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FACTORS CONTROLLING INITIATION OF VITELLOGENESIS IN A PRIMITIVELY SOCIAL BEE, *LASIOGLOSSUM ZEPHYRUM* (HYMENOPTERA : HALICTIDAE)

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SUMMARY

Hormonal, nutritional, and environmental factors influencing vitellogenesis in *Lasioglossum zephyrum* females were studied in bees maintained in plastic vials or in glass nest sites.

A protein source is necessary for initiation of egg development. Further, it appears that protein ingestion triggers juvenile hormone secretion which is followed by the initiation vitellogenesis. Social interactions are not required for ovarian development, although the nest site itself, with soil for making cells, seems to stimulate protein ingestion and therefore vitellogenesis.

Inhibition of egg development in « worker » bees exerted by the « queen » can be overcome by treating « workers » with juvenile hormone, suggesting that the inhibition is mediated through the corpora allata.

RÉSUMÉ

Facteurs contrôlant le début de la vitellogenèse chez une abeille primitivement sociale *Lasioglossum zephyrum* (Hymenoptera : Halictidae).

On a étudié les facteurs hormonaux, nutritionnels et environnementaux influençant la vitellogenèse des femelles de *Lasioglossum zephyrum* élevées dans des tubes en plastique ou dans des nids en verre.

Une source de protéines est nécessaire pour l'initiation du développement des œufs. De plus, il apparaît que l'ingestion de protéines déclenche la sécrétion d'hormone juvénile qui est suivie du début de la vitellogenèse. Des interactions sociales ne sont pas nécessaires au développement ovarien, bien que le nid lui-même, avec du terreau pour faire les cellules, semble stimuler l'ingestion de protéines et, par conséquent, la vitellogenèse.

L'inhibition du développement des œufs chez les « ouvrières » par la « reine » peut être levée par le traitement des ouvrières avec de l'hormone juvénile, suggérant que l'inhibition passe par les corpora allata.

INTRODUCTION

Females of *Lasioglossum zephyrum* which emerge in summer, reside in colonies averaging 14 individuals. Usually all intergradations between queen and workers are present, and this array is depicted in the scale of vitellogenesis from inactive ovaries to active oviposition. In the majority of nests, however, a single « queen » is responsible for much of the egg-laying (BATRA, 1966).

Two major problems are evident concerning the control and inhibition of vitellogenesis in this primitively eusocial bee: (1) the mode of vitellogenic inhibition presumably exerted by the more queen-like individual over other bees of the colony, and (2) factors which mediate initiation of yolk deposition in reproductive females. This paper deals with various environmental, hormonal, and nutritional factors which synergistically stimulate yolk deposition in this species.

METHODS AND MATERIALS

Bees were maintained under summer photoperiod and air temperatures similar to that experienced in nature. Two different experimental devices were employed. In some experiments, bees were maintained in plastic vials with a volume of 1.74 cm^3 connected to two 60 mm long plastic tubes. One tube was provided pollen and the other with a mixture of honey-water. Certain variations were used as described below.

The second apparatus, described by MICHENER and BROTHERS (1971), consisted of a 3 mm layer of soil between two glass plates. Pupae collected from the field were placed in artificial cells formed near a tunnel leading to the outside of the glass nest site. The nest was capped with a vial of the same dimensions as described above so that the bees were not permitted free flight.

Vial tubes or cups within the vials attached to glass nest sites were provided with pollen collected from various flowers gathered near *L. zephyrum* nests in the field. Honey-water mixtures of 50:50 (v:v) *Apis mellifera* honey mixed with water, or saturated sucrose-water solutions were also provided in a similar manner. In some experiments honey-water mixed with calf-serum, 1:10 (v:v), was introduced into vial tubes of nest site vials, In all experiments bees were provided with water.

In both designs the glass nest or tubes were maintained in darkness to mimic conditions in soil, whereas the vials were exposed to light.

Topical juvenile hormone applications were performed by coating the thorax of a restrained bee with 0.15 μg of synthetic juvenile hormone (JH) in olive oil. The restraining devise consisted of a vacuum-supplied disc covered with plastic mesh. The bee was held against the mesh owing to the suction beneath, and therefore use of carbon dioxide or other anesthetic was unnecessary.

Ovaries were dissected in Dietrich's (Kahle's) solution and measurements made on width and length of basal oocytes. Oocyyte volume was calculated according to BELL (1969).

RESULTS

1. Isolated bees.

Newly emerged *L. zephyrum* females maintened in plastic vials engaged in behavior patterns which were similar to those previously described for bees in the field (BATRA, 1964, 1968) and in artificial colonies (MICHENER *et al.*, 1971). Activity was confined to the plastic tubes from approximately 4 hours before sunset to 4 hours after sunrise (fig. 1), whereas the bees engaged in short flying spurts and rapid walking protruding into the vial. When disturbed the bees backed down into the tube. That bees simulated working behavior was suggested by their periodic pushing of either soil or pollen placed in the tubes. Working periods lasted 1 to 4 minutes spaced by equal or greater periods of grooming. The most common pattern observed was moving pollen from the tube into the vial using hindlegs and abdomen. Up to 10 mm³ of pollen were moved in a 6 hour period. Bees were observed on a few occasions

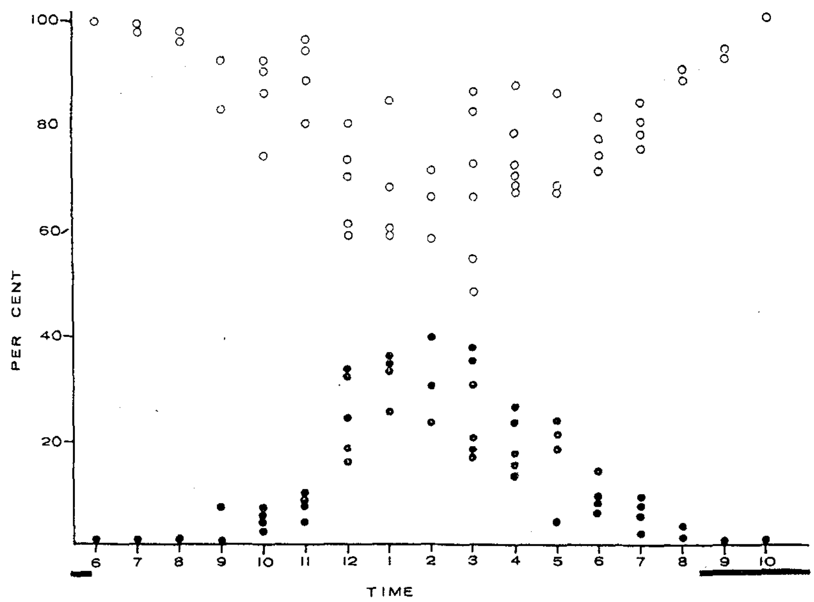

FIG. 1. — Activity of isolated bees. Open circles represent the per cent of bees in the tubes (guarding, moving pollen), closed circlees represent the per cent of bees in the vial (rapid walking, feeding). The heavy line below the lower axis depicts the dark period during the light cycle. Observations were made between 5:30 am to 10:30 pm. Each point represents one observation.

in the vials during the ramaining portion of the daylight hours. Bees in the tubes spent on the average 50 % of their time guarding tube entrances facing the vial with antennae moving pollen from one tube to another on the hind legs and abdomen, but whether this was a conscious effort is not certain. Bees were commonly observed drinking honey water, although their time in tubes was divided almost equally between the pollen tube (53 %) and honey water tube (47 %).

Several variables were introduced to determine what factor or factors might stimulate ovarian development in isolated bees not permitted to make nests. Table I depicts the results of these experiments. Bees provided with pollen and honey-water were dissected after 5 or 12 days, periods of time sufficient for initiation of egg development in bees provided with nesting sites, or after 30 days. No yolk spheres were present in the basal oocytes, as determined by phase contrast microscopy, and basal oocyte volume, 0.09×10^{-3} mm^3, showed no significant increase over oocyte volume of newly emerged *L. zephyrum* females (Table I, *a*). Starved females also had no ovarian development, but only survived for 24 to 48 hours (Table I, *b*). No pollen was observed in guts of most isolated bees provided with pollen and honey water although slight quantities were found in 5 % of the females examined, whereas the crops were filled with honey-water in all cases. On the basis of these results it was assumed that the protein consumption of isolated bees was at the most minimal as compared with pollen-filled guts of bees caught in the field or captured from artificial colonies. When honey-water diluted with calf serum was offered to isolated bees, a significant increase in mean basal oocyte volume (8.67×10^{-3} mm^3) was observed (Table I, *c* and *d*). Variation in oocyte volume among protein-fed bees (.09 to 48.40×10^{-3} mm^3) may reflect differences in the quantity of liquid ingested. No increase in oocyte volume occurred when bees were fed boiled calf serum, presumably depleted of protein, mixed honey-water.

In a variety of insect species the juvenile hormone stimulates vitellogenesis (review : ENGELMANN, 1968). It was of interest, therefore, to test this possibility in isolated *L. zephyrum* females. Juvenile hormone

TABLE I. — EFFECTS OF PROTEIN DIET AND JH APPLICATIONS ON OVARIAN DEVELOPMENT IN ISOLATED BEES

TREATMENT*	N	MEAN VOLUME OF LARGEST OOCYTE ($mm^3 \times 10^{-3} \pm$ S.D.)	PER CENT FEMALES WITH OVARIAN GROWTH
a. Fed honey-water and pollen......	40	0.09 ± .00	0
b. Starved	20	0.09 ± .00	0
c. Fed pollen and honey-water with 10 % calf serum..................	25	8.67 ± 5.48**	48
d. Fed pollen and honey-water with 10 % boiled calf serum...........	10	0.09 ± .00	0
e. Fed pollen and honey-water, topical JH application...............	25	69.52 ± 12.02**	80
f. Fed pollen and honey-water, topical olive oil application	25	0.09 ± .00	0

* Although pollen was provided, few bees ingested pollen when isolated.
** The relatively large S.D. are accounted for by the inclusion of volumes of oocytes which failed to engage in vitellogenesis.

was topically applied to provide each bee with 0.15 micrograms of synthetic hormone mixed with olive oil. The bees were also permitted to feed on pollen and honey-water. After 12 days the dissected ovaries contained basal oocytes with a mean volume of 69.52×10^{-3} mm^3, and vitellogenesis was initiated in at least 4 of the 6 basal oocytes in each female. No growth was stimulated when bees were treated with an equal quantities of olive oil, nor was pollen detected in guts of dissected bees (Table I *e* and *f*).

soil-filled plastic tubes. Apparently neither contact with other bees, nor provisions for burrowing were sufficient to stimulate pollen feeding and ovarian development.

Within 48 hours after emergence, bees in glass nest sites began increasing the length of their burrows, and within 7 days cells were constructed and provisioned. Successful cell construction was indicated by egg hatching, pupation, and emergence of male offspring.

Groups of bees in similar nest sites, but without soil, failed to develop eggs, and as in isolated bees, failed to feed on pollen. Conversely, bees in soil-filled nest sites showed various degrees of ovarian development as in normal colonies. In each of 12 nests (Table II) one individual had terminal, chorionated basal oocytes, whereas others showed some ovarian development often with one or more resorbing oocytes. Pollen-filled guts were observed in 75 % of the bees.

TABLE II. — OVARIAN GROWTH OF BEES IN ARTIFICIAL COLONIES IN GLASS NEST SITES

NEST #	BEE #1	VOLUME OF LARGEST OOCYTE ($mm^3 \times 10^{-3}$)			
		BEE #2	BEE #3	BEE #4	BEE #5
1	55.60	20.90	0.09	—	—
2	117.30	55.60	2.35	.09	.09
3	55.60	.21	.09	.09	—
4	.71	.12	.09	—	—
5	209.50	.90	.90	.22	—
6	219.50	157.20	16.30	.27	.09
7	.92	.91	.09	.09	—
8	320.00	1.23	—	—	—
9	54.60	47.50	.54	.09	—
10	160.22	27.80	—	—	—
11	8.30	.12	.09	—	—
12	119.20	1.47	—	—	—

Isolated bees in glass nests, but without soil, failed to develop eggs, and as in vials (discussed above) failed to feed on pollen. On the other hand, isolated bees in glass nest sites with soil fed on pollen and exhibited vitellogenesis.

2. Bees in artificial colonies.

Vitellogenesis and pollen feeding were not initiated in untreated groups of 3 to 5 bees maintained together in plastic vials, nor in groups of bees in vials complete with

In some nests the bees were provided with honey-water but no pollen. In these cases 8 of 12 nests contained no bees with ovarian development after 12 days, whereas one bee in one nest had moderate ovarian growth and 3 bees in 3 nests had less ovarian development (Table III, *b*). To further probe the effects of feeding protein and non-protein diets, six nests were prepared as above. Pollen was provided in addition to sucrose water in 3 nests, whereas the other 3 nests were given only sucrosewater (Table III, *c* and *d*). In these experiments no ovarian development was observed in bees without access to pollen, whereas those bees provided with pollen exhibited the normal gradation of ovarian growth from egg-laying females to females with only slight ovarian growth. Bees without pollen did, however, dig tunnels and construct cells, but did not provision or close the cells.

TABLE III. — EFFECTS OF RECENT DIET AND HORMONE APPLICATIONS ON OVARIAN DEVELOPMENT OF BEES IN ARTIFICIAL COLONIES IN GLASS NEST SITES

TREATMENT	NUMBER BEES	NUMBER NESTS	% FEMALES WITH OVARIAN DEVELOPMENT	AVERAGE VOLUME OF LARGEST OOCYTE ($mm^3 \times 10^{-3} \pm$ S.D.)
a. Nests provided with pollen and honey-water (Data from Table II).	41	12	76	109.53 ± *
b. Nests with honey-water..........	36	12	22	5.23 ± 1.85
c. Nests with sucrose-water and pollen	10	3	75	97.65 ± *
d. Nests with sucrose-water........	11	3	0	.09 ± .00
e. Nests with honey-water, pollen, topical JH application............	15	5	60	174.22 ± 23.17
f. Nests wiht honey-water, pollen, topical olive oil application.........	16	5	75	88.78 *

* Standard deviations for the means of *a*, *c* and *f* were meaningless owing to the inclusion of data from bees with no ovarian development which always occur in a nest.

As described above, JH administration was followed by ovarian development in isolated bees even in the absence of post-emergence protein feeding. Secondly, in nests provided with a protein source (pollen) some females exhibited little or no ovarian development, and usually one female usurped the egg-laying function of the colony. It was of interest therefore to investigate whether inhibition of « worker » ovarian development is mediated by curtailing the secretion of JH by the corpora allata. Nests containing 3 to 5 pupae were observed over a period of one week during which the adult females emerged, dug tunnels and constructed cells. The nests were then chilled to inactivate the inhabitants, the bees were removed, and in 3 nests the bees were treated with JH and in the remaining 3 nests the bees were treated with olive oil. The bees were returned within 4 hours to their respectives nests and left undisturbed for one week. Table III (*e* and *f*) depicts the increase in ovarian development in bees treated with JH as compared with those treated with olive oil. No credence can be given to differences in the percent of bees with ovarian development, as most bees in normal colonies have at least moderate ovarian growth, but a significant difference in volume of the largest oocyte was observed.

DISCUSSION

In general, diet and seasonal factors determine the extent of ovarian development in bees. Newly emerged worker honeybees require a protein source for initiation of yolk deposition, although the source may be experimentally provided by soybean flour (HESS, 1942), casein (VERHEIJEN-VOOGD, 1959), or pollen (PAIN, 1961). Protein requirements for vitellogenesis are common in a variety of insect groups, and in some species feeding triggers secretion of juvenile hormone (JOHANSSON, 1964). Seasonal influences on ovary development have been reported by VELTHIUS (1971), especially with regard to seasonal variation in oocyte growth during the summer months.

Inhibition of worker egg development is apparently mediated in social Hymenoptera by an effect the queen has over workers (review : BUTLER, 1964). The inhibitory factor(s) is apparently secreted by queen mandibular glands. The mechanism of action is not well understood, however, especially since the queen substance vapor causes some inhibition in addition to the strong effect of ingestion. Injections, however, failed to provide definite results.

That JH plays a role in worker honeybee ovary development is suggested by a reduction in ovary regression when corpora allata from 4-day-old queen larvae were implanted into 4-day-old worker larvae. Corpora allata from worker larvae failed to stimulate the action of queen larval glands (CHAI and SHUEL, 1970) No oocyte growth resulted from the above experiments, however, whereas farnesyl methyl ether, a juvenile hormone analogue, did stimulate oocyte development when administered to larvae. Thus, although an effect of JH is suggested, the results also indicate the possibility of different hormones or different concentrations of hormones issuing from implanted glands as compared with injection of FME. The work of CHAI and SHUEL does not suggest, however, that JH might enhance vitellogenesis when administered to adult bees.

The results of topical application of JH in *L. zephyrum* point out, although indirectly, that JH stimulates ovarian development. A protein meal is not required for this process, however, if JH is administered; but without the hormone, then protein ingestion is a requirement. Similar results have been reported by BOHM (1972) on *Polistes metricus*. The only exception to this hypothesis was the substantial number of bees with developing ovaries in nests provided with only honey water. According to WHITE *et al.* (1962), *Apis mellifera* honey contains only 0.041 % nitrogen, an obviously insufficient quantity of protein to stimulate egg development. Because no vitellogenesis was observed in bees fed sucrosewater, it is concluded that either soil or honey contamination is responsible for the above result. The findings lend themselves to the hypothesis that protein ingestion stimulates JH secretion, although the products of protein metabolism are not immediately required for synthesis and mobilization of yolk precursors. Exactly where yolk precursors are stored in female *L. zephyrum* is certainly a problem to be investigated more closely.

Interactions within a *L. zephyrum* colony apparently are not requisite for vitellogenesis, and this is in keeping with the primitive sociality of this species. The nest itself in some way is required for egg development, however, as groups of bees maintained in a space equal to the space in a nest, and with identical provisions, fail to develop eggs. In soil-filled nests, however, normal egg development ensues.

Seemingly the queen's influence on « worker » ovarian development is vulnerable to the counteraction of JH. This finding suggests the possibility that a queen effect is exerted on the secretory capacity of the corpus allatum or its control center in the brain. On the other hand, the normal stimulation of JH secretion activated by protein ingestion—and workers do ingest pollen—might be short-circuited by the inhibition exerted by the queen. In this species the mode of queen inhibition is unclear and ideed there is little exchange of materials among members of a colony (MICHENER *et al.*, 1971). Moreover, the extent of ovarian development varies among worker individuals and intergradations are observed which could be caused by varying degrees of inhibition by the queen over specific subjects or by individual variation in JH secretion by the corpora allata.

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