REGULATORY MECHANISMS IN CASTE DEVELOPMENT IN THE HONEYBEE, *APIS MELLIFERA* L.

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INTRODUCTION: It is becoming increasingly evident that various aspects of the honeybee society are regulated by complex systems of control (Free, 1966). Some features of these systems appear to be analogous to the components of a model for a negative feedback system (Hassenstein, 1971; Powers, 1973). The principle of such systems is as follows: in response to a disturbance to the system, information on a deviation from the desired value (or normal value) of a controlled variable is passed to a controlling centre which transmits a correcting signal to a controlling element, which in turn corrects the deviation. We shall examine evidence of mechanisms regulating caste development at various levels of organization, in the general context of the negative feedback model. Our review will of necessity be selective; space limitations prevent due recognition of valuable contributions by many workers, including some of our own graduate students.

The honeybee colony normally consists of one queen, some 50,000 or 60,000 workers, and a variable number of males or drones. Each of the three castes is reared in a cell of typical form, size and orientation, and is supplied with a characteristic food. Determination of the male (haploid) is genetic, but under certain conditions genetic determination is reinforced by a behavioural mechanism (Woyke, 1967). Determination of the female caste is trophogenic and mediated by a behavioural mechanism. The caste of the adult female depends on the food eaten by the larva during the first 3 days (Becker, 1925; Weaver, 1957). All larvae develop quickly during this initial "undetermined" period. Larvae determined as queens continue to develop rapidly thereafter, completing their post-embryonic development in about 12 days, whereas workers require about 17 days (Bertholf, 1925).

The subject of caste determination has been reviewed in detail by several writers in the past few years (Brian, 1965; Rembold, 1961; Shuel and Dixon, 1960; Townsend and Shuel, 1962; Weaver, 1966).

REGULATION OF QUEEN REPLACEMENT Normally a healthy, vigorous queen maintains her dominant position in the colony by means of at least 2 mandibular gland secretions: 9,oxodec-trans-2-enoic acid and 9,hydroxy-dec-trans-2-enoic acid (Butler, 1967; Pain, 1973) collectively referred to as "queen substance" (Butler, 1956). Queen substance has both behavioural (releaser) and physiological (primer) effects. Its suppresses the tendency of worker bees to construct queen cells, and inhibits the limited reactivation which can occur in some worker ovaries (Butler, et al. 1959; Butler and Fairey, 1963; Pain, 1951). The means of distribution of these pheromones to the worker bees has not yet been settled (Butler, 1956; Velthuis, 1972), and nor has their mode of action.

A limitation of the supply of queen substance due to death or enfeeblement of the queen leads to her replacement. One or more queen cells are constructed in which the queen lays eggs, and nurse bees supply the cells with royal jelly. If the queen dies, and no queen cells with eggs are available, workers rear queens in "emergency" cells converted to the shape and orientation of queen cells. Should a colony remain queenless for an extended period, the ovaries of a few workers may undergo limited development and these workers may lay a small number of unfertilized eggs. Apparently ovary development in a few workers inhibits development in the rest (Velthuis, 1970); thus the number of laying workers is restricted. In most races of bees the laying of eggs by workers is a dead end as far as the future of the colony is concerned, because only drones are produced, but A. mellifera capensis can produce females parthenogenetically from worker eggs (Anderson, 1953).

REGULATION OF WORKER AND DRONE CELL CONSTRUCTION

Construction of drone and worker cells, like that of queens, may be regulated through pheromones. The existence of a group of "construction pheromones" has been postulated by Chauvin and his colleagues (1961). They observed that no worker cell construction occurred in the complete absence of a queen, but that the presence of even a part of a dead queen stimulated comb construction. Free (1966) found the presence of larvae in queen cells to be necessary for the building of drone combs, that is the presence of developing female reproductives stimulated the production of male reproductives.

CONTROL OF EGG LAYING The next stage in caste development is the depositing of an egg of the proper genetic complement in the right cell. Evidently the matching of a haploid egg with a drone cell and a diploid egg with a worker cell is based on proprioception. Koeniger (1970) found that the queen meters the cell with her forelegs during her pre-oviposition inspection. The laying of a fertilized egg is the normal event; some special property of the drone cell inhibits this. As drone cells resemble worker cells except for their larger size, the inhibitory effect must be related to size. Why, then, does a queen not lay unfertilized eggs in queen cells, which are also larger than worker cells? The answer is not known, but it is conceivable that discrimination between drone and worker cells is based not on absolute cell size, but on a change in cell size and that perception of a size change is not operative during the rather infrequent occasions of egg-laying in queen cells. The different orientation, too, may be significant.

REGULATION OF LARVAL DIETS As little study has yet been given to the nutrition of drone larvae, and as diet is not the determining factor in drone development, discussion will be restricted to female larval diets.

Larvae developing in queen cells are abundantly supplied with royal jelly. It has been estimated that provisioning a queen cell involves 1200 to 1500 feeding visits by nurse bees (Lindauer, 1952; Habowsky, 1962). A worker larva is supplied more sparingly in the course of about 150 feeding visits. Queen and worker diets also differ with respect to composition, and the worker diet varies with age. At the nursing level, then, female caste differentiation must involve a means of recognizing both caste and age, and a mechanism for varying the diet accordingly.

Each feeding visit is preceded by an antennal inspection of the cell and its contents (though all visiting bees do not deposit food - Lindauer, 1952; Habowsky, 1962). Do the recognition cues reside in the larva or in the cell? Apparently nurse bees can distinguish between young drone and worker larvae (Haydak, 1958). This ability is impaired if the queen is missing. Young diploid drone larvae from eggs laid by highly inbred queens secrete an ether-soluble substance which causes the worker bees to recognize them as "wrong" and eat them (Woyke, 1967). Ploidy cannot provide the basis for differential feeding of newly hatched female larvae, however, because all are diploid. That diets are differentiated very early in larval life has been shown by chemical analyses and respiration measurements (Shuel and Dixon, 1959). Some physical or chemical property of the cell must provide the initial cue. In the early stages nurse bees will feed a female larva according to the type of cell which it currently occupies, regardless of its origin. As development proceeds, the nursing pattern gradually becomes fixed. Older larvae are recognized as "right" or "wrong" for the cell, and eventually an age is reached at which a larva transferred to the "wrong" cell type is not fed (Weaver, 1957).

There is considerable information relative to the mechanical basis of variation in diets. Individual dietary constituents come from at least 3 sources: proteins from the hypopharyngeal glands (Kratky, 1931), lipids from the mandibular glands (Barker et al., 1959: Callow et al., 1959; Simpson, 1960) and sugars presumably from the honey sac (Gontarski, 1960; Habowsky, 1962). One nurse bee in the course of a visit does not supply a sample of the complete diet, but rather one of three broad-spectrum components (Rhein, 1933; Jung-Hoffmann, 1966). Young female larvae in both kinds of cells receive a mixture of clear component (from the hypopharyngeal glands and the honey sac) and a milky component (from the hypopharyngeal glands and the mandibular glands). The milky component makes up a relatively large proportion of the queen larval diet. Worker larvae past three days receive a third component that is yellow in colour and probably comes from the honey sac. Jung-Hoffmann's observations are consistent with analytical data on the major constituents of the larval diets (see below). Taken in conjunction with the fact of the various glandular origins of the diet, they provide a reasonable explanation for the mechanics of dietary variation.

Less is known about the physiological basis of dietary control, probably because of the extreme tedium involved in making the relevant observations and measurements. Three general alternative nursing patterns might be visualized:

(a) a differentiation of nursing populations on the basis of age, so that nurse bees of similar age feed larvae of the same caste or age,

(b) a differentiation of nursing populations according to the glandular development of the nurse bees, and

(c) no differentiation of nursing populations, any nurse bee feeding a larva of either caste or any age.

Pattern (a) would be consistent with observations that worker bees tend to perform specific tasks according to their age (Perepelova, 1928; Rosch, 1925). Considerable flexibility, however, appears to exist within the general trend (Lindauer, 1952). A rigid system, moreover, would seem to be incompatible with the ability of the colony to respond to the failure of a queen by rearing a new queen.

There is some evidence to support pattern (b). Habowsky (1962) found a statistical difference between queen and worker nurse bees with respect to hypopharyngeal gland development, though with considerable overlap. Condition of the glands was not closely associated with age. Simpson (1960) observed that in bees with large hypopharyngeal glands the mandibular glands often contained only a watery secretion. Feeding pattern (b) would be compatible with this evidence and consistent with a system of feedback regulation. It is possible that the glands of some bees react to the failure or removal of the queen, and respond selectively to stimuli associated with queen larvae. Response to loss of the queen - an extreme deviation from the normal situation - may differ from the response to queen failure, a less serious deviation. Queenless bees rearing new queens in converted worker cells changed the diet to a royal jelly type before altering the cells to the queen cell form (Jung-Hoffmann, 1966). Recognition in this case could not have been based on the cell type. Possibly the condition of the glands of some nurse bees in the queenless situation is such that a recognition cue for queen nursing is unnecessary. Kuwabara (1947) observed that in queenless colonies large numbers of worker cells contained a diet resembling royal jelly. Perhaps a small number of larvae in such cells diverge from the worker type more rapidly than the rest and consequently have their cells transformed.

There is little evidence either for or against pattern (c). Although it would involve a rather complex system of stimuli and responses, its flexibility would be consistent with feedback regulation.

It would appear that the question of the physiological basis of diet regulation cannot be settled by observations of behaviour along, and that a physiological appraoch to receptor physiology in nurse bees is needed.

COMPARATIVE COMPOSITION OF LARVAL DIETS. Royal jelly and worker jelly, the respective diets of queen larvae and young worker larvae, differ considerably in composition. Royal jelly contains more lipids, much more sugar, and less water than worker jelly (Shuel and Dixon, 1959), and also more biopterin (Hanser and Rembold, 1960). The presence in royal jelly of a dialysable substance which supports queen development, but whose activity is rapidly lost at room temperatures, was reported by Weaver (1962). It was later extracted and tested by Rembold and Hanser (1964), but has proved difficult to purify and identify. Whether it is completely absent from worker jelly, or present in relatively low concentration, has not been reported. Modified worker jelly, fed to older worker larvae, is very high in sugar and low in lipids (Shuel and Dixon, 1959) and in vitamins (Hydak and Vivino, 1950).

HORMONAL REGULATION OF DEVELOPMENT The most obvious evidence of hormonal influence in honeybee development is in late larval development and metamorphosis. Queens pass through the pupal stage much more quickly than workers. When honeybee larvae beyond $4\frac{1}{2}$ days of age were ligated they tended to skip pupation and attempt matamorphosis within a few days (Schaller, 1952). Thus it would appear that metamorphosis was precipitated by effective decapitation.

It is reasonable to suppose that worker larvae still have an appreciable amount of juvenile hormone in circulation during the terminal stages and queens much less. The significance of the protracted worker pupal period may lie in providing time for the synthesis of a motor neuron assembly commensurate with the complex behaviour of the adult. The evidence of tissue analysis (Liu and Dixon, 1965; Tripathi and Dixon, 1968; 1969; Tsao, 1972) and the retrogression of the reproductive system (Zander et al., 1916) suggest that a reorganization takes place in the worker during the prepupal and pupal periods.

There is evidence too of hormonal regulation at earlier stages of development. Prothoracic gland and oenocyte nuclei are much larger in late instar queen larvae than in worker larvae of the same age (Lukoschus, 1952; Ritcey and Dixon, 1969). Histological studies have not established caste differences in activity of the neurosecretory cells of the brain and frontal ganglia earlier than the fourth instar, though this does not necessarily imply identity of action in the two castes. The corpora cardiaca are poorly developed in all young larvae, and prothoracic glands and oenocytes show no obvious caste differences prior to the critical period for caste determination (Ritcey and Dixon, 1969). Corpora allata do exhibit pronounced differences in both fine structure (Liu and Dixon, 1973) and function (Dixon and Moser, 1972). When assayed for juvenile hormone activity in newly emerged female cockroaches, corpora allata of 48-hour queen larvae are repressive, whereas worker allata of the same age are activating and can effectively replace the corpora allata of the roach. The caste difference in hormone balance at this time is quite distinct, and may even involve an additional repressor hormone. At about 72 hours, when the point of virtual "no return" for caste development has been reached, the relative activities of workers and queen corpora allata are reversed. Worker allata are now repressive and queen allata more active than those of the 48-hour workers (Dixon and Moser, 1972).

Presumably the concentration and timing of the corpora allata secretions are important to developing tissues of varying competence. Although we have no direct evidence of the nature of their influence in metabolism in honeybee larvae, there is indirect evidence that their effects may be important:

(1) A commercial juvenile hormone preparation* increased growth

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* Ayerst Research Laboratories, Montreal. We are indebted to Dr. A.J. Manson for supplying the sample.

rates of larvae on artificial diets (Shuel, 1973)

(2) Worker and queen allata differ in their effects on cockroach tissue; 48-hour worker allata (activating) when transplanted into newly-emerged, decapitated roaches allowed protein synthesis to proceed normally. Queen allata of the same age (repressive) totally disrupted this process in normal roaches with their allata intact and also affected the fine structure of the endoplasmic reticulum, mitochondria, and nucleopores (Liu and Dixon, 1973)

(3) Implantation of 3-day queen corpora allata (active stage) into 4-day worker larvae caused a partial arrest of ovary regression in the pupal stadium, whereas 3-day worker allata (inactive) had no effect (Chai and Shuel, 1970)

(4) Corpora allata of other insects have been shown to influence a number of physiological activities including feeding and digestion (Strangways-Dixon, 1962), and lipid and protein synthesis (Wigglesworth, 1965).

It is quite conceivable that differential corpora allatal activity could be responsible for caste-differences in respiratory metabolism. Beginning about 50 hours of age, there is a positive regression of oxygen uptake with time in queen larvae and a negative regression in workers (Shuel and Dixon, 1968). By 3 days of age the mitochondrial content of queen is higher than that of workers (Osonai and Rembold, 1968). At 72 hours there is a difference between castes with respect to the relative participation of two anaerobic pathways, glycolysis and the pentose phosphate stunt (Lerer and Dixon, 1973). As worker larvae consume less oxygen, their overall use of anaerobic pathways must be more effective than that of queens.

Hormonal conditioning might well be of great significance in the expression of dimorphism.* Different types and levels of corpora allatal activity may interact with other endocrine centres for which no differences have yet been recorded.

Differences in nutritional status may also be significant. When ligation was carried out on late instar larvae (cf. Schaller, 1952), that had been starved for 2 days, larvae failed to defecate and metamorphosis was delayed for 8 days (Dixon, 1973). Corpora allata dietary interactions are not uncommon in insects. Either hyperactive allata or a low nutritional state, for instance, can prolong the pupal period (Staal, 1972). Preferential carbohydrate feeding is caused by the allatectomy of Calliphorid flies, and protein ingestion is enhanced by active corpora allata (Strangways-Dixon). Once endocrine differences have been established, numerous possibilities for interaction exist. The question now arises, what initiates endocrine differences? What regulates the regulators?

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* There is new direct evidence of an important role of juvenile hormone in caste determination. Topical application of juvenile hormone to $3\frac{1}{2}$ day old larvae in worker cells resulted in the development of queen-like adults. (Wirtz, P., and Beetsma, J., (1972), Ent. exp. & appl. 15:517-520).

ENDOCRINE-NUTRITION RELATIONSHIPS The diets of queen and worker larvae are known to differ in respect to a number of components (see above). The candidate for corpora allatal differential activator might be either a single component or a combination of two or more. One compound that is of special interest is 10-hydroxydec-trans-2-enoic acid, which makes up the major part of the lipid fraction of royal jelly (Townsend and Lueas, 1940) and is present in higher concentration in royal jelly than in worker jelly (Shuel and Dixon, 1959). It is an effective repressor or protein synthesis in the collaterial glands of the roach; 9-oxodecenoic acid, the queen substance component which inhibits worker ovary development (Pain, 1973) has a similar though much less pronounced effect (Dixon, 1973). Both 10-hydroxydecenoic acid and 9-oxodecenoic acid inhibit cockroach corpora allata (Dixon, 1973) and 9-oxodecenoate inhibits the allata of the worker honeybee (Luscher and Walker, 1962). Both substances mimic the repressive action of 48-hour queen corpora allata on protein synthesis, an effect which is reversible with synthetic juvenile hormone. 10-hydroxydecenoate represses respiratory metabolism (Dixon, 1973), and its high concentration in the queen larval diet during the first 3 days appears to slow down the growth rate (Smith, 1960; Kinoshita, 1973).

In summary we might postulate the following sequence of events: a dietary component(s) induces different patterns of corpora allatal activity with time, the worker allata being activated around 48 hours, the queen allata 24 hours later. Target organs differ in competence at 48 and 72 hours and divergent growth patterns result - a phenomenon that Waddington (1966) has described as an epigenetic "canalization" of development.

Diet during subsequent development acts as supportive feedback stabilizing larval development along queen or worker lines, and holding it on course. One example of this is in the dietary level of sugar. Worker jelly will not support pupation; the addition of sugars to the worker diet (modified worker jelly) by the nurse bees on the third day permits normal pupation (Shuel and Dixon, 1968a).

As epigenetic control is less precise than genetic, one would expect forms intermediate between queen and worker to appear. These are of common occurrence amongst laboratory reared larvae (Weaver, 1955; Smith, 1959; Jay, 1964; Dietz, 1971). Under natural conditions they are probably recognized as abnormal and eliminated by the worker bees (Weaver, 1957).

CONCLUSIONS The aspects of caste regulation that we have examined are compatible with a model of feedback regulation. Although knowledge of many components of the regulatory system is incomplete or lacking, pheromones appear to be important in signal transmission at the social level and hormones at the physiological level. There is good evidence for endocrine control of female caste development through variation in hormone concentration and balance at critical periods. We suggest that variation in endocrine levels is under dietary control; the mechanism by which dietary differences initiate endocrine differences is under investigation in our laboratories. The development of a chemically defined diet which would permit experimental control of growth from young larva to adult, is also being pursued. Its realization would be of great help in elucidating hormone-diet relationships.

Brian (1965) has likened the queen of an insect society to a master endocrine gland or the shoot apex of a vascular plant. The latter analogy implies a dominance-repression relationship. We suggest that an important feature of the honeybee society is the repression of a tendency of a worker to develop into a reproductive. Several aspects of worker development suggest a repression: the slowing down of worker growth in the fourth instar, while the growth rate of the queen remains steady; the wide fluctuation in various haemolymph constituents indicative of a reorganization; the more anaerobic metabolism of the worker; the retrogression of the worker ovaries; and the relatively long period required for metamorphosis. The retrogression of the larval worker ovaries - apparently under allatal control- resembles the suppressive effect of the queen substance on the residual competence of worker ovaries. The two mechanisms might be regarded as complementary.

Caste-development involves social regulation, regulation of diet, and physiological regulation which includes neurophysiological, endocrine, and metabolic mechanisms. As yet only the bare outlines are discernible; the details of the mechanisms and the integration of the various levels remain challenging problems.

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