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Heritability of the Varroa-specific hygienic behaviour in honey bees (Hymenoptera: Apidae)

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Summary

The hygienic behaviour of the honey bees is considered to be a potential characteristic associated with resistance to *Varroa destructor n.sp.* In this study the heritability of the hygienic behaviour of *Apis mellifera* L. bees was estimated on the basis of the mother–daughter regression. Data were obtained from measurements of the bees' hygienic behaviour towards *V. destructor*-infested cells and towards pin-killed sealed brood. The heritability for the hygienic behaviour towards *V. destructor*-infested brood cells was $h^2 = 0.18$ ($\pm$ 0.27) and $h^2 = 0.36$ ($\pm$ 0.30) for the hygienic behaviour towards dead brood cells. The repeatability was likewise higher for the pin-killed brood assay ($W = 0.46$) compared with the assay using living mites-infested brood cells ($W = 0.24$). The genetic correlation between the behavioural responses to either the mite-infested or pin-killed brood cells was calculated to be $r_g = 0.61$ ($\pm$ 0.51) and the phenotypic correlation to be $r_p = 0.11$ ($p = 0.28$, $n = 100$). Since hygienic colonies demonstrate resistance to brood diseases such as American foulbrood and chalkbrood, it may be worthwhile to intensify the expression of the hygienic behaviour through selective breeding and thus strengthen these potential characteristics associated with resistance to *V. destructor* in honey bee stock.

Zusammenfassung

Heritabilität des Varroa-spezifischen Hygieneverhaltens der Honigbienen (Hymenoptera: Apidae)

Dem Hygieneverhalten der Honigbienen wird als potentieller Varroa-Toleranzfaktor besondere Beachtung geschenkt. In dieser Untersuchung wurde – auf der Basis der Mutter-Tochter-Regression – die Heritabilität des Hygieneverhaltens von *Apis mellifera* L. ermittelt. Als Datengrundlage dienten Quantifizierungen des Hygieneverhaltens der Bienen gegenüber mit *Varroa destructor n.sp.* infizierter und gegenüber toter ('genadelte') gedeckelter Bienenbrut. Der daraus ermittelte Heritabilitätswert lag für das Hygieneverhalten gegenüber mit Varroamilben infizierter Brut bei $h^2 = 0.18$ ($\pm$ 0.27) und bei $h^2 = 0.36$ ($\pm$ 0.30) für das Hygieneverhalten gegenüber toter Brut. Auch die Wiederholbarkeit war ähnlich höher bei dem Hygieneverhalten gegenüber toter Brut ($W = 0.46$) im Vergleich zu $W = 0.24$ ermittelt am Hygieneverhalten der Bienen gegenüber experimentell mit lebenden Milben infizierten Brutzellen. Die genetische Korrelation zwischen diesen Verhaltensreaktionen wurde als $r_g = 0.61$ ($\pm$ 0.51) errechnet und die phänotypische Korrelation als $r_p = 0.11$ ($p = 0.28$, $n = 100$). Da hygienischen Bienenvölkern eine erhöhte Widerstandsfähigkeit gegenüber Amerikanischer Faulbrut und der Kalkbrut zugesprochen wird, erscheint es lohnenswert das Hygieneverhalten durch Selektion zu fördern, um so auch diesen potentiellen Varroatoleranz-Parameter bei den Honigbienen zu stärken.

Introduction

Beekeeping with honey bees, *Apis mellifera* L., is endangered world-wide by the mesostigmatic mite *Varroa destructor n.sp.* (BRADBEAR 1988; MATHESON 1996). As *A. mellifera* colonies die from *V. destructor* infestation within a few years if the mite population growth is not regulated by the beekeeper (ROSENKRANZ and ENGELS 1985; RITTER 1988) and because chemical mite control has its problems and limitations (reviewed in MILANI 1999 and WALLNER 1999), it is of common and economic interest to breed bees with a

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higher tolerance/resistance to this mite. However, selection and breeding are long-term solutions to the present crisis in apiculture. Commercially available lines of honey bees with resistance to *V. destructor* would alleviate the need for frequent and expensive acaricide use. Before breeding bees for resistance, it is first necessary to identify the mechanism/characteristics of the bees that confer resistance and to evaluate the genetic basis, in particular the heritability. The latter is the main focus of the studies reported here. It is a useful statistic for predicting response to selection and for organizing breeding schemes

Theoretically, the most effective way to reduce the threat of the mite to the bees is to influence its reproductive capacity by changes in the biology of the host. However, any change in the biology of the bee that can reduce the reproduction of the mites may also favour an adaptation of the mites to these changes (BIENEFELD et al. 1998).

Hygienic behaviour is the dominant natural defence against brood diseases of the honey bee such as American Foulbrood (*Paenibacillus larvae larvae*) and Chalkbrood (*Ascosphaera apis*) (reviewed in SPIVAK and GILLIAM 1998a; b), and is also a natural defence against *V. destructor* mites infesting brood cells (reviewed in BOECKING and SPIVAK 1999). As a behavioural trait of the bees, it might contribute to overall resistance against *V. destructor*. Honey bees with hygienic behaviour detect, uncap, and remove diseased, infested, or parasitized brood from the comb (BOECKING and DRESCHER 1991).

The studies reported here focused on the estimation of the heritability and repeatability of the bees hygienic behaviour in relation to Varroa resistance. Moreover, we calculated the genetic and phenotypic relationship between the hygienic behaviour towards mite-infested and dead brood.

Materials and methods

General design

In order to reduce environmental variation all colonies of the experimental population were placed in a similar environment and had similar management schemes. Six bee yards were established in a large forest area near the city of Bonn. The average distance between the bee yards was about 1.5 km. The experimental population ($n = 77$ colonies) included *A. m. carnica* queens from different genetic origins (breeders) and geographic regions of Germany.

Following the first test period (1996) of the 'mother' generation these 77 colonies, placed in magazine-hives, were split to build up new colonies and subsequently re-queened with their mated 'daughters' of the F_1-generation. The terms 'mother' and 'daughter' refer to the queens of the colonies used. Virgin 'daughter' queens of the F_1-generation were placed in mating boxes and shipped to 33 different localities throughout Germany for random mating. These locations were preferably selected in areas where bee-keeping with *A. m. carnica* bees predominated and they were geographically distributed from the North to the South of Germany. This mating design allowed the genetic relationship of the 'fathers' to the offspring workers to be neglected while calculating the average genetic relationship between 'mother' and 'daughter'-queens later.

In a second test period (1997) 80 bee colonies of the F_1-generation could be included in the quantification of their hygienic behaviour during succeeding experiments.

Quantifying the hygienic behaviour

The hygienic behaviour of the bee colonies towards *V. destructor*-infested brood cells was quantified by experimental infestation of brood cells in ordinary wax combs (following the method of BOECKING and DRESCHER 1992). To test this behaviour, the caps of recently ($\approx$ 0–6 h) capped brood cells ($n = 10$) of one brood comb in each test colony were partially opened using a razor-blade, and one live *V. destructor* mite was introduced

into the cell. The partially opened cell caps were carefully closed again. Other recently capped brood cells on the same comb were opened and closed again without introducing a mite and served as control cells (n = 10). All test cells were mapped on a transparent plastic sheet temporarily attached to the top bar of the frames. Moreover, recently capped brood cells were marked on these transparent plastic sheets, without any manipulation, to monitor possible cannibalism of the bees during the experimental period. The live *V. destructor* mites used for experimental infestation were collected from a random group of four highly infested colonies (not test colonies) using the powdered-sugar method (RAMIREZ 1988; BOECKING and RITTER 1993). Ten days later, we determined how many cells were removed. In cases where the control cells were removed by the bees, for the statistical analysis the data were corrected following the formula of SCHNEIDER-ORELLI (1947).

In an interval of approximately 1 week following the end or before starting each test of the hygienic behaviour towards the mite-infested brood we quantified the hygienic behaviour towards dead brood (pin-killed) in each colony, respectively.

The hygienic behaviour of a bee colony towards dead brood cells was quantified using the pin-killed-brood-assay (following the method of NEWTON and OSTASIEWSKI 1986). We modified the method by using a specially constructed 'pin-punch' which allowed us to damage a cluster of seven neighbouring capped brood cells simultaneously. Randomly selected capped brood cells (n = 70), that contained aged brood (developmental stage = white eyes of the bee pupa), were pin-killed on one comb in each colony tested. Each central pin-killed brood cell of the seven-cell-cluster was marked on a transparent plastic sheet temporarily attached to the top bar of the frame. The removal success rate of the colonies was checked 13 (min) to 15 (max) hours following the pin-killing of the brood using the transparent plastic sheets.

Statistical analysis

The repeatability (W) was calculated using the whole data set of repeated measurements (1996 and 1997) by a restricted maximum likelihood (REML) procedure using SAS INSTITUTE (1997). The standard error (s_w) of the repeatability was calculated using the formula of DICKERSON (1960).

Heritability (h^2) was calculated on the basis of a 'mother–daughter regression' (KEMPTHORNE and TANDON 1953; OLLIVIER 1974; FALCONER 1984) as: $h^2 = b/0.5$, where b is the coefficient of the 'mother–daughter regression' and 0.5 is the genetic relationship between 'mother' and 'daughter' queens. The limited databases (as a result of the time-consuming data collection of the behavioural traits in each test colony) used for the estimation of the heritability in this investigation did not allow consideration of both genotypes of workers and queens separately as BIENEFELD and PIRCHNER (1990) suggested. Hence the traditional way was used, in which the queens' genotype represents the whole colony and the average genetic relationship between 'mother' and 'daughter' queens is assumed to be 0.5 (BAR-COHEN et al. 1978). Furthermore, if worker bees perform the hygienic behaviour they are influenced by maternal effects of the queen (pheromones, egg-laying capacity) during their life. The queen (0.5 of the worker genes and 100% maternal effects) is more likely to represent the colony compared to the average of the workers (WILLAM and EßL 1993), which are inhomogeneous from the mating design in this investigation. These are all arguments that favour the estimation of the heritability on the basis of the mother–daughter regression. Data were adjusted for season and location and for different number of observations per colony.

The standard error [$s_{(h2)}$] of the heritability estimates and the genetic correlation [r_g] between the behavioural responses to either the mite-infested or pin-killed brood cells was calculated according to FALCONER (1984).

Results

Quantifying the hygienic behaviour

The hygienic behaviour of the 'mother' generation towards mite-infested brood cells was quantified once with n = 55 colonies in August 1996, whereas in the colonies of the 'daughter' generation this test was repeated at three different times during 1997 (June and at the beginning and end of July), respectively. The average removal rate towards brood cells artificially infested with one mite ranged from 16.7 to 32.4% 10 days after the experiment was started (see Table 1). These results show that variation in this trait existed in the experimental population.

Using the specially constructed 'pin-punch' as the pin-killed-brood-assay for quantitative estimates of the hygienic behaviour towards dead brood cells we tested the hygienic tendency of the 'mother' generation twice in 1996 and at four different times during 1997 with the colonies of the 'daughter' generation, respectively. In all repetitions we found some colonies that removed 100% of the treated cells within 13–15 h (see Table 2). The results show that considerable variation in this trait existed in the experimental population used, with a range of hygienic response to pin-killed brood from 0.0 to 100% within 13–15 h.

Statistical analysis

The statistical analysis revealed that the repeatability (W) of the repeated measurements regarding the data of both the 'mother' and 'daughter' generation, was higher for the pin-

Table 1. **Average removal rates of the test colonies towards brood cells artificially infested with one living *V. destructor* mite per cell (n = 10/colony)**

Date	Colonies (n)	Removal rates (%)	Range (%)	CV[a]
August 1996	55	29.0 ± 20.5	0.0–90.0	70.7
June 1997	92	32.4 ± 19.3	0.0–90.9	59.6
July [b] 1997	77	16.7 ± 14.9	0.0–60.0	89.2
July [e] 1997	76	21.2 ± 16.4	0.0–70.0	77.4

[a] variation coefficient; [b] beginning; [e] end

Table 2. **Average removal rates of the test colonies towards pin-killed brood cells (n = 70 cells/colony)**

Date	Colonies (n)	Removal rates (%)	Range (%)	CV[a]
September [b] 1996	64	83.0 (21.6	12.9–100	26.0
September [e] 1996	58	70.4 (24.7	2.9–100	35.1
April 1997	86	72.3 (23.2	0.0–100	32.1
June 1997	90	66.0 (27.8	0.0–100	42.1
August [b] 1997	77	68.6 (21.9	5.7–100	31.9
August [e] 1997	76	54.6 (25.6	0.0–98.6	46.9

[a] variation coefficient; [b] beginning; [e] end

killed brood assay ($W = 0.46$, 1996 and 1997, six repetitions) compared with the experimental infestation with living mites ($W = 0.24$, 1996 and 1997, four repetitions).

For the hygienic behaviour towards *V. destructor*-infested brood cells we calculated the heritability to be $h^2 = 0.18$ ($\pm$ 0.27) ($b = 0.088$ coefficient of the mother–daughter regression; $F = 1.07$; $p = 0.307$; $n = 45$, number of mother–daughter pairs) and for the hygienic behaviour towards dead brood cells as $h^2 = 0.36$ ($\pm$ 0.30) ($b = 0.179$ coefficient of the mother–daughter regression; $F = 2.38$; $p = 0.128$; $n = 66$, number of mother–daughter pairs).

The genetic correlation (r_g) between the behavioural responses to either the mite-infested or pin-killed brood cells was calculated to be $r_g = 0.61$ ($\pm$ 0.51), the phenotypic correlation (r_p) to be $r_p = 0.11$ ($p = 0.28$, $n = 100$).

Discussion

The measurements of the hygienic response of the bees to either mite-infested or pin-killed brood show that considerable variation existed in this behavioural trait in the experimental population of honey bee colonies tested. However, this experiment was not designed to include colonies that were specifically high or low in their hygienic response.

The repeatability as a statistical index for the similarity of repeated measurements with the same colony is defined also as the upper limit of the heritability (FALCONER 1984). Its value can be calculated without the knowledge of the genetic relationship between the colonies tested. The calculated repeatabilities in this study ($W = 0.46$ for the pin-killed brood assay and $W = 0.24$ for the removal of mite-infested cells) correspond well with the values known. BOECKING (1994) found the repeatability of the hygienic behaviour towards brood cells infested with one living *V. destructor* mite to be $W = 0.29$ compared with brood cells infested with two mites as $W = 0.64$. In investigations by HOFFMANN (1996) the repeatability was likewise higher for the pin-killed brood assay ($W = 0.55$) compared to the experimental infestation with living mites ($W = 0.38$). THAKUR et al. (1996) who observed individually marked bees in this behavioural trait found the repeatability of uncapping to be 0.10 (duration), 0.13 (frequency) and 0.26 (intensity). The repeatability of removing was estimated at 0.04 (duration), 0.12 (frequency) and 0.17 (intensity).

The estimated heritability of the hygienic behaviour in our study ($h^2 = 0.18$ ($\pm$ 0.27) for the removal of mite-infested cells and $h^2 = 0.36$ ($\pm$ 0.30) for the pin-killed brood assay) revealed that this trait is to some extent genetically based. The values also indicate considerable influence from environment. The expression of hygienic behaviour is known to be influenced by environmental factors. For example, weak colonies, or a lack of incoming nectar have been shown to reduce the removal response to mite-infested and dead brood cells, respectively (MOMOT and ROTHENBUHLER 1971; BOECKING and DRESCHER 1993; SPIVAK and GILLIAM 1993; SPIVAK 1996). The results of this study again demonstrate that the rate of removal of mite-infested or dead brood within a particular colony is not always consistent between assays, even under the same environmental conditions (RODRIGUES et al. 1996; SPIVAK and DOWNEY 1998).

Quantitative genetic studies on the hygienic behaviour using a laboratory bioassay also showed only moderate estimates for genetic variance, with $h^2 = 0.14$ for uncapping and $h^2 = 0.02$ for removing dead brood (MILNE 1985). In contrast, HARBO and HARRIS (1999) calculated the heritability of the hygienic behaviour, based on the removal of freeze-killed brood, to be $h^2 = 0.65 \pm 0.61$. The authors used sibling analysis to determine the heritability. In general heritability calculated from sibling analysis yields higher values due to dominance and epistasis effects and due to common environment. More important, heritability is a property not only of a specific character but also of the experimental population and of the environmental circumstances (FALCONER 1984; COLLINS 1986).

The calculated standard error of the heritability values are high in our investigation, obviously because the data base was not large enough. Theoretically for any experimental design it is necessary to include measurements of as many as possible genetically different 'mothers' and one of her 'daughters'. But this competes with the capacities needed. The estimated low heritability of the hygienic behaviour towards brood cells infested with living *V. destructor* mites can also indicate problems in the accuracy of the method. Obviously the pin-killed-brood-assay method, after NEWTON and OSTASIEWSKI (1986), modified in this investigation by the use of a specially constructed 'pin-punch', is easier to standardize compared with the artificial infestation with living mites. The lower heritability value in the case of the hygienic behaviour towards mite-infested brood might also be related to the fact that in contrast to diseased brood, mite-infested larvae and pupae do not necessarily die, with the consequence that removal of mite-infested brood does not always involve a strict behavioural sequence of detecting, uncapping and then removing the parasitized brood compared with dead brood. In some cases the caps of mite-infested brood are opened and then closed again by the bees with a new wax cap without eliminating the bee brood. In those cases, the mites may leave these cells by the temporary hole in the capping (BÄR and ROSENKRANZ 1992). As a result of opening and closing the cell cap by the bee, a distinct change in the silk/wax-structure of the inner cell cap can be observed (BOECKING and DRESCHER 1994). Careful examination of 643 unremoved brood cells that had been experimentally infested with one living mite, in the first set of experiments (1996) of the investigation reported here, revealed that 69 (10.7%) of the cell caps showed clear indications that the worker bees had opened and closed those cells at least once during the 10 days of the investigation without eliminating the brood. Although the introduced mites could have left the cells while they were open, the mites were missing in only four (5.8%) of these cells.

There is a positive correlation between the rate of removal of mite-infested brood and dead brood/freeze-killed (BOECKING and DRESCHER 1992; SPIVAK and DOWNEY 1998). The genetic correlation ($r_g = 0.61 \pm 0.51$) in our investigation can indicate the extent to which both characters are controlled by the same genes. However, we can make use of the correlation between both characters by selecting for a character of secondary importance but higher heritability (here the hygienic behaviour towards dead brood), in order to improve the correlated character of major importance (here the hygienic behaviour towards mite-infested brood cells). Colonies that were pre-selected for high hygienic behaviour based on the freeze-killed brood assay removed significantly more brood cells that had been experimentally infested with *V. destructor* mites compared with colonies selected for low hygienic behaviour (SPIVAK 1996).

The overall effect of hygienic behaviour on population growth of the mites within a colony is still unknown. However, SPIVAK and REUTER (1998) demonstrated that colonies bred for hygienic behaviour (based on the rate of removal of freeze-killed brood) had fewer mites than commercial colonies not selected for the behaviour after 1 year without mite treatment. The same colonies also had lower incidences of chalkbrood and American foulbrood, and produced more honey than the commercial colonies. These results demonstrate the benefits of selecting bee colonies for hygienic behaviour. However, since hygienic colonies demonstrate resistance to brood diseases such as American foulbrood and chalkbrood, the trait may be worthwhile for incorporation into honey bee stocks. On the basis of this study it should be possible to intensify the expression of the hygienic behaviour towards mite-infested cells through selective breeding.

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