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The Fate and Effect of Hairs Removed from Honeybees with Hairless-Black Syndrome

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Honeybees, *Apis mellifera,* attacking other bees exhibiting hairless-black syndrome were found to have ingested bee hairs probably as a result of the attack. Experimental samples of bees were fed bee hairs and virus isolated from sick bees both separately and in combination. Control samples, samples fed hair, samples fed virus, and samples fed both hairs and virus averaged 8, 7, 37, and 70% mortality, respectively. Presence of hairs enhanced the effect of the virus.

INTRODUCTION

One colony sign of hairless-black syndrome, a malady of the adult honeybee, *Apis mellifera,* is an attacking behavior on certain bees by their hive-mates (Kulinčević et al., 1969; Rothenbuhler et al., 1969). This behavior involves apparently healthy bees scissoring their mandibles over the surface of sick bees (Waddington, 1971). This activity removes the hairs from sick bees and renders the underlying black integument more visible.

Kulinčević (per. comm.) found evidence that some of the removed hairs were ingested by attacking bees. The present investigation was designed to further explore this observation and determine whether ingested bee hairs influenced the mortality resulting from exposure to a virus isolated from bees exhibiting the syndrome (Rinderer, 1974).

Overall, the investigation involved counting bee hairs in the guts of bees in various categories, determining whether bee hairs could be fed experimentally to groups of bees, and studying mortality induced by feeding bee hairs and virus both separately and in combination.

MATERIALS AND METHODS

Forty attacked bees, 40 attacking bees, and 40 bees neither attacked nor attacking were collected from the entrances of free-flying colonies. Their guts were removed, squashed on microscope slides in a drop of water, and the contents were examined under phase contrast optics at 100–200×. Each item judged to be a hair or a piece of hair was counted using the criterion of the presence of at least two side branches on the hair shaft.

For determining whether bee hairs could be experimentally fed to adult bees, the hairs on the thoraxes of 102 worker bees were removed. This removal involved freezing young healthy bees from free-flying colonies, pulling away the heads, abdomens, wings, and legs from the thoraxes, and shaving the thoraxes under a dissecting microscope with a chip of razor blade. The removed hairs were collected from the microscope stage and were mixed by agitation with glass beads in 50% sucrose syrup. This mixture was fed over 3 days to 2 samples of 50 bees, 0–24 hr of age when put into laboratory cages identical to those described by Kulinčević et al. (1973). Sucrose syrup not containing bee hairs was fed to two similar samples of bees as a control. After the feeding period, 45 bees from each sample were killed and their gut contents were examined for the presence of hairs.

For the virus-hair feeding experiment, 20 samples, each of 50 0–24 hr-old bees were

TABLE 1
Bee Hairs Observed in the Guts of Adult Honeybees Collected from Free-Flying Colonies

Activity of bees	Number of bees	No. of hairs present: Mean of raw data ($\bar{X} \pm s$)	Mean of transformed data[a] ($\bar{X} \pm s$)
Attacked	40	52.7 ± 74.6	1.4 ± 0.56**
Attacking	40	21.4 ± 24.8	1.0 ± 0.63**
Neither attacked nor attacking	40	1.9 ± 3.3	0.28 ± 0.37**

[a]Transformed by log (X + 1).
**Significantly different from other means ($P < 0.01$).

put into laboratory cages. Hairs to be fed to some of these samples were shaved from thoraxes of worker bees, and virus was collected as described by Rinderer et al. (1975). The 20 samples were divided into four treatment groups and fed (1) virus and bee hairs, (2) bee hairs only, (3) virus only, or (4) neither. Each of five samples in the first treatment group received, on each of 3 consecutive days, the virus particles from eight and one-third sick bees suspended in 50% by volume ribonuclease-free sucrose[1] syrup in a 2-ml container. Samples in the first treatment group also received, on each of the 3 days, bee hairs shaved from 17 bee thoraxes in sucrose syrup in a second container. A second treatment group of five samples of bees received feedings of virus in sucrose syrup and feedings of sucrose syrup without hairs; a third treatment group of five samples received feedings of hair in sucrose syrup and feedings of sucrose syrup without virus; a fourth treatment group received paired feedings of sucrose syrup with no hair or virus. After these feedings, all samples had continual access to sucrose syrup and sterile distilled water. Each sample of bees was observed, dead bees removed, and mortality recorded daily for 14 days starting the first day treatments were administered.

All data were analyzed by analyses of variance and least significant difference tests. Counts of hair were transformed to log (χ + 1) in order to achieve uniformly normal distributions for analysis (Snedecor and Cochran, 1967).

[1]Ribonuclease-free sucrose obtained from Sigma Chemical Company, St. Louis, Missouri.

RESULTS AND ANALYSES

Of the bees collected from free-flying colonies and examined for ingested hairs, attacked bees averaged 53 hairs in the gut, attacking bees averaged 21 hairs, and bees engaged in neither activity averaged only two hairs (Table 1). The mean number of hairs was significantly different ($P < 0.01$) for each of the three categories of bees. From these observations it is concluded that hair ingestion does occur during attacking behavior. Consideration of the question raised by the large number of hairs present in the gut contents of attacked bees is presented in the discussion.

In experimentally fed groups, those bees fed hairs averaged 59 hairs per bee, while those not fed hairs averaged only 3 hairs (Table 2). This is a significant increase ($P < 0.01$) in the number of hairs present in the guts of bees fed hairs when compared to controls. The presence of large numbers of hairs still in the feeding dishes after the feeding period indicated that only a small number of the total hairs available were ingested. Nevertheless, since the hairs that were ingested were similar in number to those found to occur as a result of attacking behavior, it seemed possible to explore the effect of ingested hairs.

In the virus–hair feeding experiment, bees fed hairs without virus or fed only sucrose syrup displayed similar averages of 7 and 8% mortalities (Table 3). Bees fed virus without hairs had an average mortality of 37%. Bees fed virus and hairs showed the significantly higher ($P < 0.01$) average mortality of 70%. Treatment with only hairs does not affect

TABLE 2
Bee Hairs Observed in the Guts of Adult Honeybees After 3 Days Feeding on Sucrose Syrup Laden with Bee Hairs or Sucrose Syrup Alone

Treatment	Number of bees	No. of hairs present: Mean of raw data ($\bar{X} \pm s$)	Mean of transformed data[a] ($\bar{X} \pm s$)
Hairs in sucrose syrup	90	59.26 ± 3.6	1.65 ± 0.04**
Sucrose syrup	90	3.00 ± 3.6	0.39 ± 0.04**

[a]Transformed by log (X + 1).
**Significantly different from the other mean ($P < 0.01$).

mortality, while treatment with only virus does. Treatment with virus and hairs together results in a greater mortality than that resulting from virus alone. It is concluded that the presence of ingested hairs in their guts affects bees in ways which increase their susceptibility to the virus.

DISCUSSION

Kulinčević et al. (1969) as well as Waddington (1971) have observed in natural situations the sequence of bees first attacking, then becoming sick, and finally themselves becoming subject to attack. This sequence would account for the greater number of hairs present in attacked bees when compared to their attackers. Apparently the attacking phase of the sequence is the major period when bees ingest hairs. Attacked bees, already past the attacking phase of the sequence, would as a group have more hairs in their guts than active attacking bees not yet finished with this hair-ingesting phase of the sequence. Further, the sequence strongly suggests that bees acquire virus as they attack sick hive-mates. This acquisition may occur during the feeding events interrupting attacking behavior which were described by Waddington (1971).

TABLE 3
Average Percent Mortality of Adult Honeybees Fed the Indicated Materials in Sucrose Syrup

Materials fed in sucrose syrup	Number of replicates[a]	Average percent mortality ($\bar{X} \pm s$)
Hairs[b] + virus[c]	5	69.6 ± 7.2**
Virus[c]	5	37.2 ± 10.2**
Hairs[b]	5	7.2 ± 2.3
Nothing	5	7.6 ± 2.6

[a]50 Bees per replicate.
[b]Each replicate received the hairs collected from 51 bee thoraxes.
[c]Total virus per bee was that collected from 0.5 bees (approximately 10^8 particles).
**Significantly different from all other means ($P < 0.01$).

While the coincident ingestion of virus and hairs during attacking behavior increases the incidence of mortality, the precise mechanism is unclear. Most probably the hairs, with their branches terminating in sharp points, mechanically damage the gut as they pass through, increasing substantially the number of potential infection sites available to the virus.

Some authors have suggested that attacking behavior rids the colony of sick bees, thereby lowering the incidence of disease in the colony (Morison, 1936; Burnside, 1945). Waddington (1971), while not offering an alternative, was unsure of this interpretation when he found attacked bees remained in a colony for long periods. His observations coupled with the present data show that attacking behavior results in conditions which promote the incidence of disease within a colony. Not only does the interaction between the attacked and attacking bee result in the spread of virus, it also results in hair ingestion, which renders bees more susceptible to the disease.

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