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Relationships between resistance characteristics of honey bees (*Apis mellifera*) against Varroa mites (*Varroa destructor*)

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Abstract

Ectoparasitic mite, *Varroa destructor*, today is one of the main reasons for colony losses worldwide. This study deals with relationships between measurements of resistance characteristics and measurements of bee infestation. During the trial, 105 *Apis mellifera carnica* colonies were tested according to AGT (Arbeitsgemeinschaft Toleranzzucht) breeding program. Data of hygienic behavior and mite population growth development were obtained. Further, they were evaluated for the traits SMR (Suppressed Mite Reproduction), recapping behavior (REC) and brood infestation. Significant influences of hygienic behavior, SMR and REC on the mite infestation were considered. SMR and REC show relatively high coefficients of regression on mite infestation values. The results show, that SMR and REC might be suitable selection traits to decrease the mite population growth within the colonies.

Keywords: *Apis mellifera carnica*, hygienic behaviour, recapping, suppressed mite reproduction, *Varroa destructor*

Introduction

The ectoparasitic mite *Varroa destructor* is the major threat for apiculture. It switched from the original host *Apis cerana* (Eastern honey bee) to *Apis mellifera* (Western honey bee). On the new host, a balanced host-parasite relationship is lacking. *V. destructor* is a hemophagous parasite and reproduces in brood cells. It weakens the host through deprivation of haemolymphae and it is a major vector of different viruses (Rosenkranz et al., 2010). In the AGT breeding program, mite population development and hygienic behavior are measured in the performance test (AGT, 2013). It was considered that there are correlations between mite population growth and higher rates of non-reproduction of mites (Harbo and Harris, 1999). In naturally surviving *A. mellifera* populations, the traits SMR (Suppressed Mite Reproduction) and VSH (Varroa Sensitive Hygiene) contribute to resistance against *V. destructor* (Locke, 2016). SMR is the main part of VSH, which is a more broadly defined trait that includes removal of infested pupae (Harris, 2007). The traits SMR, respectively VSH were used to select bees, which show resistance to *V. destructor* (Harbo and Harris, 2005; Ibrahim and Spivak, 2006). The traits might be relevant to improve resistance against *V. destructor* in European populations (Büchler et al., 2010). Observations on SMR selected bees show, that this trait is often linked to intensive uncapping and recapping of brood cells (REC behavior), but the biological interactions are unknown yet (Villa et al., 2009). Recent research has shown that REC of brood cells plays a major role in surviving populations of bees in Europe (Oddie et al., 2018).

Materials and methods

Field measures of resistance characteristics

In this study, 105 Carniolan (*Apis mellifera carnica*) colonies were tested according to the AGT breeding program. Hygienic behavior was measured with the PIN-Test method, where 50 brood cells were pierced with a fine insect pin and removal of pupae was checked 8 hours later. The performance test was extended, so there were three characteristic forms (complete cleaned cells, partially cleaned cells and untouched cells) which were noted. 1st Pin-Test was performed between June 8th and June 16th, 2017, 2nd Pin-Test was performed between July 5th and July 29th, 2017. Mite population was measured through natural mite fall in spring and the adult bee infestation during summer. To measure the infestation rate of the bee samples, the powdered sugar method was used (Macedo et al., 2002). Mentioned measurements of bee infestation were performed from September 7th to September 16th, 2017 (1st measurement, n = 73) and on September 28th, 2017 (2nd measurement, n = 23). The traits SMR and REC were measured according to the RNSBB-protocol (Büchler et al., 2017). Brood samples (worker brood) were gathered when 1st measurement was performed, or 3 or 6 weeks before respectively, when a treatment threshold was transcended by a colony in earlier measurements (n = 32). These colonies which leave the test earlier, contribute data except bee infestation measurements for this analysis.

Brood cells, at a minimum development stage of 7 days post capping, were examined under a stereo microscope. The capping of the cell was removed carefully, brood cell was checked for infestation with *V. destructor* and it was noted, if part of the pupae´s spun cocoon were lacking on the inner side of the cell capping. This is an indication whether a brood cell was opened and recapped by bees during the development of the pupae. When there was a single foundress mite in the brood cell, the development stages of the offspring were noted. If the foundress mite was infertile, the offspring was too late to mature (development delay) or the male was absent, the mite was noted as non-reproductive. Together with this examination, data on brood infestation were collected (Büchler et al., 2017).

Statistical analyses

Data were analyzed with the statistic program SAS, Version 9.4. (SAS Institute Inc., 2012). The following model was used for testing the influence of PIN-test, SMR, and REC results on mite infestation:

$Y_i = \mu + b_1*nmf + b_2*it+e_i$

where are Y_i - measurements which represent mite population in the colonies (1st, 2nd bee; brood), μ - intercept, b_1 and b_2 - regression coefficients, nmf_i - natural mite fall in spring, it - influencing trait (PIN complete cleaned, PIN partially cleaned, PIN untouched, SMR, REC of infested cells, REC total, targeted REC) and e_i - residual.

Results

As shown in Table 1, there was a significant ($P<0.05$) negative influence of PIN-Test complete cleaned on the 1st measurement of bee infestation, but the coefficient of regression was relatively low (b = -0.06). The results for SMR indicated significant negative effects on both measurements of bee infestation and brood infestation, where coefficients of regression were relatively high (b = -8.79; b = -5.62; b = -0.26). REC of infested cells also showed a significant negative influence on bee infestation at the 1st measurement (b = -3.08) and a highly significant ($P<0.001$) negative influence on brood infestation (b = -0.18). Targeted REC showed a highly significant influence on the 1st measurement of bee infestation and brood infestation, with relatively high coefficients of regression (b = -7.14, b = -0.35).

Table 1. Influence of resistance traits on adult bee infestation/Coefficient of regression

Measured trait[1]	Date of sampling bees for infestation rate assessment		
	1st bee	2nd bee	Brood
PIN complete cleaned	-0.06*	0.02	0
PIN untouched	0.06	0.06	0
SMR	-8.79*	-5.62*	-0.26*
REC infested	-3.08*	-0.25	-0.18***
REC total	-0.52	0.67	0.01
Targeted REC	-7.14***	-1.4	-0.35***

* Significant $P<0.05$; *** highly significant $P<0.001$; [1]1st, 2nd (bee) - measurements of bee infestation in summer (powdered sugar method); PIN - hygienic behavior (PIN-Test); SMR - suppressed mite reproduction; REC – recapping. Units of values: SMR, REC, PIN, brood - relative 0-1; 1st, 2nd bee - Mites per 10 gram bees.

Discussion

The relationship between bee infestation and SMR was also confirmed by other studies (Harbo and Harris, 2000; Harris et al., 2003). In models of population growth dynamics, reduced mite reproduction (SMR) would have a strong influence on mite population growth (Calis et al., 1999). This relationship can be confirmed by the analysis of this data set, where relationships of SMR to bee and brood infestation were considered. Colonies selected for VSH, respectively SMR, are able to reduce brood infestation and mite fertility in highly infested combs (Villa et al., 2009). In this test it was not proven if colonies are able to reduce infestation of highly infested combs, but the significant negative correlation of SMR and REC to brood infestation was confirmed. REC of infested brood cells can have an effect on non-reproduction of mites (Villa et al., 2009). Research on naturally surviving *A. mellifera* populations in Europe (Oddie et al., 2018) show higher values of REC in contrast to susceptible colonies. A significant relationship between REC of infested cells and 1st measurement of bee infestation was found in this research. Bees selected for SMR showed higher rates of hygienic behavior (Ibrahim and Spivak, 2006). Accurate measurements are needed to identify colonies with a slightly better resistance against *V. destructor* (Harbo and Harris, 1999), because mite population will also be influenced by environmental factors. However, measurements of VSH, SMR and REC are demanding and time consuming and future research should focus on finding reliable and simple methods to test the colonies for these resistance traits. As REC is probably easier to measure than SMR, it might be an interesting trait for large scale selection programs against *V. destructor*.

Conclusions

In this study, SMR and recapping had the strongest influence on the decrease of mite population growth. They are promising resistance characteristics, which can be important for selective breeding of bee resistance against *V. destructor*.

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