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ORIENTATION BY PUPATING LARVAE OF *VESPA ORIENTALIS* (HYMENOPTERA : VESPIDAE)

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SUMMARY

Observations made on colonies of *V. orientalis* show that there are some pupae whose orientation in the comb prior to eclosion does not correspond to the geotropic orientation of the majority of pupae. Geotropic natural errors are discussed vis-a-vis their effect on the various developmental stages, and are shown to be lethal to the brood at the end of the pupae stage.

Experiments were carried out in order to separate the gravitational cues from the cues originating within the combs cells, resulting that the larvae are responding primarily to cues originating within the cell, and much less so to gravity. The number of disoriented workers is invariable smaller than the number of disoriented sexual forms.

RÉSUMÉ

Orientation des larves en cours de nymphose chez *Vespa orientalis*.

L'observation montre que, dans les colonies de *Vespa orientalis*, quelques nymphes avant l'éclosion ne sont pas orientées dans le rayon comme les autres. Nous avons analysé les conséquences des erreurs de géotropisme survenant dans les conditions naturelles sur les différents stades de développement. Nous avons montré qu'elles sont létales pour le couvain à la fin de la nymphose.

Des expériences qui ont été réalisées pour séparer les facteurs de gravité de ceux qui proviennent des alvéoles, il ressort que les larves répondent principalement aux facteurs alvéolaires et beaucoup moins à la pesanteur. Le nombre d'ouvrières issues d'alvéoles mal orientés est toujours inférieur à celui des sexués issus de tels alvéoles.

INTRODUCTION

In combs constructed by Vespinae, the cells ordinarily face downwards (SPRADBERY, 1973). However, if the combs are experimentally turned around to a position different from the norm, then the wasps alter or rotate the comb construction so as to have the cell outlets facing down (MONTAGNER, 1964; WAFA and SHARKAWI, 1973). Although the queen oviposits even in the cells experimentally turned upwards, the workers usually abandon combs that have been dispaced from the natural position by 180°, and commence building new, correctly oriented combs to which the queen transfers after a while (ISHAY, 1967).

This fact that the comb cells face down has a bearing in all nest activities. Thus, the queen oviposits and the workers feed the larvae or construct cells while their backs are toward the ground, and of course the brood is suspended upside down in the nest. The eggs and larvae at instars 1-3 must therefore be fastened to the cell walls, otherwise they would fall out. However, larvae at instars 4-5 are freely mobile within their cells and are capable of regulating their depth within the cells by muscular activity aided by various tegumentary protuberences.

Just prior to pupation the larva first spins a silk dome across the cell entrance, then inverts itself within the cell to complete its silk cocoon along the sides and base. Finally it reverts in its initial head down position within the cell. At this stage the passage from midgut to hindgut opens for the first time, permitting the meconium to be excreted and pressed against the upper end or roof of the cell. Now the larvae undergoes moult with the result that the meconium becomes situated between the silk cocoon and the moult skin. When the pupae is finally formed, its head faces the cell entrance while its ventrum is usually directed towards the center of the comb (ISHAY and al., 1972). Any larvae that does not pupate in the normal head-down position is doomed to death because it will be incapable of leaving the cell upon eclosion.

The present study was undertaken to determine what cues the larvae respond to in orienting in a head-downward position prior to pupation.

MATERIAL AND METHODS

Observations and experiments were carried out on colonies of *Vespa orientalis* collected in the field during the active season, as well as on colonies grown in a Vesparium either in an open court (with an egression to the field) or enclosed in special breeding boxes (with no egression). The collection and rearing of hornets was as described earlier (ISHAY, 1964). Entire colonies containing adults, as well as comb with brood only, were maintained in the vesparium on angles of 90° and 180° to normal in order to study the response of the larvae and the adults to the experimentally-induced conditions. Control colonies were maintained in normal position.

RESULTS

Observations on Normal Colonies.

Approximately 25 % of 300 colonies taken from the field during May-October between 1960-1974 contained some pupae which despite having completed maturation within the cell, fail to hatch. Such « aborted » pupae constitute between 0.20 to 3.33 % (in 25 % of the colonies).

Close examination reveals three reasons for this failure to eclose :

1. The pupa completes its developments to imago with its head facing up towards the roof of the combs and with excreted meconium above its head. The imago evidently cannot chew its way through the meconium and the carton of the cell roof and dies. This accounts for about 20 % of the eclosion failures.

2. The pupa develops with its head facing up towards the roof of the cell and the excreted meconium is interposed between the tip of the abdomen and the silk dome sealing the cell entrance. The developing imago cannot emerge from the cell. This accounts about 60 % of the eclosion failure.

3. The pupa is in the proper position within the cell, with its head facing down, but the excreted meconium interposes between its head and the silk dome, sealing the cell entrance and preventing agression of the imago. This accounts for the remaining 20 % of the eclosion failures.

All of these situations may occur on the same colony, or even in the same combs. In all instances the imago dies within a few days without any attempts by the aduly nest-mates to liberate it. Only following death of the imagos are the cells opened by the workers and the corpses of the dead imagos ejected to make way for new eggs.

Experiments.

a) *Rotating the comb through 90°.* During the first few days there is no apparent change, the queen continuing to oviposit in the shifted cells and the workers tending the brood as usual. Subsequently, however, there are several successive changes as follows : the cells along the upper border of the comb are modified by the workers through elongation and « bending » of their margins, so that within a few days the cell entrances face down again. At the same time, other workers commence building new stems upon the existing comb. These stems extend out wards from the comb and at their tips new comb cells facing down are constructed (fig. 1). It is interesting to note that in the course of the former building activity, i.e., the elongation of existing cells, the workers alter vacant cells but fail to add pulp to all cells occupied by brood.

The queen will continue to ovoposite in horizontal cells (at right angles to

the gravitational pull) as well as in the properly vertical cells, and colonies will persist in both types of cells, but gradually most of the population will shift to the vertical cells as they begin to produce larvae need to be fed.

b) *Rotating the combs through 180°*. In this position the comb-cell entrances face up, which is exactly opposite to the normal arrangement. Larvae at instars 4-5 are tended as normaly, but larvae at instars 1-3 hang headdown towards the base (roof) of their cells, are ignored by the workers, and die shortly after. Cells

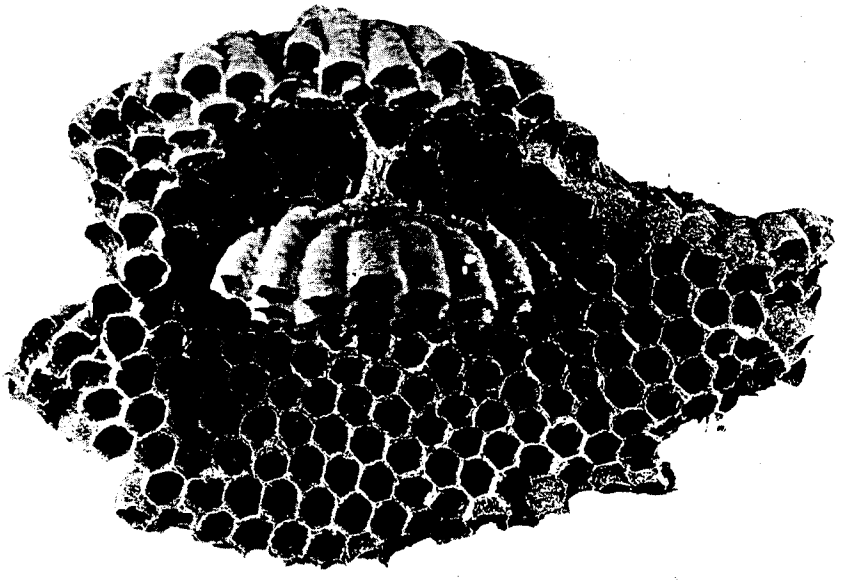

FIG. 1. — Comb rotated through 90°. The big comb has been rotated. The cells along the upper border have been modified so that their entrances face down (left side) or sealed (right side). A new comb, attached by a stem to the old one contains cells that open downwards.

FIG. 1. — Le grand rayon a subi une rotation de 90°. Les alvéoles de la partie supérieure ont été modifiés de telle sorte que leur ouverture est maintenant orientée vers le bas (à gauche) ou fermée (à droite). Les alvéoles du rayon qui a été ensuite construit s'ouvrent vers le bas.

that had been vacated are gradually sealed off by the workers (fig. 2) while concurrently new, downwards-facing cells are constructed along the margins of the comb. The queen continues for awhile to oviposite in the original cells. Most larvae pupating in such inverted cells develop normally, i.e., after spinning the silk cocoon they orient in it with their head facing the cell entrance sa that normal eclosion of the imago is made possible. Some of the larvae, however, pupate in an inverted position 98 % of these larvae facing down towards the roof of the comb and the excreted meconium interposed between the tip of the abdomen and the silk dome. In table I are summarized the data on the direction of pupation

in inverted combs during September-October (the season when worker, drone and queen larvae are in abundance).

By day 6 and subsequently, there is considerable degeneration of the 5th instar larvae, some of them dying within their cells and others ceasing to feed.

As is evident from Table I, only a small number of worker larvae pupate with their heads facing down, i.e. away from the cell entrance; while the majority orient properly with respect to the cell entrance. On the other hand, drone larvae tend to pupate more frequently in accordance with the gravitational

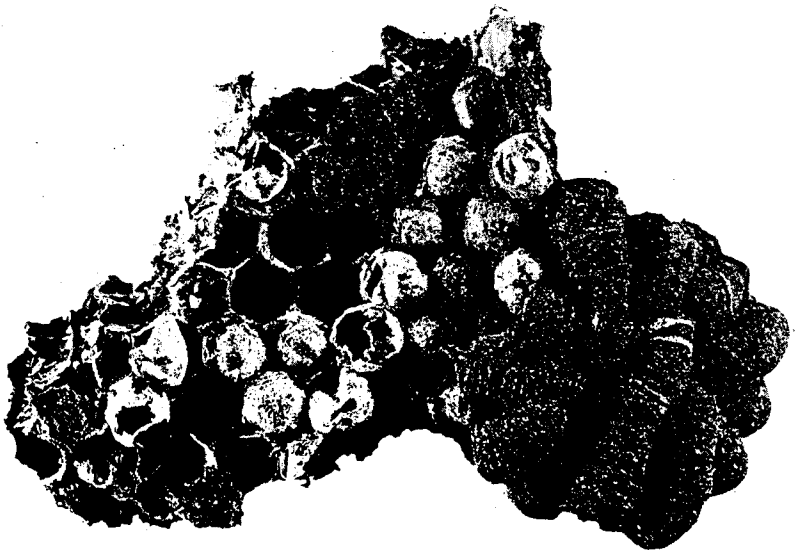

FIG. 2. — Comb rotated through 180°. Some of the empty cells of the original comb have been sealed by the adults. Attached to the right side of the old comb is a cluster of new cells oriented in the usual downward-opening direction.

FIG. 2. — Le rayon de gauche a subi une rotation de 180°. Quelques-uns des alvéoles vides ont été fermés par les Guêpes. Le rayon qui a été ensuite construit a été attaché au rayon inversé. Ses alvéoles ont l'orientation habituelle.

pull, than the workers, and queen larvae do so more frequently than drone larvae. These differences are significant ($P < 0.05$).

Larvae in the combs maintained in the vesparium in the normal position pupate properly as in natural colonies. All larvae that pupate in the inverted position are incapable of eclosing from the cell and are thus doomed to death but if artifically removed from their cells after completing maturation they appear normal. On the other hand, larvae that pupate in the normal position are unaffected by inversion of the comb and emerge normally and on time from the cells.

TABLE I. — Direction of pupation in artifically inverted hornet combs.

TABLEAU I. — Orientation de nymphes de Guêpes par rapport à la pesanteur dans des rayons qui ont été inversés expérimentalement.

	Days after Comb Inversion											
	1		2		3		4		5		6	
	N*	I**	N	I	N	I	N	I	N	I	N	I
Workers .	380	8 = 2 %	450	9 = 2 %	190	4 = 2 %	86	1 = 1 %	32	0 = 0 %	16	0 = 0 %
Drones ..	180	11 = 6 %	230	15 = 6 %	280	16 = 6 %	210	14 = 6 %	84	6 = 6 %	31	2 = 6 %
Queens ..	240	24 = 10 %	310	35 = 11 %	290	48 = 14 %	310	16 = 5 %	91	8 = 8 %	29	5 = 15 %
Total	800	43 = 5 %	990	59 = 6 %	760	68 = 9 %	606	31 = 5 %	207	14 = 7 %	76	7 = 9 %

* N : Normal pupation, where the head of the pupa, following the spinning of the silk dome, still faces the cell entrance even after inversion of the comb.

** I : Inverted pupation, where the pupa is oriented with its head toward the bottom of the cell and the tip of the abdomen toward the cell entrance.

* N : nymphes restées en position normale. Après le tissage du cocon, la tête fait toujours face à l'entrée de l'alvéole, malgré l'inversion du rayon;

** I : nymphes qui se sont retournées dans l'alvéole. Leur tête est maintenant orientée vers le fond de l'alvéole.

DISCUSSION

Hornet workers are oriented by gravitational cues when building and react promptly to changes of direction of combs when disturbed. Although the queen prefere, most probably, to lay eggs in positive geotaxis oriented combs, she continued to lay also in inverted combs. Most of the larvae do not relate to the new geotropic orientation either because they are regulated by the shape of the cell in which they grow or because the geotropic sense of the larvae is determined at early life and requires a longer period of change for its modification.

Inversion of the comb produces considerable mortality of the larvae possibly by disrupting their ability to communicate via acoustic means (ISHAY and al., 1972; ISHAY and al., 1974). It also results in a lowered pupation rate, suggesting damage incurred by the new geotropic orientation.

Failure of some of the pupae to hatch following the imaginal metamorphosis is connected with the occurrence of natural errors vis-a-vis geotropism. In the three described mechanisms of hatching failure, the larvae do not follow the usual behavioural pattern. The reason for this is not clear but may be genetically

controlled. However, all the errant, pupae are doomed to die and are thus removed from the active circle of the colony. It would seem that proper maintenance of the colony does not favor any individum whose response to geotropism differs from the norm. The number of cases in which the pupae do not behave with regard to geotropism in the ordinary manner is relatively small, although statitically significant. It is possible that the phenomena originated in the geological era during which the cells and combs of Vespidae evolved from prototypes similar to those of Euminidae in which the eggs and larvae were hanging from the roof of the cells (as in Vespidae today). However, the cell could lie horizontally, or it could stand vertically with its entrance on the top, as when the prey was supplied to the larvae intact the arrangement of the cell was of importance neither to the larvae nor to the wasp (MALYSHEV, 1968). As a matter of fact, the cases in which the meconium accumulated above the head of the emerging pupae and prevented its eclosion might not have been lethal if the Vespidae tackled the removal of the meconium similarly to Polybini of the genera *Palybioides, Ropalidia, Belonagaster* and *Parapolybia.* In these genera, the wasps remove faecal pellets from the cells by chewing out the roof of the cell then sealing the hole with a salivary secretion which dries to form a mika-like window (PARDI, 1965; EVANS and EBERHARD, 1970).

In the course of the ontogeny of *Vespa orientalis,* the change from a dependence chiefly on cellular cues of the larvae and pupae, to an absolute dependence on geotropical cues by the adult workers, leaves room for speculating that similarly ordered changes occurred also in the course of phylogeny. If this be true, then the advanced construction of a comb with a positive geotropic orientation must have been a rather relatively recent development.

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