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Jerzy Woyke

How to Determine the Sex of Living Honeybee Larvas

(Rozpoznawanie płci żywych larw pszczelich)

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Woyke, J.*

HOW TO DETERMINE THE SEX OF LIVING HONEYBEE LARVAS[1]

The necessity for the practical determination of sex of larvas arose during investigations carried out in the Apiculture Dept. of Agricultural University in Skierniewice on the development of drones from fertilized eggs under laboratory conditions (Woyke, 1962, 1963 a, b, c).

The determination of larval sex was indispensable to ensure that part of fertilized eggs have produced drone larvas and in order to know which have survived or perished, and ultimately to be able to compare the viability of hatched drone larvas with those of workers in the neighboring comb cells. In order to rear the larvas further their sex has to be determined in advance in living specimens. Every beekeeper in any case requires an easy method of identifying the sex of the larvas without resorting to a fixing and staining procedure.

Previously published descriptions of the external sexual character were based on dead, fixed and stained specimens and they proved to be absolutely inadequate for the determination of living material.

In view of the above difficulties the authors of the present paper have tried to work out a method suitable not only for the practical determination of the sex of the living specimens but one that will also avoid the damage of larvas, lowering their viability if not the outright killing of the material. The method should enable to determine the sex of larvas without taking it out from the comb cell or rearing dish. The only handling allowed could at most be the raising of the larval abdomen to examine the sexual characters. Turning over the larvas in the royal jelly or mixed food could led to clogging of larval spiracles and consequently to suffocation.

Sex of older, but not yet sealed larvas, can also be determined by their size, since worker larvas are larger than drone larvas of the same age. This method, however, cannot be applied when larvas of various age are reared under laboratory conditions which alter to a certain extent

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*Apiculture Dept. of Warsaw Agricultural University (SGGW), Skierniewice.

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their development. It has likewise no value when the drone larvas are reared from fertilized eggs since the development of such larvas is largely unknown. The only sex criteria under such conditions are the differences in the rudiments of external parts of the reproductive organs in the drone larvas and, additionally, the sting rudiments in the females. Understandably, the inner reproductive organs cannot be used in the determination of sex of the living larvas. Only the external sexual structures or those visible through the thin cuticle may be compared.

Review of the Literature

The rudiments of the external parts of reproductive organs of the drone larvas were described by Michaëlis (1900) and Zander (1900, 1016). The former author studied the entire larval specimens, which had been stained and fixed, the other investigated microtomic slides.

The female rudiments of the reproductive organs were studied on microscopic slides and described by Löschel, Meier and Zander in one common paper (1916). The sting rudiments of an older, fixed larva were also described by Nelson (1924).

A comparison of the external sexual characters of the youngest larvas was made by Zander (1914) in preliminary note to the above cited paper. A similar study but based on histological slides was also carried out by Woyke (1963 b). The descriptions of the rudiments of the external reproductive organs as well as that of the sting, can also be found in the basic handbooks dealing with the bee anatomy, by Zander (1922) or Snodgrass (1956). However, these are mostly not original descriptions but are based on works by the authors cited above.

As may be seen from the above, the descriptions of the rudiments of the external sexual organs or that of the sting of living larvas are still wanting.

To compare the external sexual character of the living larvas with those in the dead fixed ones, the descriptions of these features observed in the subsequent larval stages must be first considered.

According to Zander, Löschel, Meier (1916) and Woyke (1963) the sexes can be distinguished on microscopic slides in larvas within six hours of hatching. In the drone larvas a thickening of the epithelium which forms one elongate imaginal disk is visible on the ventral side of the twelfth segment. This disk constitutes the rudiments of the future external parts of reproductive organs. Such thickening occurs in the female on the ventral side of three abdominal segments — on the tenth and eleventh segments as paired structures and on the twelfth segment as a single plate. Woyke (1963) gives a microphotograph of a one-day-old larva of female

and male in which the differences of the abdominal structures are clearly visible. According to Meier these are visible only on the inner side of the epidermis and cannot be observed externally.

A further development of the external parts of reproductive organs is described by Michaëlis as follows: in the second larval stage a pit develops on the twelfth abdominal segment, on the wall of which a strong growth of epidermis takes place producing in the subsequent stage two posteriorly directed processes which grow gradually. Just before pupation the processes begin to protrude outward from the pits. Their apices are then situated at the same level as the margines of the "pockets" but they are still mostly hidden inside. During pupation two new processes are formed. According to Zander the initial two processes split at the apex within the sixth day of hatching. The median pair of processes becomes transformed during the further development of the drone larva into a pair of median plates, known as penis valves, which close externally the opening of the reproductive organs. The external pair of processes produces a pair of parameral plates situated externally with respect to those previously described.

According to Snodgrass (1956) the first stage of development of the external reproductive organs of the drone "appears at a later stage" of the larval life in a form of an oval disk with two processes. The development of the sting and of rudiments of the reproductive organs is, according to Löschel and Meier, similar in the worker and queen larvas. On the second day of larval life the sinking of the imaginal disks of the eleventh and twelfth segments can be observed. On the following day two directed posteriorly processes grow out of pockets of the eleventh segment and four processes appear from the pockets of the twelfth segment. These processes grow longer and adhere closely to the skin surface beneath the chitin cuticle. This stage lasts until the sixth day after hatching. In the further stage of development of the worker and queen larvas processes of the eleventh segment will become the lancets of the mature sting. The median pair on the twelfth segment will unite to form stylet, and those of the outer pair will form the oblong plates and the seath lobes. Imaginal disks of the tenth abdominal segment do not produce folds until at the end of larval development. They form a pair of oviducts and a part of the vagina.

Method

A proportion of the investigated larvas were fixed at various stages of development for identification and a comparison with descriptions of fixed and stained larvas published previously. For fixing, hot water, Carnoy or Gilson fluid were used and the larvas were subsequently

preserved in 75% alcohol. Some larvas were preserved in alcohol without a previous fixing. The fixed and preserved larvas were not stained for subsequent study.

The main attention was devoted to living larvas. First, combs with drone eggs were placed in a bee colony or were selected inside. Then a small surface, 10×15 cm large, was selected in a comb. In order to find this surface again during later investigation, a row of cells was purposedly destroyed on the two adjacent margins of the selected rectangle. This procedure was applied to all selected combs and they were subsequently placed back into the hives. The combs were taken out and checked every day under a microscope whereby hatching time and progress in the development of bee and drone larvas in particular cells was systematically noted. The sexual characters already investigated on fixed larvas were of particular interest. Any differences found in the morphology of the two sexes were the object of detailed scrutiny. For better observation sometimes the head or the posterior end of the larva were carefuly raised with the aid of a grafting or a preparatory needle. Although the main consideration was devoted to practical methods of determination of the sex of larvas within their cells and such larvas were the principal subject of investigation, some of the larvas were nevertheless taken out of their cells for a more thorough scrutiny under the microscope. Attention was, however, mainly paid to those characters which were already observed in larvas inside the cells.

The examination of larvas in comb cells was carried out under a stereomicroscope Mst 130 which was fixed on a column stand. This stand made possible an examination of comb cells even when they were placed at a certain distance from the frame bar. Illumination with an ordinary microscope lamp proved insufficient, particularly in the case of smaller larvas. A direct light could not penetrate the cells due to their depth; the bottom of the cells thus remained poorly illuminated. Excellent results were obtained with the application of a prism for vertical illumination. It made possible good illumination of the cell bottom, and the larva resting on it — of any size — could be observed perfectly. Such illumination was thus employed for the present investigation and the comb was placed horizontally under the microscope.

Results

The rudiments of reproductive organs and of the sting were clearly visible in the fixed larvas, temporarily preserved in alcohol — even in the ones that were not stained. It is probable that a scrutiny of stained specimens at an earlier stage of development could provide a better picture

of some anatomical details. Still, even the above simpler method was adequate for a comparison of the development of the said organs in particular larval stages with the existing descriptions. The examination of the fixed specimens showed that all described processes of the imaginal disks which produce the external reproductive organs and the sting in a further stage of development of the larvas, are situated beneath the external layer of the epidermis.

The sexual characters of the living larvas displayed at first sight considerable differences when compared with those of the fixed specimens.

In the young 1–2-days-old larvas of drones and bees no visible differences in external anatomy was observed. Even a close scrutiny under the microscope of larvas extracted from their cells did not reveal any distinct differences. Neither imaginal disks nor any processes were visible in this stage. Within three days of hatching, however, feebly marked imaginal disks could be seen on the ventral side of the respective segments. The disks were marked as white spots on more transparent ground on larval body. In the drone larva such white spots were visible in the posterior part of the twelfth segment. No processes under the outer layer of the skin were, however, observed. In the worker larva the most conspicuous was the last disk on the twelfth segment, likewise in the form of a whitish spot. It was found in the anterior part of the segment — thus situated closer to the head as compared with a similar disk in the drone larva. The disk on the eleventh segment was much less conspicuous and that on the tenth segment was hardly noticeable at all and sometimes completely missing. The processes — possibly sting rudiments — were likewise wanting under the outer layer of the epidermis in this period. It happened often, too, that the imaginal disk of the penultimate abdominal segment, usually in from of a whitish spot, was the only disk conspicuous in the larva in question. Thus, within the third day of hatching the only imaginal disk visible in both the drone and bee larvas was the one on the twelfth abdominal segment. Although the location of these disks along the longitudinal axis of the body differed in the drone and the female bee to a certain extent, this characteristic proved to be an insufficient criterion for the determination of sex. Thus, it seems that a realiable method for determination of sex during the first three days after hatching is still wanting.

According to Bartholf (1925) the larva undergoes four moults within 3.25–3.5 days of hatching. Thus a four-day-old larva had usually already undergone the fourth moulting. In those four-day-old larvas which had already cast the skin or which were just being in the process of doing so a strange phenomenon was observed. After illumination, all imaginal disks emitted a greenish-blueish light. Such larvas, with a fluorescence

effect, are presented in Fig. 1. All disks — those on the head, thorax or even abdomen — showed such fluorescence. In the head some fluorescent lines and spots were observed but it was difficult to distinguish definite areas here. On the ventral side of all thoracic segments, however, the imaginal disks of legs were discernible in the form of distinct marks. Similar areas which constitute the imaginal disks of wings were situated at sides of the second and third thoracic segments.

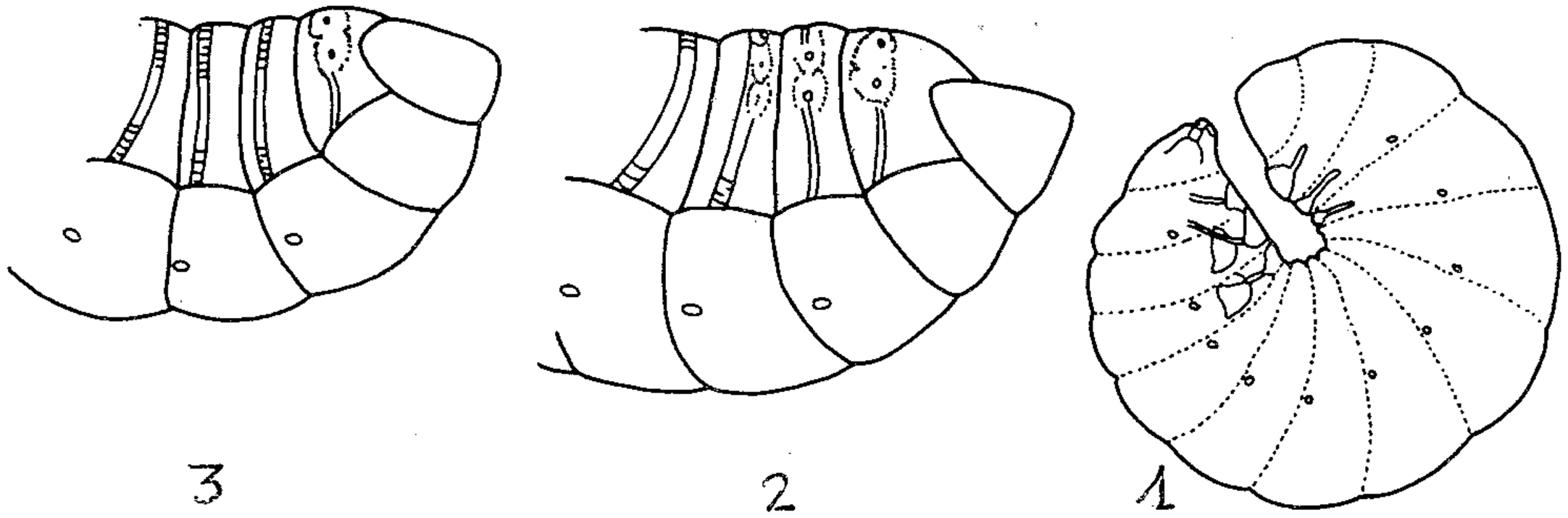

Fig. 1. Four-days-old living worker larva with fluorescent imaginal disks on the thorax and abdomen.

Fig. 2. The abdomen of four-days-old living worker larva with fluorescent imaginal disks on tenth, eleventh and twelfth segment.

Fig. 3. The abdomen of four-days-old living drone larva with single fluorescent imaginal disk on twelfth segment.

In the females the fluorescent disks of the reproductive organs and of the sting were found on the ventral side of the tenth, eleventh and the twelfth body segments (Fig. 2). The fluorescence, however, was confined here to the margins of the disks. In addition, various spots and lines were seen inside the disks. Of the abdominal imaginal disks, the pair on the tenth segment proved to be the smallest. It was partly situated in the posterior part of the segment behind a trachea. These disks were elongated and tapered toward lateral body sides.

Similar disks but considerably larger ones were found to be situated fairly symmetrically on the eleventh body segment at both sides of the trachea. The largest disk was on the twelfth segment and it was situated in its anterior part. The disk was oval in shape and slightly bent anteriorly. In its median part two fluorescent incisions — an anterior and posterior one — could be sighted: thus, it was unclear whether this was one single disk or two merged together. Fluorescence was also observed in the middle of the right and left side half of the disk. The trachea linked with the said disk is smaller than the ventral one of the tenth segment. Under the outer layer of the epidermis irregular formations could be seen but the rediments of the sting visible on fixed specimens and described in previous papers,

in the same stage of development, were not found. Thus, the greenish-blueish fluorescence induced in a pair of imaginal disks on the ventral side of the tenth and eleventh body segments and in one disk on the twelfth segment is a characteristic feature of the four-day-old female larvas and can be used in their identification.

Fluorescent disks on head and thorax, similar to those found in female larvas, can also be observed in four-day-old drone larvas. On the abdomen, however, only one such a disk is visible; it is seen on the ventral side of the twelfth segment and its shape is similar as that of the female larva (Fig. 3). The only difference is a backward bending of the disk by contrast to a forward curving in the female larva. Morover, the disk in question is situated slightly closer to the posterior margin of the segment than in the female larva. A fluorescent incision cutting into the anterior and posterior margin of the disk is likewise found. A fluorescence also occurs in the center of the right and left half of the disk. The imaginal processes — present and described in the fixed bee larvas — were, however, not visible. The abdominal disk connects with a smaller trachea than the one found on the ventral side of other segments.

Thus, the presence of one fluorescent disk on the ventral side of the twelfth abdominal segment is characteristic of the living four-day-old drone larva.

All disks described above are also seen on the subsequent days after hatching both in bee and drone larvas. However, the fluorescence becomes weaker every subsequent day.

Conclusions

(1) The imaginal abdominal disks of the living and of the fixed larvas differ considerably.

(2) The sex of living larvas can be determined easily and without error on the fourth day after hatching.

(3) The greenish-blueish fluorescence of the imaginal disks on the abdominal segments makes possible the determination of the sex of the living larvas. Fluorescent disks on the ventral side of the tenth, eleventh and the twelfth segment are characteristic of the females, whereas only one such abdominal disk on the ventral side of the twelfth segment indicates the drone larva. The fluorescence of the disks gradually wanes in both sexes on subsequent days.

REFERENCES

[1] Bertholf, L. M., (1925) — The moults of the honeybee. Journ. Econ. Ent. 18(2): 380–384.

[2] Michaëlis, G., (1900) — Bau und Entwicklung des männlichen Begattungsapparates der Honigbiene. Zeitschr. wiss. Zool. 67(3): 439–460, 1 table.

[3] Nelson, J. A., (1924) — Morphology of the honeybee larva. Journ. Agr. Res. (28(12): 1167–1213, 8 tables.

[4] Snodgrass, R. E., (1956) — Anatomy of the honey bee. 334 pp. Comstock Publ. Assoc., Ithaca N. Y.

[5] Zander, E., (1900) — Beiträge zur Morphologie der männlichen Geschlechtsonhänge der Hymenopteren. Zeitschr. wiss. Zool. 47(3): 461–489, 1 table.

[6] Zander, E., (1914) — Das Geschlecht der Bienenlarve. Zool. Anz. 44(6).

[7] Zander, E., Löschel, F., Meier, K., (1916) — Die Ausbildung des Geschlechtes bei der Honigbiene (Apis mellifica L.). Zeitschr. angew. Entomol. 3(1): 1–74, 6 tables.

[8] Zander, E., (1922) — Der Bau der Biene. E. Ulmer, Stuttgart.

[9] Woyke, J., (1962) — The hatchability of "lethal" eggs in a two sex-allele fraternity of honeybees. J. Apic. Res. 1: 6–13.

[10] Woyke, J. (1963a) — Drones from fertilized eggs and the biology of sex determination in the honey bee. Bull. Acad. Polonaise Sci., Ser. Biol. 11(5).

[11] Woyke, J., (1963b) — Drone larvae from fertilized eggs of the honey bee. J. Apic. Res. 2 (in printing).

[12] Woyke, J., (1963c) — What happens with the diploid drone larvae in the hive? J. Apic. Res. 2 (in printing).

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