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FACTORS DETERMINING HYPOPHARYNGEAL GLAND ACTIVITY OF WORKER HONEY BEES (*APIS MELLIFERA* L.)

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SUMMARY

The effects of worker age, brood stage and time of brood introduction or brood withdrawal on the hypopharyngeal (HP) gland activity of worker bees were examined. Worker age exerted a marked influence on gland activity. Only larvae affected HP gland activity, while eggs and pupae did not. Bees had inactive glands until their third day of larval feeding and they maintained high gland activity for approximately three days after larval removal. The data are congruent with the hypothesis that feeding behaviour itself causes gland activation, although the possibility that larvae produce a primer pheromone cannot be excluded.

RESUME

Facteurs déterminant l'activité des glandes hypopharyngiennes des ouvrières d'Abeille (Apis mellifera L.)

On examine différents facteurs qui pourraient affecter l'activité des glandes hypopharyngiennes (HP) des ouvrières d'Abeille, tels que l'âge des ouvrières, les stades de développement du couvain et le moment d'introduction ou de retrait du couvain. L'âge des abeilles exerce une influence marquée sur l'activité des glandes HP ; celle-ci est corrélée avec la croissance et la résorption des glandes décrites dans la littérature. Seules les larves sont aptes à activer les glandes HP, alors que les œufs et les nymphes ne le sont pas. Les glandes de l'abeille restent inactives pendant trois jours après le début du nourrissage des larves ; elles conservent leur activité pendant trois jours environ après le retrait des larves. Ces données sont en accord avec l'hypothèse que c'est le comportement de nourrissage lui-même qui active les glandes ; cependant, on ne peut exclure la possibilité que les larves produisent une phéromone stimulante.

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INTRODUCTION

In most social insects, interactions between adults and brood are universal and important parts of the social organization (MICHENER, 1974). In the honey bee society, the brood plays several important roles in its social organization. It has been reported to mediate pollen collecting activity (FREE, 1967), maintain the normal social structure by inhibiting worker bees' ovarian development together with queen substance (JAY, 1970; KROPACOVA and HASLBACHOVA, 1971) and initiate warming behaviour of worker bees through a pheromone (KOENIGER and VEITH, 1983, 1984).

The hypopharyngeal (HP) glands are paired food glands located in the heads of worker honey bees. The glands are of primary importance to the brood-adult interactions, as they provide secretions rich in protein, which are fed to larvae of all three castes, and also to adult queen and drones (RIBBANDS, 1953; SNODGRASS, 1956).

It has been shown that honey-bee brood can have a profound effect on the hypopharyngeal (HP) gland activity of adult worker bees (BROUWERS, 1982, 1983). After hypothesizing that threre might be a larval signal to activate the protein synthesizing process of HP glands in worker bees, a series of studies was undertaken to investigate the properties and mode of action of the signal (HUANG, 1988; HUANG, submitted; HUANG *et al.*, in press). In this study, several factors which could potentially affect the HP-gland activity were examined.

The age of worker bees could have a large effect on the HP-gland activity. It is well known that the HP glands are undeveloped when bees just emerge, and develop to full size within 6-12 days (OTTO, 1955) if pollen is supplied. They usually degenerate when bees start foraging (RÖSCH, 1925; SOUDEK, 1927; HASSANEIN, 1952; HAYDAK, 1957). Even though BROUWERS (1982) showed that summer bees with fully developed glands and high gland activity were at least 6-7 days old, no systematic studies have been conducted to monitor the change of HP-gland activity as the worker bees age. It is also of interest to determine the effect of age on the differentiation of HP-gland activity under broodless and brood-right conditions.

The term "brood" generally refers to any of, or a mixture of, eggs, larvae and pupae. A previous study has demonstrated that bees must have direct access to brood to activate the protein synthesis of the HP gland (HUANG *et al.*, in press), but it is not known which brood stages have the ability to do so. Results from a study to compare the relative effects of different stages of brood would provide some clues as to the property of the brood signal. For example, if egg and pupal stages both were to have the ability to activate the HP glands, the signal would be more likely to be chemical than behavioural. Two other important questions also remain unanswered: the latency of HP gland activation after brood is introduced and how long

the HP glands sustain their elevated gland activity after brood is withdrawn. Answers to these two questions would let us know whether brood is immediately effective on gland activation or some intermediate steps are necessary.

We report in this paper several experiments conducted to examine (1) the HP-gland activity of differently aged bees under broodright and broodless conditions, (2) the relative effectiveness of various brood stages in activating the HP glands, and (3) the HP-gland activity of bees at different times following introduction of brood as well as after removal of brood.

MATERIALS AND METHODS

Experiments were carried out from May to August, 1985 and 1986. The honey bees used were from the stock maintained by the Apiculture Field Laboratory, University of Guelph, which represents a mixture of European bee races (*Apis mellifera* L.).

The HP-gland activity was measured by an *in vivo* bioassay (Huang, submitted). Radioactive leucine (337 mCi/mMol, New England Nuclear) was used as a tracer by injecting one μl (0.1 μCi) of it into the thoraces of sampled bees. After injection, they were incubated in room temperature for 2 hours, which was found to be the optimal duration. The bees were then killed by immersing in 20% TCA (trichloroacetic acid) and their HP glands removed in 7% TCA. The glands of each bee were washed three times individually with 7% TCA and dissolved in 0.2 ml 1 N NaOH. Finally 10 ml of Aqueous Counting Scintillant (ACS, Amersham) was added to the dissolved glands and radioactivity was measured with a liquid scintillation counter (Packard Scintillation Counter 460C). HP-gland activity was expressed as disintegrations per minute (DPM) per bee, a measurement of radioactivity recovered from the dissected HP glands. Statistical analyses were conducted after logarithm transformation of data, as it was found that he responses were not normally distributed and their means and standard errors were correlated. All statistical models (General Linear Models Procedure) were constructed following FREUND and LITTLE (1986), and performed by SAS (SAS Institute Inc., 1985).

HP-Gland Activity of Differently Aged Bees Under Broodless and Brood-right Conditions

Four colonies, each kept in a five-frame hive with Langstroth frames, were established in early May of 1987. In early June, two of these were randomly selected to become broodless colonies. This was done by confining the queen in a wire-gauze cage, through the apertures of which bees could feed and communicate with the queen. Therefore the colonies became broodless yet no laying workers appeared because the queen still exerted her inhibitory effect on the ovarian development of worker bees (BUTLER and FAIREY, 1963).

After the two colonies with caged queens had been broodless for two weeks, 200 newly-emerged bees (a mixture from two other colonies) were introduced to each of the four colonies (two broodless and two brood-right). These bees were marked within 20 hours after emergence with Testor's paint on the thorax and sprayed with sugar syrup prior to introduction. Because different colours were used for bees introduced to broodless and brood-right colonies, bees that drifted between treatment colonies were distinguished and avoided during sampling.

Five bees were sampled daily from each colony, starting from the day the marked

bees were introduced (day 0). HP-gland activity was assayed within half an hour after bees' removal from the colony. For unknown reasons, more bees introduced into the broodless colonies were rejected relative to those added to brood-right colonies. Therefore, further samples could not be taken from the broodless colonies after 16 days of sampling, while for brood-right colonies sampling continued until 27 days after bee introduction. A total of 398 bees were sampled and assayed for HP-gland activity.

Since the age factor here was nested within each colony, the data conformed to a split-plot design and were analysed as such. The brood treatment was treated as the main unit and age as the subunit, colonies within each main unit (broodless or brood-right) treated as replicates. Correlation analysis was also performed between the means of the HP-gland activities of bees from broodless and brood-right colonies over different ages.

Effects of Various Brood Stages on HP-Gland Activity

A 6×6 latin square design was used to examine the effectiveness of various brood stages. This design eliminated possible variation caused by colony conditions or seasonal factors. Six 10-frame colonies, each with its queen caged, were used in this experiment. Six replicates were used, whereby six treatments were allocated to the six colonies for each replicate. The six treatments used were broodless control, eggs, capped brood, larvae younger than 3 days, larvae older than 3 days, and a mixture of eggs, pupae and various ages of larvae (with about 1/4 eggs, 1/4 capped brood and 1/2 larvae). Each colony therefore received all six treatments, but in a different order. Larvae of known age were obtained by confining the queen in a compartment made of queen-excluder material within the hive.

The experiment started when all colonies had been broodless for two weeks. Thirty newly emerged bees were marked with Testor's paint and introduced into each colony (one different color for each colony). On the fifth day after bee introduction, a frame of comb containing the appropriate brood stage was put into the centre of each of the six colonies. The colonies were checked daily to ensure that the stage of brood had not changed. For example, the egg comb was replaced daily with newly laid eggs to be certain no eggs would hatch into larvae. For the broodless control treatment, an empty frame was replaced daily to make it comparable to other treatments. After three days of treatment (the 8th day after bee introduction), the colony-specific colour-marked bees (aged 8 days) were sampled. Five bees were sampled from each colony, and their HP-gland activity assayed as described earlier. Only marked bees on "the treatment frame" (the frame with eggs, larvae, capped frood, or mixture) were sampled. A total of 180 bees were sampled and their HP-gland activities measured.

HP-Gland Activity of Bees at Different Times After Introduction and Withdrawal of Brood

To determine how quickly the HP glands become activated after introduction of brood, two experiments were carried out.

In the first experiments. 30 newly emerged bees were placed in a Liebefeld cage (size $10.5 \times 9 \times 7.5$ cm) with the front side made of glass and the lower side of screen gauze. These cages were similar to that pictured in the study of KULINCEVIC and ROTHENBUHLER (1973; *figure 1*). Distilled water and sugar syrup (50% by weight) were provided *ad libitum* through the two holes on the top of the cage; pollen was provided in a vial cap (diameter 2.5 cm) on the bottom of the cage in the form of pollen-sugar syrup mixture. The cage was kept in an incubator at 35 ± 1°C and relative humidity approximately 50%. When the caged bees were 5 days old, a piece of comb (6×6 cm)

containing larvae about 1-2 days old was put into the cage. Five bees each were sampled and their gland activity assayed at 1, 2, and 3 days after larval introduction.

A second experiment was conducted with bees in a 10-frame Langstroth hive as follows. A frame of brood with eggs and larvae up to 2 days old on one side and larvae 2-4 days old on the other was introduced to a colony which had been broodless for 2 weeks, as a result of caging the queen. Five bees were sampled at time 0 (just before introduction of bee brood), 5 min., 15 min., 30 min., 1 h, 2 h, 3 h, 5 h, 10 h, 24 h and 72 h after the introduction of the brood. Only bees actually visiting larval cells were sampled. The majority of these bees were found in a preliminary trial to be nurse bees (with greatly elevated HP-gland activities). HP-gland activities were measured for the 53 bees sampled (only three bees were sampled at 3 h).

Another experiment was designed to investigate the maintenance of HP-gland activity following removal of all larvae. Four cages of bees under the same condition as above were maintained in the incubator. Each cage had about 30 bees (aged 5 days) and a piece of comb (6 × 6 cm) containing larvae 1-2 day old. After the bees had fed the larvae for three days, the comb containing larvae withdrawn, and the bees were sampled subsequently to monitor their HP-gland activity. Three to five bees were sampled from each cage 0, 3, 4, and 5 days after the larvae were removed. A total of 43 bees were sampled.

RESULTS AND DISCUSSION

HP-Gland Activity of Differently Aged Bees Under Broodless and Brood-right Conditions

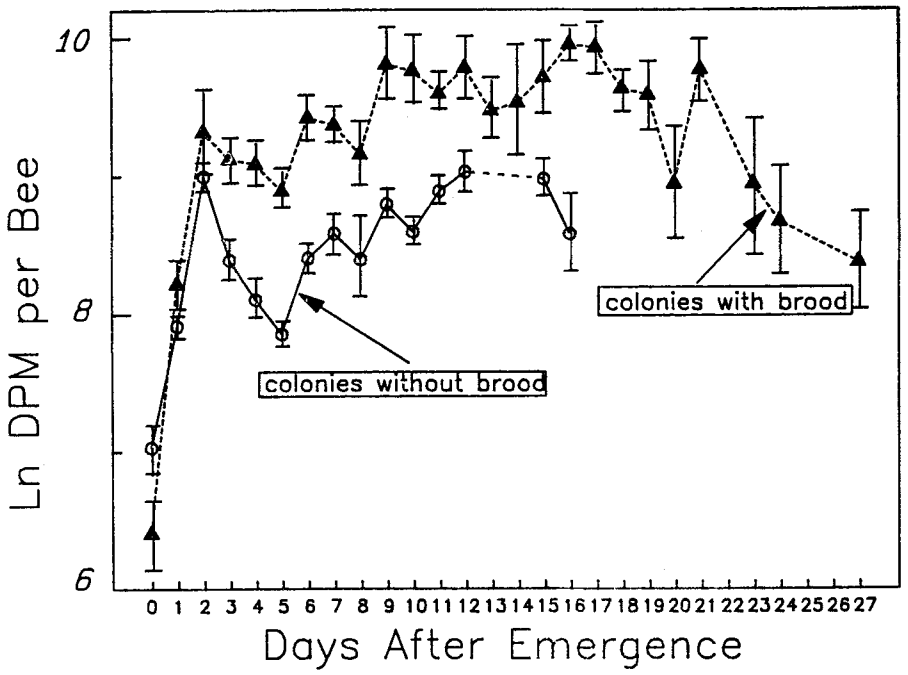
The statistical results of this study are presented in the ANOVA table (*table I*) as obtained with SAS. It can be seen that both brood and woker-bee age have significant influences on the HP-gland activity ($F_{1,1} = 186.65$, $P = 0.047$; $F_{24,38} = 6.48$, $P = 0.0001$, respectively). Replicate effect and interactions between brood and age are not significant ($F_{1,1} = 0.09$, $P = 0.818$; $F_{14,38} = 1.09$, $P = 0.39$, respectively).

Table I. — ANOVA table for experiment studying the change of HP-gland activity with bee age under brood-right and broodless conditions.

Tableau I. — Tableau d'analyse de variance (ANOVA) pour l'expérience sur la variation de l'activité des glandes HP d'abeilles élevées avec ou sans couvain.

Source	DF *	SS #	Mean Square	F. Value	Pr > F [d]
Replicate	1	0.01	0.01	0.09	0.8178
Brood	1	57.44	57.44	186.65	0.0465
Error A	1	0.18	0.18		
Age	24	159.32	6.64	6.48	0.0001
Age × Brood	14	15.58	1.11	1.09	0.3936
Error B	38	38.68	1.02		
Sampling Error	318	138.47	0.43		
Total	397	409.58			

Note: * DF: degrees of freedom.
SS: sum of squares.
[d] Pr > F: figures presented are actual probabilities of obtaining a larger F while the null hypothesis is true. Therefore, when Pr is smaller than 5%, it is significant at 5% level, and so on.



Fif. 1. — Change of HP-gland activity of bees as they age under broodless and brood-right conditions.
Solid triangles represent the mean HP-gland activities of 10 bees sampled from two brood-right colonies on each day (connected with dotted line). Circles represent similar data from bees in broodless colonies (solid line). The vertical bar above and below the two mentioned lines represent the standard error of the mean.

Fig. 1. — Activités des glandes HP en fonction de l'âge d'abeilles élevées en présence ou en l'absence de couvain.
Les triangles pleins (reliés par des lignes pointillées) représentent chacun une moyenne de 10 abeilles prélevées dans deux colonies contenant du couvain. Les cercles (reliés par des lignes pleines) représentent des données similaires pour des colonies sans couvain.
Les barres verticales représentent l'erreur type de part et d'autre de la moyenne.

HP-gland activity under broodless and brood-right conditions is presented graphically in *fig. 1.* It appears that except for the first three days, HP-gland activities from broodless and brood-right colonies maintained a consistent difference. Bees from brood-right colonies maintained elevated HP-gland activity from 9 to 19 days of age. There is a substantial decline at day 20 and a subsequent increase of HP-gland activity on day 21. A similar peak of HP-gland activity was previously recorded at about the time bees began to forage (HUANG, unpublished data), using the modified *in vitro* method of BROUWERS (1982). It is possible that the drop indicates the termination of nursing duties, while the next peak in activity represents

the production of forager-specific proteins such as invertase, which coincides with the onset of foraging.

Although the HP-gland activities of bees from broodless colonies are consistently lower than those of bees in brood-right colonies, a gradual increase in glandular activity is evident in both groups between days 5-15. A correlation analysis shows that the two means are highly correlated over 0 to 16 days (data were missing from days 13-14 for broodless colonies, so 15 pairs of means were analyzed; Pearson correlation coefficient $R = 0.894$, $df = 13$, $P = 0.0001$). This probably indicates that bee age has a marked effect on the pattern of change in HP-gland activity, no matter whether bees are under broodless or brood-right conditions. However, other factors common to the four colonies, such as nectar and pollen availability and weather conditions, could also have played roles in influencing the HP-gland activity. The experiment could not exclude the latter temporal factors because the age of bees was confounded with them. The experiment would have to be repeated over different seasons and different resource conditions to separate the effects, if any, of these variables.

The general pattern of HP-gland activity of bees from brood-right colonies a shown in *figure 1* matches the three phases of glandular development (HASSANEIN, 1952; HAYDAK, 1957). The activity was minimal in newly emerged bees which have undeveloped glands. Then it rose sharply in the first three days after emergence as the glands developed to full size. The activity stayed at a high level while the bees were engaged in brood rearing, and declined when bees started foraging. The glandular activity of bees from broodless colonies had a similar trend, but as there was no brood for bees to care for, their glands displayed a much lower level of activity. The gland activity of these bees actually declined steeply 3 days after emergence. The peak of activity on day 2 probably represents synthesis of "structural proteins" which are necessary for glandular development but would not appear in the brood food.

It is unfortunate that the shortage of marked bees in broodless colonies obviated comparison of gland activity betwen bees under the two brood conditions at older ages. Nevertheless a decline in HP-gland activity from day 23 on was observed for bees in the brood-right colonies (*fig. 1*), possibly due to the start of foraging. The HP-gland activity of bees from the two brood conditions may converge again at older ages. A similar decline in HP-gland activity for broodless bees would not be expected, because 1) the bees from broodless colonies already had a lower gland activity compared to that of brood-right bees; and 2) the shift of tasks to foraging in broodless bees may be less distinct because the absence of brood reduces foraging activity (FREE, 1967) and broodless bees have greater longevity (MAURIZIO, 1950; FLURI *et al.*, 1982). We observed that generally foraging was much less intensive in broodless colonies than in brood-right ones.

Effects of Various Brood Stages on the HP-Gland Activity

This experiment was designed to determine the relative effectiveness of different brood stages in elevating HP-gland activity. The results of the ANOVA analysis are presented in *table II*. Both the effects of treatment and of replicates were highly significant ($F_{5,20} = 4.29$, $P = 0.0082$; $F_{5,20} = 25.21$, $P = 0.0001$, respectively). The colony effect ($F_{5,20} = 2.29$, $P = 0.09$) was not significant at the 5% level. The HP-gland activity as affected by various brood stages is presented in *figure 2*. Together with eight planned contrats shown in *table III*, we can summarize the following results. The effects of pupae and eggs were not different from the broodless control (contrasts 1 and 2 in *table III*), indicating their inability to activate the HP glands of the sampled bees. The effects of larvae younger than 3 days (LA1) and older than 3 days (LA2) were not significantly different from each other (contrast 3 in *table III*). Larval treatments considered together were significantly more effective than either the control, or eggs and pupae combined in triggering HP-gland activity (*figure 2;* contrasts 4, 5, 6 in *table III*). A mixture of all brood stages, on the other hand, was intermediate between eggs/pupae and larvae: it was more effective than eggs/pupae but less effective than larvae (*figure 2,* contrasts 7 and 8 in *table III*).

Table II. — ANOVA table for a 6 × 6 latin-square design to study the effect of various brood stages on HP-gland activity of worker bees.

Tableau II. — Tableau d'analyse de variance (ANOVA) avec un plan d'expérience en carré latin 6 × 6 pour l'étude des effets des différents stades de développement du couvain sur l'activité des glandes HP d'ouvrières d'Abeilles.

Source	DF	SS	Mean Square	F Value	Pr > F
Replicate	5	1.79	0.36	4.29	0.0082
Colony	5	0.96	0.19	2.29	0.0848
Treat	5	10.53	2.11	25.21	0.0001
Error	20	1.67	0.08		
Total	35	14.95			

Table III. — Planned contrasts between various treatments or treatment combinations of the latin-square experiment.

Tableau III. — Tableau des contrastes entre différents traitements ou entre combinaisons de traitements dans le plan d'expérience en carré latin.

Contrast	df	Mean Square	F Value	Pr > F
1. control vs pupae	1	0.01	0.11	0.7415
2. control vs eggs	1	0.00	0.02	0.8818
3. control vs mixture	1	1.42	17.05	0.0005
4. old larvae vs young larvae	1	0.01	0.15	0.7013
5. control vs both larvae	1	6.01	71.91	0.0001
6. eggs + pupae vs both larvae	1	8.43	20.14	0.0001
7. mixture vs eggs + pupae	1	1.68	100.86	0.0002
8. mixture vs both larvae	1	1.15	13.78	0.0014

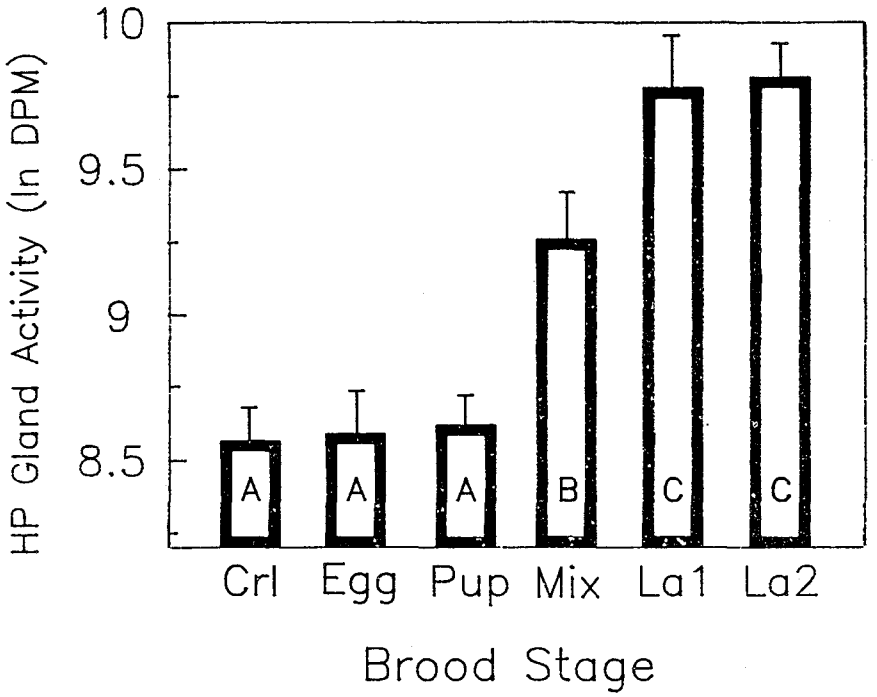


Fig. 2. — Means and standard errors of HP-gland activity of bees from various brood-stage treatments.

Each bar represents the data from 30 bees.

Legend for x-axis label:

Crl: broodless control;

Egg: egg stage;

Pup: capped brood;

Mix: a mixture of all brood stages;

La1: larvae younger than 3 days;

La2: larvae older than 3 days;

Bars with the same letters are not significantly different from one another at 5 % level, tested by planned contrast.

Fig. 2. — Moyennes et erreurs types de l'activité des glandes HP d'abeilles ayant été en présence de couvain à divers stades de développement. Chaque colonne représente la moyenne des données de 30 abeilles.

Légendes de l'axe des abscisses:

Crl. : témoins sans couvain ;

Egg : œufs ;

Pup : couvain operculé ;

Mix : mélange de tous les stades de développement du couvain ;

La1 : larves de moins de 3 jours ;

La2 : larves de plus de 3 jours.

Les valeurs des colonnes portant la même lettre ne sont pas significativement différentes au seuil de 5 % pour les contrastes planifiés du tableau 3.

It is of interest to note that a mixture of all brood stages resulted in intermediate levels of HP-gland activity. The only explanation we can provide for this is that possibly fewer nurse bees would be needed to care for the mixture (because only about half of the cells contained larvae that needed to be fed), so that a smaller proportion of bees in the samples had activated HP glands.

BROUWERS (1983) suggested that eggs alone seem to have the ability to activate the HP glands of bees kept in Liebefeld cages, but failed to present statistical evidence to support that conclusion. In contrast, no effect by eggs was detected in this experiment. It may be seen from *figure 2D* of BROUWERS (1983) that among 50 bees sampled from the egg treatment, only 3 bees had gland activity above, and another 3 bees had activity about the same level as, the "minimal gland activity displayed by known nurse bees". Iy seemed that the egg stage has only a slight, if any at all, effect on HP-gland activation.

HP-Gland Activity of Bees at Different Times After Introduction and Withdrawal of Brood

Table IV presents the means and standard errors of HP-gland activity of bees sampled at different times after larval introduction. In the cage experiment, only bees sampled three days after larval introduction showed an elevated HP-gland activity (upper part, *table IV*). The results from the

Table IV. — HP- gland activities of bees sampled at various time intervals after brood was introduced. Top: experiment carried out in cage. Bottom: experiment carried out in hive.

Tableau IV. — Activité des glandes HP d'Abeilles prélevées à des moments différents après introduction du couvain.

	Time *	N	Mean DPM #	Std error
Cage:	1 d	5	3291.3	700.1
	2 d	5	5932.3	2030.4
	3 d	5	11357.2	1933.6
Hive:	0 m	5	6674.9	793.0
	5 m	5	5801.3	1614.8
	15 m	5	7271.2	1367.2
	30 m	5	5562.0	1475.0
	1 h	5	4162.3	861.7
	2 h	5	7089.1	1225.2
	3 h	3	7608.3	2024.5
	5 h	5	4238.5	1295.8
	10 h	5	3248.6	848.0
	24 h	5	7267 4	1262.8
	72 h	5	17071.7	5739.5

Note: * for time intervals, d denotes day, m minute, and h hour.
DPM: disintegrations per minute per bee.

hive experiment studying the precise timing of HP-gland activation are presented in the lower part of *table IV*. No sign of elevated HP-gland activity was found until 72 hours after brood introduction. Unfortunately, the status of the glands at 48 hours could not be determined. However, it is clear that bees did not have their glands activated within 24 hours, even though the larvae were undoubtedly being fed in this period, for otherwise they would have starved to death. (Most larvae 3-5 days old move out of their cells when not fed for more than 10 hours; personal observation by Z.-Y.H). Therefore, there is a time lag between the initiation of actual feeding behaviour and gland activation. The similarity in results between this experiment and the cage experiment seems to suggest that the time lag is about three days. This result is compatible with that of BROUWERS (1983), who noted that HP glands "demonstrated the highest activities *after* 3 days", when eggs and young larvae were present. The time lag here probably indicates that the HP glands are activated after, rather than before the larval feeding, i.e. the elevated HP-gland activity is a result of, rather than a pre-condition of, larval feeding.

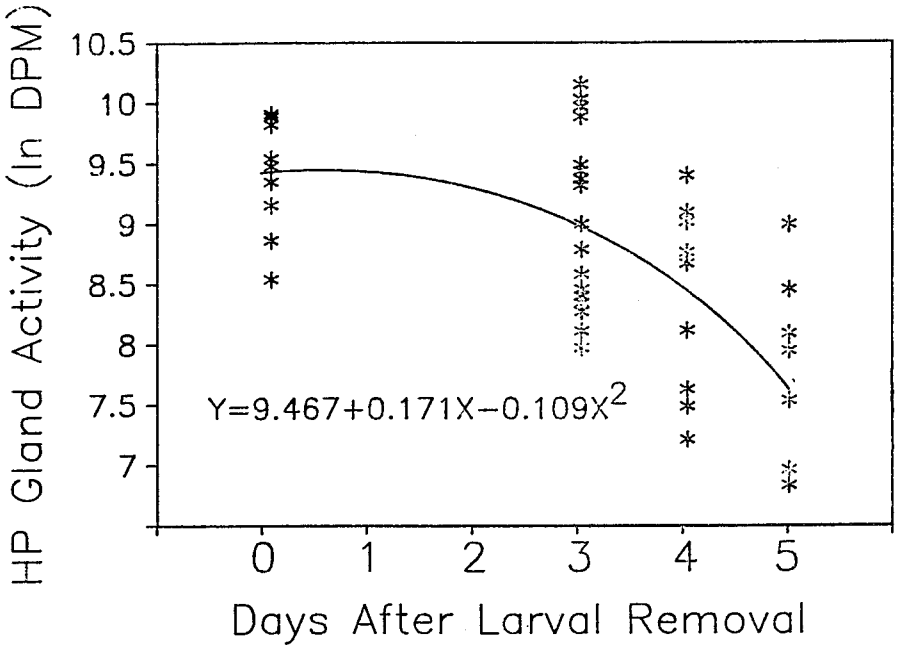


Fig. 3. — HP-gland activity of caged bees sampled at different times after brood was removed.

Fig. 3. — Activité des glandes HP d'abeilles prélevées à des moments différents après le retrait du couvain.

No difference in HP-gland activity was found between different cages ($F_{3,31} = 0.33$, $P = 0.80$), but larval removal reduced the gland activity dramatically ($F_{8,31} = 2{,}71$, $P = 0.0214$). Further analysis showed that there was a quadratic regression between the number of days after larval withdrawal and HP-gland activity of caged bees ($F_{2,40} = 13.54$, $P < 0.001$). *Figure 3* depicts this relationship graphically. This result demonstrates once again that the elevated HP-gland activity is dependent on the presence of larvae, but also there is a time lag between the removal of brood and the decline of gland activity. The gland activity returned to approximately the same level as that of bees from broodless colonies about 4 days after the larvae were withdrawn.

This time-lag phenomenon for gland activation can have several explanations. One possibility is that the brood indeed has a signal to activate the HP gland, but the signal has a "primer" effect instead of a "releaser" effect, so some time is required for the signal to effect a response. Alternatively, one can postulate that feeding behaviour itself is sufficient for gland activation, if there is a simple negative-feedback mechanism. One inevitable result of the feeding is that clusters of HP glands cells would become smaller as glandular secretions are transported through the ducts to the mouthparts. With operation of a negative-feedback mechanism, the glands could start protein production if they are smaller than a threshold size, and conversely stop protein production if glands are larger than an upper threshold. Such a mechanism could be accomplished through stretch receptors or a direct biochemical feedback system.

Both the above hypotheses can satisfactorily explain the time lag before gland activation. However, the negative-feedback model also predicts that 1) once larvae are removed, the size of glands would increase because there is no protein output and 2) the glands would therefore stop protein production after a certain time (while the glands reach the threshold size) as a result of signals from stretch receptors. The predictions are met by the facts that 1) glandular hypertrophy occurs in broodless or winter bees and 2) there is also a time lag for gland "de-activation" (i.e. for the elevated HP-gland activity to return to the level of inactive glands). According to the hypothesis, the time lag for activation and "de-activation" should be of about the same length, which is what the experiments showed (three-four days).

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