Abstract
Recent studies have proven defensive abilities of the Western honeybee (Apis mellifera) against the ectoparasitic mite, Varroa spp. For a quantitative genetic study of individual uncapping and removing of Varroa-infested brood cells, 1436 individually marked bees from 22 unrelated colonies from three different genetic groups were continuously observed using an infra-red video technique. The variance between colonies, the intra-class correlation coefficient, and heritability was estimated with a Bayesian threshold model approach. Differences between genetic groups were significant. Colonies within groups differ significantly with respect to uncapping and removing. The variance between colonies was more than tenfold the residual variance for both traits, and heritabilities were found to exceed the normal parameter space (>1). The very high correlation of workers within colony cannot be exclusively explained by additive genetic resemblance. The possible impact of common prenatal environmental (maternal) effects, and non-additive inheritance as well as the influence of a putative major gene are discussed.

Introduction
The ectoparasitic mite Varroa destructor has caused extensive damage to the Western honey bee (Apis mellifera). In the Eastern honeybee A. cerana, there is no evidence of epidemic level parasitization because a balanced host-parasite relationship exists due to defence mechanisms, such as ability to catch and kill mites, nest-mate grooming and self-grooming (PENG et al., 1987a), efficient removal of Varroa-infested brood (PENG et al. 1987b) and non reproduction in worker brood (KOENIGER et al., 1983). Some studies have also indicated the natural defence mechanism in A. mellifera, but to a lesser extent than in A. cerana (PENG et al., 1987a). THAKUR et al. (1997) found that Varroa resistance traits in Apis mellifera, e.g. uncapping and removing, are performed at different levels of intensity within a colony. Some workers are very active, whereas the majority is inactive. These results may be explained by the composition of a colony where various patrilines with different distributions of behavioural response thresholds are present. Consequently, we started (unpublished results) to select worker bees showing an extraordinary active uncapping behaviour toward varroa-infested brood cells during 12 days infrared video observation. These workers, normally infertile, were induced to lay eggs. In the honeybee, unfertilized eggs develop into drones, whose sperm can be used for insemination. To evaluate the possible success of this breeding concept, information on the heritability of such individual behaviour is necessary.

Materials and Methods
To compensate for age effects on Varroa defensive characters, only freshly emerged worker bees (0-12h old) were used. The bees came from 22 unrelated colonies and were individually labelled with numbered plates on the thorax region. 3 samples of bees came from pure Apis mellifera mellifera (2 from Poland, 1 from Norway), 14 from A. m. carnica colonies from different breeders in Germany and 5 carnica-influenced but not purebred colonies ("land-race"). The colonies were not pre-selected according to hygienic behaviour. About 65 (±10) marked bees (total n = 1436) from each colony were kept in a frame cage with a sliding panel of glass on one side and metal gauze on the opposite side. The cage contained a brood frame with both sealed and unsealed brood, honey and pollen. The queen was also placed within the cage so as to provide harmony and stabilisation during the long term observation. The six frame bee colony was kept in a polystyrene beehive and the caged brood frame was inserted with the gauze facing the rest of the colony (allowing contact), and the glass panel against the wall of the beehive. Through a small hole in this wall (sealed off from normal light) observations could be made with an infrared-video camera (with a black/white CD-chip). We used infrared light because it does not disturb bees and they do not react to it during long observations.

80 freshly capped worker brood cells from the observation comb were each infested with phoretic mites. Parallel control cells (n = 20) were prepared as above but without mites. Recordings were analysed for the number of brood cells where individual workers were involved in uncapping and removal. Bees that died during the course of the investigations were excluded from statistical analysis.

Individual uncapping and removing behaviour is not normally distributed. Bees either execute a special behaviour or do not and because this kind of data generates no normal distribution, traditional methods
of heritability estimation cannot be applied. Like many other characters of biological interest and economic importance, this behaviour may also have an underlying continuity with a threshold, which imposes a discontinuity on the visible expression. The assumption that all or no traits have an underlying continuity on a non-visible physiological basis, enables heritability estimates of such characters (FALCONER and MACKAY, 1996). For the estimation of the heritability ($h^2$) we used a Bayesian threshold model (SÖRENSEN et al., 1995).

Results

34 of the 80 Varroa-infested cells were uncapped during the 12d capped brood phase. 88% of those cells were uncapped during the 4th and 7th day after capping. Only 2 control cells were opened, which were excluded from the calculations. On average 6.8 bees were involved in uncapping of one infested brood cell. Individual uncapping activity differed widely. 83.7% of the bees were not involved in uncapping, the remaining 16.3% (234 bees) were involved in uncapping of at least one infested brood cell, whereas 0.3% were involved in uncapping of more than 5 (max. 9) cells.

The difference between the three bee origins was highly significant with regard to uncapping (error probability 0.0033) and removing (error probability 0.0001), respectively. The average percentage of bees involved in uncapping was 5.9% in A.m.mellifera, 12.5% in the “land-race” bees and 19.5% in A.m.carnica. The corresponding values for removing infested brood were 14.7%, 24.4% and 32.4%. The colony effect was highly significant for uncapping and removing Heritability was estimated as 3.1± 0.25 for uncapping and 3.0 ± 0.23 for removing.

Discussion

Until now hygienic behaviour against experimentally killed, ill (e.g. Ascosphaera apis, Paenibacillus larvae larvae) or parasitized (Varroa) brood has been observed according to the colony performance. Percentages of removed cells were used in breeding programmes or to estimate genetic differences between honeybee strains. Quantitative genetic studies on hygienic behaviour using experimentally killed brood showed moderate to high heritabilities. Similar to our experimental approach, BOECKING et al. (2000) estimated the heritability of the mean percentage of removed Varroa infested cells to be 0.18. In general, hygienic behaviour is known to be strongly affected by environmental influences such as colony size or nectar flow age of workers and the interaction between different patrilines within a colony which influence colony expression, increase phenotypic variation and consequently tend to lower $h^2$-estimates.

The present study is the first, which has tried to estimate heritability of individual behaviour. Individual response to the unknown stimuli and the initiation of uncapping and removing of infested cells was found to be more genetically determined than the corresponding behaviour of the whole colony. Standardisation with respect to environment and age demography may partly explain this result, however the present estimates for individual uncapping and removing clearly exceed the parameter space (0 to 1).

All individuals were tested simultaneously and each had an equal chance of participating in uncapping and removing. However, offspring descending from the same colony shared the same environment during their prenatal development (maternal effects), which may affect their behaviour in their adulthood. Also the genetic makeup of bee colonies tends to overestimate the heritability, because the genetic variance contained additional dominance and epistatic components. In addition, the impact of major genes (genes with large effects on the expression of a trait) often result in $h^2$-value larger than 1 (FALCONER and MACKAY,1996).

The absolute values of the heritabilities of individual uncapping and removing of Varroa infested brood cells are overestimates, however the highly significant difference between the three bee origins also indicates a strong genetic determination. As shown in other studies, behaviour in the honeybee and in other species seems to be often influenced by major genes and if a major gene is involved in this detecting and removing of Varroa infected cells, it may someday be used in advanced techniques, such as marker assisted selection. But even traditional breeding methods can profit from the strong genetic determination of the observed traits. Especially selection based on the individual performance of specialist bees and using the reproductive ability of normally infertile honeybee workers seems to be an efficient way of achieving genetic progress in such characteristics (unpublished results).

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REFERENCES


