

## Sequential hygienic behavior in Carniolan honey bees (*Apis mellifera carnica*)

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Genet. Mol. Res. 8 (2): 655-663 (2009)

Received December 12, 2008

Accepted January 26, 2009

Published June 9, 2009

**ABSTRACT.** We examined the sequence, order or steps of hygienic behavior (HB) from pin-killed pupae until the removal of them by the bees. We conducted our study with four colonies of *Apis mellifera carnica* in Germany and made four repetitions. The pin-killing method was used for evaluation of the HB of bees. The data were collected every 2 h after perforation, totaling 13 observations. Additionally, for one hygienic colony and another non-hygienic colony, individual analyses of each dead pupa were made at every observation, including all details, steps or sequences of HB. The bees recognize the cells containing dead pupae within 2 h after perforation, initially making a hole in the capping, which is the beginning of HB. Uncapping of the dead brood cell reached maximum values from 4 to 6 h after perforation; after 24 h, practically all cells were already uncapped. Another variable, called brood partially removed, was analyzed 4 h after perforation, after the cells had been perforated, which involved uncapping, followed by partial or total removal of the brood. Maximum values of brood partially removed were found 10 h after perforation, though such cells could be found up to 48 h after perforation. The most frequent sequence of events in both colonies

was: capped cell → punctured cell → brood partially removed → empty cell. A new model of three pairs of recessive genes (uncapping u1, u2 and remover r) was proposed in order to explain the genetic control of the HB in *Apis mellifera*. We recommend evaluating HB 24 h after perforation and using a correction factor to compensate for control removal levels. We found a series of details of HB, which allow a study of how various factors may affect the sequence of the activities involved in HB and investigation of the genetics that controls this process.

**Key words:** Hygienic behavior; Honey bees; *Apis mellifera carnica*; Sequences of hygienic behavior; Carniolan bees