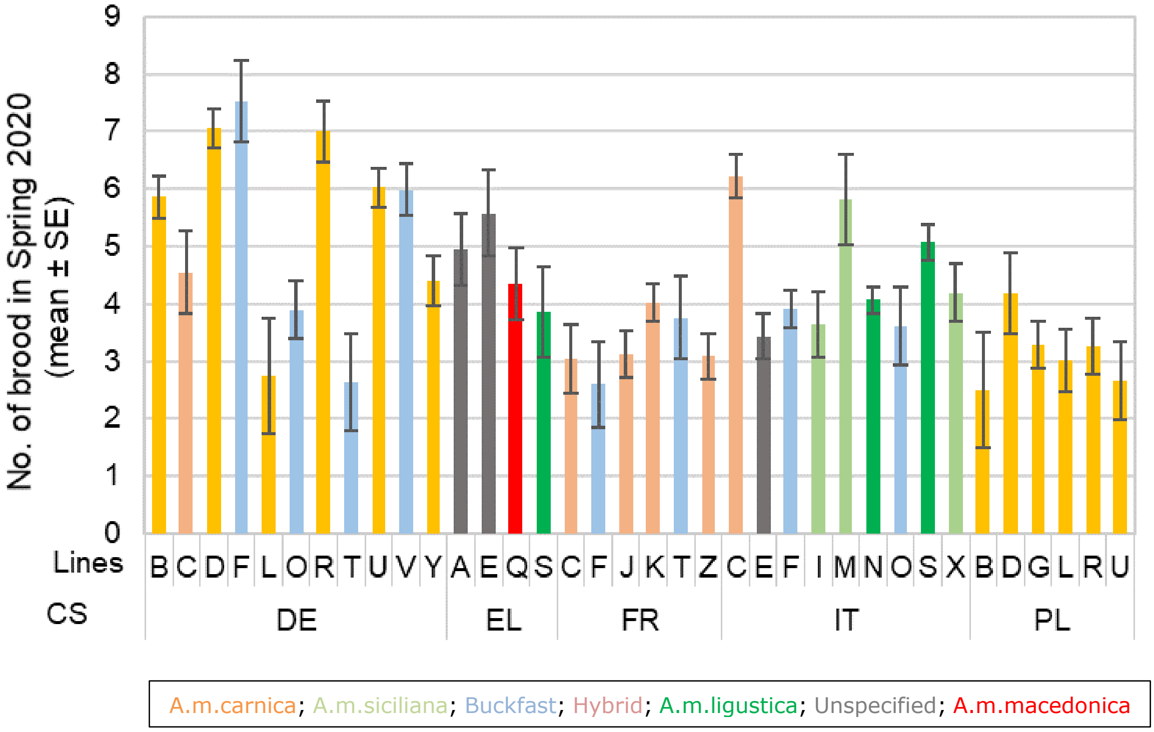
6.3.1.3. Colony strength in spring 2020

Figure 6.8. Number of bees (expressed as number of frames occupied by adult bees) at the beginning of the productive season (spring 2020), per line and per case study (CS) in PT apiaries. Bars represent the adjusted mean number of bees of lines present in the apiaries of each case study.



The variability in number of bees in the spring (measured as number of frames occupied by bees) (Fig. 6.8) was entirely influenced by the apiary ($F = 25.39$, $p < 0.001$), while none of the other factors tested in the statistical model (line, origin, subspecies, selection effort) showed any significant effect on this measure of colony development. Although the average overwintering index in the German CS was lower, lines tested in the German case study generally had higher numbers of bees than lines tested in other case studies. This shows the high dependency of the honey bee colony cycle on environmental conditions. Furthermore, beekeeping practices and colony management strategies common within the German case study probably contribute to the higher strength of colonies in the spring.

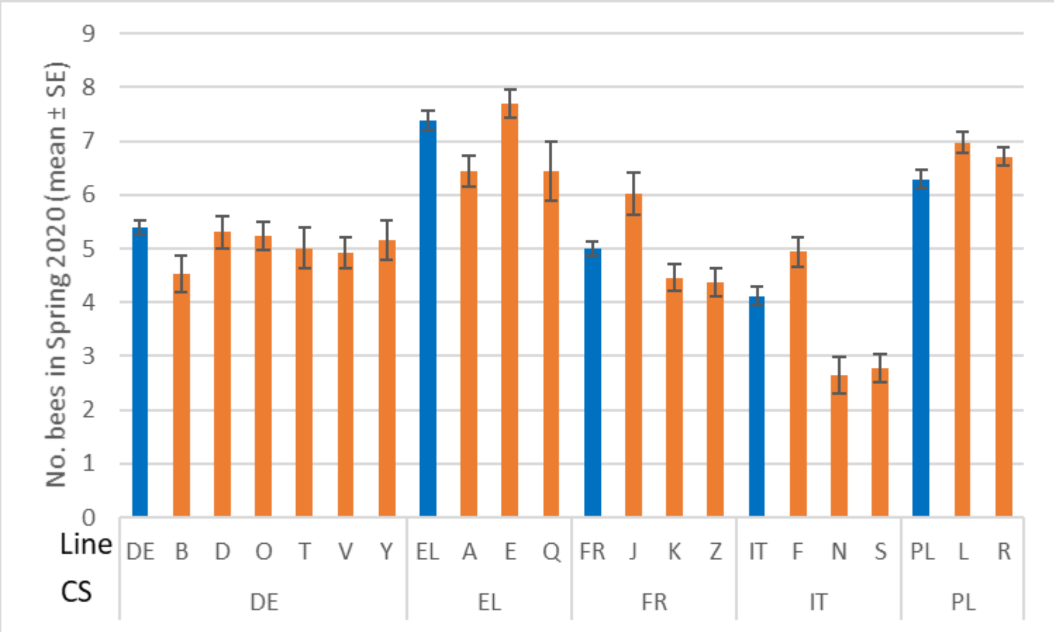
Figure 6.9. Amount of brood (expressed as number of frames with brood) at the beginning of the productive season (spring 2020), per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.



The differences between number of brood combs in spring (Figure 6.9) are also significantly affected only by the apiary in which the test was performed ($F = 21.61$, $p < 0.001$). The strength of the lines within PT apiaries, as measured by amount of brood, mostly reflects the strength given by number of adult bees, apart from a few exceptions, such as line F in the German CS, which was the strongest in terms of amount of brood, but not when assessed as number of bees. This specific line, which originates from a queen producer in Italy, seems to have reacted differently to the environmental effect compared to the other lines.

The influence of the environment on colony strength in the spring, measured as number of combs with adult bees, was confirmed in the CB apiaries, where differences between EurBeST lines and beekeepers' own stock was strongly affected by apiary ($F = 64.88$, $p < 0.001$). In this part of the case studies, the interaction between the apiary and the genotype, both in terms of specific line ($F = 1.88$, $p < 0.001$) and of EurBeST stock versus beekeepers' own stock ($F = 2.12$, $p < 0.001$), was found to have a significant effect on the colony strength with the beekeepers' own stock showing by trend greater strength than the EurBeST lines (Figure 6.10).

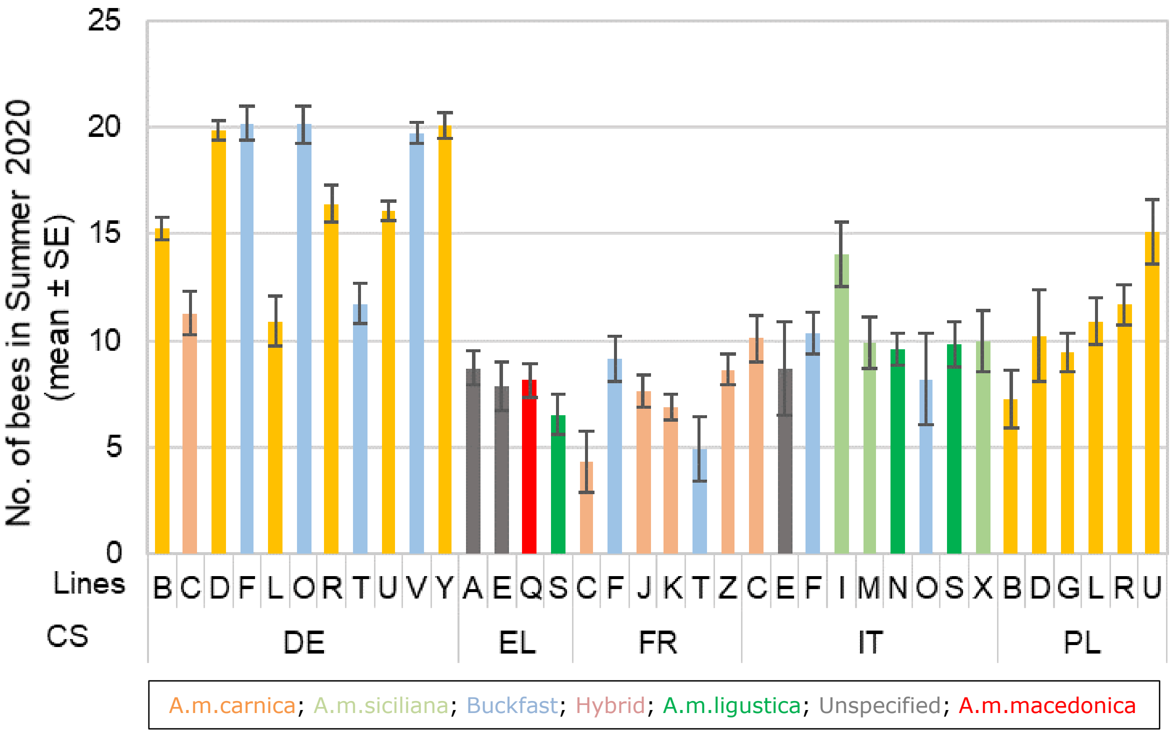
Figure 6.10. Number of bees (expressed as number of frames occupied by adult bees) at the beginning of the productive season (spring 2020) of the tested lines in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).



6.3.1.4. Colony strength in summer 2020

On average, in the 2020 inspections following the first inspection, colonies were stronger than in the first spring census. We observe the same overall pattern, with the colonies in the German case study showing the highest strength (more than half the lines with >15 frames of bees, value not achieved in any other case study). In terms of number of bees, the lines tested in Southern European conditions, especially France and Greece, tended to be smaller compared to the German and Polish case studies (Figure 6.11). Statistical analysis shows that colony strength in summer was significantly affected by apiary ($F = 24.96$, $p < 0.001$) and line ($F = 1.74$, $p = 0.019$), but also by genetic origin ($F = 15.05$, $p < 0.001$), with local colonies displaying significantly higher numbers of bees compared to non-local ($p < 0.001$) and intermediately local ($p < 0.01$). As the amount of brood is not statistically affected by origin (reported below, Fig. 6.13), we hypothesize a higher life expectancy of adult bees in their area of origin. The effects of genotype-environment interactions on colony strength are also highlighted by the statistically significant interaction between line and apiary ($F = 1.61$, $p < 0.05$).

Figure 6.11. Number of bees (average of several measures of number of frames occupied by adult bees) during the productive season (summer 2020), per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.



Statistical analyses showed that in CB apiaries line (F = 3.89, p < 0.001), apiary (F = 58.78, p < 0.001) and their interaction (F = 2.86, p < 0.001) were all significant factors for variations in colony strength (number of bees) in the summer. In most case studies, some EurBeST lines were stronger than CBs' own stock (Fig. 6.12). However, overall the CBs' own stock tended to have slightly higher number of bees, although this difference was not significant. Instead, the interaction between origin (own stock vs. EurBeST stock) and apiary was statistically significant (F = 2.65, p < 0.001), highlighting the effect of the genotype-environment interactions on colony development.

As for the spring inspection, the strength assessed during the productive season by number of brood combs (Figure 6.13) mirrored the strength expressed by number of frames occupied by adult bees. In the Italian case study, the lowest ratio between adult bees and brood was noticed, similar to findings reported in a previous study on the European level for colonies in Southern Europe (Hatjina et al., 2013[4]). On average, in the late spring/summer inspections, colonies were

[4] Hatjina, F & Costa, C; Büchler, R; Uzunov, A; Drazic, M; et al (2014) Population dynamics of European honey bee genotypes under different environmental conditions. Journal of Apicultural Research, 53(2): 233-247. http://dx.doi.org/10.3896/IBRA.1.53.2.05.

stronger than in the first spring census. Statistical analysis shows that number of brood combs in summer was significantly affected by apiary ($F = 19.07$, $p < 0.001$), but differently from number of bees, not by line or origin. Furthermore, neither subspecies, nor breeding effort towards good colony development, were factors that influenced the measured variability of the trait.

Figure 6.12. Number of bees (expressed as number of frames occupied by adult bees) in summer 2020 in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).

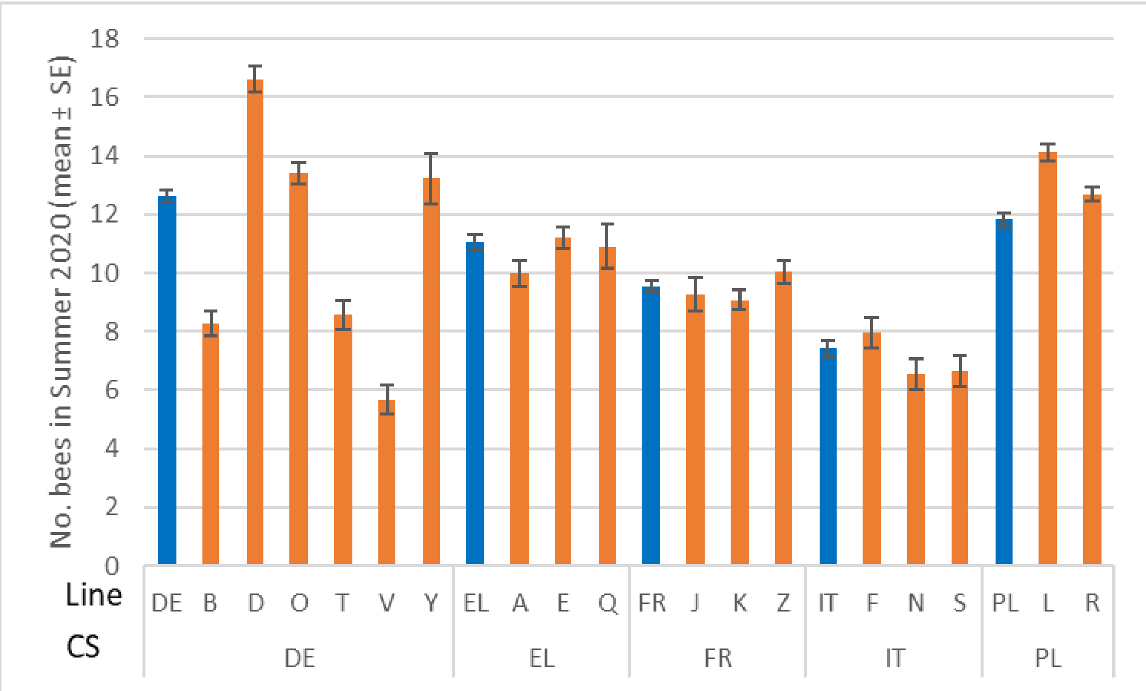
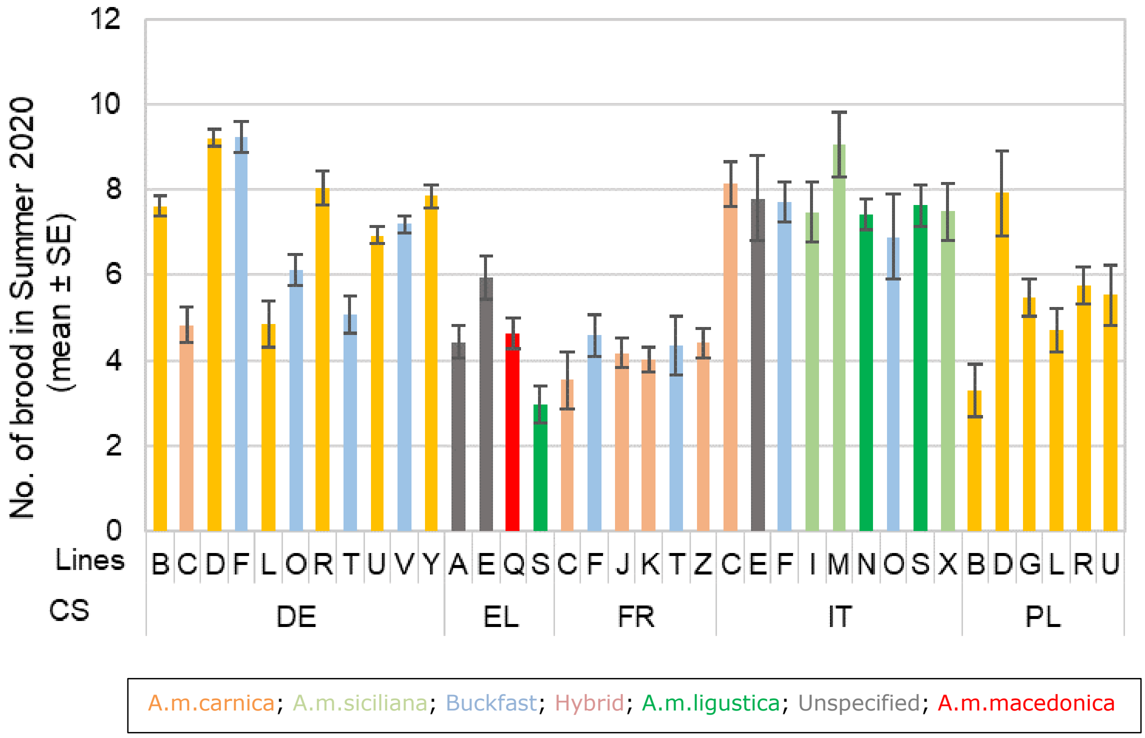


Figure 6.13. Amount of brood (expressed as number of frames with brood) in summer 2020, per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.

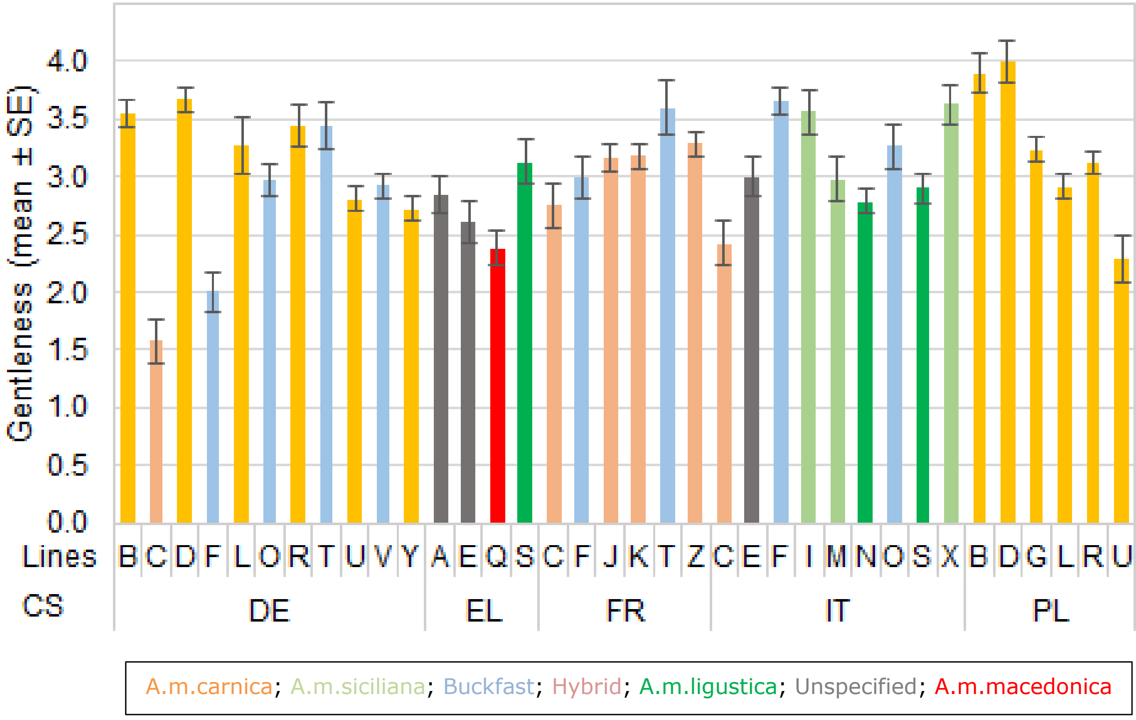


6.3.2. Traditional apicultural traits

The behavioural and productive traits, traditionally measured in honey bee breeding programs, were all significantly influenced by genetic line and by apiary (statistical analyses performed at the PT level). We found indication of effect of origin (local VS non-local or intermediately local) for gentleness (at the PT level) and for honey production in PT and CB apiaries. In these cases, performance of local lines was better (more gentle, less prone to swarming, higher honey yield) than when lines were non-local.

6.3.2.1. Gentleness

Figure 6.14. Gentleness (average score of all measurements from Sep. 2019 until Sep. 2020) per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.

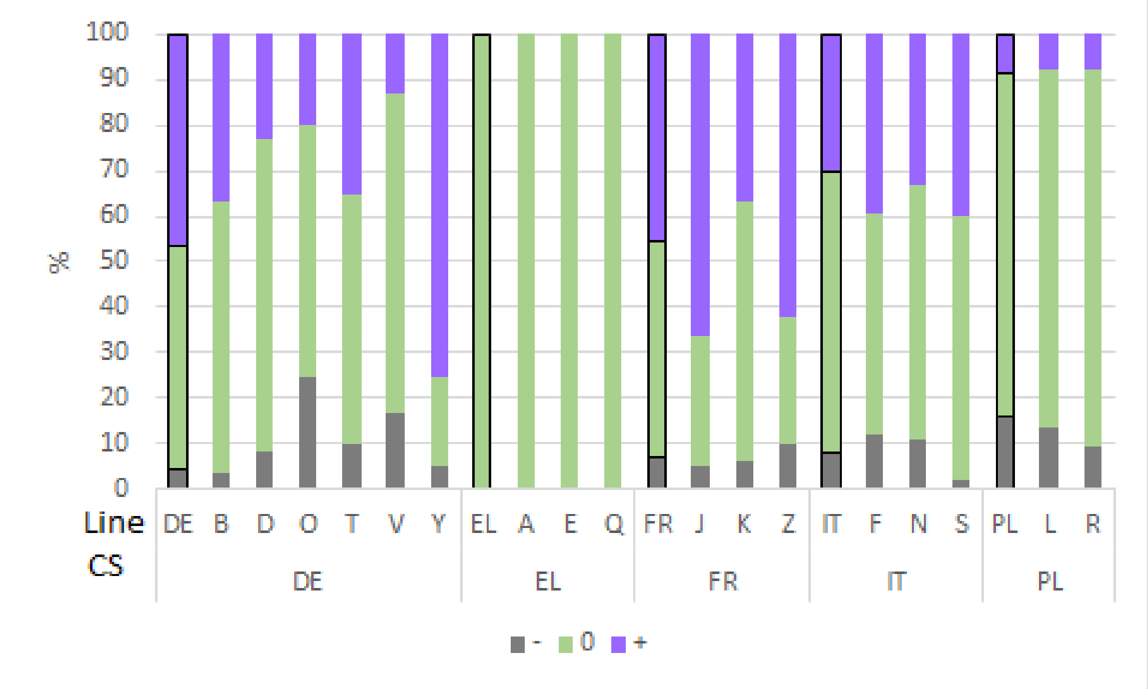


There was significant variability in scores for gentleness between lines ($F = 9.79$, $p < 0.001$) and between PT apiaries ($F = 33.81$, $p < 0.001$). Average scores from the whole productive season (Figure 6.14) ranged from a minimum of 1.57 (± 0.19 SE) for the C line in the German case study to the maximum value of 4.00 (± 0.18 SE) for the D line in the Polish case study. We found that the selection effort behind the trait is significant ($F = 9.59$, $p < 0.001$), and that scores of lines in which three or more generations of selection effort towards breeding for gentleness have been enacted are significantly higher ($p < 0.001$) than scores of lines in which no selection towards gentleness was performed. Some lines that were tested in different case studies showed consistency across locations, highlighting the success of the selection effort behind the trait (*e.g.* lines B and D), while for others (*e.g.* C and F) there were noticeable genotype-environment interaction effects. Overall, the interaction between line and apiary was statistically significant ($F = 3.25$, $p < 0.001$). It is interesting to notice that results from a previous pan-European study (Uzunov et al, 2013[5]) are confirmed, in terms of better performance of local stock: if we include

[5] Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., et al (2014) Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. Journal of Apicultural Research, 53(2): 248-260. 10.3896/IBRA.1.53.2.06

classification of lines according to origin, where origin is either local, non local, or intermediate, we find that local and intermediate lines have significantly higher gentleness scores than non-local lines ($F = 15.54$, $p < 0.001$). Subspecies / race to which the lines belonged also affected expression of gentleness ($F = 13.30$, $p < 0.001$), with Buckfast and *A. m. siciliana* displaying greater gentleness than other subspecies or races.

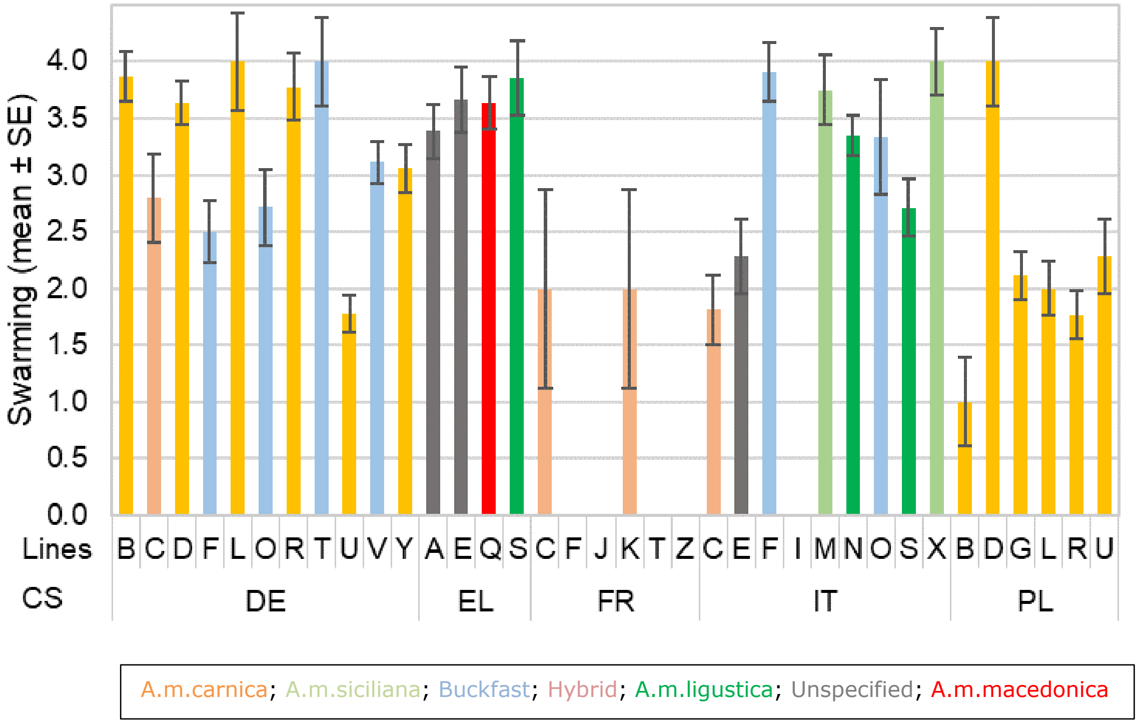
Figure 6.15. Gentleness per line and per case study in CB apiaries. Each bar shows the frequencies of positive (+), equal (0) or negative (-) evaluations of each EurBeST line for this trait. As a comparison, the average frequencies of evaluations in CB stock, per each case study, is reported (with the relevant country code).



When the EurBeST lines were evaluated by CBs for gentleness, with a simple "plus" (more gentle than average stock used by the beekeeper), "minus" (less gentle than average stock to which the beekeeper is accustomed) or "zero" (gentleness not different from the one displayed by usual stock) the overall result is that the test lines were mostly found to not be different from the stock that beekeepers are accustomed to using (high frequency of colonies scored with "0") (Fig. 6.15). In several cases (lines J and Z in French CS, line Y in German CS, all lines in Italian CS) the EurBeST lines were considered better than usual stock compared to the CB own stock (which was also assessed in the same way). In the Greek and Polish CS there were high frequencies of the "no difference" evaluation, possibly reflecting beekeeping practices and management styles (*e.g.* in which the gentleness trait is not normally noted).

6.3.2.2. Swarming

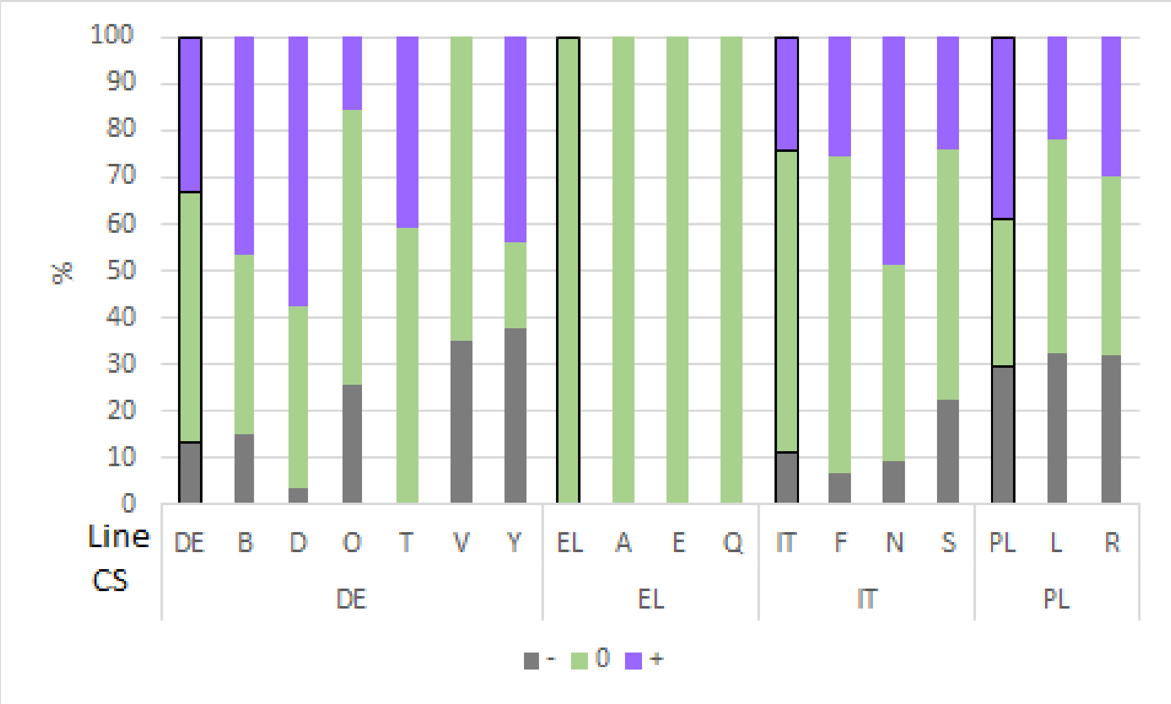
Figure 6.16. Swarming tendency (lowest score in 2020) per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.



Large differences among lines and case studies can be noticed for swarming behaviour (Fig. 6.16). Low scores indicate a high tendency to swarm, whereas high scores mean a reduced swarming tendency, which is traditionally considered a favourable apicultural trait. The variability in lowest swarming score throughout the season between lines and between PT apiaries was statistically significant, and ranged from a minimum of 1 (± 0.39 SE) for the B line in the Polish case study to the maximum value of 4, registered in lines L (± 0.44 SE) and T (± 0.39 SE) in the German case study, in line X (± 0.29 SE) in Italy and in line D (± 0.39 SE) in Poland. The interaction between line and apiary was statistically significant ($F = 2.32$, $p < 0.01$), and indeed it can be noticed that the lines present in different case studies exhibited very diverse scores: for example, line B scored 3.87 (± 0.22 SE) in the German case study and 1 (± 0.39 SE) in Poland. Differently from the gentleness trait, no effect of origin (local or not) was noticed ($F = 1.35$, $p = 0.25$). Instead, subspecies / race was found to affect swarming behaviour ($F = 4.46$, $p < 0.001$), with the hybrid origin showing a significantly higher swarming tendency compared to the other groups, and *A. m. siciliana* the lowest tendency.

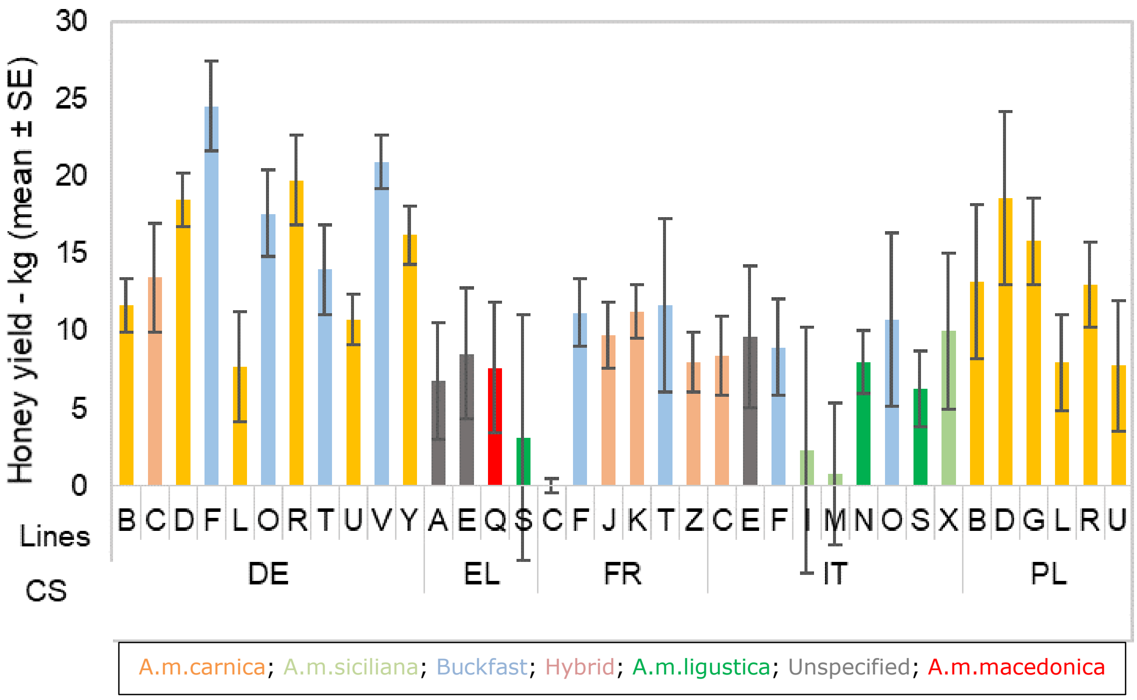
When the EurBeST lines were evaluated by CBs for swarming tendency, with a simple "plus" (less prone to swarming than average stock used by the beekeeper), "minus" (more inclined to swarm than average stock to which the beekeeper is accustomed) or "zero" (swarming tendency as expected), the overall result is that the test lines were mostly found to not be different from the stock that beekeepers are accustomed to using (high frequency of colonies scored with "0") (Fig. 6.17). However, there were several cases in which the EurBeST lines were scored as more prone to swarming ("-") with a higher frequency compared to the average of the CB own stock (e.g. lines O, V and Y in the German CS, line S in Italy).

Figure 6.17 Swarming tendency per line and per case study (CS) in CB apiaries. Each bar shows the frequencies of positive (+), equal (0) or negative (-) evaluations of each EurBeST line for this trait. As a comparison, the average frequencies of evaluations in CB stock, per each CS, is reported (with the relevant country code).



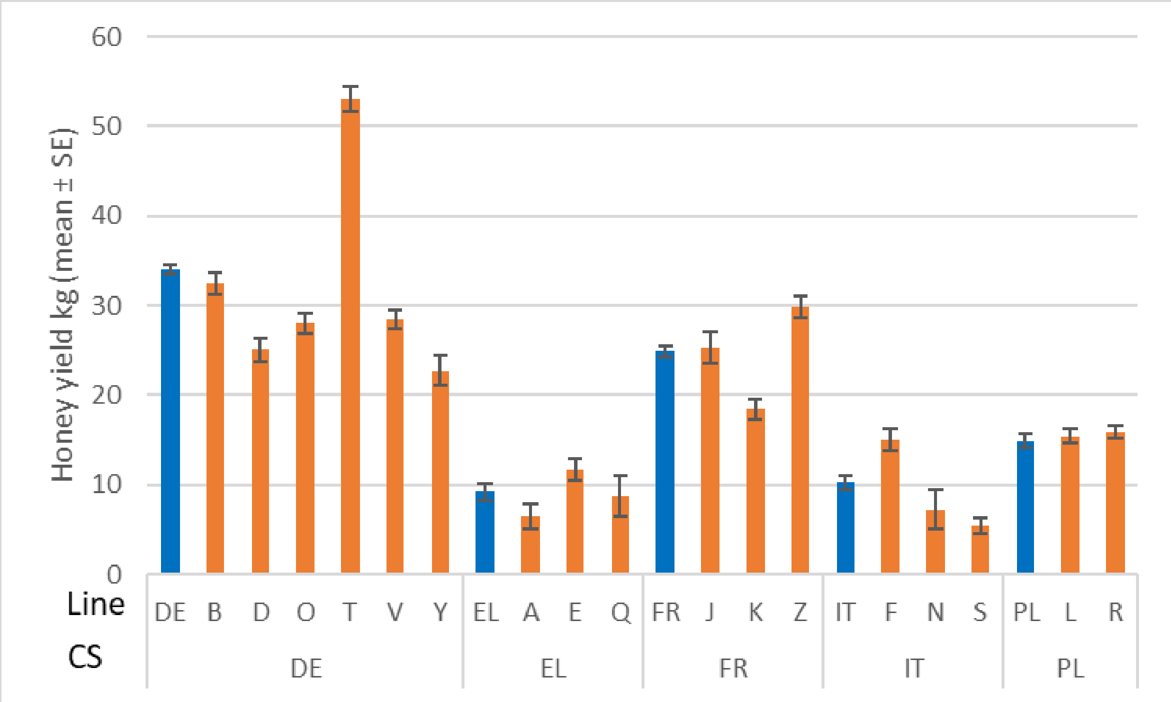
6.3.2.3. Honey production

Figure 6.18. Honey yield per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.



Average honey production per line within case study, in PT apiaries, ranged from a maximum of 24.51 Kg (± 2.88 SE) of line F in the German case study, to a minimum of 0.00 (± 0.45 SE) of line C in the French case study. Variability within each line was higher than for the behavioural traits, as can be noticed by the dimension of the error bars in Fig. 6.18. However, differences between lines were significant ($F = 2.00$, $p < 0.01$), as were differences between apiaries ($F = 12.11$, $p < 0.001$). If the lines are classified according to local or non-local origin, a significant difference is noticed ($F = 5.20$, $p < 0.01$), with local lines having significantly higher yield than intermediate lines and tendentially higher yield than non-local lines, in the respective apiaries.

Figure 6.19. Honey yield of the tested lines in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).



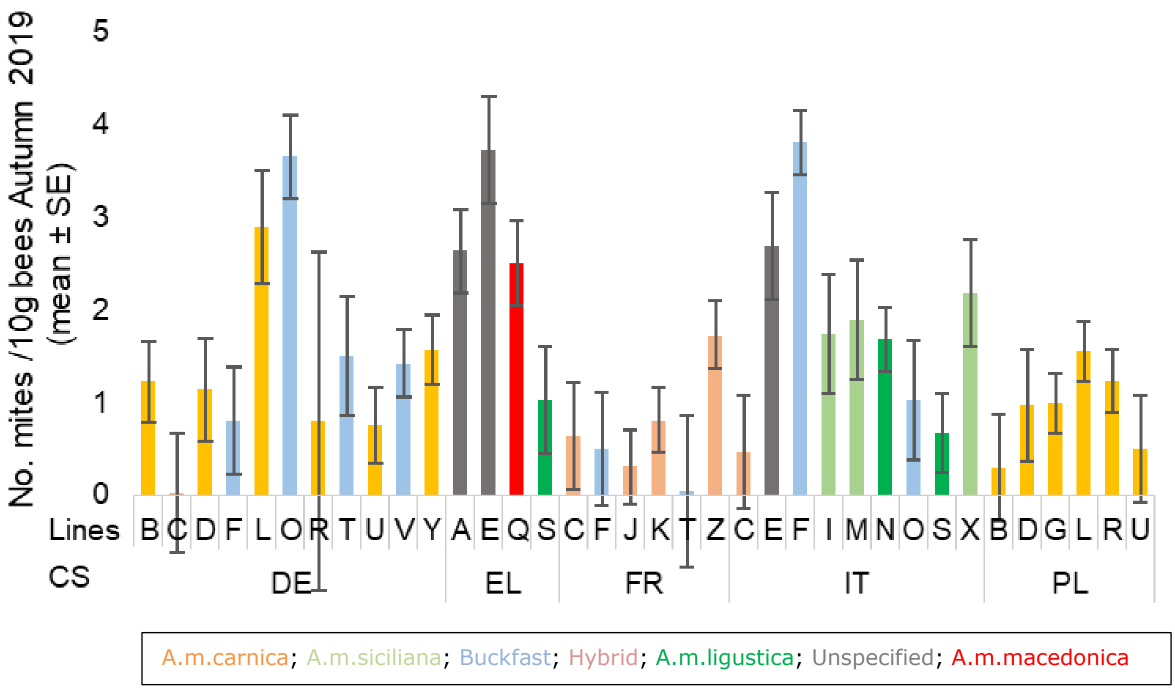
When comparing the EurBeST lines to stock used by commercial beekeepers in terms of productivity, the CB stock outperformed the EurBeST lines, with an average of 19.15 kg of honey produced by CB stock against 18.38 kg produced by EurBeST stock. Although this difference was statistically significant ($F = 10.79$, $p < 0.01$), there were some exceptions, with the Polish EurBeST lines showing slightly greater average honey yield than the beekeepers' stocks (Figure 6.19). In the German, Greek and Italian case studies, the majority of the EurBeST lines produced on average less honey than the beekeeper lines. This could be due to the fact that breeding / queen production activities have been present for a long time in these countries, and that most commercial beekeepers tend to use stock selected for apiculturally favourable traits, prioritising honey yield rather than varroa resistance. Similarly to the PT apiaries, differences in honey yield found by commercial beekeepers were significantly affected by line ($F = 11.32$, $p < 0.001$), by apiary ($F = 42.27$, $p < 0.001$) and by their interaction ($F = 6.66$, $p < 0.001$). The interaction was significant ($F = 4.73$, $p < 0.001$) also for origin (own stock vs. EurBeST stock) and apiary, indicating the role of genotype-environment interactions on the productive trait.

6.3.3. Varroa destructor infestation

The measured varroa infestation traits were all significantly influenced by genetic line and by apiary, both at the PT and CB level. An effect of queen origin (local/non-local for PTs, EurBeST/own for CBs) was only significant for the summer infestation in the CBs, with EurBeST stock displaying lower mite loads than CBs' own stock. Where it was analysed, selection effort on mite population development (MPD) was significant, with MPD lines showing lower mites levels than unselected colonies.

6.3.3.1. Autumn 2019 mite infestation

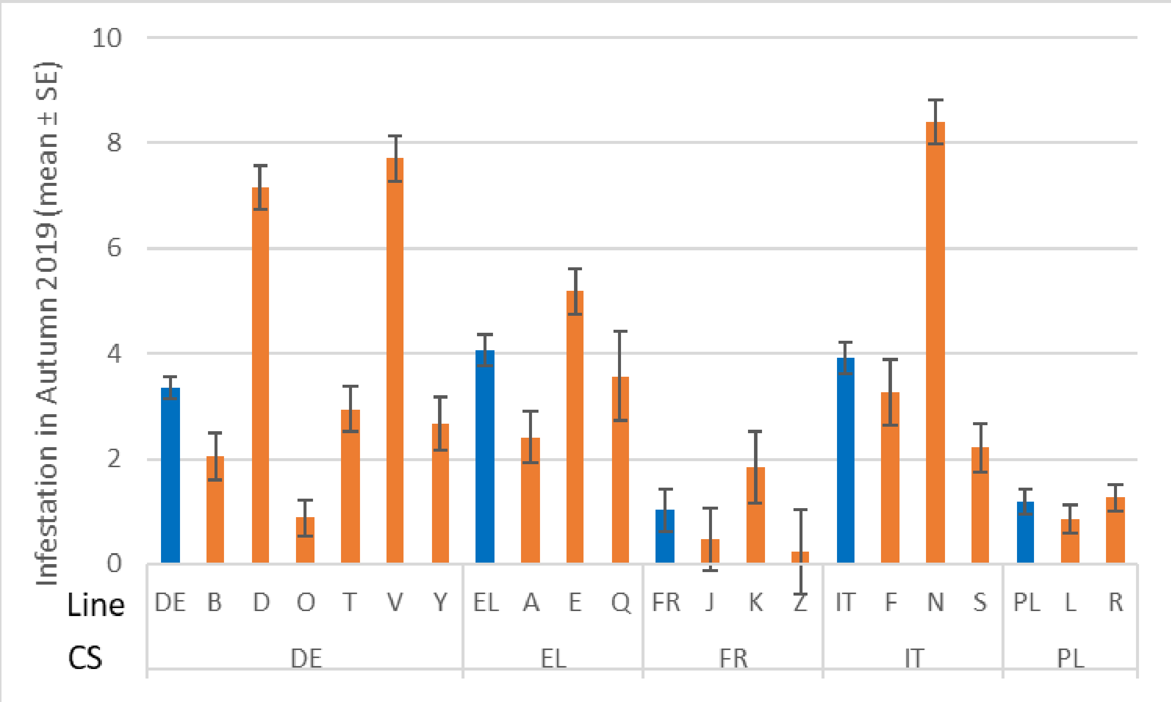
Figure 6.20. Autumn 2019 adult bee mite infestation (mites / 10 g bees) per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) tested in the apiaries of each case study.



Average adult bee mite infestation rates ranged from 0.4 (± 0.82) in line T tested in France to 3.1 (± 0.35) in line F in Italy, illustrating a huge variation in mite load only a few months following queen introduction (Figure 6.20). These results highlight significant differences between lines ($F = 3.69$, $p < 0.001$) as well as between apiaries ($F = 13.04$, $p < 0.001$), but the interaction between these genetics and environment factors was not significant ($F = 1.34$, $p = 0.13$). This is reflected in the fact that lines tested in different case studies mostly harbour similar infestation levels. If we consider the threshold of 3 mites / 10 g of bees, five lines exceed these loads and could be considered at risk for overwintering (in Germany, L and O, in Greece E, in Italy E and F). However, no line exceeds the economic threshold of 5 mites / 10 gr of bees, as expected, considering that the test queens were introduced into colonies treated against varroa (to have a uniform starting point and to reduce losses in the first winter).

Lines with a selection history based on the use of mite infestation as a criterion (mite population development) display lower rates in the autumn than lines not selected on this trait (F = 4.93, p = 0.002). The queen origin (local or not) did not influence the autumn mite load of the colonies.

Figure 6.21. Autumn 2019 mite infestation rate (mites / 10 g bees) per line and per case study in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).

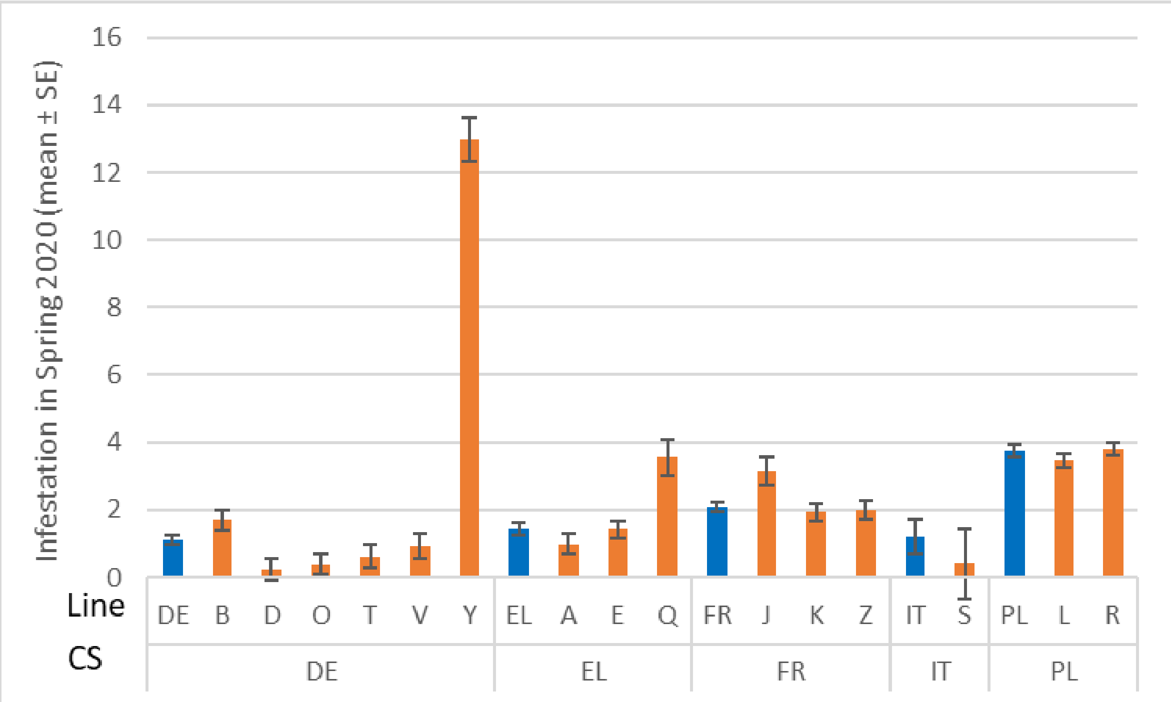


In commercial beekeeper apiaries, a majority of EurBeST lines show lower mite infestations than the average of own beekeeper stock (Figure 6.21). On average, the difference between own and EurBeST stock was not significant (F = 1.03, p = 0.31). Similarly to the PTs, the infestation was significantly different between lines (F = 6.65, p < 0.0001) and apiaries (F = 33.84, p < 0.0001). For the CBs, the interaction between the line and apiary factors is significant (F = 3.09, p < 0.0001), indicating that the same line tested in different apiaries can have significantly different loads as compared to the average of beekeepers' own stock.

6.3.3.2. Spring 2020 mite infestation

In the spring evaluation, a great variability in infestation is also observed between different stocks for the PTs; several lines also harbour mite loads that can be considered as critical, but no obvious correspondence can be made between high mite loads in the autumn and high mite loads in the spring. On average, colonies display lower loads in the spring than in the autumn; this is expected considering the natural cycle of mite population growth along the season, which initiates with few mites in the spring and a peak of infestation in the fall.

Figure 6.22 Spring 2020 mite infestation rate (mites / 10 g bees) per line and per case study in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adjusted mean of the CB stocks across each case study (blue).



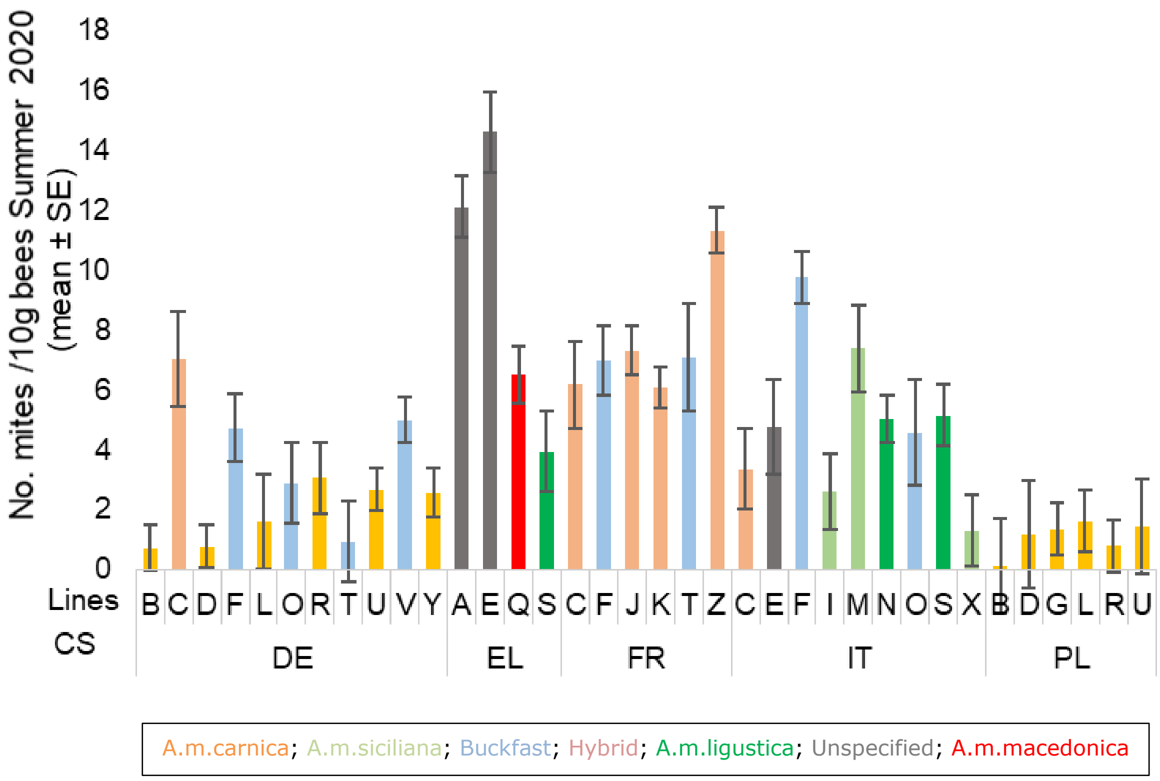
In commercial beekeepers' apiaries, and contrarily to what was observed in the autumn, most evaluated EurBeST lines display higher mite loads than the CB's own stock (Figure 6.22). However, on average the difference between own and EurBeST stock, similarly to the autumn situation, is not significant ($F = 0.053$, $p = 0.81$). It might be important to note that the extremely high value for line Y is based on the results from just two CBs one of which had on average 25 mites/10 g bees, while the other one had on average 0.91 mites/10 g of bees. Nevertheless, significant differences can be seen between lines ($F = 13.61$, $p < 0.0001$), and between apiaries ($F = 55.79$, $p < 0.0001$). Similarly to the situation in the autumn, the interaction between the line and apiary factors is significant ($F = 6.46$, $p < 0.0001$).

6.3.3.3. Summer 2020 mite infestation

Average adult bee mite infestation rates in the summer (May to August) ranged from 0.11 (± 1.59) in line B tested in Poland to 14.63 (± 1.34) in line E in

Greece (Figure 6.23). Compared to the spring evaluation, all lines but one (line Y in Germany) harbour an increase in mite infestation, which can be related to the natural seasonal increase in mite infestation levels that follow an exponential tendency. However, several lines that were measured for mite loads on adult bees both in spring and during the summer do not show such big increase in load, with absolute differences of less than 3 mites / 10 g of bees: this is the case for line O (2.88 mites / 10 gr of bees increase) and line V (2.67) in Germany, line S in Greece (2.08), and line E (2.28) in Italy. Noticeably, the summer score of all of these lines remains below the economic threshold of 3 mites / 10 g of bees.

Figure 6.23. Summer 2020 adult bee mite infestation (mites / 10 g of adult bees) per line and per case study in PT apiaries. Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.

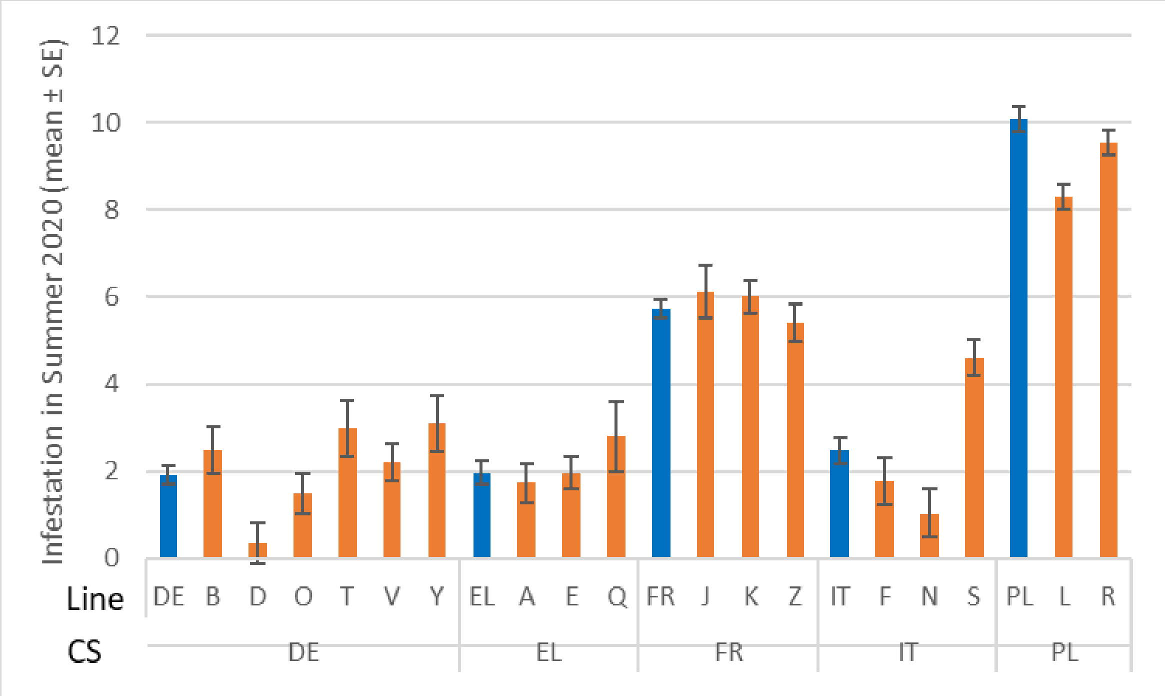


The great variability in mite load results from significant differences between lines ($F = 3.75$, $p < 0.001$) as well as between apiaries ($F = 4.55$, $p < 0.001$). Interestingly, at this point of the season, the interaction between these genetic and environmental factors is significant ($F = 1.96$, $p = 0.005$). On average, colonies in the three Mediterranean countries (EL, FR, IT) harbour higher mite loads than countries from Northern Europe (DE, PL). However, if we consider the commonly accepted threshold of 3 mites / 10 g of bees for treatment requirement, this mite load was exceeded in a majority of testing apiaries, and colonies could be considered at risk for overwintering. Differently from what was seen in the autumn, queens of local origin displayed significantly lower infestations in the summer as compared to queens of non-local origin ($F = 0.29$, $p = 0.002$).

Similarly to what was seen in autumn, lines with a selection history based on the use of mite infestation as a criterion (mite population development) display lower rates than lines not selected on this trait ($F = 0.29$, $p = 0.002$). As a result, all lines selected for low MPD displayed lower varroa loads than lines unselected for this trait. Of the lines originating from natural selection ("SUR" in Table 6.2), only a few of them displayed noticeably lower varroa loads than the other lines (e.g. line X in Italy), and in some cases loads were very high compared to other lines in the case study (e.g. line C in German CS, line A in Greek CS).

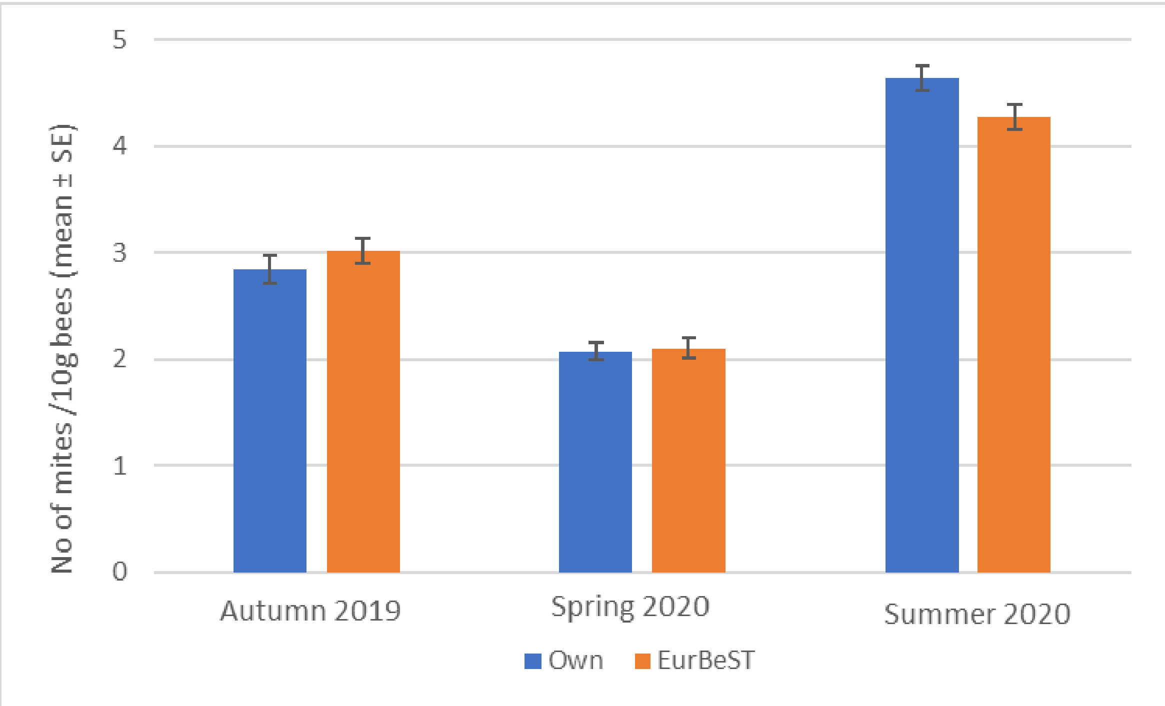
Similarly to the rest of the measurements done in autumn and spring, in CB apiaries (Fig. 6.24) the difference in mite load was significant between lines (4 = 4.57, $p < 0.0001$) and apiaries ($F = 52.85$, $p < 0.0001$); the interaction between these factors was significant ($F = 3.62$, $p < 0.0001$). Interestingly, and contrarily to what was observed in the spring and autumn, the overall average mite load in summer is significantly lower (- 0.4 mites/10g of bees) in EurBeST stock than in own stock ($F = 3.45$, $p = 0.033$, Figure 6.25).

Figure 6.24. Summer 2020 mite infestation rate (mites / 10 g bees) per line and per case study in CB apiaries. Each bar represents the adjusted mean value of EurBeST tested lines (orange), in comparison to the adj



usted mean of the CB stocks across each case study (blue).

Figure 6.25. Average mite infestation rate in CB apiaries of the EurBeST tested lines (orange) and the own CB's stock (blue), in autumn 2019, spring 2020 and summer 2020.



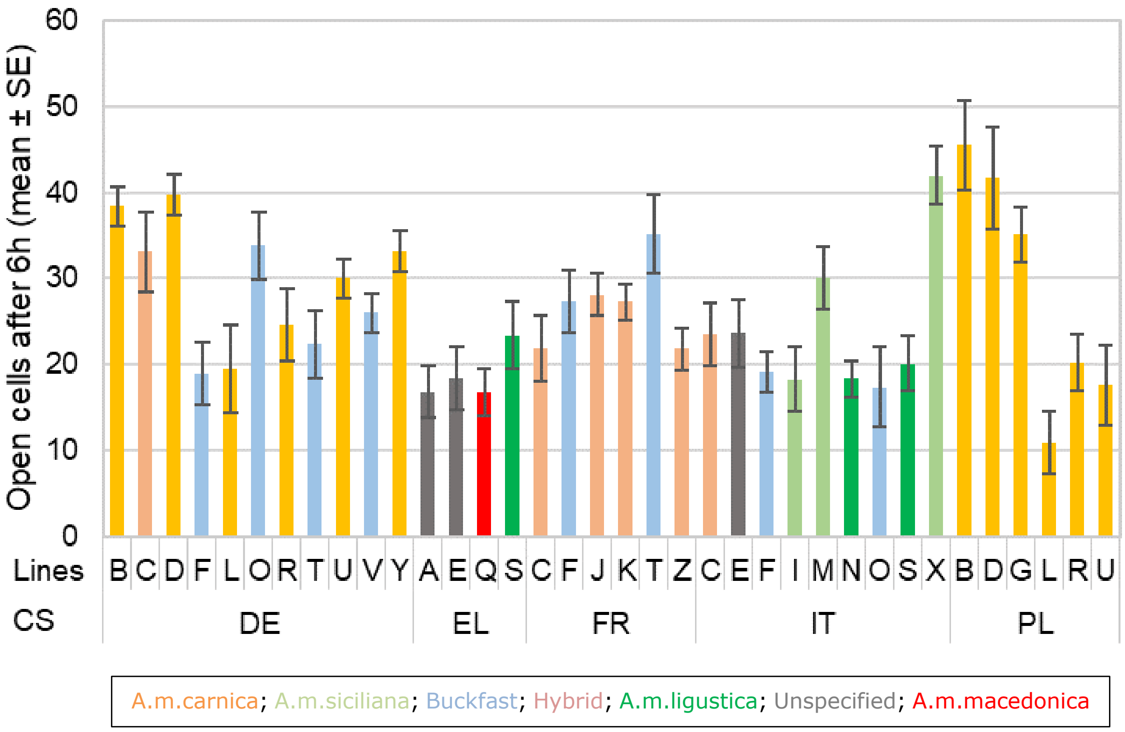
6.3.4. Specific traits of Varroa resistance

The most common traits linked with varroa-resistance (described in 4.2.3 and Figure 4.1.) were tested within the EurBeST project: hygienic behaviour, VSH, REC and SMR. For VSH, 160 colonies were tested and a total of 4 921 brood cells were artificially infested with varroa mites, while 4 556 cells were opened and closed and served as control cells. For SMR and REC, 252 brood samples from all case studies were collected and a total of 103 131 brood cells were opened and examined, out of which 8 114 were infested with varroa mites. In the end, there were 210 valid evaluations (samples with at least 10 brood cells infested with a single foundress varroa mite) used in the statistical analysis.

Environment had a major effect on the expression of all resistance traits. The only trait that differed between lines and subspecies is hygienic behaviour. Further, origin of the queen (local or non-local) was not a major factor, and in some cases local lines performed better, while in others non-local lines were more successful. A summary table of significant effects on the different traits is available in the Annex ("Mean data and statistical parameters of PT & CB testing.pdf"). Positive correlations are found between VSH, REC and hygienic behaviour, while VSH and hygienic behaviour were negatively correlated with infestation rate of adult bees.

6.3.4.1. Hygienic behaviour (Pin test)

Figure 6.26. Hygienic score (number of opened cells 6 hours after the test) per line and per case study (CS) in PT apiaries (percentage of opened cells after 6 hours ± SE). Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.

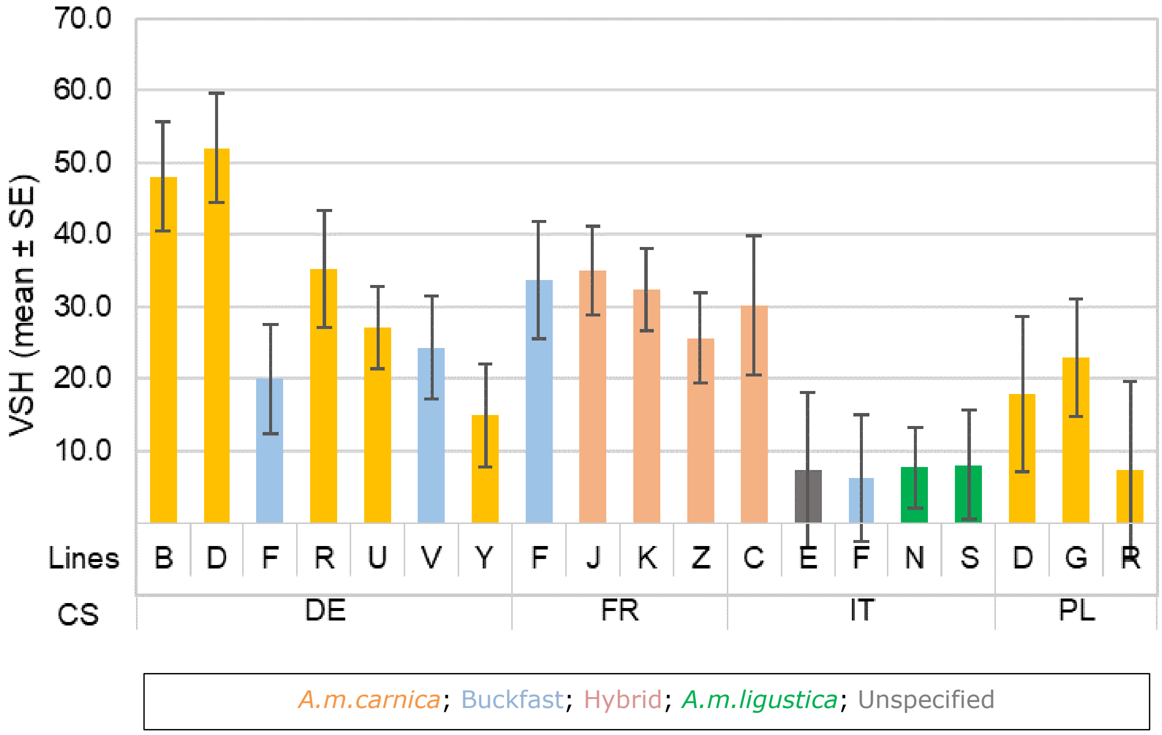


Hygienic behaviour was tested in all PTs at least twice during the course of the case study (Figure 6.26). Hygienic scores range from 11 (± 3.65) for Line L in Poland, to 45.5 (± 5.16) for line B in Poland and vary greatly between different lines ($F = 2.91$, $p < 0.001$), and apiaries ($F = 4.81$, $p < 0.001$). Most lines that were tested in two case studies showed similar expression of hygienic behaviour at several locations, as shown by the absence of significant interaction between the lines and the apiaries ($F = 1.24$, $p = 0.22$). This is especially evident for lines from highly selected stock; for instance, lines B and D showed highest hygienic behaviour both in the German and Polish CSs. We also observed a significant effect of queen origin ($F = 6.29$, $p = 0.002$).

There were significant differences between subspecies recorded ($F = 2.31$, $p = 0.033$), with *A. m. carnica* lines showing higher hygienic removal comparing to all others but *A. m. siciliana*. This could be expected, as most *A. m. carnica* lines are coming from long-lasting selection programs, which include selection for increased hygienic behaviour. The *A. m. siciliana* lines originate from a previous project focusing on varroa-resistance, in which hygienic behaviour was considered an important trait.

6.3.4.2. Varroa sensitive hygiene (VSH)

Figure 6.27. VSH (removal of varroa-infested brood) per line and per case study (CS) in PT apiaries (adjusted mean ± SE). Each bar represents a line (depicted by letter code) present in the apiaries of each case study.

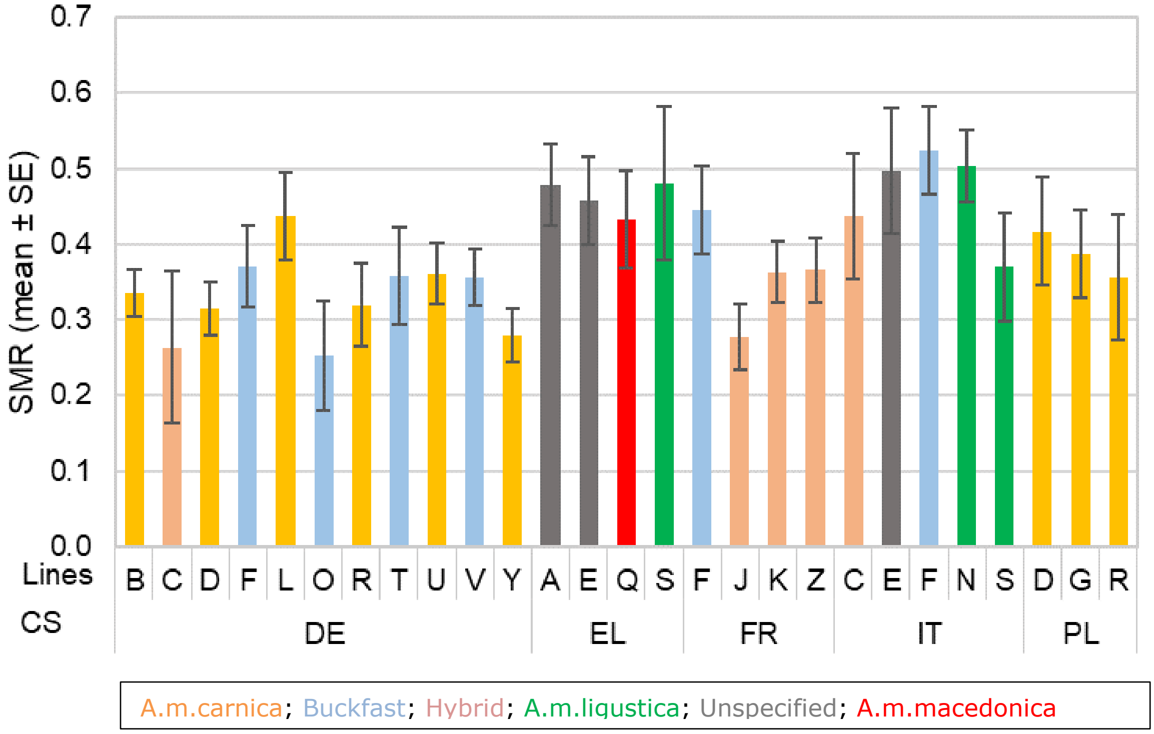


The average removal of pupae from brood cells artificially infested with varroa (mean ± SE) was 25.05 ± 24.02 %, ranging between 7.37 ± 8.58 % in line E to 48.03 ± 22.79 % in line B. No significant differences between lines are found ($F = 0.762$, $p = 0.708$) which is not surprising, as only one of the lines tested was selected for VSH. The high VSH scores recorded for the lines B and D (Figure 6.27) could be the consequence of a high selection effort for hygienic behaviour in German and Austrian breeding programs. These lines also had the highest scores for hygienic behaviour (Figure 6.26), and the significant positive correlation found for VSH with hygienic behaviour supports this (Table 6.5).

Environment had a significant effect on the expression of VSH, and lines tested in several apiaries usually had different scores ($F = 3.66$, $p = 0.001$). For instance, line D in the German CS had more than 50% of cells removed, while in the Polish CS it had less than 20%. Line F, tested in three CSs (Germany, France and Italy) and line R, tested in two CSs (Germany and Poland), harbour the same tendency. There were no differences in VSH between the different subspecies / races tested in the study ($F = 0.68$, $p = 0.64$).

6.3.4.3. Suppressed mite reproduction (SMR)

Figure 6.28. Results of SMR (proportion of non-reproducing mites) per line and per case study (CS) in PT apiaries (mean ± SE). Each bar represents the adjusted mean value of a line (depicted by letter code) present in the apiaries of each case study.

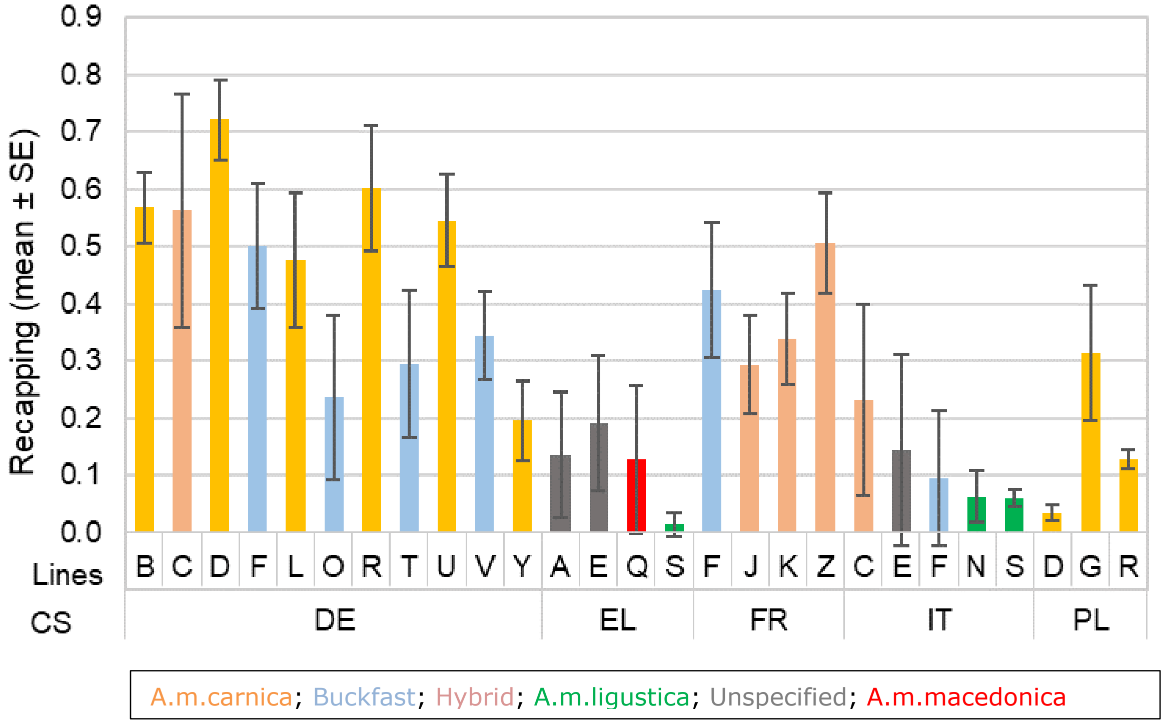


The average proportion of non-reproducing mites (mean ± SE) was 36.90 ± 15.13 %, with the range of 25.25 ± 14.06 % in line O to 50.33 ± 12.14 % in line N. There were no significant differences between lines ($F = 0.760$; $p = 0.751$). Location of testing (apiary) had the most significant effect on SMR ($F = 4.15$, $p < 0.0001$). The lack of significant interaction between line and apiary ($F = 1.68$, $p = 0.05$) implies that a given line mostly achieved similar results in different apiaries (Figure 6.28) and thus suggests that change of environment did not have an effect on SMR. This is confirmed by the fact that origin of the queen (local or non-local) was not a significant factor. Furthermore, no difference in mite non-reproduction was found between different subspecies ($F = 0.25$, $p = 0.94$). Lines selected on SMR did not display statistically different scores from lines not selected on this trait ($F = 0.27$, $p = 0.85$).

It is interesting to notice that line N with the highest average score for SMR attained very low VSH (Figure 6.27), REC (Figure 6.29) and relatively low hygienic behaviour (Figure 6.26). It is likely that factors other than VSH or REC are the cause for low reproduction of mites for this line.

6.3.4.4. Recapping of infested brood cells (REC)

Figure 6.29. Recapping (proportion) of brood cells infested with varroa per line and per case study (CS) in PT apiaries (adjusted mean ± SE). Each bar represents a line (depicted by letter code) present in the apiaries of each case study.



The average recapping rate of brood cells infested with varroa (mean ± SE) was 36.38 ± 33.68 %, ranging from 6.00 ± 6.98 % in line S to 59.05 ± 31.73 % in line D. There were no significant differences between tested lines ($F = 1.20$, $p = 0.26$). The main source of variation is apiary ($F = 4.144$, $p < 0.001$). A high standard deviation in most of the cases reveals high variability of this trait within the line, which is not unusual (Büchler et al. 2020[6], Kovačić et al. 2020[7]) as most of the lines were not selected for recapping. There was no interaction between line and apiary ($F = 0.90$, $p = 0.58$), but still it is worth mentioning that lines D and R in the German CS had much higher recapping in comparison to the same lines in the Polish CS. Furthermore, line F had similar scores in German and French CS, but scored much lower in the Italian one from where it originates. In general, queens tested in Greece and Italy showed lower recapping rates in comparison to German and French CS.

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[6] Büchler, R., Kovačić, M., Buchegger, M., Puškadija, Z., Hoppe, A., Brascamp, E.W. (2020) Evaluation of Traits for the Selection of *Apis Mellifera* for Resistance against *Varroa Destructor.* Insects, 11(9).

[7] Kovačić, M., Puškadija, Z., Dražić, M.M., Uzunov, A., Meixner, M.D., Büchler, R. (2020) Effects of selection and local adaptation on resilience and economic suitability in *Apis mellifera carnica*. Apidologie (2020).

As for SMR, there were no differences between subspecies (F = 0.62, p = 0.68) or queen origin (local or non-local, F = 1.43, p = 0.11).

6.3.4.5. Correlation of resistance traits

Analysis of correlations presents mutual relationships between different varroa resistance traits and their relationship with honey bee varroa infestation. The calculated values for the coefficient of correlation range from 0 to 1, and higher numbers represent higher relationships. Only colonies with samples for which there are at least 35 brood cells infested with single foundress varroa are used in this analysis.

Table 6.5. Coefficients of correlation between different varroa resistance traits and their correlation with adult bee and brood infestation.

Trait		REC	SMR	Brood infestation	Bee infestation	Hygienic behaviour
VSH	Correlation Coefficient	0.379**	-0.119	0.008	-0.169*	0.264**
	Sig. (1-tailed)	0.000	0.117	0.469	0.045	0.006
	N	101	101	101	101	89
REC	Correlation Coefficient		-0.069	0.092	-0.005	0.101
	Sig. (1-tailed)		0.248	0.180	0.482	0.173
	N		101	101	101	89
SMR	Correlation Coefficient			-0.024	0.030	-0.154
	Sig. (1-tailed)			0.405	0.383	0.075
	N			101	101	89
Brood infestation	Correlation Coefficient				0.581**	-0.277**
	Sig. (1-tailed)				0.000	0.004
	N				101	89
Bee infes-tation	Correlation Coefficient					-0.518**
	Sig. (1-tailed)					0.000
	N					89

The highest positive relationships were found between VSH and recapping, and between VSH and hygienic behaviour (Table 6.5), which suggests a good mutual relationship between these traits. While for recapping there was no correlation with adult bee infestation, both VSH and hygienic behaviour were negatively correlated with the infestation, meaning that colonies with high levels of VSH or hygienic behaviour have lower infestation rates of bees. This is especially noticeable in relation to hygienic behaviour with adult bee infestation ($p < 0.001$) and brood infestation ($p < 0.001$). Thus, for example, lines B, D and X, which had the most pronounced hygienic behaviour (Figure 6.26), had among the lowest rates of bee infestation at the end of the testing (Figure 6.23).Therefore, selection toward those traits may contribute to reduce infestation of colonies. Correlations of VSH, REC and hygienic behaviour with SMR were not significant. As expected, a positive correlation was found between brood infestation and adult bee infestation.

7. Economic aspects

One of the aims of the EurBeST study was to analyse and valorise the process of honey bee selection through an economic analysis of the costs and expenses of organising and realising the procedures of queen production, colony evaluation, followed by identification and selection of the preferable genotypes (in this case queens) as parents of the next generation. In our study, we estimated costs and expenses for the four basic elements of the breeding cycle: queen production, mating, colony evaluation and estimation of breeding values. In addition, we estimated the costs and benefits of use of stock selected for improved varroa resistance vs. the commonly used own stock in commercial beekeeping operations.

To our knowledge, this is the first study addressing the economic aspects of breeding for genetic improvement of honey bee stock, in particular those incurring through selection towards improved varroa resistance.

7.1. Costs of queen production and mating

The number of queens produced annually in EU has been estimated as close to two million, out of which about one third are produced in three countries participating in the EurBeST study (Italy, Poland and France). Queens produced in Greece and Germany also provide a significant proportion of the overall EU queen production. Moreover, in most of these countries, selection for improved varroa resistance has already been established, or is becoming more popular in breeding programs as one of the prime traits of interest (for more details please see chapters 3 and 4).

The calculation of the costs for queen production is based on the costs for queen rearing (labour, transportation, feeding, protection from pathogens and required equipment), marketing, value of assets and other costs (Figure 7.1).

Before marketing them, newly produced queens usually are mated, where three main types of practices (open mating, mating with use of mating stations and instrumental insemination), with differing levels of effort and costs are relevant.

The average costs for queen production amount to 22.58 € per queen, ranging from 8.22 € in Poland to 37.3 € in France. The main share of the costs comes from the labour costs, which significantly vary between the CSs.

Here, we include the calculation of the costs for mating as a part of the total costs, based on the labour, transportation, value of assets, and other costs. Concerning the costs for mating, the variation is even more pronounced, ranging from 0.39 € to 2.5 €, with the overall average of 2.01 € per queen. Still, these costs do not include the maintenance of the mating station and those of the drone colonies involved. Often, fees of about 4 - 6 € per queen are charged for the use of the mating station.

The difference between the selling price and the production price is on average 3.08 € per queen, ranging from 15.86 € to -12.3 €. A positive balance per queen was calculated for Germany (15.86 €), Poland (3.82 €) and Greece (1.26 €), while the balance was negative in France (-12.3 €) and Italy (-3.82 €). The negative balance results from the combined effect of high production costs and other costs (administration, insurance, taxation) and a low selling price per queen.

Table 7.1 Parameters and estimated costs for queen production and mating per one queen

Queen rearing	DE	EL	FR	IT	PL	Avg.
Labour costs	17.17	5.75	20.90	8.44	4.97	11.87
Transport costs	3.39	0.73	1.45	0.34	0.17	1.47
Feeding	1.84	1.72	4.62	2.12	0.23	2.05
Protection (Disease treatment)	0.25	0.17	0.22	0.98	0.11	0.35
Equipment (1-year use)	0.56	0.58	0.25	0.38	0.05	0.41
Total queen rearing costs	23.21	8.96	27.45	12.26	5.53	16.15

Marketing	DE	EL	FR	IT	PL	Avg.
Package, transport, labelling	0.75	0.83	0.00	0.23	0.48	0.51
Promotion and marketing	0.00	0.13	0.10	0.03	0.08	0.06
Total marketing costs	0.75	0.96	0.10	0.26	0.56	0.57

Other costs	DE	EL	FR	IT	PL	Avg.
Veterinary services	0.00	0.04	0.00	0.02	0.02	0.01
Other services and support	0.41	0.00	0.19	0.17	0.09	0.20
Water, electricity, heating	0.22	0.16	0.29	0.59	0.23	0.29
Insurance	0.07	0.01	1.81	0.22	0.07	0.35
Other general costs (administration, telephone, accounting, etc.)	0.13	0.12	0.52	2.63	0.18	0.69
Income tax	0.00	0.58	1.38	0.78	0.28	0.52
Total other costs	0.82	0.91	4.20	4.40	0.87	2.07

Assets	DE	EL	FR	IT	PL	Avg.
Total depreciation queen production	4.47	4.16	5.55	2.99	1.26	3.79
(A) Total cost queen production	29.24	14.99	37.30	19.91	8.22	22.58

Mating	DE	EL	FR	IT	PL	Avg.
Labour cost	0.45	1.05	0.08	0.72	0.10	0.51
Labour transport time	1.04	0.25	0.00	0.18	0.00	0.40
Total labour cost mating	1.49	1.30	0.08	0.90	0.10	0.91
Transport costs	0.14	0.48	0.00	0.13	0.00	0.17
Other costs for mating except labour and labour transport, if occurred (insurance, feeding, protection, transport of mating boxes, etc.)	0.39	0.04	1.05	0.12	0.05	0.31
Total mating costs	2.02	1.82	1.13	1.15	0.15	1.39
Depreciation mating (assets)	0.48	0.71	1.68	0.22	0.24	0.62
(B) TOTAL COST MATING	2.50	2.52	2.81	1.37	0.39	2.01

(C) Average queen selling price	45.10	16.25	25.00	16.63	12.04	23.32
Difference of AVERAGE selling PRICE (C) and QUEEN production COST - price (A)	15.86	1.26	-12.30	-3.28	3.82	3.08

7.2. Costs of colony evaluation

The methodology is based on the calculation of costs for performance tests for colony evaluation. All data required for this calculation (described below) were recorded by 20 performance testers who participated in the study. In addition, a separate estimation of costs for breeding value estimation was included based on experts' experience and current prices for this kind of service.

The colony evaluation is based on the recommended methodology for basic performance testing, varroa infestation monitoring and tests for varroa specific traits, which also includes number of tests, time needed for one test and number of apiary visits based on the experts' experience and estimations (Table 7.2). The total costs consist of the labour costs, the transport costs and additional costs, such as depreciation of the equipment needed for performing SMR, REC and VSH tests.

Labour costs are calculated including labour time for carrying out colony tests, travelling time to and from the apiary (based on estimation of one hour as average time needed for travelling 50 km distance) and labour time for data management (recording and entering data) with hourly payment fees. Transport costs are calculated based on the apiary distances and average fuel costs.

Table 7.2. Recommended performance testing methodology, methods, number of tests and expert estimation of time needed, and number of apiary visits

Testing method	Number of tests	Time for one test (minutes)	Total time per test (minutes)	Percent of total time	No of apiary visits	Percent of total visits	Comments
Basic performance testing	19		61	11%	9	41%	
1. Colony strength (bees) – occupied combs with bees	4	3	12	2%	4	18%	
2. Colony strength (brood area) – number of combs with brood	4	3	12	2%		0%	4 visits combined with 1, 5
3. Honey production (net weight)	2	5	10	2%	2	9%	
4. Swarming (scoring)	5	3	15	3%	3	14%	2 visits combined

Testing method	Number of tests	Time for one test (minutes)	Total time per test (minutes)	Percent of total time	No of apiary visits	Percent of total visits	Comments
							with 1,2 & 5
5. Gentleness	4	3	12	2%		0%	4 visits combined with 1, 2
Varroa infestation monitoring	4		45	8%	5	23%	
6. Adult bee infestation	2	10	20	4%	1	5%	1 visit combined with 1, 2 & 5
7. Brood infestation	1	15	15	3%	1	5%	
8. Natural mite mortality	1	10	10	2%	3	14%	1 test, 3 visits per test
Varroa specific test methods	4		450	81%	8	36%	
9. Hygienic behaviour	2	15	30	5%	4	18%	2 tests, 2 visits per test
10. SMR & REC	1	180	180	32%	2	9%	1 test, 2 visits per test
11. VSH	1	240	240	43%	2	9%	1 test, 2 visits per test
Total	27		556	100%	22	100%	

The proposed methodology additionally recommends 12 colonies per testing apiary and investment in equipment of 2 000 €, with 10 years of life-cycle.

However, if the queen producer / beekeeper decides not to perform the colony evaluation by himself, but rather contracts an external beekeeper as provider of this service, the costs for queen production (multiplied by the number of sent queens) should be regarded as additional costs (please see Table 7.1).

– Total costs

Colony evaluation costs (Table 7.3) are highest in Germany and France (273 € and 265 € per one colony), while the lowest ones are noticed in Greece and

Poland (85 € and 87 € per one colony). The differences are primarily the result of national labour market conditions and labour costs (Figure 7.1).

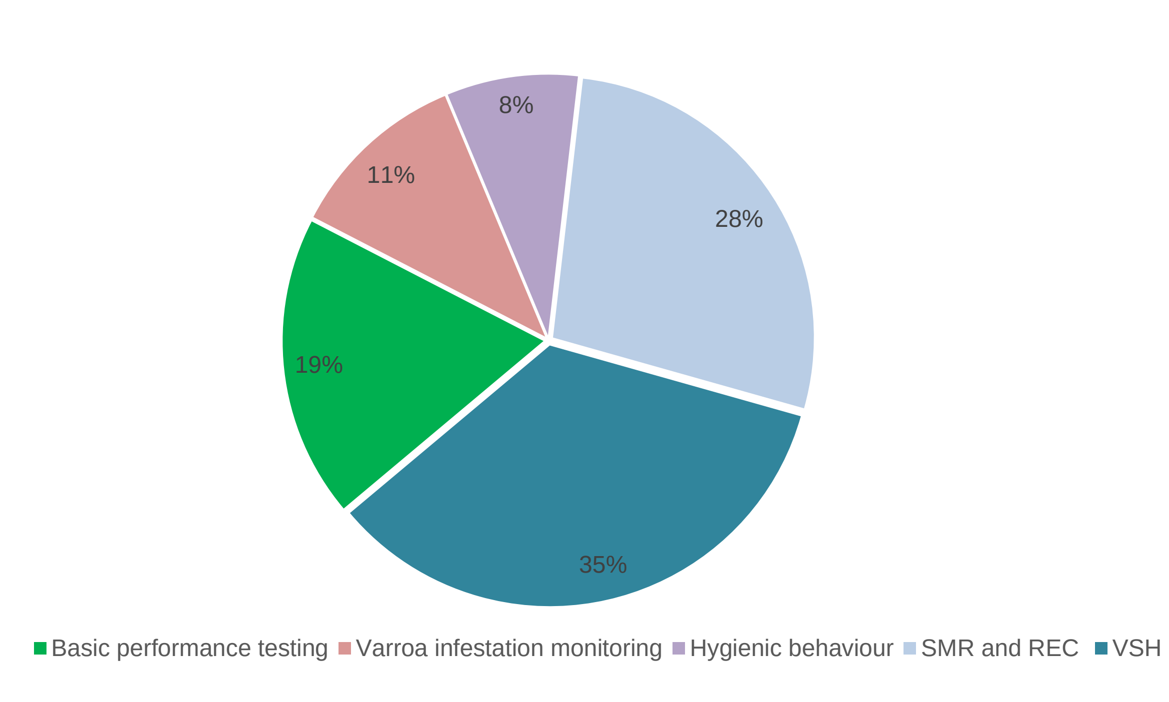
Table 7.3. Average colony evaluation costs per one colony in €

	DE	EL	FR	IT	PL	Average
Number of performance testers	6	2	3	6	3	
Labour costs	247	54	236	126	64	162
Transport costs	9	14	12	25	6	14
Depreciation of equipment	17	17	17	17	17	17
Total costs	273	85	265	168	87	193

Figure 7.1. Average colony evaluation costs per one colony in €

The colony basic performance testing costs amount to about 20% of the total colony evaluation costs (Figure 7.2). The main costs derive from the monitoring and testing for varroa resistance. Varroa infestation level and hygienic behaviour together amount to almost 20%, while the highest share of the colony evaluation costs, with more than 60% of the total, results from assessing the SMR & REC and VSH traits.

Figure 7.2. Share of different testing methods in colony evaluation costs



– Costs for specific elements of performance testing

The highest costs per different testing methods incur in Germany and France, where in general costs are three times higher compared to Greece and Poland (Figure 7.3).

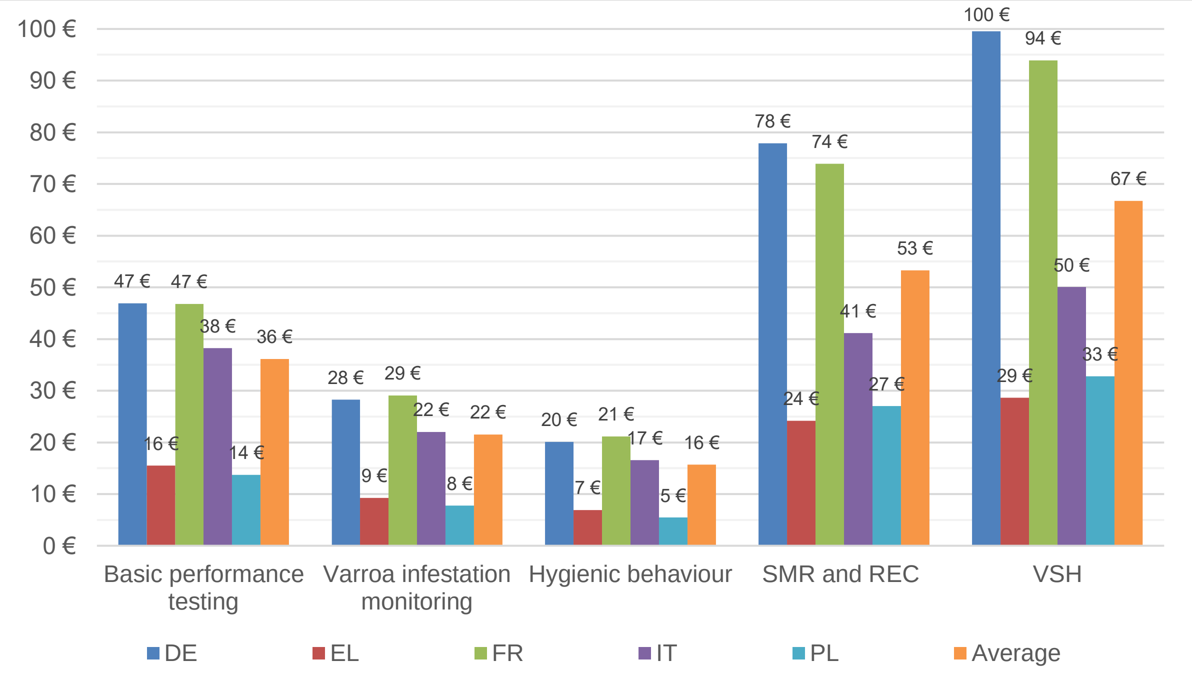
Basic performance testing

The basic performance testing costs are calculated based on the labour, labour transport and transport costs for performing the testing of five traits: colony strength in terms of bee population (number of occupied combs), colony strength in terms of brood area (number of combs), honey production (net weight), swarming (scoring) and gentleness (scoring). Labour costs for data management are allocated according to the proportion of these basic tests to the total number of tests conducted. On average, the basic performance testing cost per one colony amounts to 36 €, with the highest costs in Germany and France, and the lowest ones in Poland and Greece (Figure 7.3).

Selection for traits of varroa resistance

Costs for selection for traits of varroa resistance, as part of colony evaluation costs, are calculated based on the labour, labour transport and transport costs for performing varroa infestation monitoring and specific test methods. Additionally, labour costs for data management were allocated according to the proportion of these specific resistance tests to the total number of tests conducted, plus the share of depreciation of equipment necessary for SMR & REC and VSH (Figure 7.3).

Figure 7.3. Average costs in € per different testing methods and countries



- .Varroa infestation monitoring

Varroa infestation monitoring costs are calculated based on the individual costs for monitoring three different parameters: adult bee infestation, brood infestation and natural mite mortality (Figure 7.3). Average costs for monitoring varroa infestation per one colony are 22 €.

- Varroa specific test methods

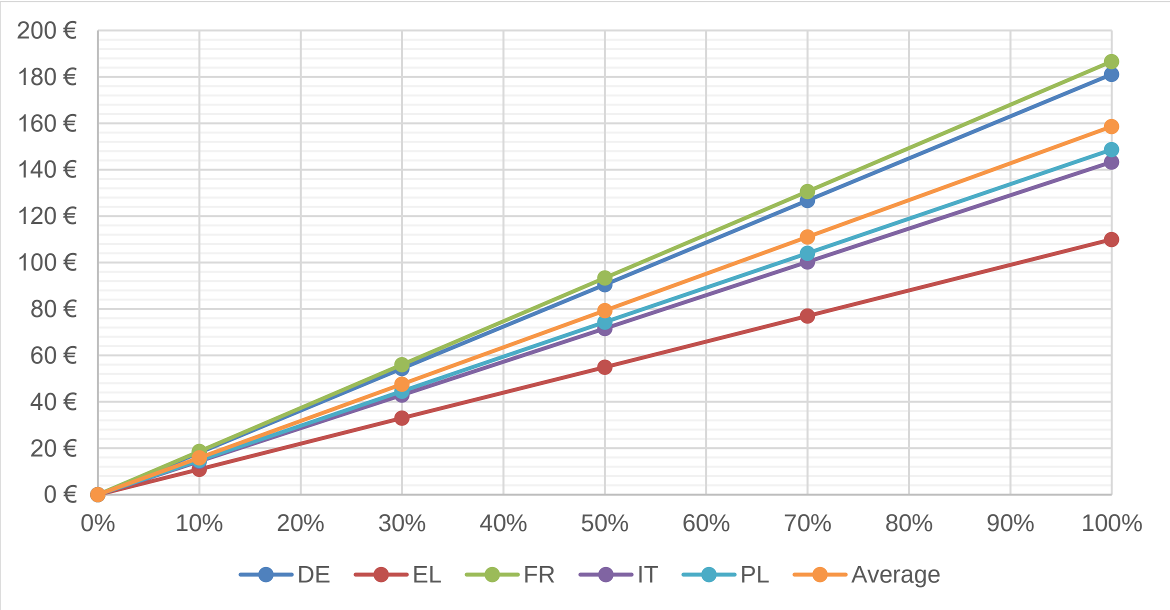
Testing costs for hygienic behaviour, SMR & REC and VSH, as varroa specific test methods, are calculated based on the individual costs for performing each test. In addition, the relative amount of labour for data management, and 50% of the total depreciation of equipment for performing SMR & REC and VSH tests, is allocated to the costs of these tests. The average hygienic behaviour testing costs per one colony are 16 €, for SMR & REC testing 53 €, and for VSH testing costs are 67 € per one colony (Figure 7.3).

- Survival test

Although the colonies in the PT apiaries were managed without varroa treatment, the duration of the evaluation does not allow us to consider the EurBeST study as a survival test for the colonies involved. Nonetheless, the data obtained allow us to calculate the average costs (value of lost colony) based on the average value of the colonies for different loss rates.

The costs of running a one-year survival test show huge variation across the different case studies, caused by different loss rates and by differing values per colony (Fig. 7.4). The country where the costs for the survival test are lowest is Greece.

Figure 7.4. Average survival tests costs (Y-axis) per different loss rates (X-axis) per one colony in €



– Costs for estimation of breeding values

The methodology is based on the average national labour costs, exemplarily increased by the factor four, as the breeding value estimation cannot be performed by the performance testers themselves, but requires staff with specialised skills and expertise that is usually more expensive. According to experts' experience, the breeding value estimation of 200 colonies (queens) on average requires around 30 hours of labour time. Currently, only few breeding programs in Europe rely on breeding value estimation as a routine procedure in their selection strategy (see remarks on Table 6.2 regarding the genotypes tested in the EurBeST case studies). However, there is an increased interest in using this methodology as selection tool in well established systematic breeding programs for genetic improvement and conservation of honey bees.

Table 7.4. Estimation of breeding value evaluation costs in €

	DE	EL	FR	IT	PL	Avg.
Average hourly fee (€/hour) (4 times fee)	73	47	66	41	35	54
Time needed (average 200 colonies) in hours	30	30	30	30	30	30
Costs breeding evaluation (4 times) in €	2 178	1 410	1 974	1 239	1 040	1 618
Costs breeding evaluation (4 times) per queen	10.89	7.05	9.87	6.19	5.20	8.09

The average costs for breeding evaluation per one queen are 8.09 € based on a model of labour costs increased by a factor of four (specialised expert labour; Table 7.4). Additionally, the software licence costs are around 1 000€ per year, which means extra costs of 1 € per queen for performing the breeding value estimation for a total of 1 000 queens per year.

Finally, the costs for selection per queen is the product of the costs for queen rearing, mating, colony evaluation and the costs for estimation of breeding values (Table 7.5).

Table 7.5. The costs for selection per element and queen in €

Element	DE	EL	FR	IT	PL	Average
Queen production	29.24	14.99	37.30	19.91	8.22	22.58
Colony evaluation	273	85	265	168	87	193
Costs breeding evaluation per queen	10.89	7.05	9.87	6.19	5.20	8.09
TOTAL COST FOR SELECTION PER QUEEN	313.13	107.04	312.17	194.1	100.42	223.67

7.3. Costs and benefits of selected stock for commercial beekeepers

The methodology for costs-benefit analysis of selected stock for commercial beekeepers is based on honey yield values as benefit minus costs of winter losses, which include the country-specific price of the lost colony and honey value, plus the value of next year's losses estimated based on the different varroa infestation leves determined in the study summer period. The honey yield for the next year was assumed to be equal to the one used in the EurBeST study for each of the CBs.

The final cost-benefit results present the differences between the tested selected lines and the commercial beekeepers' local stocks.

As basis for the costs-benefits analysis, the biological data for honey yields per colony, colony losses and level of summer mite infestation, provided by the commercial beekeepers participating in the case studies of the respective countries, were used. Calculation of the value of honey and colony was based on the data for the honey selling price and the value of one colony collected from the commercial beekeepers who participated in the study.

To calculate the economic consequences of different levels of mite infestation, a 10% increase of colony mortality in the following winter per infestation increase of 1 mite/10 g of bees in summer was assumed.

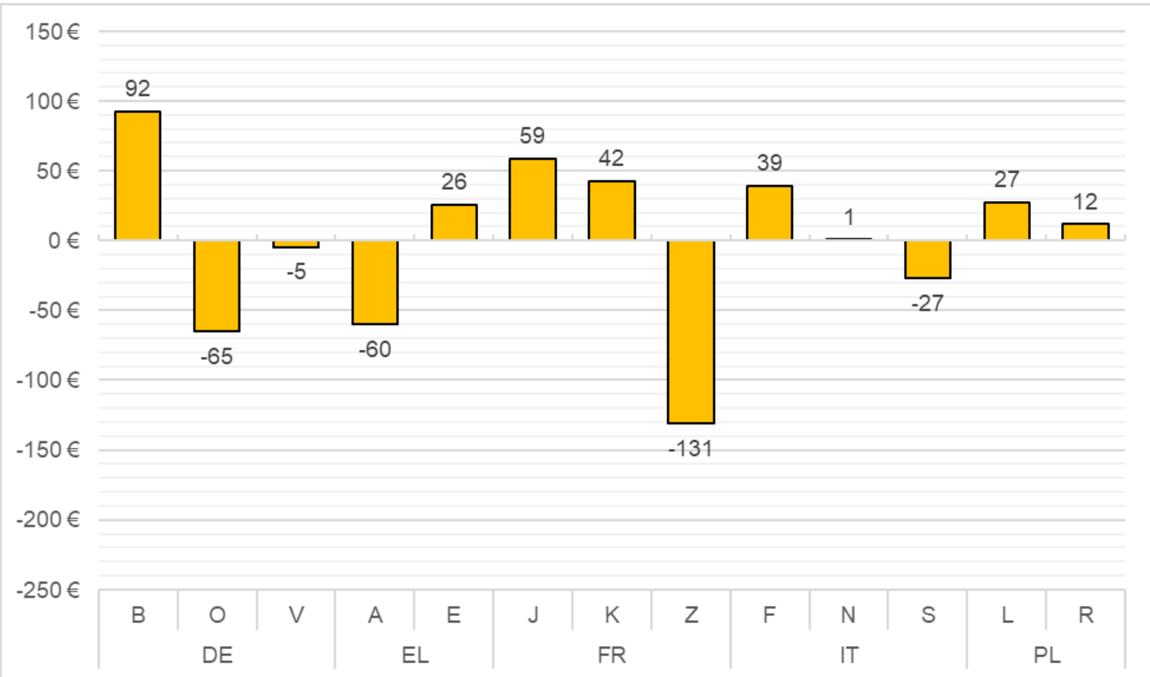
Table.7.6. Estimated loss rate based on mite infestation level

Summer mite infestation level (mites/10 g bees)	< 1	1	2	3	4	5	6	8	10	20	30 >
Expected next year loss rate	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%

The model demonstrates the cost-benefit effects of different mite susceptibility based on the assumption that the beekeeper is realising a treatment-free colony management concept. However, beekeepers are nowadays used to apply chemical treatments to keep their colonies below economically relevant infestation thresholds.

The highest positive difference can be noticed in the German CS, where line B has positive economic results that are by +92 € higher than the local stock. In contrast, the economic results of line Z are lower by -131 € than the local CS line in France. Substantial positive differences are also noticed with line E in Greece (+26 €), line J and K in France (+59 € and +42 €), line F in Italy (+39 €), and line L in Poland (+27 €). Considerable negative differences can be noticed with line O in Germany (-65 €) and line A in Greece (-60 €).

Figure 7.5. Cost-benefit results expressed as income difference between the tested selected lines and the commercial beekeepers' local stocks



8. Conclusions and denouement

The EurBeST study evaluated the production and performance data of more than 3 500 honey bee colonies in total, recorded by more than 130 beekeeping operations in seven different countries. In addition to the traditional and widely recognised traits of commercial interest, the study placed a strong focus on comparing colonies of different genotypes in different environments regarding their expression of traits related to varroa resistance under commercial beekeeping conditions. While the resistance potential of several such traits (hygienic behaviour, VSH, SMR and REC) is well known from the scientific literature, there are currently few data available that would allow an assessment of the suitability of such traits in the European beekeeping environment. The EurBeST study provides a significant contribution to closing this knowledge gap. In addition, the study for the first time provides insights into the economic aspects of honey bee selection, including improvement of varroa resistance, and queen production by producing cost-benefit analyses of the relevant operation elements.

While the quality of the currently available honey bee breeding stock in Europe to a high degree meets the demands and requirements of the beekeepers regarding its production and performance traits, our customer survey with almost 400 replies indicates that the level of beekeeper satisfaction with mite resistance traits of commercially available queens is much lower. Thus, the survey confirms the success of long-term selection efforts in several countries, but the outcome also demonstrates the need to intensify selection efforts and improve the commercial availability of stock with improved varroa resistance to meet the increasing demand of the beekeeping community.

Analysis of the EU market for reproductive material of European honey bees, including data on production and trade of all member states, shows that there is huge diversity of this sector across the EU member states. The number of breeders and the amount of reproductive material produced and traded varies greatly between countries, even between closely neighbouring ones. While the breeding structure and production volume in a few countries appear sufficiently well developed to satisfy their own demand on high-quality reproductive material, this is not the case in all countries across the EU. In agreement with this observation, trade data show that the within-EU market for reproductive material has been steadily increasing for the past years.

However, despite an apparent and growing demand, there exists to date no well-established market for varroa-resistant stock in Europe. While several varroa-resistant European honey bee populations resulting from natural selection are known, and breeding programs focusing on varroa resistance are being conducted in a number of countries, the replies to our questionnaire circulated among experts on varroa resistance selection confirm that the supply of stock marketed as resistant, if available at all, is mostly very limited and small-scale. Moreover, reliable experience or experimental evidence regarding the resistance of such stocks, together with their suitability for beekeeping, under different environmental and management conditions, is still lacking.

The results of the EurBeST study now provide such evidence, which was collected based on an experimental design that was developed with contributions from a diverse and representative expert team of European beekeepers and bee scientists. The investigation scheme was realised in five large-scale case studies on two different levels, performance testing (PT) apiaries and commercial beekeepers (CB), and was run for one full apicultural season.

Although the colonies of the PT apiaries were not treated against varroa in the winter 2019/20, and losses therefore had to be expected, some of the lines showed consistently low to moderate loss rates, and summer mite infestation levels of several tested lines did not exceed the economic treatment threshold of 3 mites /10 g bees. In a few lines only, the increase of mite infestation from spring to summer 2020 reached a level that would have required immediate beekeeper action under commercial conditions. Surprisingly, colonies headed by queens from "survivor populations" did not show outstandingly superior survival rates or low mite levels that would support a higher level of varroa resistance. In general, none of the differences observed in any of the traits could be explained by genetic effects of subspecies or queen origin alone. Instead, our results indicate that environmental factors (apiary effects) had a significant effect on both overwintering success and mite infestation development in the 2020 season; and a strong interaction between genotype and environment (GEI) was observed for most traits that were considered, including mite infestation, hygiene behaviour and SMR. Here, we also observed significant correlations between some of the different parameters used to measure varroa resistance, while in turn higher scores in these traits resulted in a significant negative effect on the mite infestation level of adult bees.

Thus, the results of the EurBeST study are in agreement with those of previously conducted large-scale experiments (*e.g.*, Büchler et al. 2014[8]) in that they underline again the importance of and need for locally established testing and selection efforts to improve the performance traits of adapted honey bee genotypes. The overall highly satisfactory performance data of lines that had been subject to selection for at least two generations also demonstrate the potential of selective breeding to improve traits of both commercial interest and varroa resistance.

In the test apiaries comparing EurBeST lines with beekeepers' own stock, run by commercial beekeepers, the overall loss rates were consistently lower than in the performance testing apiaries, and overwintering success, estimated by the overwintering index, was higher, with little difference between beekeepers' own stock and the EurBeST selected lines. The performance of beekeepers'

[8] Büchler R; Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, Uzunov A, Berg S, Bienkowska M, Bouga M, Drazic M, Dyrba W, Kryger P, Panasiuk B, Pechhacker H, Petrov P, Kezic N, Korpela S, Wilde J (2014) The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. Journal of Apicultural Research, 53(2): 205- 214. http://dx.doi.org/10.3896/IBRA.1.53.2.03

stock and selected lines also did not differ widely in regard to behavioural traits, such as gentleness and swarming. However, the beekeepers' stock on average outperformed the EurBeST lines in terms of honey production; nonetheless, the productivity of a few of the test lines across the case studies was significantly higher. Furthermore, effects of local adaptation were to the advantage of the beekeepers' own stock in this regard, whereas the EurBeST test lines were distributed among test apiaries regardless of regional closeness.

Average mite infestation rates in the autumn of 2019 and spring of 2020 did not differ significantly between the EurBeST test lines and the beekeepers' own stock in the commercial apiaries. However, according to the routine colony management practised by these beekeepers, the colonies in the commercial apiaries were mostly treated during the winter. In contrast, mite levels in the summer 2020 were significantly lower in the selected test lines compared to beekeepers' own stock. Unquestionably, maximising their profit by using queens from genetic stock selected for high performance of productivity traits is in the genuine and relevant commercial interest of professional beekeepers. Our results therefore indicate that beekeepers seem to be well aware of the benefits of selection and are motivated to use stock with good production qualities. However, given the current market situation with very limited availability of varroa resistant stock, beekeepers are oftentimes not able to direct their purchase decisions towards including stock with improved mite resistance.

As integral part of the EurBeST study, we performed the first economic analysis of the costs and benefits of honey bee selection, taking into account all relevant aspects of colony evaluation and queen production, also including the costs incurring by selection for improved varroa resistance. In the five countries assessed, the costs for basic performance testing in the selection process for commercially relevant traits average around 36 € per colony, and so they can be justified by selling a sufficient number of queens produced from these sources at a reasonable price. In contrast, with a European average of more than 150 €, it is extraordinarily expensive for a queen producer to assess varroa resistance traits of colonies, especially those directly related to estimating mite reproduction such as hygienic behaviour, VSH and SMR. Seen that queens selected for these traits do not necessarily produce significantly more honey, so these traits do not immediately result in a direct advantage to the commercial beekeeper client, it seems unrealistic to expect that beekeepers would pay a price that would not only compensate the production costs but also grant a profit to the queen producer.

In conclusion, the results of the EurBeST study confirmed the existence of significant differences in the expression of mite resistance traits between several potentially varroa resistant European lines of honey bees. The results also highlight that selection for improved mite resistance in a given line is not necessarily in contradiction with selection for high production potential, as a few lines in the study showed high performance in both these aspects. While these examples demonstrate the principal possibility of accomplishing good performance success by selection, the high expectations of professional beekeepers, especially in regard to varroa resistance, are currently not met in all aspects by the available stock.

The results of the EurBeST study underline the importance of selective breeding of honey bees to achieve the goals of the EU Green Deal, specifically of the Farm to Fork and Biodiversity strategies. Increasing the varroa resistance of European honey bee populations will contribute to halting or even to reversing the decline of pollinators and, at the same time, to reducing the use of critical chemotherapeutics in beekeeping. The strengthening of local breeding activities contributes directly to preserving honey bee biodiversity on a European level. In addition, the utilisation of well-selected and locally adapted genotypes increases the economic return of professional beekeepers, and it ensures sustainable pollination services and the supply of customers with healthy and wholesome bee products. Furthermore, selective breeding efforts will also contribute to providing sustainable solutions to the challenges of climate change and ecological transitions, and to the emergence of new exotic pests and pathogens.

Based on the summarised findings of the study, we develop the following recommendations for:

Queen breeders:

- As customers primarily look for quality and have high expectations in terms of the honey production potential and manageability traits of queens, queen producers should invest in high-quality output and continuously improve their knowledge and skills to optimise their production routines.
- Our results show that in some countries the production costs for queens are not covered by the sale price, so prices for breeding material need to be increased or subsidised.
- Enhanced cooperation between queen breeders, performance testers and scientific breeding centres is needed to substantially improve the genetic traits of reproductive material and to ensure that breeding stock with good local adaptation is made available to the customers.

Commercial beekeepers:

- The use of well-selected breeding stock is a major factor of economic success in commercial beekeeping!
- The “one best bee for all beekeepers" does not exist; instead, strong interactions between genotypes and local conditions prevail. Each beekeeper has to identify the most suitable stock for the specific conditions in his/her business.
- Because of these strong interactions, it is recommendable to obtain stock from breeders in the same region, selected under similar colony management conditions.
- From an economic point of view, most of the colonies in a commercial apiary should be headed by queens from stock with long-proven quality;

however, promising new genetic sources could also be evaluated in a small number of colonies.

Selection programs and performance testers:

- The overall good performance of lines that were selected for more than two generations underlines that, to be successful, breeding programs need to be clearly defined and consistently followed over long periods.
- Cooperation with other breeders and breeding centres will contribute to achieving a sufficient population size for testing.
- Selection has to address the local environmental and management related conditions to develop well-adapted genotypes.
- Considering the correlation found between reduced varroa infestation and hygiene behaviour and VSH, together with the comparatively easy to perform and economic testing methods for this trait, it seems worthwhile to promote hygiene behaviour and VSH for wide-scale testing.
- The effects of other traits like SMR were less conclusive, and these traits need to be further developed.

Politicians and public authorities:

- Selection of honey bees in general, and selection for improved mite resistance in particular, is an efficient way to increase the productivity, to reduce colony losses and to improve bee health. It will also improve the ability of colonies to cope with environmental and climatic changes.
- Support of regional breeding programs is needed to utilise the strong genotype-environmental interactions with regard to bee health and productivity.
- Improvement of the breeding sector highly depends on scientific support. Selection criteria for parasite and disease resistance can be further optimised, and introduction and implementation of new techniques, like selection based on genetic markers or breeding value estimation, can contribute to an increased selection success.
- The market structures for honey bee breeding material should be improved in most of the member states and need to be better harmonised between the countries.
- As the costs for specific selection methods for improved mite resistance are quite high and difficult to cover with the market price of queens, public funding of some well defined selection activities is recommendable to enhance and accelerate selection success.

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