Evidence for inhibition of corpora allata activity in workers of *Bombus terrestris* by a pheromone from the queen's mandibular glands[1]

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Summary. Queens of *Bombus terrestris* inhibit the activity of worker corpora allata by means of a pheromone which is produced in their mandibular glands. Exstirpated and homogenized glands as well as an extract of the queen's body surface show the same inhibitory effect as a living unmutilated queen. The pheromone remains on the body only for 1 day after the queen has been killed. The activity of the corpora allata of workers was determined volumetrically as well as by means of a juvenile hormone synthesis in vitro assay.

In several papers it has been shown that oogenesis in queenright workers of *Bombus terrestris* (L.) is inhibited during the first days after their emergence[2,3]; but egg formation is not completely suppressed, so that workers older than 30 days can start to lay eggs[4]. The sterility of young workers is a consequence of a low juvenile hormone (JH) titre in their haemolymph[5]. This titre is regulated by the rate of the JH synthesis by the corpora allata (CA)[6]. In presence of a queen the synthesis of JH is suppressed. In queenless workers, the JH synthesis increases[6], the hormone titre in the haemolymph is elevated[5], and the workers are able to lay eggs as early as the 5th day after their emergence[3]. But the stimuli by which a queen influences the activity of CA in workers has hitherto remained unexplained. The present studies were undertaken to test whether queens of *B. terrestris* inhibit the synthesis of JH in workers by pheromones, thus controlling the sterility of the workers.

Material and methods. Bumblebees. Colonies of *B. terrestris* were started with single queens which we collected in the field or with queens which emerged from our captive colonies and had hibernated in a refrigerator. The colonies were kept in a climate room at 28-30 °C[7]. For the experiments we used queens from the 40th day after their first oviposition onward.

Extraction of queens. The queens were killed by deep-freezing (−25 °C, 20 min). The extraction of the queen's body surface was performed twice with 2 ml of chloroform/methanol 3:1 for 5 min. The extract was concentrated under nitrogen, applied on a dead queen's body, and then dried by an air-stream.

Mandibular glands. The glands were excized under CO_2-narcosis after cutting off the mandibles, immediately deep-frozen, and stored at −25 °C. For the experiments the glands were homogenized in 400 µl chloroform/methanol 3:1 containing some wax from a nest involucrum, because the queen's body extract also contains wax. The solution was applied to a dead queen and dried. Furthermore, 5 pairs of glands were stored in acetone in sealed capillaries and homogenized as described before they were used.

Activity of ovaries. The ovaries were dissected in Ringer solution and the lengths of all 8 terminal oocytes were measured with an ocular micrometer. The mean value of all oocytes was used for the evaluation of ovarian activity.

Volume of CA. The CA were taken from the workers' heads in Ringer solution and transferred into a droplet of Grace's insect T.C. medium (GIBCO), in which the adhering tissue was removed. Then the glands were placed in a blood corpuscle counting chamber (depth 0.1 mm) containing a droplet of the medium. The CA volume was calculated from the plane drawn with drawing equipment for the microscope, and from the depth of the chamber. The mean value of both glands was used.

In vitro culture of CA. The synthetic activity of CA was determined using the short term in vitro assay developed by Tobe and Pratt[8] and modified for bumblebees as described in detail elsewhere[6,9]. The CA of 4 1-day-old workers were incubated together in glass vials containing 0.1 ml Grace's insect T.C. medium (GIBCO), and 1.25 µCi L-(methyl-^{14}C)methionine (sp. act. 57.2 mCi/mmole, Amersham-Buchler) was added to a final concentration of 0.55 mM methionine. The glands were gently shaked in the dark for 3 h at 30 °C. The synthesized amount of JH was determined by extraction with ethyl acetate, TLC separation, and liquid scintillation counting. In order to avoid any adsorption of synthesized JH on the glass[10], all incubation vials were rinsed with cold JH III before the incubation was started. By this method we obtained values somewhat higher than in earlier investigations.

Results. The inhibition of oogenesis in workers by the queen. In queenright workers, the terminal oocytes grow up to 0.15 mm until the 5th day after emergence. When newly emerged workers are put together in small queenless groups, a rapid oogenesis takes place and the workers can lay the first eggs (ca. 2.7 mm) on the 5th day[3]. These results showed that oogenesis is inhibited in queenright colonies, but it remained unclear, whether only the queen or other stimuli of the colony could inhibit egg formation.

In order to elucidate the influence of a queen, the following experiments were carried out. In 9 queenright colonies oogenesis was determined in 5-day-old workers. Then the queen was removed, the newly emerged workers were individually marked with colour spots, and the extent of their oogenesis was determined after 5 days. For comparison oogenesis in 5-day-old workers was once more measured 10-14 days after removal of the queen. The results are shown in figure 1. In the presence of a queen egg formation is inhibited in workers. After removal of the queen a rapid oogenesis starts; 5-day-old workers have maturing eggs. 2 weeks later, however, oogenesis is again suppressed in 5-day-old workers.

The results indicate that the inhibition of oogenesis in workers is caused by an egg-laying queen, but also by egg-laying workers[2]. Egg formation, therefore, is inhibited again in 5-day-old workers 2 weeks after removal of the queen, when egg-laying workers are present.

The influence of a queen's pheromone on the activity and the volume of CA in workers. By means of the CA in vitro assay we quantitatively monitored the ability of a queen to inhibit the activity of CA in workers. We determined the JH synthesis in 1-day-old workers, since on the very 1st day after emergence there exist clear differences between queenright and queenless workers[6]. In a previous investigation, by measuring histological sections, we could not observe a corresponding enlargement of the glands[5]. However, when we measured the volume of whole glands in a counting chamber clear differences in the volume of CA between both groups were observed on the 1st day as well. The increased JH synthesis in queenless workers, therefore, corresponds with an enlargement of the glands on the 1st day. The relationship between CA volume and synthetic activity is shown in figure 2. Since we found

measuring the volume of CA in a counting chamber to be a rapid method, we determined the activity of the glands only by this method in a 2nd group of experiments in this investigation.

First we tested by the CA in vitro assay, whether a queen inhibits the activity of the CA by a pheromone; whether the presumed pheromone is present on the queen's body surface; and how long this pheromone remains active after the queen has been killed. The experiments were carried out in the following manner: 4 freshly emerged workers were put together with a pupal comb (10–20 pupae) in a closed nesting box supplied with pollen and honey water. A living queen was added, and the activity of the CA of the workers was determined after 24 h. Then the queen was killed by deep-freezing and fixed on the comb with a pin without damaging the pupae. 4 newly emerged workers were added, and the activity of their CA was determined after 24 h. In this way the influence of 13 killed queens was tested; each of them was separately examined 1, 2, 4, 6 and 10 days after she had been killed.

In 7 control experiments we added a 1-day-old queenright worker and in 7 other controls a freshly killed 1-day-old queenright worker instead of a queen. In all experiments in which we used a dead queen or worker we added a 1-day-old queenright worker to the 4 newly emerged workers. She was replaced by another one after 10 h. By this method the influence of the activity of a living queen or worker itself on the newly emerged workers should be compensated in the controls. On the 1st day after emergence workers begin to exhibit great building activities on the comb or the nest cover.

In the presence of a living queen the CA of 1-day-old workers produced about 10 pmole JH per pair CA and h (figure 3). The same synthetic rate of the CA of workers was found when a freshly killed queen was added instead of a living queen. But 1 day later, JH synthesis was increased up to 14 pmoles JH per pair CA and h, reaching a level which was not statistically different (t-test) to the control experiments, in which the workers were together with a living or dead worker. The results show that the queen presumably produces a pheromone, which inhibits the activity of CA in workers, and, furthermore, that the activity of the pheromone is lost 1 day after the queen has been killed.

To test whether the pheromone is present on the body surface of the queen, 4 queens were killed by deep-

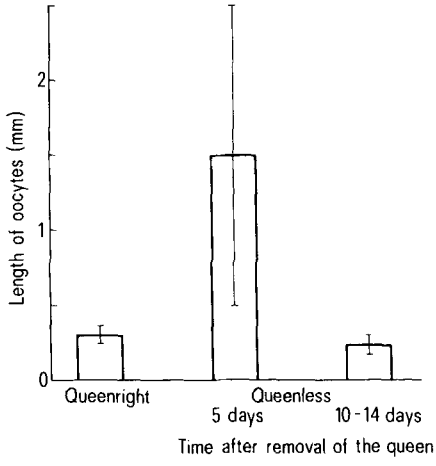

Fig. 1. Length of terminal oocytes in 5-day-old workers in queenright colonies and in queenless colonies 5 days and 10–14 days after removal of the queen. Vertical bars indicate SD.

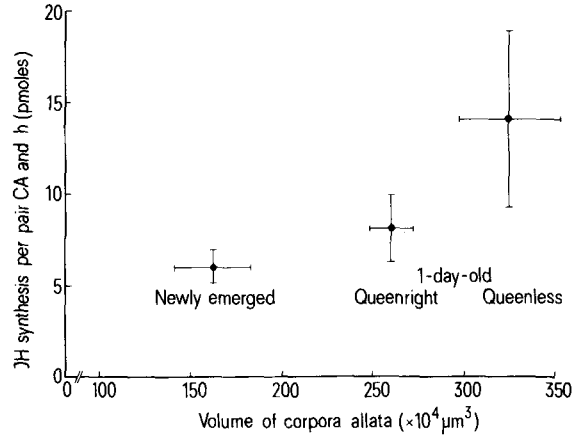

Fig. 2. Relationship between CA volume and JH synthesis in bumblebee workers. Bars indicate SD.

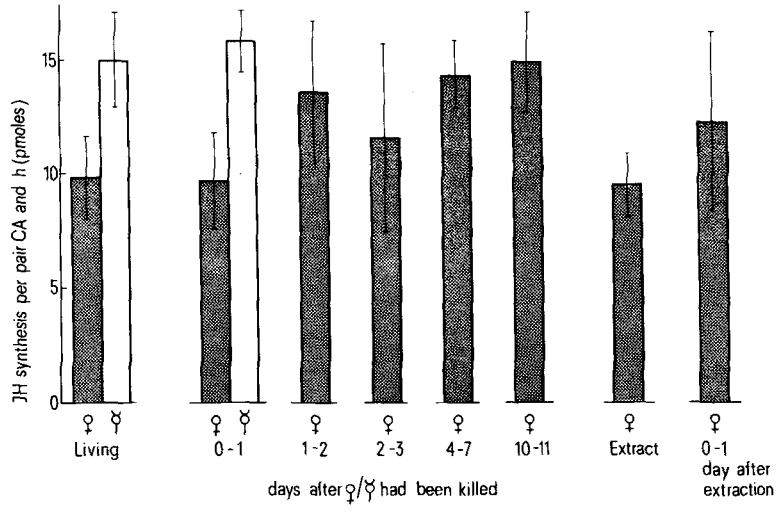

Fig. 3. The effects of living queens, dead queens, washed queens (0–1 day after extraction), and of the queen's solvent extract (♀ extract) on the JH synthesis by CA in 1-day-old workers, in comparison with the effects of workers. Vertical bars indicate SD.

freezing, washed with the extraction solvent, and dried. The extracts were applied to the bodies of queens which had been dead for over 10 days, and which had shown no inhibiting effects in former experiments. The results are presented in figure 3. In the presence of a queen's body freshly washed by the solvent (♀ 0-1 day after extraction), the JH synthesis in workers was strikingly increased, though the deviation of the values we found was fairly large. The solvent extract of the body surface (♀ extract), in contrast, had the same inhibiting effect as a living queen.

In a 2nd group of experiments, we tested whether the mandibular glands of a queen were the source of the pheromone. The experiments were carried out as in the 1st series, but we determined the activity of the CA by measuring their volumes. 11 queens were tested alive. Then, after 1 day, we excized the mandibular glands, killed the queens by deep-freezing, and extracted their body surfaces. The washed bodies, the solvent extracts, and the mandibular glands were tested separately. Furthermore, we examined 5 pairs of mandibular glands which had been exstirpated 3-4 months before and had been stored in acetone. Since we found the results between freshly excized and stored glands not to be different, we pooled the values. In 3 experiments, we tested whether the medium for homogenizing the mandibular glands had any influence on the activity of CA. In 12 controls we added a 1-day-old queenright worker instead of a queen to the 4 newly emerged workers.

The results are shown in figure 4. In the presence of a living queen the mean CA volume of 24-h-old workers was about 270×10^4 µm^3; in the presence of a worker about 330×10^4 µm^3. When we tested a freshly killed and extracted queen, the volumes of the worker glands were strikingly enlarged, but only the values obtained one day later were statistically higher than the controls with a living queen ($p < 0.001$). That freshly washed queens still showed some inhibiting effect, was also observed in the 1st series. This could be a hint that we either did not completely extract the pheromone from the surface, or that contamination in the extraction fluid may have exhibited some influence. The extract of the queen's body surface inhibited the activity of CA in workers like a living queen did. The homogenized mandibular glands were also found to show the same influence. The medium for homogenizing the glands did not inhibit the activity of the CA.

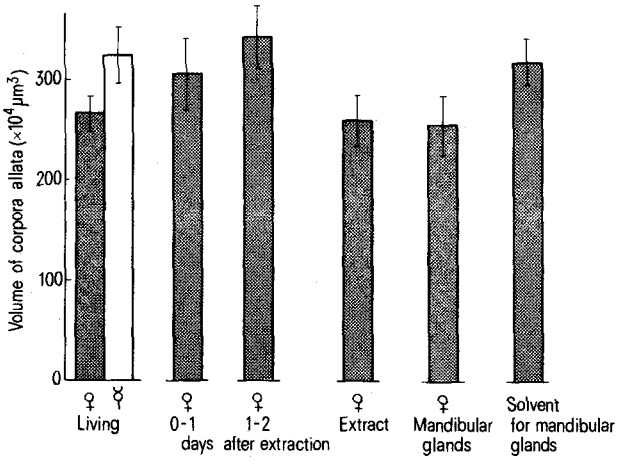

Fig. 4. The effects of living queens, washed queens (♀ after extraction), the queen's solvent extract (♀ extract), and of the mandibular glands extract and its solvent on the volume of CA in 1-day-old workers, in comparison with the effect of workers. Vertical bars indicate SD.

Discussion. In social insects the dominant females are the only egg-layers. These so called queens are able to suppress egg formation in other females which consequently become workers. The inhibition of oogenesis is caused either by a specific dominant behaviour as it has been shown in *Polistes* wasps[9,11,12] and halictine bees[13,14] or by pheromones well known from the honeybee[15-20]. In the present study we have demonstrated that also in the bumblebee *B. terrestris* activation of CA, and with it oogenesis, is inhibited in workers by a pheromone from the queen.

The pheromone is produced by the mandibular glands and it is presumably spread over the body by the queen when she is grooming. Whereas in the honeybee the corresponding pheromone was found to be persistent over years[17], the pheromone in bumblebees loses its effect 1 day after the queen has been killed. The mandibular glands have shown the same effect as a living queen in preventing activation of CA, but it cannot be excluded that glands on the abdomen produce pheromones contributing to the inhibition effect as the abdominal pheromone in the honeybee queen does[21]. Apparently, the mandibular glands also have multiple functions like the glands in the honeybee queen[20]. In the secretion of the glands in *B. lapidarius* queens Cederberg[22] could identify some ketones thought to be a defensive secretion, and van Honk et al.[23] have shown that the mandibular glands of young bumblebee queens produce a sex pheromone.

In bumblebees, egg laying workers are also able to inhibit egg formation in younger workers[2]. This inhibition might primarily be brought about by an agonistic behaviour which can be regularly observed in queenless groups. But it is as yet unknown whether queenless workers can also produce the same pheromones as a queen. In the honeybee, however, it has been demonstrated that queenless workers synthesize oxodecenoic acid, a main component of the queen's pheromone[24].

In bumblebees the pheromone of the queen inhibitis the activity of CA in workers. This is manifested in the volume of the glands, which do not enlarge as in queenless workers, and in the suppressed synthesis of JH. In this way the JH titre in haemolymph remains low and no eggs are formed. In the honeybee it has also been shown that queenless workers have enlarged CA and that the queen's pheromone inhibits enlargement of the glands[25-27]. In the wasp *Polistes gallicus* we have demonstrated that JH synthesis is inhibited in subordinated females by the behaviour of the dominant female[9]. So it can be concluded that the pheromone as well as the dominant behaviour at first influence the regulatory centre which controls the activity of the CA. But the mode of regulation of CA activity in bees and wasps is yet undefined.

In queenright colonies of *B. terrestris* egg formation in workers is not suppressed completely, but oogenesis is going on at a low level, so that old workers can have mature eggs in their ovaries[2,3]. In the absence of a queen oogenesis is accelerated. Recently it has been shown that in colonies headed by a queen whose mandibular glands were exstirpated the workers were younger on their first oviposition than workers in colonies with intact queens[4].

The sterility of workers among bumblebees is caused by a low JH titre in the haemolymph. Egg formation, therefore, can be induced even in the presence of a queen, when the titre is increased by injection of synthetic JH[5]. In the honeybee, in contrast, the sterility of workers seems not to depend on a low JH titre, since injection of JH does not induce egg formation, but releases foraging behaviour[28]. The regulation of fertility by the JH titre only is likely to be a basic system, which is also true for halictid bees[29] and for *Polistes* wasps[30,31]; for in both groups oogenesis in subordinated females can be induced by exogenous JH.

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Karyotypes of six species of African Cichlidae (Pisces: Perciformes)[1]

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Summary. Diploid numbers of 40–46 were found among the 6 species of African Cichlidae included in this karyotypic study. More species must be studies before any generalized conclusions regarding correlations between karyotypic morphology and phylogeny can be made.

Recent major taxonomic revisions of the African Cichlidae have resulted in extensive rearrangements of the genera within this very speciose family[3,4]. The osteologically based *Haplochromis-Tilapia* dichotomy of Regan[5] was invalidated by Greenwood[6]. It was indicated that, based on the structure of the pharyngeal apophysis, there may be 4 or more major generic groupings within the African cichlids. Trewavas[3] divided the large genus *Tilapia* into 2 genera, *Tilapia* and *Sarotherodon*. This split was based on both morphology and reproductive behavior. Karyotypes for a considerable number of neotropical cichlids have been published[7] but are available for only a few of the numerous African species[8–11]. Karyotypes for 5 species of African cichlids representing both of Trewavas' *Tilapia*-like genera and 2 of Greenwood's apophyseal types, *Haplochromis (Astatotilapia)* and *Tilapia (Tilapia, Sarotherodon)* are presented. Additionally, a species not discussed by Greenwood but probably of the *Haplochromis* type, *Melanochromis auratus*, is included.

Materials and methods. Somatic C-metaphase karyotypes were constructed from gill epithelial squash preparations according to Thompson[7]. All preparations were stained with acetic orcein.

Colchicine treatment of small chromosomes often results in very condensed preparations for which accurate arm ratio measurements are difficult. Thus, some chromosomes may be assigned to different categories by different workers[7]. Consequently, to allow for easier comparison with the results of others, the probable range of the arm or fundamental number (FN) is given rather than an absolute number.

Nomenclature is that of Levan et al.[12]. Metacentric-submetacentric chromosomes (msm) are those with arm ratios less than or equal to 3 and subtelocentric-telocentric (stt) are those with arm ratios greater than or equal to 3. Those in which ratios were 3 were assigned at the discretion of the observer. To calculate FN the msm chromosomes are counted as 2 while all stt chromosomes contribute but 1 to

Diploid chromosome numbers and material examined

	Specimens examined	Chromosomes per cell 37	38	39	40	41	42	43	44	45	46	Total cells
Astatotilapia burtoni	2 males	3	3	2	16	1						25
	4 females	3	1	2	17	4						27
Melanochromis auratus	2 females				1			2	4	3	10	20
Sarotherodon mossambica	2 males						2	2	17			21
	1 female						2	1	8	1		12
Sarotherodon aureus	5 males		1	1	1	4	6	5	33	1		52
	5 females	2			1	1	5	7	39	1		56
Tilapia sparrmanii	7 males	3	1	1	6	9	64	8	3			95
	3 females	4	1		3	1	28	2	1			40
Tilapia mariae	5 males		2	8	41	2						53
	3 females	3	3	2	19	2		1				30