Does the genotype of honeybee brood influence the attractiveness for *Varroa jacobsoni* and/or the reproduction of this parasite?

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Colony infestation by *Varroa jacobsoni* is the most serious problem for beekeeping worldwide. To study whether different genotypes of the host influence attractiveness for *Varroa*-mites or the reproduction of this parasite, a honeybee queen (*Apis mellifera carnica*) was inseminated with sperm from 4 drones from different Carnica-stocks. Shortly after egg laying, the brood combs were transferred to a colony which was infested with *V. jacobsoni*. After capping of cells, the brood was transferred from the foster colony to an incubator and was examined for infestation and reproduction by mites. The paternal descent of the bee brood (n = 400) was ascertained by DNA-analysis. No significant differences were found between the single bee patrilines according to their attractiveness (average infestation/cell) for the parasites. Out of the 99 brood cells which were infested with one mother-mite, 13% of the parasites proved to be infertile. There were no significant differences between bee patrilines in any of the reproduction parameters of *Varroa*. The use of these characters in breeding programmes should be critically examined.

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The ectoparasitic mite *Varroa jacobsoni* Oud. is at present the most dangerous parasite of the western honeybee (*Apis mellifera*). Without a consistent application of acaricide the survival of honeybees in temperate climates is seriously endangered. To reduce the use of pesticides and the possible contamination of honey intensive work is now being done worldwide to select *Varroa* tolerant honey bees. These efforts have become acutely relevant since an increasing parasite resistance to the most common acaracide has been demonstrated (LODESANI et al. 1995).

There are four main strategies for breeding *Varroa* resistant honeybees, namely:

1. Selection to increase brood-removal behaviour of *Varroa*-infested brood cells (PENG et al. 1987b; BOECKING and DRESCHER 1992).
2. Selection to increase grooming behaviour. This causes injuries and death of the parasites, which are easily recognised and reached by the bees (PENG et al. 1987a; RUTTNER and HÄNEL 1992).
3. Selection to shorten the duration of the postcapping stage, which gives less time for the mite development and consequently, less fertile offspring (MORITZ 1985).
4. Selection to create host brood which is less attractive to parasites or which can cause female *Varroa* mite infertility.

Several authors consider the last alternative as particularly promising (ROSENKRANZ and ENGELS 1994) because in simulation model calculations, reducing the fertility of parasites has been determined to be the most significant resistance factor (FRIES et al. 1994). There are already several experimental studies available on this subject (BÜCHLER 1989; FUCHS 1994; DE GUZMAN et al. 1995; GUZMAN-NOVOA et al. 1996). Simultaneously introduced bee brood of different origins into host colonies which were heavily infested with *Varroa* have been found to differ in their attractiveness to the parasite. (BÜCHLER 1989; DE GUZMAN et al. 1995; GUZMAN-NOVOA et al. 1996). In the study by GUZMAN-NOVOA et al. (1996), it was shown that the proportion of fertile female mites was significantly influenced by the origin of the bees, whereas FUCHS (1994) did not observe this phenomenon. It should be noted, however, that the above described experiments are methodically problematic, because brood transferred in this way are likely to differ with respect to age, rearing conditions and scent of brood and wax, which may significantly affect attractivity for *Varroa* and perhaps its reproduction. The experiment described below is an attempt to limit the source of errors, and to ascertain how far the genotype of a bee brood is responsible for differential attractiveness for *Varroa* and reproduction of the parasite.

MATERIALS AND METHODS

A honeybee queen (*Apis mellifera carnica*) was inseminated with the sperm from 4 drones. In order to provide a large variability within the offspring, the drones were selected from 4 origins (B, E, S, U) which had been selected in isolation over many generations. The sperm (0.75 µl per drone) was slightly diluted and mixed so as to ensure, as far as possible, a representative distribution. After egg laying had begun, the honeybee queen was caged to the comb for 12 hours to ensure more or less simultaneous egg laying. This procedure was repeated on another comb 3 days later. Shortly after egg laying, both combs were transferred to a host colony which was highly infested with *V. jacobsoni*. Shortly after cell capping, the combs were taken from the donor colony and transferred to an incubator (34.5°C, 60% RH). This was necessary because brood cells which have been repeatedly infested are exposed to a higher risk of being emptied by the host bees (BOECKING and DRESCHER 1992) which may influence the results. The combs were frozen two days before the emergence of the bees so as to be able to distinguish between the mother mites and their offspring, and consequently multiple infestation from the reproduction of mother mites. Two days before the emergence of the bees, the development stages of the mites can clearly be distinguished from the adults. Each cell was opened and the number of *Varroa* females and the different development stages were recorded. Those cells in which only one adult *Varroa* female was found could be used for the quantification of the reproduction success of each mother. Since the average infestation and reproduction data deviated immensely from the normal distribution, they were analysed using variance analysis for categorical data (Proc Catmod, SAS 1988). Both the paternal descent of the brood and the distribution across both combs were taken into consideration in the model.

Paternity determination for the work bees was in principle accomplished by microsatellite genotyping typing (TAUTZ 1989). DNA was extracted according to a standard protocol (SAMBROOK et al. 1989) from 400 worker bees of the sample and the four males (B, E, S, U) used for artificial insemination of the queen. Eighteen different primer pairs for microsatellite loci (ESTOUP et al. 1994) were tested on a few workers and the four males. At one locus, A76 (ESTOUP et al. 1994), all four drones were shown to have different alleles (allele length in base pairs: 349, 231, 254, 271 for B, E, S, and U, respectively). Two additional alleles (325, 211) showed up in the workers, which have to be the queen alleles. Therefore, genotyping at locus A76 provides enough information to unambiguously determine one of the four drones as the father of a particular worker bee. Polymerase chain reactions (PCR) were performed with about 50 ng of bee DNA in 10 µl solution containing 50 mM KCl, 10 mM Tris pH 8.3, 1 mg/ml gelatine, 1.5 mM MgCl2, 200 µM of each dNTP, 300 nM of each primer, 0.5 U of Taq Polymerase and one of three fluorescent dye labelled dUTPs (0.4 µM R110 or R6G, or 1.6 µM TAMRA; Perkin Elmer). 30 cycles with a 1 min plateaux each of 94°C, 58°C and 72°C were performed. PCR products were run on an ABI Prism 377 Sequencer (Perkin Elmer) with GS ROX 350 size standards (Perkin Elmer) in each lane. Microsatellite alleles were scored as fragment length in base pairs and paternities of the worker bees were determined.

RESULTS

184 and 216 brood cells on each brood comb, respectively, were available for the analysis and evaluation. Infestation ($\chi^2 = 2.26$, df = 1, n.s.), proportion of *Varroa* females which reproduced ($\chi^2 = 1.36$, n.s.), number of offspring per reproductive *Varroa* female ($\chi^2 = 0.30$, n.s.) and percentage of *Varroa* females without male offspring ($\chi^2 = 0.06$, n.s.) in the two combs did not differ significantly so the results in Fig. 1 and 2 were combined. The infestation of single cells with *V. jacobsoni* differed greatly. Approximately 50% of the cells were not at all parasitized, barely 1% of the cells were infested with 5 female mites.

Although the insemination was carried out with an equal amount of sperm and in representative proportions, the patrilines were unequally represented in the random sample (B: 11%, E: 25%, S: 11%, U: 54%). There were no significant differences between the patrilines ($\chi^2 = 5.54$, df = 3, $p > 0.10$) according to their attractiveness (average infestation/cell) for the parasites (Fig. 1). Of the 99 brood cells which were infested with only one mother mite, 13% of the mites proved to be infertile. The proportion of infertile *Varroa* females in the brood cells of origin S tended to be higher with 28% (Fig. 2), but the differences were not significant ($\chi^2 = 4.51$, df = 3, $p > 0.10$) but it should be taken into consideration that the size of the sample of this origin was low (n = 14). No significant differences were found concerning the average reproduction rate i.e., number of offspring per fertile *Varroa* female ($\chi^2 = 4.15$, df = 3, $p > 0.10$).

DISCUSSION

In this study, an unequal distribution of *Varroa* females within a colony did not appear to be influenced by the different genotype of bee brood. This is

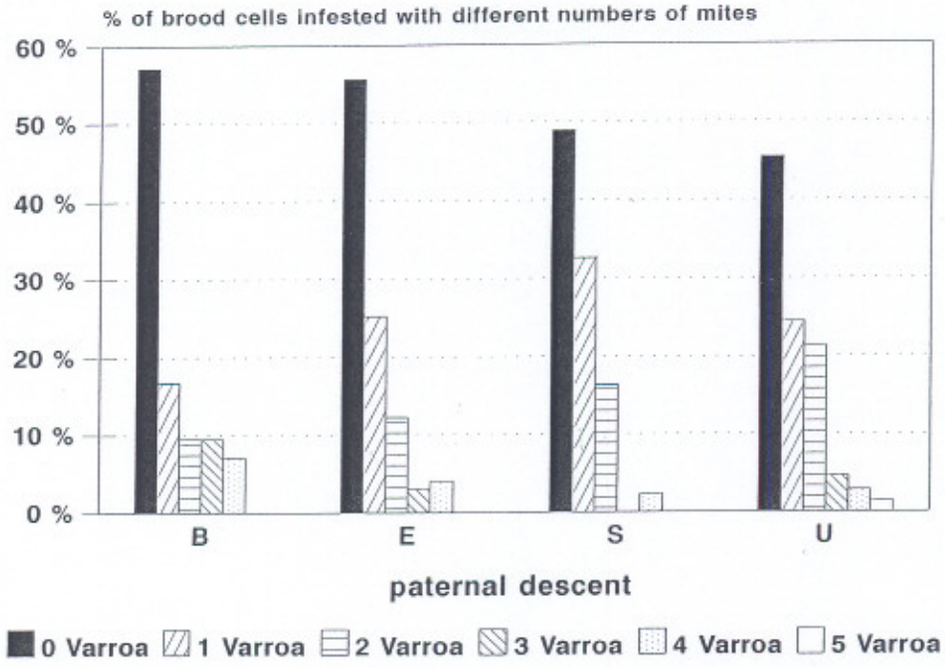

Fig. 1. Attractiveness of bee brood from different origins to *Varroa jacobsoni* (distribution of not, single and multiple infested brood cells from different paternal descent).

in contrast to the results of others (BÜCHLER 1989; DE GUZMAN et al. 1995; GUZMAN-NOVOA et al. 1996), who found that simultaneously introduced brood of different origin had significantly different level of attractiveness for the parasites. In the above studies, the mites were not only provided with the transferred colony characteristics (scent, wax, different rearing of brood) but also with a greater genotypic variability of the bee brood. In our study the offspring of different drones within a colony are maternally half-sisters ($r = 0.25$) and are, therefore, genetically more homogenous than in the other experiments. On the other hand, the brood from different races or origins within a colony do not occur naturally, so that any selection according to recognition of and reaction to possibly higher levels of differences in attractiveness is unlikely. In the case of a change of host (infection of new colonies), a fixation on certain genotypes of the host would be more of a hindrance for the parasite. While the change of host within a colony is unproblematic (infestation of new brood cells), the parasitization of new colonies through infested bees is the problem the parasites face. Any *Varroa* mite that has reached a new colony profits if it has only slight preference for specific host genotypes. The adaptability of *V. jacobsoni* has been impressively demonstrated by the unproblematic change from the original host (*A. cerana*) to *A. mellifera*.

Also DE GUZMAN et al. (1995) found—in contrast to GUZMAN-NOVOA et al. (1996)—that the reproductive success of the parasite is not significantly influenced by the genotype of the bee brood. Any bee genotype-specific effects would obviously be a very efficient means of achieving a *Varroa*-tolerant bee (ROSENKRANZ and ENGELS 1994; FRIES et al. 1994). The proportion of infertile *Varroa* females is significantly higher in Africanized bees (AUMEIER et al. 1996), which along with other resistance factors explains their high level of resistance. In a breeding programme with an European race (*A. mellifera carnica*) in Austria, after 10 years of selection, there was no demonstrable selection success concerning the pro-

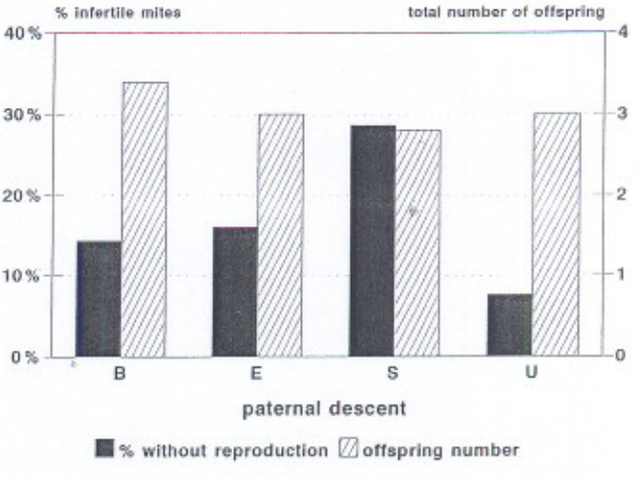

Fig. 2. Percentage of infertile *Varroa* females and average number of offspring per fertile *Varroa* female depending on the paternal descent of the bee brood.

portion of *Varroa* females without reproductive offspring (PECHHACKER et al. 1996). RUTTNER et al. (1984) and RITTER (1990) found a high rate of infertile *Varroa* females in *A. mellifera* in Uruguay and Tunisia respectively, but in an equivalent study 5 years later, Ritter (1997), (personal communication) no longer observed this phenomenon.

Varroa mites in general have only the choice between different patrilines within one colony for reproduction. We focused therefore on the parasite reaction within the colony and designed our study to analyse any mite ability to distinguish between maternal host halfsibs and to see any possible different reaction in reproduction to them. It seems unlikely that results differ with regard to dependence on the maternal background, provided that genetic variation between patrilines do not differ. To ensure that non-significant results are not caused by too small genetic variation between patrilines, we used drones from very different origins, that provide a genetic variability between patrilines larger ever naturally present within a local population. However, assuming we just have not observed significant genetic differences due to sampling error, or even if we had found a significant genetic basis for the influence of bee brood on parasite fertility, there are theoretical reasons which offer little promise to select for this trait due to system inherent counter-selection of the parasite. *Varroa* females which cannot cope with the changing situation (haemolymph composition etc.) of the bee brood will die out, whereas the females which are capable of reproduction will be mothers of the following (and ten times shorter generation interval) parasite generation. However, the distinguishing feature of the original host, *A. cerana* along with its specific resistance behaviour (selfgrooming, nestmate grooming, uncapping and removing of infested brood) is that the parasites reproduce almost exclusively in the drone brood (KOENIGER et al. 1981). Studies of *A. mellifera* prove that the fertility of the parasites is influenced by seasonal and environmental changes (OTTEN and FUCHS 1990). FUCHS (1994), in his studies of *A. mellifera*, also demonstrated that the bee brood had no influence on the fertility of the parasites; any influence is more likely associated with the physiological status of the mites. Further studies are needed to show how far the different reproduction behaviour of *V. jacobsoni* in *A. cerana*, *A. mellifera* or in European and African origins of *A. mellifera* can be explained by different climatic conditions in the regions of origin (KRAUS and VELTHUIS 1997), origin of the parasite (ANDERSON and FUCHS 1998) and/or genotype environmental interactions.

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