

Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers

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Summary. Individual worker dominance correlated with trophallactic behavior, which affects several social behaviors related to colony fitness, shows a high genetic variance in worker bees. In a bioassay we tested trophallactic behavior of workers and selected dominant (receiving) and subordinate worker bees (offering) of *Apis mellifera capensis* to establish genetic lines of both kinds. Queenright test colonies were experimentally composed of 100% subordinate workers, 100% dominant workers, 50% dominant plus 50% subordinate workers, and 100% hybrid workers from the two genetic lines. The chosen test parameters were brood-rearing, comb building, and hoarding behavior. In all cases, the colonies of pure subordinate bees showed the best colony performance, whereas the colonies composed of only dominant bees were nearly unproductive. The mixed colonies (50% dominant + 50% subordinate) ranked in the middle and did not differ significantly from the hybrid colonies. The results indicate that colony performance under queenright conditions depends on the proportion of subordinate workers. This result supports a selection model based on the combination of individual selection and on group selection at the colony level, which explains the high genetic variance of individual worker reproduction.

Introduction

Division of labor in the honeybee colony is strongly age dependent (Rösch 1925, 1927; Lindauer 1952). Corresponding to a *life program* of

polyethism, each worker bee runs through a sequence of different tasks during its lifetime. This general life plan of the honeybee worker leads from cell cleaning through brood tending and comb building to foraging. More detailed studies show that this division of labor is not a fixed pattern (Rösch 1930; Sakagami 1953) and is normally affected by the status and needs of the colony (Seeley 1982; Winston and Punnett 1982). Nevertheless, it seems that workers of the same race are more or less uniform and that they are determined and regulated mainly by environmental (colony) conditions. Genetic differences among workers and individual characteristics supposedly have little or no impact. The first results on genetically directed polyethism were reported by Moritz and Hillesheim (1985) and recently by Frumhoff and Baker (1988) and Robinson and Page (1988). Moritz and Hillesheim (1985) showed that there are different degrees of reproductive dominance among worker honeybees of *Apis mellifera capensis* that are genetically determined. Dominant workers have more developed ovaries, an earlier onset of oviposition, lay more eggs, and produce more 9-Oxo-2-decenoic acid (9-ODA). This classical queen pheromone (Butler 1954; Crewe 1982), which suppresses ovary development in workers (Butler and Fairey 1963) is common in laying worker bees of the race *A. m. capensis* (Hemmling et al. 1979; Crewe and Velthuis 1980). Furthermore, Korst and Velthuis (1982) showed that for the same race, certain properties of trophallactic behavior are positively correlated with reproductive dominance, such that dominant workers are mostly fed by others, whereas the subordinate bees regularly offer food. Furthermore, the difference in trophallactic behavior, and, therefore, the different individual dominance levels persist under queenright colony conditions (Hillesheim 1986) and may affect polyethism in general.

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The fitness of individual worker bees is not zero. Under queenless conditions the dominant workers develop their ovaries and suppress oogenesis in subordinate bees. Dominant workers have a higher individual fitness than subordinate ones and, consequently, reproductive worker dominance should lead to fixation in the population. However, Moritz and Hillesheim (1985) found a large genetic variance of individual worker dominance in a natural population. A model in which group or colony level selection balances individual selection most plausibly explains this phenomenon. In this paper we try to clarify the following:

1. Does the degree of dominance effect the division of labor and, therefore, influence colony efficiency?
2. Is there a difference in colony efficiency in queenright colonies that varies strongly in relation to their composition of dominant and subordinate bees?
3. Is colony selection likely to maintain the high genetic variance of individual worker dominance in natural populations?

Methods

1. Selection and breeding scheme

Laying worker bees of *Apis mellifera capensis* produce female offspring by thelytokous parthenogenesis (Verma and Ruttner 1983). Worker bees from six unrelated *A. m. capensis* queens imported from South Africa were selected for the following experiment. Figure 1 shows the steps of the disruptive selection. Sealed brood combs from colonies headed by the original *capensis* queens were placed in an incubator (34° C; 70% r.H.). The newly emerged workers were collected every 24 h and kept in small cages (at 27° C; sugar cake, pollen, and water were given *ad libitum*) for two days. The bees were then tested for their trophallactic behavior according to Moritz and Hillesheim (1985). Cape worker bees showing extreme dominant or subordinate behavior were introduced into small queenless colonies of *Apis mellifera carnica* where they started to oviposit. The eggs developed into workers or were introduced into breeder colonies for queen rearing. From each offspring group, several workers (28 ± 10) were tested for their trophallactic reaction, which showed the typical behavior of their worker mothers. For both directions of selection, i.e., dominant as well as subordinate, we produced six different offspring groups (named D1 to D6; S1 to S6) descended from twelve selected egg-laying worker bees. Queens were reared in each group. Some of these queens were used for drone production. Queens from each selected line were instrumentally inseminated with semen of drones from other selected lines. A sample of worker bees produced by each inseminated queen was again tested for trophallactic behavior, production of 9-ODA, first day of oviposition, number of eggs laid, and ovary developmental stage according to the methods of Moritz and Hillesheim (1985). They retained the behavior of their origin worker line. Sister bees from these strains were used for the subsequent colony tests. The following nomenclature was used:

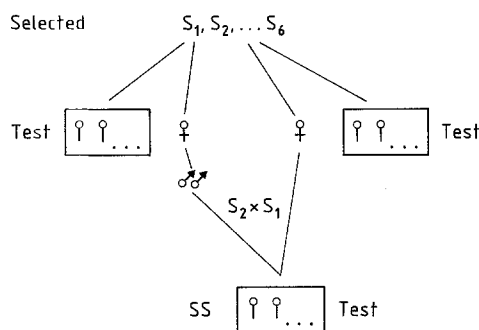


Fig. 1. Scheme of the disruptive selection of worker genotypes within a colony with an example from one subordinate line. Test = trophallactic behavior. Selected = selected worker bees, each one characterized by a number (1, ..., n); S = subordinate line; ♀ = worker bee; ♀ = queen; ♂ = sperm from drone producing queens. Detailed description of the breeding process is given under *Methods: 1. Selection and breeding scheme*

Table 1. Composition of the tested colonies

No.	Type of composition	Abbreviation
1.	100% dominant worker bees	DD + DD
2.	100% subordinate worker bees	SS + SS
3.	50% dominant and 50% subordinate worker bees	DD + SS
4.	100% hybrid worker bees	DS + DS

DD = worker bees from dominant queens inseminated with semen of drones from another dominant line

SS = worker bees from subordinate queens inseminated with semen of drones from another subordinate line

DS = worker bees from a dominant queen inseminated with semen of drones from a subordinate line

DD queen = queen of a dominant line inseminated with sperm from another dominant line. The queen produces DD workers.

SS queen = queen of a subordinate line inseminated with sperm from another subordinate line. This queen produces SS workers.

DS queen = queen of a dominant line inseminated with sperm from a subordinate line. This queen produces hybrid workers.

2. Composition of queenright test colonies

Sealed worker brood from the selected *capensis* queens (see Fig. 1 for example $S_2 \times S_1$) was placed in an incubator (34° C; 70% r.H.). A total of 1500 to 2000 newly emerged workers were collected daily over a two-week period and placed in a four frame small nucleus (*Kirchhainer Begattungskästchen*) with a caged virgin Carniolan queen. For each composition we used a queen from an established line of another race to minimize possible effects of the queen. The unrelated queen was caged to make sure that none of the eggs laid by the *own* queen (in our case only unfertilized eggs) would lead to potential egg preferences. The different types of composition are shown in Table 1. For the composition of the DD + SS colonies (Table 1, 3), we had to mix unrelated worker bees from two different queens; therefore, the pure colonies (see Table 1: 1, 2, and 4) were also composed of workers from two different selected lines with similar dominance behavior.

3. Colony tests

Brood rearing. For measuring brood rearing capacity of the test colonies, we used brood from a well established *A. m. carnica* line in order to equalize the rearing material with regard to relatedness and dominance degree, which is very low in this line. Combs with eggs and freshly hatched larvae from Carniolan queens were introduced into broodless test colonies (at any one time no more than two combs per colony). After 2 days (control interval), when eggs and larvae were completely removed by members of the test colonies, the empty brood combs were replaced with new ones. The total partial removal of larvae by the bees has its reason in dead larvae, no acceptance of alien different racial brood and more or less undeveloped pharyngeal glands which lead to starving brood. After 12 days the remaining sealed brood cells were counted. In 28 different test colonies 93 brood combs, each with on average 210 ± 28 eggs and larvae, were tested.

In order to control for the effect of rearing alien brood, colonies of *A.m. carnica*, under the same conditions as the test colonies, were given combs containing eggs and freshly hatched larvae from unrelated *A. m. carnica* queens. Any differences between test and control colonies were attributed to racial differences.

Comb building. Each colony was provided with four small strips of wax foundation (24 mm^2) attached to a perspex holder of known weight. After 2 days the colonies were placed in an incubator (27° C ; 60% r.H.) for 4 days and fed 2 M sucrose *ad libitum* by a gravity feeder. After removing the stored sugar solution by carefully washing the wax strips in water and drying them in an incubator for 24 h at 36° C , the weight of the wax strips was measured to an accuracy of $\pm 1 \text{ mg}$. The area of newly built cells (mm^2) was determined using a contact area integrator (Digiplan MOP-AM 02 KONTRON, Munich).

Hoarding behavior. Four empty combs were supplied to each colony. The test colonies were kept in the incubator (27° C ; 60% r.H.) and were fed a 2 M sugar syrup for 4 days. Combs were weighed and the sugar concentration (60–75%) of the stored syrup in the combs was measured with a refractometer. Using this information, the absolute amount of stored sugar (g) was calculated, demonstrating the hoarding performance of the different types of test colonies.

Results

Brood rearing

Table 2 shows the results of the brood-rearing test. The large discrepancy between the rearing rate of the Carniolan colonies (69%) and the best *A. m. capensis* colonies can be explained by an intraracial effect between nurse bees and brood and/or indicates that workers of *capensis* are less capable brood rearers.

All four tested colony compositions differ significantly at the 5% level in their rearing efficiency ($\chi^2 = 449.1$, $df = 3$). Not only the proportion of reared larvae (Table 2: rearing efficiency), but also the quality of the caps of the sealed brood cells (see Fig. 2), reveals a big difference in brood care ability between the *dominant* and the *subordinate* colonies.

Table 2. Results of the brood care test

Colony type	<i>n</i>	offered brood	sealed brood	rearing efficiency %
100% <i>SS+SS</i>	9	7780	1166	15.0
50% <i>DD+50% SS</i>	5	4471	532	12.0
100% <i>DS+DS</i>	10	6886	444	6.5
100% <i>DD+DD</i>	4	2921	54	1.9
Carniolan (cont.)	3	648	447	69.0

Abbreviation for the colony types same as in Table 1

n = number of tested colonies

offered brood = total number of offered brood (eggs and newly hatched larvae) for all colonies used in the specific colony type

sealed brood = total number of sealed brood for all colonies used in the specific colony type

rearing efficiency % = the percentage of sealed brood to offered brood

Carniolan (cont.) = 100% Carniolan bees as control group for the intraracial brood rearing efficiency

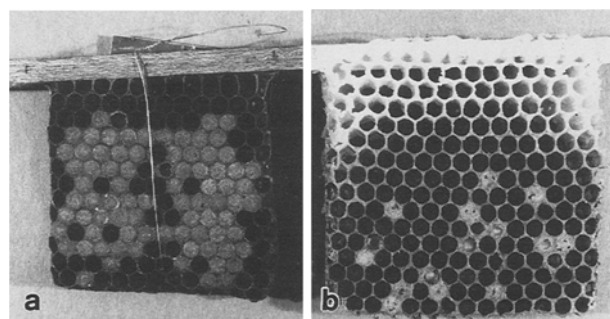


Fig. 2a, b. Appearance of two typical brood combs removed from different types of colonies. **a** Colony of 100% subordinate worker bees (*SS+SS*). **b** Colony of 100% dominant worker bees (*DD+DD*)

Comb building

Two different parameters of comb building behavior were analyzed. Weight increase was used as a measurement of wax production, and surface area of constructed comb as an indicator of construction capacity, independent of wax production, since sufficient wax was available for building the first cells. Figure 3 shows the results for both criteria. An analysis of variance on the comb surface data ($F = 10.25$, $P \leq 0.01$) and on the wax production data ($F = 8.36$, $P \leq 0.01$) reveals significant differences among the tested colonies. The results for cell-building capacity (comb area) show that the dominant bees did not construct even a single cell (Fig. 4). The *DD+SS* and the *DS+DS* colonies did not differ from each other, whereas the *SS+SS* colonies were significantly more efficient (Fig. 3). The negative values for the comb weight of the *DS+DS* and the *DD+DD* colonies demonstrate that these colonies not only failed to produce wax but also deformed the wax strips (Fig. 3 and 4)

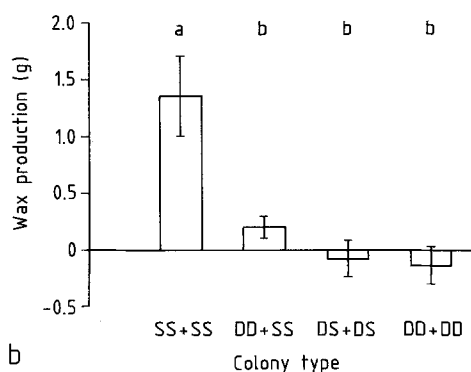
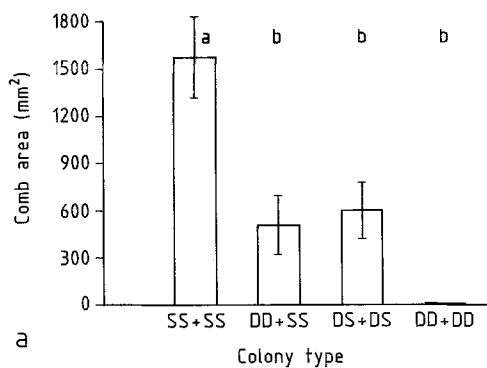


Fig. 3a, b. Mean values for building behavior using two measures. **a** The area of wax built, measured in mm². **b** Mean values for wax production, measured in g. *SS+SS*: $n=9$; *DD+SS*: $n=7$; *DS+DS*: $n=8$; *DD+DD*: $n=4$; n =number of measured colonies; each colony contained 4 combs; I =standard error of mean (*SEM*). Columns headed with the same letter did not differ significantly (Tukey multiple comparisons test)

that were provided. Only the *SS+SS* and the *DD+SS* colonies showed wax production during the test period.

Hoarding behavior

The results from the hoarding behavior test are displayed in Figure 5. The *DD+DD* colonies did not store any sugar syrup. The *DD+SS* and the *DS+DS* colonies show intermediate hoarding values. The *SS+SS* colonies are again the best types. An analysis of variance reveals significant differences among colony types at the 5% level ($F=4.027$), with *DD+DD* differing from the other groups.

Discussion

Our results show highly significant differences in colony efficiency among the colony types tested. The ranking sequences of the different colony types in all of the tests are unexpectedly uniform, especially considering the different behavioral and

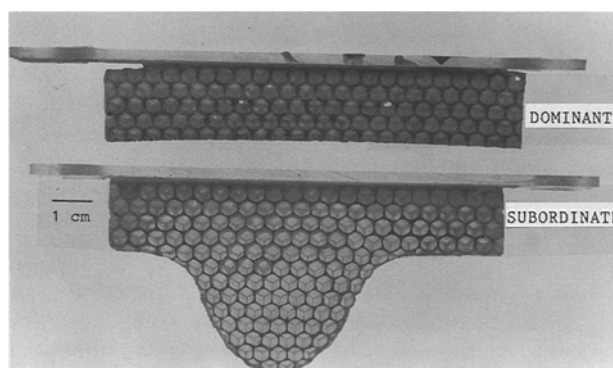


Fig. 4. Appearance of wax strips after test period. Dominant = wax strip from a colony of 100% dominant bees (*DD+DD*). The holes in this strip were bitten by the bees during the test period. Subordinate = wax strip built up by a colony composed of 100% subordinate bees (*SS+SS*)

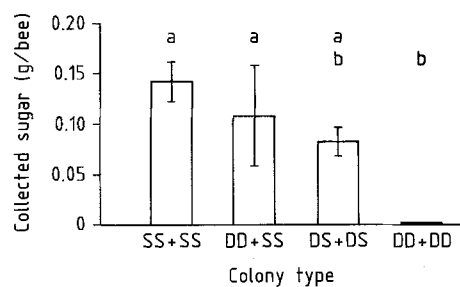


Fig. 5. Mean values for the hoarding behavior of colonies measured in gram per bee. *SS+SS*: $n=10$; *DD+SS*: $n=4$; *DS+DS*: $n=7$; *DD+DD*: $n=5$; n =number of tested colonies; I =standard error of mean (*SEM*); Columns headed with the same letter did not differ significantly (Tukey multiple comparisons test)

physiological features that are involved in the different tests. In all tests the pure subordinate colonies were the most efficient, whereas the dominant colonies were the most inferior. The mixed and the hybrid colonies had intermediate phenotypes between the two pure colony types. Consequently, it can be stated that colony efficiency is negatively correlated to the proportion of dominance genes and dominant bees in the colony.

The work activities of the dominant worker bees with respect to their *age dependent* polyethism tasks seems to be very low. They probably follow another strategy (Schmid-Hempel 1989; Schmid-Hempel and Wolf 1988), i.e., saving their energy and *waiting* for the chance to become an egglayer. The degree of individual worker reproduction is determined by strong gene effects (Moritz and Hillesheim 1985). The individual reproductive fitness of the worker caste is not zero, especially in the case of the dominant worker bees. Whenever the colony loses its queen during mating flights (ca.

19–30% in Europe and Middle East, see Tiesler 1972 and Lensky and Demter 1985), and there are no eggs or young larvae to rear into a new queen (e.g., after swarming), the dominant worker bees, which have a higher individual fitness than the subordinate ones, develop their ovaries and start egg laying (diploid eggs: workers and queens in the case of *A. m. capensis*; haploid eggs: drones in other *A. m.* races). Because of the individual fitness of the workers, genes for dominant behavior in the population should increase and go towards fixation. However, in queenless colonies with laying workers, dominant bees need subordinate worker bees to rear their brood. Hence, reproductive success of the dominant workers and colony efficiency still depend on the proportion of subordinate workers. Egg laying alone is not sufficient to transmit genes to the next generation. Without a sufficient percentage of subordinate, phenotypically altruistic bees that work, a colony of pure dominant bees will collapse. Therefore, the spread of reproductive genes is limited by adverse effects on the efficiency of the colony.

If our results obtained with small experimental colonies are also valid for natural honeybee colonies, they will have implications on colony level selection. Our results suggest that colony fitness strongly depends on the genotypic composition and the proportion of subordinate *altruistic* bees. Under queenright conditions, natural selection at the colony level should favor colonies composed entirely of subordinate workers; therefore, the frequency of subordinate genes in the population will increase. Only well-developed colonies with a large number of individuals and a high level of food stores will be able to contribute genes to the gene pool. Such colonies must consist mainly of subordinate bees. Only then will they show a high brood-rearing rate, good comb building ability, and well-developed foraging behavior. As a result, they will increase colony size quickly at the beginning of the season and rear a high number of successful reproductives (queens and drones). Furthermore, only strong colonies produce swarms of sufficient size to ensure the survival of the newly created colonies. However, in the case of queen loss and absence of young female larvae, a colony with dominant workers is favored by natural selection, because these bees will have a higher individual reproductive fitness, whereas a colony consisting only of subordinate workers will have a low colony fitness. Subordinate workers will not start oviposition quickly and the colony will therefore dwindle, because no brood will be produced to compensate for the natural death rate of bees. With increasing

time until worker oviposition, successful rearing of reproductives becomes less likely. Under those conditions, the presence of reproductive workers will guarantee an earlier start of oviposition. Evidently, it seems to be *profitable* on the colony selection level to invest in a low portion of *idle*, dominant, but potentially reproductive, worker bees. Such a mechanism explains the large genetic variance of reproductive dominance among worker bees.

A combination of genes for worker dominance and genes generating facultative subordination (e.g., better brood-rearing as documented by Cole 1986 for the genus *Leptothorax allardycei*) and *vice versa*, would also be a good worker strategy. In other words, under queenright colony conditions, the workers behave subordinately and through this behavior guarantee a high colony efficiency. Under queenless conditions, the gene or genes for worker dominance are responsible for a high individual fitness connected with rapid ovary development, whereas the facultative gene or genes ensures a sufficient brood-rearing rate. The combination of genes for dominance with genes generating subordination in one individual will be an *autarc* strategy, because under queenright as well as under queenless conditions this type will show the best adaptation. Such a combination will go to fixation and result in a small genetic variance with respect to worker dominance. In our selection regime, workers were tested under queenless conditions. The facultative model predicted very low variability in dominance related behavior, because each individual had genes for worker dominance, fixed or facultative. If the facultative system were not yet established in the tested population, then one could explain the observed variability of dominance behavior under queenless test conditions. One would, however, expect higher variability in the colony tests in lines selected for dominance behavior, because this group includes pure dominance as well as the combination of dominance connected with facultative subordination. This is clearly not the case in our experiments.

Although we cannot rule out that such genes do exist in nature (Cole 1986), we have no evidence for them in our bee material. Hence, we conclude that genetic variance in dominance behavior is maintained through counteracting selection pressure. Colony level selection favors subordinate alleles with a low proportion of dominant individuals, and individual selection supports dominant alleles with maintenance of subordinates in order to guarantee the rearing potential of the colony.

These results experimentally support a selection

model based on a combination of the competitive group selection model of Wilson (1975) and classical individual fitness recently suggested by Moritz (1989).

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