Effects of selection and local adaptation on resilience and economic suitability in *Apis mellifera carnica*

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Abstract — Modern techniques of selective breeding show high potential to improve economically important traits of honey bees. However, breeding may neglect fundamental rules of natural selection. The objective of this study was to analyze the effects of selection and local adaptation on some relevant breeding traits of *Apis mellifera carnica*. We compared open mated queens from three different origins: local Croatian genotypes that are under controlled selection since five generations (MS), a local genotype that was never under selective breeding (NS) and, finally, a non-local genotype from a long-lasting German breeding program (HS). Generally, colonies with queens from breeding programs had better scores for defensive behavior, calmness, and swarming. In contrast, colonies from the NS group showed higher expression of *Varroa destructor* resistance traits. Although the HS group showed the highest score in most of the behavioral traits, the low overwintering index of colonies from this group after both wintering periods indicated a lack of local adaptation. This research underlines the relevance of breeding activities for the resilience of honey bee populations and the achievement of local adaptation.

*A. m. carnica* / Selective breeding / Local adaptation / Resistance / Resilience

### 1. INTRODUCTION

Selective breeding of honey bees (*Apis mellifera*) has a long tradition in many European countries, where it has been practiced in various ways and to differing extents (Lodesani and Costa 2003; Hatjina et al. 2014a). Today, bee breeding predominantly aims at improving traits of apicultural interest that have an important impact on the beekeeping industry, such as reducing defensive behavior, increasing honey production, or reducing the swarming tendency (Ruttner 1972; Möbus 1983; Büchler et al. 2013; Tiesler et al. 2016; Uzunov et al. 2017). The benefits of selection are well recognized and can strongly affect the economic success of beekeeping. For instance, based on selection program employing breeding value estimation, honey production within the German Carnica population increased by 0.7 kg per year since 1996 (Bienefeld 2016), which translates into an annual increase of the production value of about 3 million Euros just for the German beekeepers.

However, current efforts in selective breeding of honey bees are also questioned, as they sometimes may neglect important life history traits...
In consequence, negative selection response may be expected for traits that are directly selected for but are correlated unfavorably. For instance, selection for high brood productivity and low swarming tendency, with the purpose to reach a higher honey production, may reduce the social immunity of the colony (Meunier 2015) and its natural resilience against pathogens. In natural habitats, honey bees inhabit comparatively small nest cavities, which leads to frequent and early swarming and smaller colonies. As a consequence, such colonies have fewer problems with the ectoparasitic mite *Varroa destructor* (Fries and Bommarco 2007; Loftus et al. 2016). On the other hand, modern beekeeping with effective control of *V. destructor* minimizes the natural selection pressure on bees to develop resistance mechanisms (Fries et al. 2006) and to establish a balanced host–parasite relationship.

However, there may also be a trade-off between life history traits (such as reproduction) and parameters of health (Webster and Woolhouse 1999; Schwenke et al. 2016). Evans and Pettis (2005) report that colonies with bees resistant to foulbrood disease caused by *Paenibacillus larvae* showed lower productivity compared to more susceptible colonies, presenting a possible trade-off between resistance and productivity. Another example for a trade-off between resistance and reproduction is given by a natural survivor population in Sweden, where colonies produce less brood as part of their parasite adaptation (Fries and Bommarco 2007). Similar observations were reported for varroa-resistant “Primorsky bees” in Germany (Rosenkranz et al. 2010) and the USA (Tarpy et al. 2007) where those bees produce less brood and honey compared to non-resistant local stock, but appear to be more resistant to mites. In contrast, a trade-off between behavioral traits (like defensive behavior or swarming tendency) and honey yield seems to be less relevant, as a positive genetic correlation of those traits has been shown in several research reports (Bienefeld and Pirchner 1991; Brascamp et al. 2016). Selection for increased hygiene behavior appears to not adversely affect productivity (Spivak and Reuter 1998) or individual innate immunity of bees (Harpur et al. 2014).

Recently, the effects of genotype–environment interactions on behavior, productivity, and survivability of bees have been well recognized and studied, resulting in a general recommendation for the use of locally adapted bees (Costa et al. 2012a; Büchler et al. 2014; Hatjina et al. 2014b; Uzunov et al. 2014). Although, in general, no difference in pathogen loads was observed between colonies of local and non-local genotypes (Meixner et al. 2014), a higher propensity to disease is expected for bees introduced from differing regions (Francis et al. 2014). Further, the resistance mechanisms toward diseases that colonies may show in one environment may not be expressed in a different environment (Corrêa-Marques et al. 2002; Seeley 2007).

The aim of this study was to compare behavior, productivity, and resistance to *V. destructor* in different *A. m. carnica* genotypes under the aspects of local versus non-local genotypes and varying selection regimes. Specifically, we compared two local genotypes that either (1) were never under artificial selection, or (2) originated from a breeding program, to (3) an introduced and highly selected genotype. The results are discussed with regard to the benefits of selective breeding, its effects on the resilience of bees, and the impact of local adaptation.

**2. MATERIALS AND METHODS**

**2.1. Study area and colony management**

The study was conducted in Osječko-baranjska County, Čeminac, Croatia (N 45°40′12″; E 18°40′40″; 90 m altitude) from October 2015 to February 2017. Altogether, 60 colonies of three different Carniolan genotypes in standard Langstroth hives were included. All colonies were established at the beginning of May of 2015 with four combs of capped brood occupied with bees, two combs of pollen and honey, and a mated queen. During August 2015, all colonies were treated with CheckMite® strips in order to reduce and equalize their
infestation rates with *V. destructor*. Afterwards, treatment of specific colonies was performed on an individual scale whenever the infestation rate of the adult bee population reached 10%. Colony inspections started on 5 October 2015, when most of the worker bees in the colonies represented daughters of the test queens (Büchler et al. 2013).

2.2. Genetic origin of groups

The first genotype (*N* = 13, no selection effort, NS group) originated from a population which, to our knowledge, had never been subjected to any selective breeding. Queens of this group originated from an isolated area in central Croatia, where most beekeepers still keep their bees in traditional skep hives. A survey conducted among beekeepers confirmed that in this area neither migratory beekeeping nor trade with swarms nor queens are present. The queens from this group were half-sisters and open mated within the area of origin.

The second genotype (*N* = 35, medium selection effort, MS group) derived from a local population in north-eastern Croatia selected for the main api-cultural traits (defensive behavior, calmness, swarming, honey yield) since five generations. The test queens originated from three mother queens that were half-sisters and mated on a mating station saturated with local drones of a different genotype, but originating from the same breeding program. Queens from the third genotype (*N* = 12, high selection effort, > 10 generations, HS group) were half-sisters originating from a single breeder queen (DE-7-45-73-2013, see pedigree in www.beebreed.eu) of the highly selected Carnica population (for productivity, gentleness, calmness, low swarming, hygienic behavior, and low mite propagation) of the Bee Institute in Kirchhain (Germany) and open mated with local drones in Rijeka (Croatia).

The differences in selection effort between MS and HS are (1) the number of generations under selection, (2) the way of estimating breeding values, and (3) the control of queen mating. All generations of the MS queens were open mated in a non-isolated mating station saturated with drones bred from the selected lines, while the mating of the mothers of HS queens for generations was realized on an isolated island mating station with complete control of drone origin.

2.3. Tested parameters

All regarded parameters are listed in Table I, together with the number and date of measurement and the methods used. The overwintering index (OI) was calculated as the ratio between the number of bees at the first spring inspection after winter and the number of bees at the last autumn inspection before the winter. The first wintering period (as measured from the beginning of significantly reduced activity of bees until the first larger pollen intake) lasted 163 days, and the second one 122 days. The amount of net extracted honey for each colony was calculated by measuring the weight of individual honey boxes before and after extraction. Adult bee samples for the determination of the *V. destructor* infestation rate were taken from the honey supers or from the upper brood box in September and October (Costa et al. 2012b; Büchler et al. 2013). Although the colonies were regularly inspected for mite infestation, only those sampling dates were used for further analysis (dates 1 to 7 in Table I) where all colonies had comparable conditions for mite development (colonies were not treated from July 2015 until end of July 2016).

The analysis of the proportion of *V. destructor* mites that failed to reproduce (SMR), and uncapping and recapping of brood (REC) were determined according to the RNSBB protocol (Büchler et al. 2017). Briefly, brood samples with pupae in the developmental stage between 7 and 12 days post capping were opened until 35 or at least 10 cells infested with single foundress mites were found. A foundress mite was considered non-reproductive (1) if it failed to produce any offspring, (2) if there was no male or exclusively male offspring, or (3) if the eldest daughter was too young to reach the adult stage and get mated until eclosure of the bee. Brood samples for analysis were collected once per month during June, July, and August.

2.4. Statistical analysis

General linear model (GLM) was used in the analysis of all tested parameters. The Genotype and Year or Month and interactions between them were considered fixed effects. The differences
between groups were determined using the Bonferroni post hoc analysis test. All means are presented as adjusted mean values (LS means ± SE). If a colony died, any data collected prior to this event were used in the statistical analysis. All statistical analyses were performed using the STATISTICA 10 (StatSoft 2011) software.

3. RESULTS

3.1. Colony strength

GLM analysis showed that Genotype and Month as fixed factors had significant \( p < 0.01 \) effects on the number of bees, while there was no effect \( p = 0.448 \) of the Month × Genotype interaction (Supplement Table I). The MS group had a significantly higher number of bees in comparison to the NS and HS groups (Table II). The analysis also showed a significant effect of Month \( (p < 0.01) \) and Genotype \( (p < 0.05) \) on the number of brood cells (Supplement Table I). The interaction Month × Genotype did not have an effect \( (p = 0.153) \) on the amount of brood. As with the number of bees, the MS group had the highest number of brood cells (Table II). From mid-March to late April, the number of bees (mean average phenotypic value of all colonies) increased from 6275 to 16,667, and the number of brood cells from 6338 to 26,700. The highest number of bees (38,942) was recorded in a colony from the NS group during June, while the highest number of brood cells (36,960) during the study was recorded at the end of April in a colony from group MS.
3.2. Defensive behavior, calmness on the comb, and swarming tendency

Defensive behavior was significantly \( p < 0.01 \) affected by the Genotype and by Month (Supplement Table II). The highest score, i.e., the least defensive bees, was recorded in group HS, which was significantly different \( p < 0.01 \) from the other experimental groups (Table II). Genotype, Month, and their interaction also showed a significant effect \( p < 0.01 \) on calmness (Supplement Table II). The highest score was recorded in group MS and the lowest one in group NS, which had a significantly lower score \( p < 0.01 \) than the other two groups (Table II). Swarming tendency was affected by Genotype (Supplement Table III), and the NS group had a significantly \( p < 0.01 \) lower score, i.e., higher swarming tendency, compared to the other two groups (Table II).

3.3. Honey production

Due to unfavorable climatic conditions during the main nectar flow, the test colonies achieved an unusually low honey production in 2016. The average amount of extracted honey was 10.9 kg. This is very low compared to the long-term average of honey production/colony in Croatia, which is in the range of 18.2 kg for hobby beekeepers to 41.4 kg for professional beekeepers (Štefanić et al. 2004). The highest amount of honey was extracted from the HS and the lowest one from the MS group, but these differences were not significant (Table II, Supplement Table V).

3.4. Survivability and overwintering ability

All colonies survived the first winter period. However, after the second winter, different colony losses among groups were recorded: 75% of the NS colonies survived, as did 70.4% from the MS group and 90% from the HS group. The overwintering index (OI) was significantly affected \( p < 0.01 \) by Genotype, but not by the Year (Supplement Table IV). Only one colony with a V. destructor infestation rate of more than 8% on adult bees during the previous year survived the winter with more than 6000 bees. The overall adjusted mean value of OI after the first winter was 75.38 ± 2.61%, and 66.15 ± 3.82% after the second winter. The local MS and NS colonies had an almost identical OI, significantly higher than colonies from the HS group (Table III).

3.5. V. destructor infestation and resistance traits

The GLM analysis for adult bee infestation with mites showed a significant effect of Month as a fixed factor (Supplement Table III). No significant differences of the adult bee infestation rate between groups were observed (Table III). Although there was no significant difference in hygienic removal of pupae from brood cells (Table III, Supplement Table VII), the highest tendency was recorded in the HS group. As for SMR, there was no effect of any of the factors in the model (Supplement Table VI). The highest proportion of non-reproducing mites was recorded in group NS, but it was not significantly different from the other two groups (Table III). The factors Genotype and Month had a significant effect on recapping of brood cells (Supplement Table VI). Significant differences were recorded among groups, with the highest recapping activity in group NS and the lowest one in HS (Table III).

| Group | N bees      | N brood    | DB (1–4)   | CAL (1–4) | SWA (1–4) | HE (kg)      |
|-------|-------------|------------|------------|-----------|-----------|-------------|
| NS    | 11,677 ± 410a | 11,072 ± 485a | 3.05 ± 0.05a | 2.80 ± 0.05a | 3.31 ± 0.09a | 11.870 ± 1.970a |
| MS    | 13,173 ± 226b | 12,441 ± 268b | 3.15 ± 0.03b | 3.39 ± 0.03b | 3.68 ± 0.06b | 10.094 ± 1.039a |
| HS    | 12,197 ± 375a | 11,478 ± 445a | 3.39 ± 0.05c | 3.27 ± 0.04c | 3.94 ± 0.09b | 12.486 ± 1.721a |

The different letters indicate significant differences between means within columns \( p < 0.05 \), Bonferroni test

Table II. Adjusted mean values (LS mean ± SE) for number of bees (N bees), number of brood cells (N brood), defensive behavior (DB), calmness on the comb (CAL), swarming behavior (SWA), and honey extraction (HE, kg)
Also, the highest variability of SMR and REC was found in the NS group (Figures 1 and 2).

4. DISCUSSION

The results of our experiment clearly show the benefits of long-term selection and breeding on the behavior of bees, especially for the traits defensive behavior, calmness on the comb, and swarming tendency. The scores of these three traits were significantly higher in the groups that originated from the breeding programs. In a comparative study investigating behavioral traits of 16 different genotypes (Uzunov et al. 2014), one of the best-rated ones was the *A. m. carnica* originating from Kirchhain, corresponding to the HS genotype in this study (Table IV). At the same time, these authors found that colonies headed by local queens were on average less defensive than colonies with non-local queens, thus postulating that genotype–environment interactions had a significant effect on this trait. However, we could not confirm this observation in the current investigation. On the contrary, the non-local group HS showed the least defensive behavior, indicating that the genetic effect of selection may dominate over a potential negative effect for this trait due to the change of environment. Both calmness on the comb and defensive behavior were significantly affected by the period of the year, and the lowest scores were recorded during late winter (February and March) and autumn (October). This could be the effect of

![Figure 1. Box plot of *V. destructor* non-reproduction for the three groups.](image-url)
weather conditions at that time of the year or the consequence of the bee age structure when mostly elder bees are in the colony.

High propensity for swarming was one of the main traits observed in the NS group, and a clear influence of the genetic origin of bees could be recognized here. Queens from the group NS originated from traditional skep hives which offer limited space for development and thus support earlier and frequent swarming (Simpson and Riedel 1963). Although in early spring the group HS, due to its low overwintering index, was weaker than the other two groups regarding the number of bees and brood cells, no significant difference of colony strength was recorded during the swarming season. Moreover, this group tended to be the strongest one during the second peak of swarming at the end of June. Consequently, differences in colony strength probably cannot be the reason for the lower expression of swarming behavior in HS group.

But yet, differences in colony strength between the groups were observed. If colony development, overwintering ability, and honey production are taken as measures of local adaptation, clear negative effects of the change of environment could be observed in the non-local HS stock.

Table IV. An arrow display of the group differences between all measured parameters: number of bees (Bees), number of brood cells (Brood), overwintering index (OI), defensive behavior (DB), calmness on the comb (CAL), swarming behavior (SWA), honey extraction (HE), adult bee infestation with *V. destructor* (BI), percentage of non-reproducing mites (SMR), recapping of brood cells (REC), and hygienic behavior (HYG)

| Group | Bees | Brood | OI | DB | CAL | SWA | HE | BI | SMR | REC | HYG |
|-------|------|-------|----|----|-----|-----|----|----|-----|-----|-----|
| NS    | ↓    | ↓     | ↑  | ↓  | ↓   | ↓   | →  | →  | →   | ↑   | →   |
| MS    | ↑    | ↑     | →  | →  | →   | →   | →  | →  | →   | →   | →   |
| HS    | ↓    | ↓     | ↓  | ↓  | →   | ↓   | →  | →  | →   | ↓   | →   |

The type of arrow presents significant differences within the column (p < 0.05, Bonferroni test)
Honey bees in commercial apiaries are today expected to survive and show high productivity in a variety of environmental conditions throughout the world. The desirable traits of just few economically preferred subspecies have influenced the distribution of these commercially successful stocks at the expense of native honey bees (Ruttner 1988; Moritz et al. 2005). However, not least initiated by discussions about honey bee health and colony losses in the past decades, local adaptation is now increasingly recognized as one of the important factors that influences behavior, productivity, and survival of bees (Costa et al. 2012a; Büchler et al. 2014; Hatjina et al. 2014b; Uzunov et al. 2014). It is noticeable that in our experiment colonies headed by non-local queens (HS group) tended to reach the peak of development about 1 month later as the local NS and MS groups. This could indicate a maladaptation to the local environmental conditions in our study area, where the flowering periods of plants start a few weeks earlier than in Germany, from where these queens originated. A similar phenomenon was observed by Dražić et al. (2014) who reported that in Croatia colonies headed by queens from Austria reared less brood during spring compared to the local colonies. The significantly smaller number of bees and brood cells observed in the NS group could be the consequence of its origin. Traditional skep hives offer small and limited space, which does not allow the overall potential growth of the colony. On the other hand, queens from the MS group were kept in larger hives and systematically selected for colony growth, and the benefits of selection could be seen in our results. Another important indicator of maladaptation is overwintering success, which was measured by calculating the OI. While the factor year did not affect the OI (although the two winters were very different in terms of length and intensity), significant differences occurred between the genotypes, mainly due to the low OI recorded in group HS. As no significant difference in colony strength was recorded during the last fall inspection of colonies in both seasons, apparently other factors affected the overwintering ability. Infestation of colonies with *V. destructor* reduces the lifespan of winter bees (Kovac and Crailsheim 1988; van Dooremalen et al. 2012; Steinmann et al. 2015). However, this cannot explain the low OI of the HS group since there was no difference in mite infestation between groups. Even if the underlying mechanisms cannot be identified by our results, we speculate that the lower OI of the non-local HS group recorded after both winters might indicate a lack of adaptation. The local bees are adapted to the local climatic conditions not only by behavior, i.e., brood development during the year (Strange et al. 2007; Dražić et al. 2014), but also at the metabolic level (Parker et al. 2010). Furthermore, local bees can cope more easily with the local pathogens (Francis et al. 2014; Hatjina et al. 2014b; Salmela et al. 2015). The lack of adaptation may also have affected the production of honey in the HS group, which did not perform so well and produced less honey than expected, perhaps due to a lower degree of exploitation of environmental resources. Together with the extremely bad weather conditions recorded during the blooming period of *Brassica napus* and *Robinia pseudoacacia* (21 rainy days recorded from April 15 to May 15), this may have affected the nectar intake during these two main honey flows in the research area. Consequently, the first honey extraction only occurred relatively late, after the *Tilia* sp. and *Helianthus annuus* bloom. However, it is possible that this under-average season masked the potential difference in honey production ability between the groups.

Hygienic behavior is a trait with high heritability (Rothenbuhler 1964; Pérez-Sato et al. 2009; Facchini et al. 2019), and it was expected that the HS group would show a significantly higher expression than the other two groups. The fact that these differences were not significant could be a consequence of the open mating of the queens from the HS group with unselected drones of unknown origin, highlighting the paternal influence on the inheritance of this trait (Pérez-Sato et al. 2009).

Although there was no significant difference between groups regarding the suppression of *V. destructor* reproduction, a surprisingly high proportion of non-reproducing mites (> 30%) was recorded in the NS group. This could be a consequence of brood recapping, which was most significantly expressed in this group. Opening and recapping of brood cells has been shown to play a
pivotal role in mite-resistant populations (Oddie et al. 2018; Martin et al. 2019). The population from which the NS queens originated is treated against mites to a substantially lower extent (once per year during October). This probably contributes to an increased selection pressure towards *V. destructor* resistance, for example, due to a higher fitness of drones from less susceptible colonies under natural mating conditions (Büchler et al. 2006). The higher recapping behavior of this group might therefore indicate that this population could have developed some degree of mite resistance. The fact that these differences in one of the relevant resistance characters did not correlate with differences in bee infestation with mites could be explained as a consequence of drifting, as colonies in the research apiary were arranged close to each other in rows (Jay 1966; Seeley and Smith 2015). The evidently higher variability of REC in the NS group in comparison to the HS group indicates a greater possibility to select for this trait in the NS population.

Nowadays, the necessity of breeding and keeping resilient bees is designated as the most promising sustainable solution (Büchler et al. 2010; Uzunov et al. 2017) to overcome the Varroa mite problem. The economic costs of colony treatments and colony losses are high (Neumann and Carreck 2010; Brodschneider et al. 2018), while chemical treatments, although they lose importance because of mite resistance against various components (Martin 2004; Sammataro et al. 2005), may contaminate bee products (Bogdanov 2006). One possible way to achieve sustainable beekeeping in the presence of mites is to allow the co-evolution of bees and *V. destructor* with as little interference as possible, but observing the damage threshold levels of mite infestation (Liebig 2001; Currie and Gatien 2006; Genersch et al. 2010). Based on a more “near to nature” approach of keeping a reasonably high infestation pressure of *V. destructor* during the swarming/mating period, the drones from the fittest colonies will have a higher chance to mate and pass on their genes, and consequently will contribute to increasing the resilience of future generations. Ultimately, the identification of the mechanisms that lay behind resistance against mites could be used in breeding programs to promote the selection process. However, this needs to be done on a regional scale, taking into account the adaptation of local bees to the specific environment they inhabit.

In conclusion, colonies headed by queens that originate from breeding programs showed significantly better scores for the main commercially recognized behavioral traits. On the other hand, colonies from the group without any selection showed higher expression of resistance traits (SMR and REC), indicating a possible improvement of these traits as consequence of natural selection and local adaptation. In addition, a low overwintering index and a slower spring development indicate a lack of adaptation of the HS group. This research provides further evidence on the importance of breeding bees under aspects of resilience and for the relevance of local adaptation for the performance of colonies.

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**AUTHORS’ CONTRIBUTION**

M.K., Z.P., and R.B. conceived and designed the research; M.K. and Z.P. performed the field and laboratory work; M.K., A.U., and M.M.D. analyzed the data; M.K., Z.P., M.M.D., A.U., M.D.M., and R.B. wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

**COMPLIANCE WITH ETHICAL STANDARDS**

**Conflict of interest**  The authors declare that they have no potential conflict of interest in relation to the study in this paper.
Effects of selection on Carniolan bee

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