

CASTE DIFFERENTIATION IN BEES : THE INFLUENCE OF BEHAVIOUR, PHEROMONES AND FOOD ON THE ENDOCRINE ACTIVITY.

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A. - INTRODUCTION

Social organizations have repeatedly developed in different systematic groups of bees (*Apoidea*). We find them mainly in the halictine bees, bumblebees, stingless bees, and in the honeybees. The structure of social organisations are different in the different groups, sometimes different even in closely related species. The different types have been classified by terms as eusocial, semisocial, parasocial, etc. (MICHENER, 1974). But apart of these varieties we find in all societies, that females are separated into two castes usually called as queens and workers (BRIAN, 1980). That traditional classification originated from the social organization of the honeybee, in which a sharp distinction exists between both castes separated by a number of morphological, physiological and behavioural features.

A general classification, however, must reduce all the different modifications to some important basic features valid for all of them : a queen is that female which lays all the eggs or at least most of them. She tries to monopolize the reproduction of the colony by preventing the other females from egg-laying. This reproductive dominance is a central process in all species leading to monogyne societies. Connected with this, the queen has a high value of social presence, spending most of the time within the nest. Additionally, a queen is characterized by her longevity, she is willing to mate, and able to hibernate. Workers, in contrast, collect food, take care of the brood, and defend the nest, in this way aiding the dominant female. The attributes of castes listed here are not exclusive for one caste, but they are mostly relative frequencies.

The differentiation of females into queens and workers can take place at the adult stage, for instance in semisocial groups in which some females of the same age live together in one nest ; or the castes are differentiated during the larval development, for instance in the honeybee. The caste specific activities of the adults do not consist of an irreversibly fixed program, but the activities are modified by the other caste, by the social environment. The workers, in general, retain the potentiality to become fertile. The differentiation into infertile workers is the result of the presence of a dominant queen. After removal of the queen one worker will become the dominant egg-layer.

At the adult stage, social signals are responsible for caste differentiation : agonistic behaviour and/or pheromones. At the

larval stages, caste differentiation is induced by the food the larvae obtain. All these exogenous signals, however, cannot act directly on the differentiation, but the exogenous signals must be primarily transformed into endogenous signals which control the differentiation. As far known, a modulation of the endocrine activity is the main response to the exogenous signals.

## B. - RESULTS

### 1/ Agonistic behaviour

Caste differentiation by behavioural mechanisms has been reported to exist in the halictine bees. The structure of social organisations show an enormous variability among the species and even among populations of the same species. Sharp differences between queens and workers do not exist. The castes are morphologically indistinguishable and differ only in physiology and behaviour. But the functions of the castes can broadly overlap so that intergradations exist between both of them.

Usually, the female with the most developed ovaries is called as queen. She lays all or most of the eggs, and she has the highest value of presence in the nest. The other females mainly act as guards or as foragers. In the presence of a dominant queen their reproduction is reduced. An ontogenetic sequence of behaviour does not exist, but the behaviour of a worker remains about the same during the adult life. Sometimes, the subdivisions of workers are called as subcastes (PLATEAUX-QUENU, 1973 ; MICHENER, 1974).

Caste differentiation occurs at the adult stage. Queen removal experiments have shown that at least in some species all the females of a colony have the potentiality to become a dominant female (MICHENER and BROTHERS, 1974 ; BREED, 1977 ; PLATEAUX-QUENU, 1978). But PLATEAUX-QUENU (1974) has reported that in *Evylaeus calceatus* caste differentiation can be irreversible : the inhibiting effect of the queen can persist, when workers have spent some time with the queen. After removal of the queen, the workers do not establish a functioning group.

In the majority of species, the queen is one of the largest females of a colony, but the size itself is not the cause for caste differentiation, the size favours a female for becoming a queen ; other attributes are the age and the copulation. The caste differentiation itself, however, is largely brought about the behavioural interactions as it has been postulated by MICHENER and BROTHERS (1974), BREED (1977), and MICHENER (1977). Pheromones are not involved, so far known. A queen is that female which exhibits the highest activity. She frequently nudges the other females. It is thought that the repeated nudging of the queen inhibits ovarian development in other females. In experiments on *Lasioglossum zephyrum* nudging was imitated by an iron ball moved up and down in the burrows with a magnet. In these colonies ovarian development was inhibited, and no queen developed. But, of course, the result of this fairly artificial situation only shows that mechanical disturbances can inhibit egg-formation.

The agonistic behaviour of the queen, on the other hand, stimulates the workers to perform the characteristic activities (PLATEAUX-QUENU, 1974 ; MICHENER, 1977). After having nudged the worker the queen backs away, the worker follows, and the queen draws the worker down to nest areas located deeper in the earth. It is assumed that in this way foragers are lead from the nest entrance to cells, in which the pollen can be placed. If this specific behaviour is common for halictine bees, remains to be investigated, but, in general, the presence of a queen promotes worker activities. After removal of the queen workers become sluggish and inactive.

Up to date, it has not been investigated, in which way the behaviour of the queen influences the egg-formation in other females. But despite of the lack of analyses of hormone titres in the castes, we can conclude that by the agonistic behaviour of the queen the endocrine system, which controls oogenesis, is more or less suppressed in workers. BELL (1973) has shown in some experiments that the inhibition of oogenesis might be performed by curtailing the secretion of juvenile hormone (JH) ; the inhibition of oogenesis could be overcome by JH treatment.

Also in the eusocial bumblebees, agonistic behaviour plays a role in caste differentiation at the adult stage (van HONK, 1981). Normally, a queen governs her colony by pheromones. But the pheromone is effective only within a short distance of few centimeters. The larger a colony becomes, the less the single worker is in the vicinity of the queen and experiences her pheromones. Finally, in a large colony the workers can lay eggs.

It has been shown by quantitative analyses that the characteristic behaviour of a worker is the result of the presence of a dominant queen. In large colonies, the behaviour of some older workers become more and more queenlike. These workers exhibit higher activities than other workers do, they frequently interact with their nestmates, and they attack workers neighbours in the hierarchy. Against the queen they behave less submissive and, finally, they attack the queen. These queenlike workers remain in the nest and do not collect food. The difference in the behaviour between the workers occurs just in the first days of adult life. Why some workers are more queenlike than others, is not known ; it seems that the differentiation of the worker caste does not result in an uniform caste structure.

In queenless groups, a dominance hierarchy is established by agonistic behaviour within few days (RÖSELER and RÖSELER, 1977). The egg-formation in subordinated workers is retarded or completely inhibited by worker-worker interactions. The attacks are especially made upon competitors for dominance as though they know the ovary status of the others. But after the hierarchy has been established, aggressive interactions are seldom observed. There after, the queenless group is probably controlled by pheromones.

## 2/ Pheromones

In large colonies, caste differentiation cannot be controlled by behavioural stimuli. The reproductive dominance of the queen is chemically controlled by pheromones extensively investigated in the honeybee.

The honeybee queen produces in the mandibular glands besides other substances a mixture of fatty acids, of which the 9-oxo-2-decenoic acid (9-ODA) is the main component controlling caste differentiation at the adult stage. This acid attracts workers, induces the retinue behaviour, and inhibits egg-development in workers and the construction of new queen cells. In the tergal glands of the queen's abdomen a pheromone is produced with other properties, but supplementing the mandibular pheromone (VELTHUIS, 1976a).

The queen's pheromone is necessary for normal worker behaviour found only in queenright colonies. If the queen is removed and no queen-replacement possible, agonistic interactions increase, and the workers show egg-formation and start egg-laying. But not all the workers of a queenless group have developed ovaries, and only some of them lay eggs. The egg-laying workers are also able to inhibit egg-formation in other workers. In addition, some of them even induce retinue behaviour in other workers like a queen.

The basis for this queenlike behaviour of queenless workers is the capability of workers under queenless conditions to produce the same pheromones as a queen. Studies on mandibular glands by CREWE and VELTHUIS (1980) have shown, that in fact queenless workers produce 9-ODA previously thought to be exclusively produced by the queen ; but the 10-hydroxy-decenoic acid remains the dominant product in laying workers. Not all egg-laying workers of *Apis mellifera mellifera*, however, seem to be able to produce 9-ODA. These workers lay eggs, but they do not elicit retinue behaviour in others. In contrast, all the workers of *Apis mellifera capensis* are capable to produce 9-ODA which even predominates in the secretion. The more queenlike pheromone produced by *Apis m. capensis* workers enables them to become always the dominant female, when placed in a group of *Apis m. mellifera* workers. What it has been shown for halictine bees and for bumblebees, is true also for the honeybee : workers are not uniform, some of them are more queenlike than others, and there are differences between different races. Modifications of worker's physiology can occur at the adult stage induced by the social environment.

It is yet an unsolved problem, in which way the queen's pheromone could inhibit egg-formation in workers. The pheromone is spread through the colony by queen-worker and worker-worker contact (SEELEY, 1979). Since egg-formation in queenright workers is suppressed and queenless workers, on the other hand, were found to have enlarged corpora allata, it was thought that the pheromone might inhibit the activity of corpora allata. JH treatment, however, did not result in egg-formation, but, surprisingly, released foraging behaviour. RUTZ *et al.* (1976) have shown that the JH titre increases during the adult life and that the titre is elevated

at the age of changing to foraging activities. If in the honeybee the ovaries are controlled by the corpora allata, oogenesis must be blocked at an early stage, at which the ovaries have not yet acquired competence to respond to JH. Thus, JH became available for controlling the tasks of workers.

In bumblebees, the differentiation of the adult castes is also controlled by pheromones. The queen inhibits oogenesis in workers and she stimulates normal activities of workers. After removal of a dominant queen workers become sluggish and inactive.

In queenless groups, one worker becomes the dominant egg-layer and then this one is also able to retard or to inhibit egg-formation in other workers. Aggressive worker-worker interactions play an important role in establishing a dominance hierarchy during the first days, there after they are seldom observed (RÖSELER and RÖSELER, 1977). Probably, the reproductive dominance is then also controlled by pheromones. But if in fact egg-laying workers become capable to produce queen pheromones as it has been shown for the honeybee, has not yet been investigated.

The pheromone is effective only in the vicinity of the queen. It persists only for one day after the queen has been killed. Since bumblebees do not feed or lick one another, the pheromone cannot be transmitted by contact. The queen disperses the pheromone by frequently changing her position within the nest. Since the pheromone is effective only in the vicinity of the queen, the individual worker experiences less the pheromone, as the colony becomes larger, and more and more workers with enhanced oogenesis develop. Finally, the queen loses her dominant position and can be pushed off the nest.

Some studies on the mode of action of the queen's pheromone has been done in *Bombus terrestris* (RÖSELER and RÖSELER, 1978 ; ROSELER *et al.*, 1981). The pheromone lowers the activity of corpora allata in workers. Primarily, the pheromone may influence the neurosecretory centres controlling corpora allata activity. The JH synthesis is decreased, the JH titre in the haemolymph remains low, and no eggs are formed. Workers kept queenless after their emergence are able to lay eggs at the fifth day. On the very first day the corpora allata activity is found to be higher than in workers of the same age under queenright conditions. Experimentally it has been shown that oogenesis is in fact controlled by a lowered JH synthesis ; after injection of synthetic JH queenright workers form eggs to the same extent as queenless workers.

In stingless bees, the regulation of fertility is not yet understood and the role of the queen is unclear (ENGELS and ENGELS, 1974 ; VELTHUIS, 1976). Workers of all species, with only few exceptions in the primitive ones, form eggs in the third week of their adult life, when they work as nurse bees. After four weeks the ovaries degenerate. The workers mostly lay trophic eggs on the rim of newly provisioned cells, and the queen eats the eggs before she starts depositing her egg in the cell. After removal of the queen no more trophic eggs are laid, and in some species workers lay

reproductive eggs. Summarizing the few data : workers of stingless bees become temporary fertile, yet this fertility seems to supply the queen's food demand.

### 3/ Food

In many social bees, the differentiation of castes occurs at the larval stages. The different development is induced by the food the larvae obtain. The best known example is the caste differentiation in the honeybee.

In the honeybee, the female larva has during the first three days the potentiality to develop either into a queen or into a worker (WIRTZ, 1973 ; BEETSMA, 1979 *et al.*). The sensitive period ends with the fourth day, thereafter the caste specific development is fixed. The larval age of about 3,5 - 4 days, during which the caste development is switched on, is called the critical period. In this period, the determination into the castes is thought to occur. The determination, however, does not switch on the caste specific program as a whole, but the development of caste characters is under control of modulating factors. When the balance is changed either by grafting worker larvae older than 4 days in queen cells or by hormone treatment, intercastes can develop.

Many expensive investigations were undertaken to analyze in the royal jelly a specific factor, which should induce queen development, the so-called determinator or the queen-determining principle (REMBOLD *et al.*, 1974 ; RAMBOLD, 1976). But studies by DIETZ and HAYDAK (1971) and ASENCOT and LENSKY (1976) have shown that the amount of food consumed by larvae seems to be responsible for the differentiation of castes. The increase of food consumption is induced by the sugar content of the food, which might be perceived by chemoreceptors in the mouthparts. The moisture content of food is probably necessary to prevent crystallization of sugars which cannot be consumed by the larvae. The importance of the endocrine system for the caste differentiation in the honeybee has early been postulated, but WIRTZ (1973) was the first who presented exact quantitative data. He showed by histological methods that the activity of corpora allata was higher in queen larvae than in worker larvae after the end of the critical period. Correspondingly, he found by means of the *Galleria* assay that the JH titre in the haemolymph of the queen was higher than in worker larvae after the end of the critical period. In the fifth instar he detected two hormone peaks in the queen with a minimum in the spinning larva, but in workers only the second peak in the post-feeding larvae.

In this first analyses REMBOLD (1976) could not confirm the results of WIRTZ (1973), he found in workers also one JH peak, but in the fifth instar feeding larvae (fig. 1a). Later on, however, he obtained results similar to those of WIRTZ (1973). REMBOLD and HAGENGUTH (1981) determined the hormone content in total larvae using a physico-chemical method (gaschromatography combined with mass-spectrometry). They found also two JH peaks in the fifth instar of queen larvae and only one, the second peak, in the worker

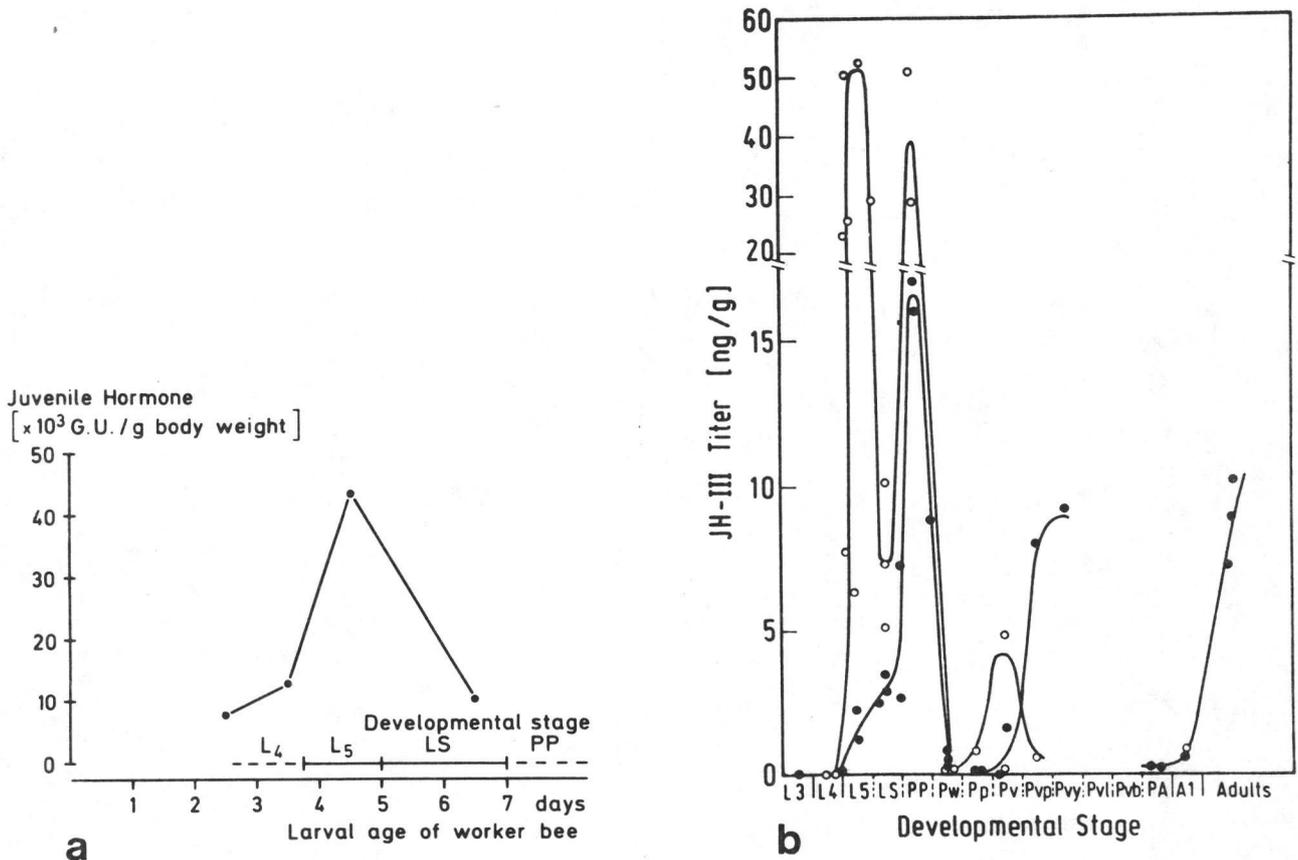


Fig. 1. - JH titres during the postembryonic development of the honeybee

a) of workers obtained by *Galleria* assay (after REMBOLD, 1976) ;

b) of queens (open circles) and workers obtained by physicochemical methods (after REMBOLD and HAGENGUTH, 1981).

L = Larval stages ; PP = Praepupae ; P = Pupae ; A = Adults.

postfeeding larvae (fig. 1b). The absolute values for queens they found to be 2-3 times higher than for workers. LENSKY *et al.* (1978) using a radioimmune-assay for JH determined also two JH peaks in the queen larvae, but the first peak they found to be in the fourth instar.

The results on the JH titres have been confirmed by several experiments, in which queens were obtained from larvae in worker

cells after application of JH (BEETSMA, 1979). The main difficulty is to find exactly that phase which is sensitive to JH. When JH I is used, which is not the natural occurring JH in the honeybee, only one application of 1 µg JH to larvae of 3,5 days resulted in an induction of queen characteristics. Application of JH III, however, the natural occurring JH in all developmental stages, resulted in queen differentiation only after repeated doses have been applied. It is assumed that JH I is more slowly released by the cuticle or that JH I is less removed from the haemolymph than JH III. In any way, JH I seems to persist longer in the haemolymph thus covering the sensitive period.

The ecdysteroid titre was determined by a radioimmuno-assay. HAGENGUTH and REMBOLD (1978) and REMBOLD and HAGENGUTH (1981) found in both castes a high level until the fourth instar (fig. 2). In the fifth instar the titre decreases to a minimum in the spinning larva. In the praepupa the ecdysteroid level again increases and remains high during the pupal development. The difference between both castes exists in a time shift: in worker larvae the decrease starts earlier and the increase later than in queen larvae, and the minimum in spinning larvae is lower in workers than in queens. The validity of the time shift depends on how exactly the larvae are dated. A comparison between two publications of the same data show (fig. 2), that the authors themselves offer different interpretations.

Also LENSKY *et al.* (1978) determined a lowered ecdysone titre in the fifth instar and a subsequently increase in the praepupa. But; in contrast, they found that in workers the ecdysone level starts increasing earlier than in queens. A second increase follows at the time of imaginal moulting.

In stingless bees, the quantity of food plays also an important role in the differentiation of larvae. In *Trigona* species, it has been shown by DARCHEN and DELAGE-DARCHEN (1971) that only the amount of food is responsible for caste differentiation. Larvae after having finished the food were grafted in new cells provisioned with food, and developed into queens. The results were confirmed by *in vitro* rearing.

In *Melipona* species, a sufficient amount of food for larvae is also necessary for queen differentiation. But it seems that the queen development can be induced earlier than in the last instar similar to the honeybee. Larvae of *Melipona beecheii* taken out of the nest at the fifth instar and reared *in vitro* under different food regimes resulted sometimes in dwarf queens and giant workers (DARCHEN and DELAGE-DARCHEN, 1975). The prolonged food intake in the last instar seems to activate the endocrine system resulting in an elevated JH titre which induces queen development. This was experimentally confirmed by VELTHUIS (1976). Application of JH induced queen development. KERR (1950) and his co-workers, who have proposed since years a genetically determined caste differentiation, now postulate that sex determining genes should be necessary for controlling the JH production and with it the caste differentiation (KERR *et al.*, 1975; CAMARGO, 1977). But this theory is difficult to understand and it offers no possibility to test it.

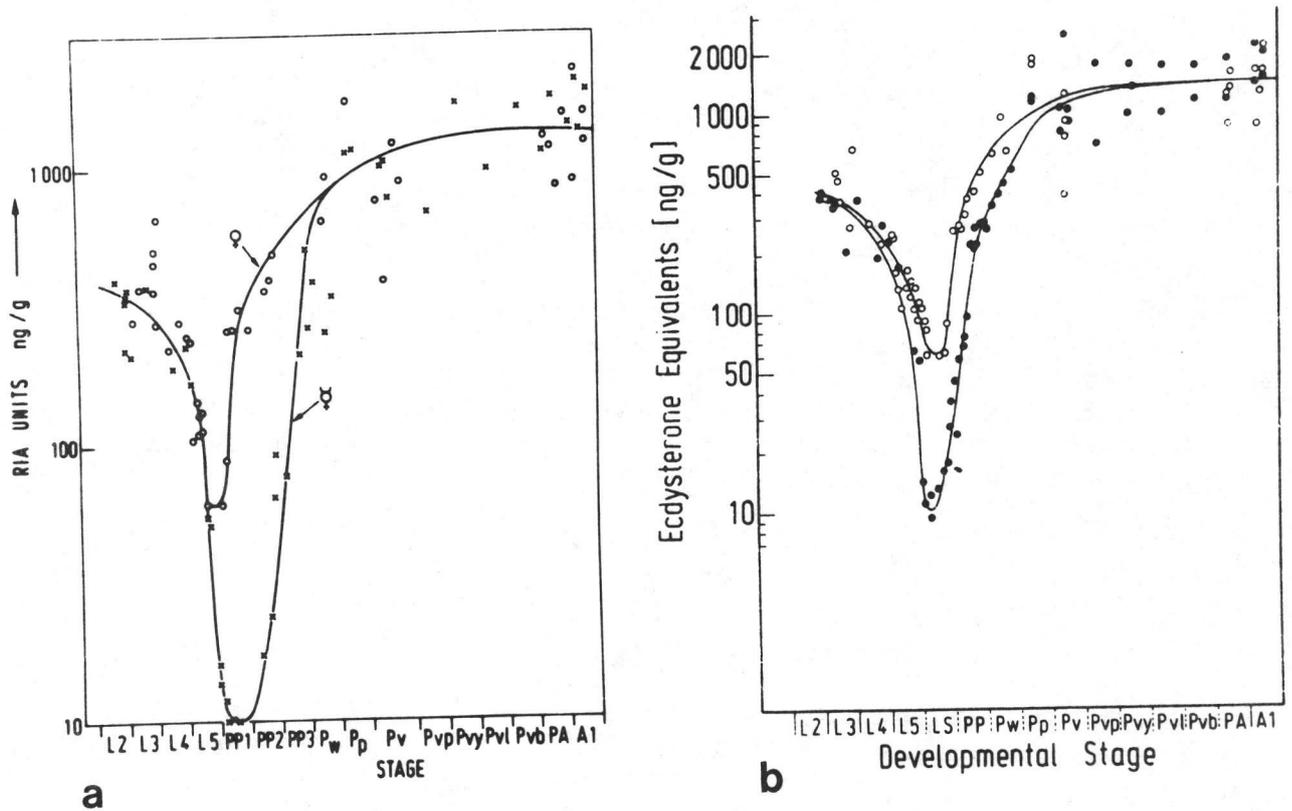


Fig. 2. - Ecdysteroid titres during the postembryonic development of queens (open circles) and workers of the honeybee  
 a) after HAGENGUTH and REMBOLD, 1978 ;  
 b) after REMBOLD and HAGENGUTH, 1981.  
 L = Larval stages ; PP = PRAEPUPAE ; P = Pupae ;  
 A = Adults.

In bumblebees, no morphological differences exist between queens and workers, but only differences in size. There are some species with castes clearly different in size, queens being always larger than workers. In other species caste sizes overlap so that a small queen can be smaller than a large worker. In all species, however, castes are well separated by physiological and behavioural attributes : only queens are able to hibernate, they store large energy depots, and only queens are willing to mate.

In *Bombus hypnorum*, the caste differentiation is induced by the amount of food the larvae obtain in the last instar (ROSELER, 1977, 1978). The increased consumption activates the endocrine system. In praepupae the JH titre in the haemolymph increases in both castes, but in queens twice as high as in workers. At that period the development becomes sensitive to JH : about 24 h. after the larvae have started spinning the cocoon, the differentiation of presumptive workers into queens can be induced by application of JH. These induced queens, of course, are of worker size, but they exhibit the physiology of queens : they store large energy depots in the fat body and do not participate in the worker tasks.

Recently, STRAMBI (unpubl.) has determined the titre of ecdysteroids in the haemolymph during praepupal development. Despite of the lack of some data one clear difference at least exists between both castes : the titre increases in the praepupae, but in workers strikingly earlier than in queens. The level of both hormones is elevated in the praepupa. A comparison between them both show (fig. 3) that the increase of JH precedes the increase of ecdysteroids. This finding is in good agreement with the hypothesis that JH at first programs the DNA and ecdysone triggers the expression of the genetic program (see below).

### C. - CONCLUSIONS

The differentiation of larvae into castes is apparently a modification of the regular insect development. Thus, at first it is necessary to know the control of insect development, before the differentiation of castes can be understood. It is well established that hormones control the insect development. But the mode of action of hormonal control is yet rather unclear, though in the last years first results have been presented on cytoplasmatic and nuclear hormone-receptor complexes regulating gene transcription, and first ecdysteroid stimulated proteins have been characterized.

Differentiation is the expression of specific sequences of the genome, the expression is coordinated by hormones resulting in a temporal and spatial pattern. The response of DNA to morphogenetic hormones is modified during the development, in the early development other effects are induced than in later stages by the same hormone level. It is, therefore, assumed that the development is based on a fixed program, the single steps are modulated by hormones. The modification of the DNA program is thought to be influenced by JH, so that certain gene groups can be transcribed, whereas others do not respond. The transcription itself is controlled by ecdysone. But, of course, the control on a posttranscriptional level is also possible and has been confirmed.

In studies on hormonal influences on the development, the activity of the hormone system is determined during the development, either the activity of the gland or the hormone level in the system. Thereafter one tries to correlate developmental steps to changes in the hormone titre. But the hormone level must not immediately regulate developmental steps, the cells can only acquire competence to

### Hormone concentrations in the haemolymph of *Bombus hypnorum* - Queens

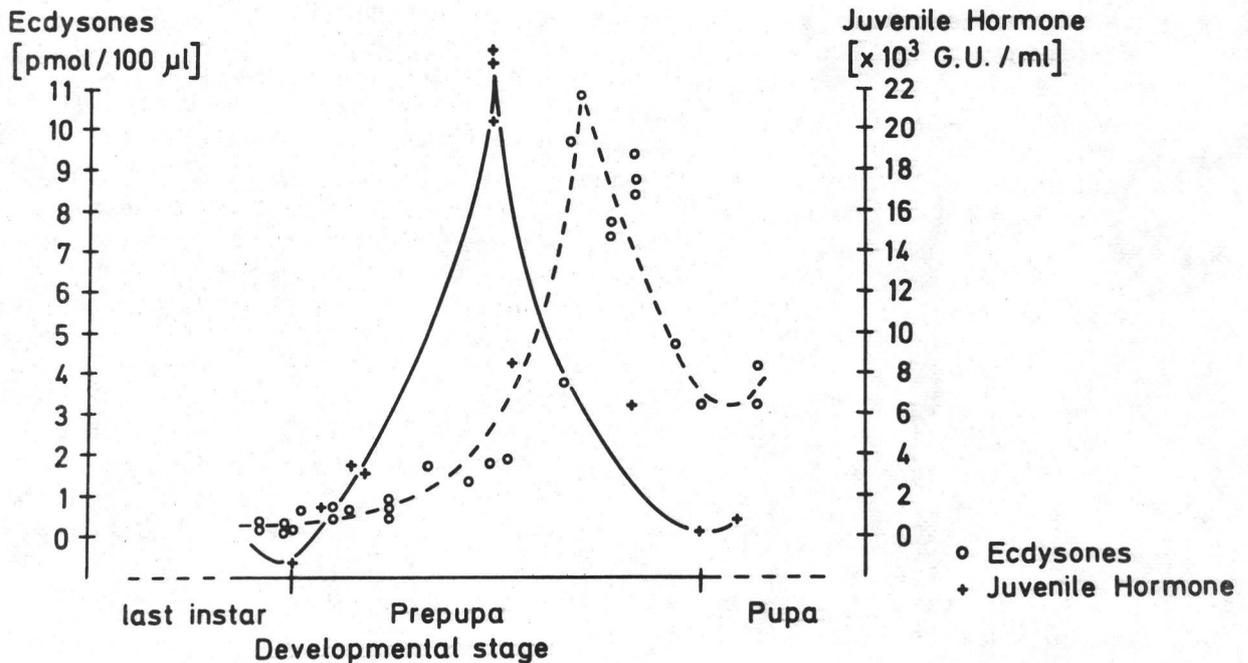


Fig. 3. - Titres of ecdysteroids and juvenile hormone (obtained by *Galleria* assay) during the praepupal development of queens of *Bombus hypnorum*.

respond later to hormones. In any case, the validity of the results is finally proofed by hormone treatment, which should induce the specific developmental steps.

In the view of a molecular level social insects must have alternative gene patterns in their genome for queens and workers, the different expression should be controlled by a specific balance of hormones. Thus, caste differences in the hormone titre are studied. The caste, of which the development seems to be connected to a lowered titre, is treated with hormone. In this way one tries to increase the hormone level and to induce the development of the other caste. The validity of the results depends on the methods used, their sensitivity and specificity, and depends on how exactly the age of the developmental stages have been determined. Moreover, it is without any doubt a difference, if the hormone content is

analysed in body extracts, in which the hormone pool is determined, or if the hormone content is analyzed in the haemolymph, by which the actual titre is determined. The different methods however, should not be discussed here, but they must be considered.

The results on caste differentiation in bees can be summarized as follows : in bees, the differentiation of larvae into either a queen or a worker is induced by the amount of food. This is true also for halictine bees, in which caste differentiation occurs at the adult stage. Queens are larger than workers, the size favours a bee to become the dominant female, indicating that probably physiological attributes are connected to the size. In species with mass provisioning of cells, a different quantity of food can become effective only at the end of larval development, when one larva has finished the food, whereas the other continues eating. This mechanism has been found in stingless bees and in bumblebees, despite in the latter the larvae are fed progressively. Consequently, differentiation into the castes can occur only in the praepupa after cessation of feeding.

The signal "quantity of food" must be transformed in an endogenous signal which controls the development. This is brought about by an activation of the endocrine system. The amount of food is thought to activate at first the neurosecretory centres which control the activity of hormone glands. The activation of the endocrine system by feeding stimuli is not exclusively limited to social bees, but this phenomenon was found to exist also in the adults of other insects (ENGELMANN, 1970).

The last instar is characterized by dramatically changing of hormone activity. But the hormone peaks found in the praepupae of social bees are not restricted to them. They have been found also in other insects, for example in the butterflies *Pieris brassicae* (MAUCHAMP *et al.*, 1981) and in *Galleria mellonella* (HSIAO and HSIAO, 1977). In solitary species the last instar is also sensitive to exogenous signals which influence the development. The dimorphism of some butterflies, for instance, is induced by the day-length in the last instar resulting in different hormone levels. In *Iphiclides podalirius* the last instar of the spring form has a higher ecdysone level than the summer form (SCHELLER *et al.*, 1981). Social bees have additionally used the sensitive period at the end of the last instar for caste differentiation. In highly evolved species, the determination is shifted to earlier stages so that morphologically well separated castes can develop. But despite their determination at the larval stage, the caste specific functions of the adults must be regulated by means of behaviour and pheromones.

The different hormone titres between both castes and the caste specific response to JH treatment is a good evidence for hormonal control of caste development. Also the behavioural and pheromonal stimuli, by which the function of the adults are regulated, seem to act via the endocrine system. Thus, the theory of hormonal control of caste differentiation in bees is well established.

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