Insectes Sociaux, Paris
1979, Volume 26, n° 2, pp. 155-164

© Masson, Paris, 1979

SOME SOCIAL AND DIETARY PREREQUISITES OF OOCYTE DEVELOPMENT IN *VESPA ORIENTALIS* L. WORKERS

M. MOTRO *, U. MOTRO **, J.S. ISHAY *** and J. KUGLER *

* *Department of Zoology, George S. Wise Faculty of Life Sciences.*
** *Department of Statistics.*
*** *Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.*

Reçu le 7 septembre 1977. Accepté le 8 mai 1978.

SUMMARY

Removal of the queen induced ovarial development in workers of the Oriental hornet, *Vespa orientalis* L. This occurred in workers kept in groups and mainly during the first five days after removal of the queen. Cooling of the workers or their subjection to a protein-free diet inhibited their ovarial development, but in either case, the inhibition disappeared once normal conditions were restored. For workers living in a group, ovarial development occurred only in workers of high hierarchal rank. However, the ovaries developed in all workers which were isolated singly *after* having lived in a group. On the other hand, inhibition of ovarial development occurred in about 50 % of workers isolated singly from the moment of emergence, which suggests that social contact acts as a releasing stimulus for ovarial development in queenless hornet workers. Development of the ovaries was observed also in worker groups prevented from building, and the connection between building and ovarial development is discussed.

RESUME

Quelques conditions sociales et alimentaires requises pour le développement des ovocytes chez les ouvrières de *Vespas orientalis L.*

Le fait d'enlever la reine entraîne le développement des ovaires des ouvrières chez le Frelon oriental *Vespa orientalis* L., surtout dans les cinq jours suivants. Le refroidissement des ouvrières ou la fourniture à celles-ci d'un régime sans protéines inhibent leur développement ovarien, mais, dans les deux cas, l'inhibition disparait dès que ces conditions artificielles sont levées.

Dans les groupes d'ouvrières, le developpement des ovaires apparait seulement chez les ouvrières du haut de la hiérarchie. Cependant, on l'observe chez toutes les ouvrières

qui ont été isolées après qu'elles aient vécu en groupe. Par ailleurs, l'inhibition du développement ovarien se produit chez 50 % environ des ouvrières qui ont été isolées depuis leur éclosion imaginale, ce qui suggère que les contacts sociaux stimulent le développement ovarien des ouvrières orphelines. Le développement ovarien a été aussi observé chez les ouvrières qu'on empêchait de construire : nous discutons la relation entre l'activité de construction et le développement ovarien.

INTRODUCTION

In previous papers (ISHAY, 1976 ; KUGLER *et al*, 1978) we have shown that workers of the Oriental hornet that are kept apart from the queen are capable of building a comb and ovipositing in its cells. In each group a hierarchy of workers was found to be formed wherein one or more workers oviposit, occupy the comb and engage in building and maintenance (e.g., cell construction, larval feeding, etc.). Some do not oviposit, but occupy the comb and engage in building and maintenance activities, while others still do not participate at all in the nest duties and do not occupy the comb.

These findings have raised several questions :

a - What are the conditions that stimulate or inhibit ovarial development in owrkers kept queenless from the moment of emergence (either singly or in groups) ?

b - Under what physiological or social conditions is it possible to influence ovarial development in workers that have already spent some time in association with the queen (which, as is known, inhibits ovarial development in workers) ?

c - Is there a correlation between building activities of the workers and the state of their ovaries ?

d - Is there a correlation between the weight of the worker and the state of its ovaries ?

e - Is there a correlation between group size and the number of egg-laying workers ?

Several of these points have been studied in other species, e.g. in *Polistes* sp. (PARDI, 1948 ; DELEURANCE, 1957 ; GERVET, 1964 ; EBERHARD, 1969 ; TURILLAZZI and PARDI, 1977), in *Paravespula germanica* (F.) and in *Paravespula vulgaris* (L.) (MONTAGNER, 1966), and in *Halictus* sp. (KNERER and PLATEAUX-QUÉNU, 1966).

The present paper describes experiments which were carried out in order to investigate the mentioned points as they apply to *V. orientalis* workers.

MATERIALS AND METHODS

Experimental hornets were collected from colonies found in nature. The collections were made from May to August, when building activities of the hornets are at a peak.

To obtain hornets of known age, pupa-containing combs with or without adult populations were placed in boxes. Each day, all workers emerging on the same day, i. e., not more than 24 hours apart, were removed and maintained in breeding boxes (ABB), as described by ISHAY (1964). It should be pointed out that newly emerged workers are of a lighter color than adult workers, and their wings are not yet folded lengthwise, so that they are easily distinguishable. Experimental hornets were marked by a number on the back of the thorax, and kept in the vesparium singly or in groups, under the following conditions :

a - Ordinary conditions, i.e., in breeding boxes (ABB). They were offered a diet including proteins (honeybees or meat morsels) and a 30 % sugar solution, as well as building materials (usually a clod of clay soil) as described by ISHAY (1964, 1976). In some of the experiments newly emerged workers were used, while in others, workers of various ages collected from natural colonies were employed. The experimental hornets were maintained either singly or in groups of 10-15 workers per ABB.

b - All conditions as in (a) except that building materials were denied. For this purpose the hornets were kept in special ABBs made out of glass so that they would not be able to scrape building materiel off the walls of the container.

c - Under refrigeration — the workers were kept in a refrigerator in the dark at 4° C for several days and then maintained under the laboratory conditions described in (a).

d - With a protein-free diet, i.e. all conditions as in (a) except that protein food was withheld.

The activity of the workers was observed daily. The workers were weighed on the day of emergence, as well as before dissection. The state of ovarial development was assessed as follows : Workers that had undergone one of the above treatments were anesthetized with diethyl-ether, then killed, dissected under a dissecting microscope, and the largest oocyte within the ovarioles separated and measured by ocular micrometer. Dimensions of the largest oocyte were considered as criteria for ovarial development in the various experimental groups.

Table I. — Oocyte development in workers maintained in queenless groups from the moment of emergence.

Tableau I. — Développement des ovocytes d'ouvrières qui ont été maintenues dans des groupes orphelins depuis l'éclosion imaginale.

Age (days after emergence)	No. of workers * in the group	Mean length of oocyte (mm)	Mean coefficient of variation +
0	5 (1)	0.2560	0.1284
1	10 (1)	0.3540	0.1643
2	11,12 (2)	0.3939	0.2495
3	7,12 (2)	0.6674	0.3438
6	6,21 (2)	0.8496	0.7293

* In parentheses - number of groups.

+ Coefficient of variation = $\frac{\text{standard deviation}}{\text{mean}}$

RESULTS

In the first phase of the investigation we measured ovarial development in young workers that had been kept in queenless groups from the moment of emergence. As can be seen from Table I, the mean length of the oocytes increased over the test period and there was a more marked increase in the coefficient of variation with time, which reflected the increasing variability in oocyte size with increased age of the workers.

In a parallel experiment we measured ovarial development in older workers that had been separated into queenless groups only after growing up in a normal nest (i.e., in a nest containing a fertilized, ovipositing queen as well as adults and brood at various ages). The results, as summarized in Table II, are clearly similar to those in Table I, i.e., there is an increase

Table II. — Oocyte development in workers that had been separated at age 2-8 days from normal queenright colonies.

Tableau II. — Développement des ovocytes d'ouvrières qui, entre 2 et 8 jours depuis l'éclosion imaginale, ont été retirées de colonies « normales » (avec une reine).

Days after separation	No. of workers * in the group	Mean length of largest oocyte (mm)	Mean coefficient of variation
0	8,10 (2)	0.3391	0.0883
1	9,14 (2)	0.3896	0.1310
2	12,15 (2)	0.4052	0.1791
3	11,11 (2)	0.4454	0.2464
4	10,10 (2)	0.6060	0.4975
5	14,17 (2)	0.5658	0.6836
6	16,16 (2)	0.7950	0.8754
7	14 (1)	1.4267	0.7983

* In parentheses - number of groups.

in the mean length of the oocytes and a steeper increase in the coefficient of variation with age.

The average length of the largest oocyte of workers which were separated after being in a queenright colony for 2-8 days (0.3391 mm), was significantly greater ($P < 0.005$) than the average length of the largest oocyte from newly emerged workers (0.2560 mm). The average length (0.3939 mm) of the largest oocyte of 2-day-old workers which lived in queenless groups is significantly larger ($P = 0.01$) than the average length (0.3391 mm) of the largest oocyte from workers aged 2-8 days that lived in groups with a queen and were dissected immediately after separation from her.

Another experiment was done with workers emerging from combs containing pupae, but no adult population. These workers had not been exposed to the possible influence of a queen and adult workers for 10 days before their emergence. The results (Table III) are similar to those from

Table III. — Oocyte development in newly emerged workers. These workers emerged from combs which were without queen and adults for 10 days before the emergence.
Tableau III. — Développement des ovocytes d'ouvrières qui viennent d'éclore. Ces ouvrières proviennent de rayons sans reine ni autres catégories d'imagos, et ceci depuis 10 jours avant l'éclosion.

Days (age)	No. of workers in the group	Mean length of largest oocyte (mm)	Coefficient of variation
0	7	0.2657	0.0569
1	9	0.3733	0.1104
2	10	0.4188	0.2123
3	10	0.7240	0.4243
5	7	0.8800	0.8072

nests containing adults prior to emergence of the test workers (Tables I and II).

No positive correlation was found between worker body size (expressed as weight) and the state of ovarial development. The Spearman Rank Correlation test was used. The correlation coefficient (calculated from a sample of 50 workers) was 0.1495, which is not significantly different from zero. Examining the connection between body weight and hierarchal state, it appears that the weight at emergence is not an important factor in determining dominance. In Table IV, three groups of sibling workers, which

Table IV. — Correlation between weight at emergence and hierarchial state.
Tableau IV. — Corrélation entre le poids à l'éclosion imaginale et le rang social.

	GROUP No. 1	
Dominant (egg-laying)	Subdominant	
3	16	(n) sample size
0.40367	0.35422	(x) mean
0.0000003	0.0025986	(s^2) variance
t = 3.8787		P < 0.005
	GROUP No. 2	
Dominant (egg-laying)	Subdominant	
6	9	(n) sample size
0.29380	0.34716	(x) mean
0.0003140	0.0033450	(s^2) variance
t = — 2.4451		P < 0.05
	GROUP No. 3	
Dominant (egg-laying)	Subdominant	
8	44	(n) sample size
0.39375	0.39273	(x) mean
0.0023125	0.0033331	(s^2) variance
t = 0.0535		

emerged on the same day are given as examples. The average weight of dominant workers in group 1 is significantly higher than of the subdominants. The average weight of the dominants in group 2 is significantly lower than that of the subdominants. In group 3 there is no significant difference between the means. In each case we used the t-test for examining equality of means (unequal variances).

The correlation between group size and the number of egg-layers in the group is not significantly positive (Table V). Oocyte size was measured in

Table V. — No. of egg-layers in large and medium sized groups.

Tableau V. — Nombre de pondeuses dans des groupes de grande taille ou de taille moyenne.

Group number	No. of workers per group	No. of egglayers
1	12	3
2	14	2
3	15	2
4	16	3
5	17	5
6	19	3
7	20	4
8	20	2
9	44	4
10	45	4

workers maintained from emergence in glass ABBs, thus devoid of building materials. It is clear from Table VI that oocytes do develop in workers that are prevented from building.

Table VI. — Oocyte development in workers deprived of building materials (bred in glass containers).

Tableau VI. — Développement des ovocytes d'ouvrières qui ne disposent pas de matériaux de construction (elles ont été élevées dans des récipients en verre).

Age (days)	No. of workers * in the group	Mean length of largest oocyte (mm)	Mean coefficient of variation
5	10,12 (2)	0.6036	0.7617
10	18 (1)	0.6498	1.1486
21	11 (1)	0.7491	0.9659

* In parentheses - number of groups.

In order to study the influence of cooling on oocyte development, workers immediately after emergence were exposed for 2-6 days to low temperature (4°C in the dark). From the data presented in Table VII, the estimated slope of the linear regression line (of oocyte length to duration of

cooling) is 0.0018 mm/day, and a 95 % confidence interval for the slope is (— 0.0030, 0.0066). It is clear, then, that the cooling prevents oocyte development. However, when the cooled hornets were restored to optimal temperature conditions (27°-28°C), their ovaries developed, and oviposition was observed on the 4th-5th day after the change in conditions.

Table VII. — Oocyte development in cooled workers *

Tableau VII. — Développement des ovocytes d'ouvrières « refroidies » (maintenues à la température de 4°C pendant quelques jours, un jour après l'éclosion imaginale).

Days of cooling	No. of workers in the group	Length of oocyte (mm)
2	5	0.28, 0.32, 0.32, 0.32, 0.32
3	6	0.28, 0.32, 0.32, 0.32, 0.32, 0.32
4	5	0.28, 0.28, 0.32, 0.32, 0.32
5	6	0.28, 0.28, 0.32, 0.32, 0.32, 0.32
6	7	0.28, 0.32, 0.32, 0.32, 0.32, 0.36, 0.36

* The workers were kept at low temperature (4°C) for several days, from one day after emergence.

Workers that had been kept in groups (7 groups) or singly (12 workers) on a sugar diet only, neither developed oocytes nor engaged in building over 6 successive days of observation. Normal oocyte development was restored in these hornets by adding proteins to their diet, following which they started egg-laying on the 4th-5th day.

Another experiment was done on single workers that had been isolated : a) after spending 3-4 days in a queenright colony ; b) after spending 3-4 days in a queenless colony ; and c) immediately after emergence. Results of oocyte size measurements in these workers, as given in Table VIII, lead to the following conclusions :

1 - Oocytes develop in some of the singly isolated workers.

2 - Mean oocyte length in workers isolated after being in queenright nests is not significantly different from that of workers isolated after being in queenless nests, but in both cases the mean oocyte length is significantly greater than in workers kept in isolation from the moment of emergence ($0.025 < P < 0.050$).

3 - The coefficient of variation in the last group is significantly greater than in either of the first two groups ($0.025 < P < 0.050$ between the first and the third groups and $P < 0.025$ between the second and the third groups). Means and coefficients of variation compared by Student's t-test for unequal variances).

It is noteworthy that while ovarial development occurred in all workers isolated after living in a group, it occurred in only 10 of 21 workers isolated immediately upon emergence.

Table VIII. — Oocyte development in singly isolated workers.

Tableau VIII. — Développement des ovocytes d'ouvrières isolées après ou peu de temps après l'éclosion imaginale.

Source of workers	Isolated from queenright nests, 3-4 days after emergence	Isolated from queenless nests, 3-4 days after emergence	Isolated from queenright nests, immediately after emergence
Number of workers	12	8	21
Mean length of oocyte (mm)	1.7967	1.7850	1.2181
Standard deviation (mm)	0.6942	0.6017	0.9150
Coefficient of variation	0.3864	0.3371	0.7512

A positive correlation was found between building activity and mean oocyte length. The data (Table IX) show that the mean oocyte length in workers engaged in building is significantly greater than is non-building workers ($P < 0.0025$ by Student's t-test for unequal variances).

Table IX. — Correlation between building activity and mean oocyte length in workers kept in single isolation from the moment of emergence.

Tableau IX. — Corrélation entre l'activité de construction et la longueur moyenne des ovocytes d'ouvrières qui ont été isolées depuis l'éclosion imaginale.

	Building workers	Non-building workers
Number of workers	10	11
Mean length of oocyte (mm)	2.0520	0.4600
Standard deviation (mm)	0.6143	0.0908

DISCUSSION

Under natural conditions in queenright colonies, workers of *Vespa orientalis* are from the moment of emergence exposed to the founding queen, the reproductive agent of the colony. The workers, whose ovaries are usually undeveloped, assume the various tasks of building and brood maintenance. Only at the end of the season do some workers lay unfertilized eggs (WILSON, 1971 ; SPRADBERRY, 1973 ; ISHAY, 1976). When worker-groups without a queen were introduced into the ABBs, their activities followed a uniform pattern. During the first two days after their introduction, the workers concentrated in a dark corner without building. Subsequently, however, they constructed a pedicel, then a comb on its lower end, and finally commenced ovipositing in the comb cells.

Although morphologically all workers emerging on the same day are quite similar, they show considerable physiological and behavorial difference. Thus, not all of them build and only some of them develop their oocytes and oviposit; again, while most engage in the various nest maintenance duties, some do not participate at all in the group activities.

When the degree of ovarial development (as measured by the size of the maximal oocyte) and the process of founding a nest were studied in queenless workers of *Vespa orientalis*, it was found that there is no clear correlation between weight and ovarial development or hierarchal status. This is unlike the situation in *Polistes gallicus* (L.), where TURILLAZZI and PARDI (1977) found a positive correlation between size of the females, ovarial development and the hierarchal position. A positive correlation between body size and fertility was also found in *Halictus ligatus* Say, *H. scabiosae* (Rossi) and *Evylaeus linearis* (Schenck) (KNERER and PLATEAUX-QUÉNU 1966).

The number of egg-laying workers in queenless groups of *Vespa orientalis* was variable. In groups ranging from 12 to 45 workers, the correlation coefficient between size of the group and the number of egg-laying workers was not significantly positive.

The degree of ovarial development was quite similar in the following: a) Workers of uniform age kept in a group from the moment of emergence; b) Groups of workers of different ages, separated from the queen 2-8 days after their emergence; and c) groups formed from workers emerging from adult-free combs. In each of the three experiments it was found that the mean size of the largest oocyte increased with the time which passed from the group formation. Also the variability of ovarial development among group members increased with time (as seen by the rapid increase in the coefficient of variation). Such variability is a result of the establishment of the hierarchy between the members of the group. This corresponds to the development of behavorial differences between the queenless workers of *Paravespula vulgaris* and *Paravespula germanica* according to hierarchal status (MONTAGNER, 1966).

The fact that oocyte development in queenless workers which passed the last 10 days before emergence in combs with or without adults was quite similar, shows that the adults do not influence oocyte development in workers which are in the final stage prior to emergence.

Refrigeration, or the withholding of proteins from the diet of the workers, produces reversible depression of ovarial development.

The experiments with singly isolated workers showed that living in isolation from the moment of emergence has a deteriorating effect on the development of the oocytes. Only part of these workers showed ovarial development. It would seem, therefore, that in order to acquire the capacity to develop oocytes, workers require social stimulation at least during the

first days after emergence. In this connexion, it should be mentioned that a positive influence of social stimulation on the normal development of the individual was found in social animals, even in man (SPITZ, 1946).

There was a positive correlation between worker ovarial development and the building activities, so that in the building workers, the average oocyte length was significantly greater than in non-building workers. A positive correlation between cell initiation and ovarial state was reported also in *Polistes* spp. (PARDI, 1948 ; DELEURANCE, 1957 ; EBERHARD, 1969).

The question, however, is whether in *Vespa orientalis* workers there is, indeed, a causal relationship between ovarial development and building activity. In queenless groups, all building workers had developed oocytes. In the queenright nests, the building activities are undertaken by the workers having underdeveloped ovaries. In the experiments summarized in Table VI, we found ovarial development also in workers deprived of a chance to build. In other words, the ovaries of workers can develop in the absence of building, and vice versa.

References

DELEURANCE E.P., 1957. — Contribution à l'étude biologique des *Polistes* (Hymenopteres-vespidae). I. L'activité de construction. *Behaviour, 11,* 67-84.

EBERHARD M.J.W., 1969. — The social biology of Polistine wasps. *Misc. Publ. Mus. Zool.,* Univ. Michigan, No. 140, 101 p.

GERVET J., 1964. — Essai d'analyse élémentaire du comportement de ponte chez la Guêpe Poliste *P. gallicus* L. (Hymen. Vesp.). *Ins. Soc., 11,* 21-40.

ISHAY J., 1964. — Observations sur la biologie de la Guêpe orientale *Vespa orientalis. Ins. Soc., 11,* 193-206.

ISHAY J., 1976. — Comb building by *Vespa orientalis. Anim. Behav., 24,* 72-83.

KNERER G., Plateaux-QUÉNU C., 1966. — Sur le polymorphisme des femelles chez quelques Halictinae. *C.R. Acad. Sci., 263,* 1622-1625.

KUGLER J., MOTRO M., ISHAY J., 1979. — Comb building abilities of *Vespa orientalis* L. queenless workers. *Ins. Soc. 26,* 147-154.

MONTAGNER H., 1966. — Sur le déterminisme des castes femelles chez les Guêpes du genre *Vespa. C.R. Acad. Sci., 263,* 547-549.

PARDI L., 1948. — Dominance order in *Polistes* wasps. *Physiol. Zool., 21,* 1-13.

SPITZ R., 1946. — Psychoanalytic study of the child. *Int. Press University,* N.Y. 11, p. 313-342.

SPRADBERY J.P., 1973. — Wasps. Sidgwick and Jackson, London, 408 p.

TURILLAZZI S., PARDI L., 1977. — Body size and heirarchy in polygynic nest of *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Monitore Zool. Ital.* (N.S.), *11,* 101-112.

WILSON E.O., 1971. — The Insect Societies. *Harvard Univ. Press,* edit., Cambridge, Mass., 548 p.

© 1979. *Masson, Paris.* *Le Directeur de la Publication :* Dr J. TALAMON.

Tous droits de traduction, d'adaptation et de reproduction par tous procédés réservés pour tous pays.

La loi du 11 mars 1957, n'autorisant aux termes des alinéas 2 et 3 de l'article 41, d'une part, que les copies ou reproductions strictement réservées à l'usage privé du copiste et non destinées à une utilisation collective et, d'autre part, que les analyses et courtes citations dans un but d'exemple et d'illustration, « toute représentation ou reproduction intégrale, ou partielle, faite sans le consentement de l'auteur ou de ses ayants droit ou ayants cause, est illicite » (alinéa 1er de l'article 40).

Cette représentation ou reproduction, par quelque procédé que ce soit, constituerait donc une contrefaçon sanctionnée par les articles 425 et suivants du Code pénal.

Masson, éditeur, Paris. - Dépôt légal : 1979 - N° d'ordre : 5156 - 3e trimestre 1979.
Imprimé par S.E.I.L.C. (France) — Commission paritaire : n° 60787 - Printed in France