1966

Sex Characters of Larval Bees (Hymenoptera: Apoidea)

Ross A. Nielsen

George E. Bohart
Utah State University

Recommended Citation

Nielsen, Ross A., and G. E. Bohart. 1966. Sex Characters of Larval Bees (Hymenoptera: Apoidea). Ann. Ent. Soc. Am. 60(2): 414-419.

Sex Characters of Larval Bees (Hymenoptera: Apoidea)[1,2,3]

ROSS A. NIELSEN AND G. E. BOHART
Entomology Research Division, Agr. Res. Serv., USDA, Logan, Utah

ABSTRACT

A structure diagnostic of the male sex was found on the 12th sternite of all species of larval bees studied; it usually took the form of a narrow transverse slit on the posterior part of the segment. Structures diagnostic of the female were found on the same area of most species, but they were less well defined. Other characters situated on the posterior ventral area of the abdomen also were found useful in distinguishing the sexes of certain species. Differences between genera and species suggest the potential usefulness of these characters in taxonomic studies of larval Hymenoptera. Determination of sex by external structures was verified by waiting for pupation and by dissecting for the gonadal rudiments.

Since 1957, studies of diapausing prepupal bees have been in progress at the Wild Bee Pollination Investigations Laboratory in Logan, Utah, under the direction of G. E. Bohart. Experimental designs could have been improved if the investigators had been able to sex the prepupae involved. Later determination of sex based on pupae sometimes sufficed, but this procedure did not permit equal distribution of the sexes in the various treatments, and usually caused months of delay. Also, pupation was often difficult to induce under laboratory conditions, and many prepupae died before pupating. The delay became serious, especially for treatments that increased mortality.

The sex of honey bee larvae is usually determined on the basis of cell type (worker, drone, queen). However, bees in colonies with recently mated queens frequently raise workers in drone cells, and the intermediate-sized cells which sometimes occur may be used for rearing either sex. Apparently the diploid drone eggs that are sometimes produced are laid in worker cells. Woyke (1963), who was concerned with the production of diploid drone larvae, found it necessary to rear the larvae until they were old enough to sex by using differential fluorescence of the imaginal discs. Also, many solitary bees develop through the egg and larval feeding stages for a week or two and then enter a quiescent prepupal period lasting through the winter months. When the diapause is broken, development proceeds to the pupal stage in which sex can be determined easily, but sex distinction is not obvious in the earlier stages.

Michaelis (1900) studied the postembryonic development of the genital organs in fixed specimens of larvae of the honey bee, *Apis mellifera,* and this study was extended by Zander (1900) to include larvae of the genera *Vespa* and *Bombus.* Although neither author was primarily concerned with the use of genitalic rudiments to distinguish the sexes of bees, Michaelis' descriptions of developing organs in the honey bee demonstrated that sex differentiation was possible in this species, and Zander (1916) demonstrated a characteristic male invagination on the 12th sternite. The information obtained by these investigators seems to be generally unknown, and those who do know about it have failed to see its usefulness in distinguishing the sex of bee larvae.

The primary objective of this study was to dis-

[1] In cooperation with Utah Agricultural Experiment Station. Accepted for publication June 13, 1966.
[2] This work was facilitated by a National Defense Education Act Fellowship to the Department of Zoology, Utah State University.
[3] Acknowledgments are due to Dr. Datus M. Hammond for providing invaluable assistance in the photomicrography, and to William P. Nye, for providing the photographs of living alkali bee larvae.

cover morphological methods of distinguishing the sexes of prepupal bees.

METHOD AND MATERIALS

For this study, I first selected live prepupae to provide a cross section of 7 families. Except for the honey bee, all are solitary and have quiescent prepupal stages; the honey bee develops from egg to adult without diapause. The following 12 species were chosen:

Colletidae
  Colletinae
    Colletes ciliatoides Stephen
  Hylaeinae
    Hylaeus cressoni (Cockerell)
Andrenidae
  Panurginae
    Nomadopsis anthidius (Fowler)
    N. scutellaris (Fowler)
Halictidae
  Nomiinae
    Nomia melanderi Cockerell
    N. nevadensis Cresson
    N. triangulifera Vachal
Nomadidae
  Epeolinae
    Triepeolus dacotensis (Stevens)
Megachilidae
  Megachilinae
    Megachile rotundata (F.)
Anthophoridae
  Anthophorinae
    Anthophora occidentalis Cresson
  Emphorinae
    Diadasia enavata (Cresson)
Apidae
  Apinae
    Apis mellifera L.

After the study of living prepupae was completed, I examined earlier stages of *Apis, Bombus, Megachile, Nomia,* and *Osmia.* Finally, I examined various larval stages of *Halictus, Andrena,* and *Nomia* preserved in alcohol to increase the number of genera and to determine whether young and preserved specimens could be sexed as well as living mature larvae.

External Examination of Live Prepupae

While they were in diapause, the larvae of each species were tentatively divided by sex. The larger average size of females furnished a rough guide in most species, but never produced an accurate separation. In *M. rotundata* the relative position of larvae in the cell series was also used as a basis for preliminary sorting, since the females were usually deeper in a cell series than the males.

The 2 groups were then examined externally for any morphological characteristics tending to follow a similar bimodal distribution. These characteristics were designated as possibly sex differentiating and were checked by pupation or dissection.

Since pertinent characters were situated in the posteroventral area, this was my principal area of search.

Because new characters found in later studies were always rechecked in the species examined earlier, not all bees were incubated immediately after their first examination. When the prepupae in incubation started to pupate, they were observed for transitional changes in structure that linked the sexual characteristics of pupae and prepupae. Re-examination of the prepupae with these transitional characteristics in mind was then useful in locating additional sex differentiating characters. Trypan Blue, rubbed lightly on the area being examined, emphasized cuticular conformations without preventing subsequent pupation.

Check of Accuracy of Determinations

Pupation.—Pupation was induced to check the validity of sex-differentiating characters. Incubation at constant conditions of 28°C and 75% RH was used for most species. However, for *M. rotundata* a temperature of 32°C was more successful, and *D. enevata* pupated best at room temperature (about 22°C).

My past experience indicated that pupation would be difficult, if not impossible, to induce in some species; in these species the gonadal rudiments were located by dissection as positive proof of sex. Dissection also was used to some extent on all species to obtain immediate verification. However, the technique was tedious. Whenever possible, verification of large numbers of specimens was done by sexing pupae.

Dissection.—Since prepupal tissues are largely liquid, the dissections were done on specimens immersed in normal saline solution. An incision made through the dorsal vessel was spread slightly to expose the underlying tissues, and the gonadal rudiments were located ventral to the dorsal diaphragm of the seventh and eighth segments. Stain was then injected at the midline, just ventral to the gonadal rudiments. About 1 min later, the excess stain was rinsed away by using an eyedropper to gently apply saline solution. Most larvae had gonadal rudiments large enough for the ovaries to be distinguished from the testes with a stereoscopic microscope (×45). Because of its flabbiness, *A. occidentalis* was dissected by making a deep longitudinal cut along the seventh, eighth, and ninth segments just dorsal to the spiracles; then when a deep transverse cut was made anterior to the seventh segment and another posterior to the ninth, the resulting flap could be raised to expose the gonads. Gonads of *T. dacotensis* were very small and closely attached to a mass of large fat bodies below the dorsal diaphragm of the eighth segment. When Trypan Blue was injected among these fat bodies, it stained the gonads and fibrous tissue but generally left the fat bodies white.

Three stains (Aniline Blue, Trypan Blue, and Basic Methylene Blue) were used. Basic Methylene Blue was the most satisfactory after I learned not to overstain, but the other dyes were nearly as helpful.

Imaginal discs were observed by removing the sternites from the 10th segment to the anus. When Aniline Blue was applied, the discs remained white against a blue background. Basic Methylene Blue stained them blue against a generally white background. However, I found that imaginal discs could

be used only with difficulty for sexing larvae because their appearance was variable, depending upon the stage of development of the larva.

Cuticle Mounts.—As the study progressed and structures associated with one of the sexes were found, slides of cuticle were made from the critical region. In most cases diagnostic structures could be seen without staining. Temporary mounts were made by placing the sternum of the 12th segment in glycerine or water on a slide and examining it under the compound microscope. Permanent mounts were made by staining with Fuchsen Acid or a mixture of equal

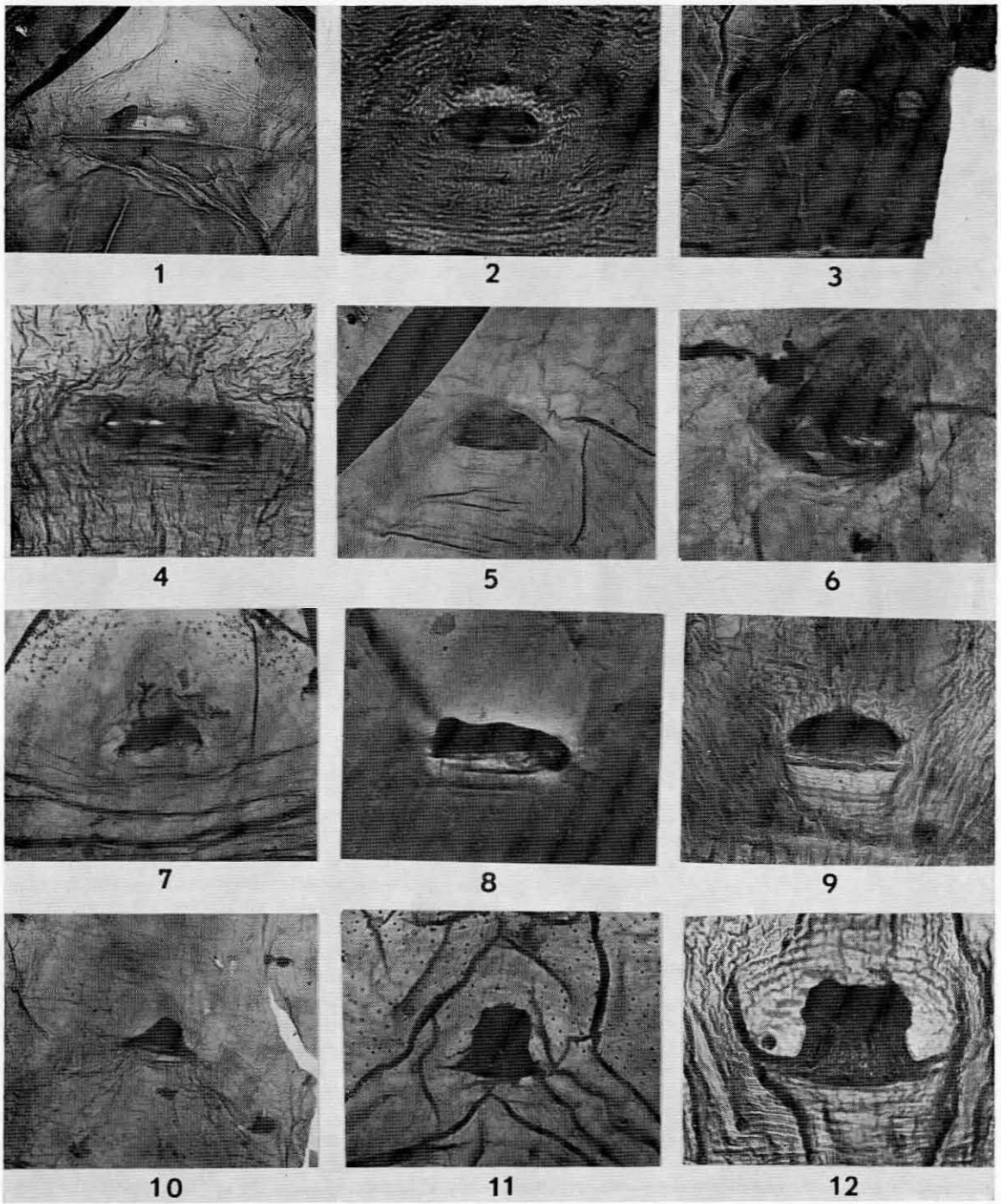

FIG. 1–12.—Male structure on 12th sternite. 1, *Hylaeus cressoni.* 2, *Nomadopsis anthidius.* 3, *N. scutellaris.* 4, *Nomia melanderi.* 5, *Anthophora occidentalis.* 6, *Apis mellifera.* 7, *Colletes ciliatoides.* 8, *Triepeolus dacotensis.* 9, *N. nevadensis.* 10, *Megachile rotundata.* 11, *Diadasia enavata.* 12, *N. triangulifera.*

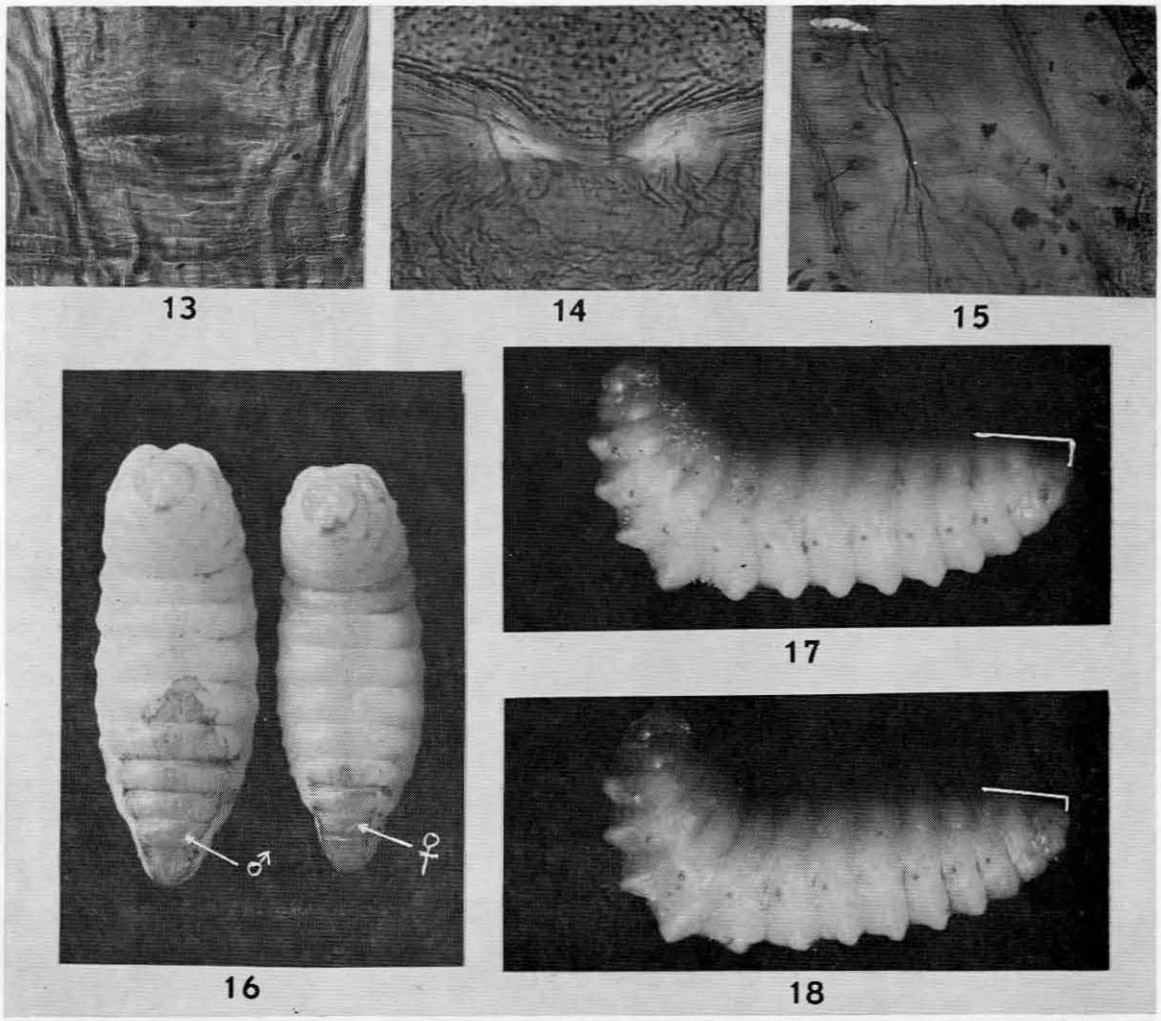

FIG. 13–15.—Female characteristic on 12th sternite. 13, *N. triangulifera.* 14, *N. melanderi.* 15, *M. rotundata.* FIG. 16–18.—Characteristics of living *N. melanderi.* 16, ventral view of male and female. 17, profile view of male. 18, profile view of female.

amounts of Fast Green, Orange G, and picric acid in 70% alcohol. Both these stains differentiated the critical structures adequately for study and photography.

RESULTS

Sex Differentiation by Secondary Sex Characters

Males. — External Characters. — A morphological character universally diagnostic of males of all species included here was found on the 12th sternite (Fig. 1–12): it had the form of a narrow, transverse slit and was never situated anterior to the middle of the sternite. Examination with a compound microscope revealed that it was an apparent opening in the outer cuticle, associated with an invagination of the body wall. Each side of the slit was sclerotized in varying degrees, making the area easier to see on some species than on others. Some specimens of *N. melanderi, N. triangulifera,* and *T. dacotensis* could be sexed by this character without magnification; on other specimens of the same species it was less conspicuous but was easily seen under the stereoscopic microscope, especially when the contours were accentuated with Trypan Blue. The structure was disclosed better on all species (except *H. cressoni*) when I rubbed Trypan Blue lightly over the area and when I used high magnification (×160), though lower magnification usually sufficed. *H. cressoni* was more difficult to study than the other species because it was smaller and had poor staining properties.

When Trypan Blue was rubbed on the critical area of *N. triangulifera,* both sexes appeared to have a transverse slit. However, examination under the compound microscope showed the male structure to be a deep invagination (Fig. 12); the female had only a slight infolding (Fig. 13). Also, the female was equally sclerotized both anteriorly and posteriorly to the invagination; the male was more heavily sclerotized anteriorly. In *D. enevata,* the wrinkled

surface of the cuticle made sex diagnosis by the presence or absence of the male structure difficult but not impossible.

Examination was made of all larval stages of both sexes of *A. mellifera* and of *Bombus griseocollis* De Geer. As early as the first instar, larvae of *Apis* and *Bombus* males had a rough area on the 12th sternite which, by the second instar, changed to the characteristic slit. When the male honey bee entered the stage at which the pupa was forming under the prepupal cuticle, a definite swelling caused by formation of the genitalia was observed.

Young larvae of *M. rotundata, N. melanderi, N. triangulifera, Osmia cornifrons* (Radoszkowski), and *O. ligneria* Say clearly exhibited the male structure as early as the second instar. Some specimens could be sexed in the first instar but a degree of accuracy was lost, especially in preserved specimens.

Prepupae of the genera *Andrena* and *Halictus* preserved in alcohol had the male structure clearly defined as in living material.

Internal Characters.—Two types of apodemes that extended internally from the male structure at the 12th sternite, were observed in all species studied.

One type appeared as a narrow slit through the external cuticle, with an invagination extending inward. The apodeme was sufficiently sclerotized to present a rather constant shape in each species; that is, it opened up from the narrow slit in such a way that it resembled a transverse figure 8. Internal tissues were attached to the sclerotized area.

The second type of apodeme was less heavily sclerotized than the first, but had a characteristic length or shape for each species. It appeared as a flexible flap extending inward from the external slit.

Females.—External Characters.—External female characters were less well defined than male characters. When stained preparations of the 12th sternite were mounted on slides, the female character could be seen on most but not all species (Fig. 13–15). The female of *H. cressoni* (difficult to sex because of size) was distinguished by a dent in the center of the 12th sternite. Of 287 prepupae sexed by this method, only 4 were wrongly determined.

The female of *N. melanderi* had a less ovate 12th sternite than the male (Fig. 17, 18). The straight edge that touched the 11th and 12th sternites fell much closer to the caudal end of the female than the male. Also a V-shaped crease on the anterior half of the 12th sternite of the female was a reliable differentiating characteristic (Fig. 16). *N. nevadensis* had a similar V-shaped crease, but it was less evident than on *N. melanderi.*

Females of *C. ciliatoides* and *Anthophora occidentalis* tended to have a little longer 12th sternite than the males. In addition, segmentation was less marked between the 11th and 12th sternites.

A. occidentalis had a mandibular character diagnostic of sex which also appeared in essentially the same form in the adult. In the female, the cuticle on the extreme lateral base of the mandible was more pronounced than in the male. This characteristic was not always apparent; of 24 bees examined, 5 were wrongly sexed by this character.

Internal Characters.—Stained slides made from the 12th sternite of female prepupae showed a differentiated area that was usually anterior to the position occupied by the slitlike structure in the male (Fig. 13–15). Muscular tissue was normally attached to this area. *N. melanderi* had an attachment of muscles at about the center of the sternite, and the sternite was depressed at that point. *N. nevadensis* also had a depression at a position anterior to that seen on the male, and muscular tissue was attached to this area.

The female of *H. cressoni* had a depression anterior to that occupied by the invagination on the male, and slides showed muscle attachment at that point. Females of *C. ciliatoides, M. rotundata,* and *A. occidentalis* had muscle attachment anterior to the position taken by the apodeme in the male, but females of *N. triangulifera* had their muscle attachment at or posterior to the position taken by the apodeme in the male.

Sex Differentiation by Primary Sex Characters

Gonadal Rudiments.—A remarkable similarity existed between the gonadal rudiments of male and female larvae in the solitary species studied. The rudiments of ovaries and testes in any given species were similarly situated when the prepupae were in deep diapause. When the prepupae were nearly ready for pupation, the gonads were displaced slightly to the rear but not by more than 1 segment. An exception to the rule of constant location within a species was *N. triangulifera,* in which the gonads were situated in either the seventh or eighth segment. The rudiments of ovaries tended to be more slender and slightly longer than testes. Suspensory ligaments also helped to distinguish the sexes but tended to make the ovaries difficult to distinguish from the surrounding tissues.

As the larvae started breaking diapause, male and female gonads developed and became less similar. The ovaries enlarged faster than the testes, and the suspensory ligaments became more evident. The developing spermatophore sacs tended to divide the testicular lobes into tightly spaced, equal-sized compartments.

DISCUSSION

Although the universal male characteristic was diagnostic of sex in all species included in this study, other characters that I found also were useful. *H. cressoni,* because of its small size and weakly sclerotized male structure, was sexed more easily by the female character. No errors were made on individuals sexed by the male method, but rigorous requirements of magnification and lighting were necessary. External female characters on *N. melanderi* were almost as reliable as the male structure and were easily seen with little or no magnification. Mandibu-

lar differences that existed in the sexes of *A. occidentalis* are interesting but of little practical importance. Perhaps useful mandibular differences or other differences not associated with sex organs will eventually be found.

Probably the male structure can be used to sex all groups of Hymenoptera. Zander (1900) described the later developments of the male genital pocket in *Vespa* and *Bombus,* but he did not discuss the earlier larval rudiments except to say that they were similar to those of the honey bee as described by Michaelis (1900).

It seems strange that recent students of Hymenoptera larvae have not made use of Michaelis' and Zander's fundamental studies of the ontogeny of sex in honey bees and several other species. Even though their studies did not emphasize sex differentiation, the essential external structures were described. Woyke (1963) indicated awareness of their work, but stated that he could not use it to sex living material because Michaelis' and Zander's methods required fixatives. The present studies show that this is not so. Both living material and specimens preserved in alcohol can be sexed without difficulty. Apparently we can sex bee larvae of all species, whether the specimens are alive, preserved or represented merely by exuviae. The male structure of honey bees and bumble bees can be distinguished in the earliest larval stage and that of all other bees studied in the second instar.

Secondary sex characteristics are of primary importance in the taxonomy of adult Hymenoptera, but larval taxonomy has been slow because the poorly defined structures make separation difficult. But recently students have placed considerable emphasis on the comparative morphology of larvae to test the taxonomic categories erected on the basis of adult morphology; this work has been centered mostly on characteristics of the head capsule. This study shows that secondary sex characteristics differ between taxa and probably can be used in phylogenetic investigations.

REFERENCES CITED

Michaelis, G. 1900. Bau und Entwicklung des männlichen Begattungsapparates der Honigbiene. Z. Wiss. Zool. 67: 439–60.

Woyke, J. 1963. Rearing and viability of diploid drone larvae. J. Apicult. Res. 2: 77–84.

Zander, E. 1900. Beiträge zur Morphologie der männlichen Geschlechtsanhänge der Hymenopteren. Z. Wiss. Zool. 67: 461–89.

1916. Die Ausbildung des Geschlechtes bei der Honigbiene (*Apis mellifica* L.). Z. Angew. Entomol. 3: 1–74.

Reprinted from the
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA
Volume 60, Number 2, pp. 414–419, March 1967