Morphological changes in the male accessory glands and testes in *Vespula vulgaris* (Hymenoptera, Vespidae) during sexual maturation

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Abstract. The present study documents the pace of accessory gland and testes degeneration in the wasp *Vespula vulgaris* by means of a histological and metric approach, that has not been carried out for social wasps so far. To a certain extent, comparison is made with the degenerative processes of the mucus glands of the honeybee drone. In *V. vulgaris*, no generative tissue is left by the end of 9 d of age, and so degeneration is a fast process. The three different parts of the accessory glands (muscle layer, gland epithelium, and lumen) change with respect to age. The secretory cells of the epithelium reach their maximum activity during the first days of adult life, which results in a maximally filled gland lumen by 9 d. We also provide, for the first time, a histological study of testes degeneration for this species. At eclosion, well-defined cystic structures are still visible, whereas at 9 d, it is no longer possible to distinguish different cystic structures. The diameter of the testes decreases with respect to age.

Additional key words: reproductive biology, social wasps, mating, ontogeny, male

Social insects are well known for their amazing variety of exocrine glands, of which the secretions play a role in numerous aspects of colony organization (Billen & Morgan 1998). As is the case with honeybee drones, male wasps belong to the neglected gender in scientific studies, as is reflected in the few reports on their biology. However, males are also equipped with a number of exocrine glands with variable functions. Examples include sex pheromone production of the mandibular glands in a number of ant species (Hölldobler & Maschwitz 1965), the territorial scent marks from the labial glands of bumblebee males to attract conspecific females for copulation (Kullenberg et al. 1970), and mating sign formation by the secretion of the mucus glands in honeybees (Koeniger 1986, 1991). The male reproductive system consists of paired testes that are responsible for the production of sperm, which is transferred to the females during copulation. Different from mammals, sperm synthesis in males of social hymenopteran species is not continuous, as the testes degenerate before the males become sexually mature. Testes degeneration is actually a remarkable feature

in social hymenopterans, although it has never been documented at the histological level in wasps.

Zander (1916) and Bishop (1920) mentioned that most of the sperm was found in the seminal vesicle of adult males in honeybees. But in ants, spermatogenesis stops shortly before or after eclosion, and sperm is stored in the seminal vesicle (Forbes 1954; Hölldobler 1966; Ball & Vinson 1984). In wasps of *Polistes* spp., the maturation of the sperm occurs in the larval stages (Machida 1934). Generally speaking, insects sustain significant levels of sperm competition because of their tendency to keep sperm internally in their spermathecae corresponding with special storage organs (Simmons 2001). The intensity of sperm competition is determined to a considerable extent by the absolute number of different males involved in competition for the ova of a single female (Parker 1998). Genital adaptations for manipulating rival sperm or to influence the duration of copulation are only expected in polyandrous species. Also, holdfast devices associated with the male genital apparatus are to be expected only in species with polyandrous females.

In most insects, the male genital system also consists of accessory glands of mesodermal or ectodermal origin, which open into the deferent or ejaculatory ducts, respectively. Among insects, the accessory glands vary notably in size, shape, number, and embryological origin (Adiyodi & Adiyodi 1975; Leopold 1976; Grassé

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1982; Chapman 1998). Among the functions of the secretions of these glands is the contribution to the seminal fluid and activation of the spermatozoa (Chen 1984; Davey 1985). The secretory cells of these male accessory glands are arranged as a monolayered epithelium and correspond with "Class-1," according to the classification of Noirot & Quennedey (1974, 1991).

A comparative study of the male genital apparatus of 51 bee species, excluding *Apis* spp., was provided by Ferreira et al. (2004). Among these, the male stingless meliponine bees were characterized by the absence of accessory glands (Dallacqua & Cruz-Landim 2003). In *Apis*, the accessory glands, called mucus glands, play an important role during sperm transfer (Woyke 1958; Woyke & Ruttner 1958; Koeniger et al. 1989; Koeniger & Hänel 1996). The secretion of the mucus glands, together with the cornual secretion, also contributes to the visibility of the mating sign in honeybees (Koeniger 1991; Koeniger et al. 1996; Colonello & Hartfelder 2005). Moors et al. (2005) described how the morphological structure of the mucus glands in honeybees changes with respect to age. Cruz-Landim & Dallacqua (2005) found that all the secretion of the drone accessory gland is produced before the sexual maturation of the male. They interpret this aspect as justified, because during mating the genital apparatus is lost and the drone dies. Colonello & Hartfelder (2003, 2005) focused on the male accessory gland products and their function in the reproductive biology of social bees. During the first 5 d of the mature drone, the mucus protein content increases enormously (Colonello & Hartfelder 2003).

In vespine wasps, mating occurs around noon, preceded by nuptial flights of males, which frequently congregate around prominent vegetation like trees or bushes. Mating occurs mostly while the queen is supported by a substrate (ground, grass, leaves, or branches) instead of during flight (Donisthorpe 1917; Thomas 1960). At the beginning of copulation, the male mounts the queen and inserts his penis into the queen's sting chamber. Afterwards, he releases the queen and assumes a freehanging position, supported only by their locked genitalia (Post 1980; Ross 1983). Finally, the last phase of the copulation involves reversal of the positions, like the S-position described by Schremmer (1962).

Important, but so far unanswered, questions are why testes in males of *Vespula* spp. mature that early during development, why hooks can occur near the aedeagus tip, and why mucus glands are huge. The answer for multiple mating in those species could be linked to the fact that their window of time for mating is narrow, because males emerge late in the season when winter approaches.

In male wasps, the reproductive organ is similar to most insects (El-Deeb et al. 1966). It contains a pair of testes, each linked to a median ejaculatory duct by a vas deferens, that is relatively short and leads into the large seminal vesicles. The seminal vesicles open into the base of the male accessory glands, which are probably associated with sperm maintenance or spermatophore production. Besides these structures, the male genital organ consists of a phallus (Kluge 1895).

In this contribution, we report on the development of the male accessory glands and the testes degeneration during sexual maturation in the wasp *Vespula vulgaris* LINNAEUS 1758, which is the first histological study of testes degeneration in social wasps.

Methods

Samples

Males of *Vespula vulgaris* were obtained from colonies in Leuven and Bree (Belgium). Accessory glands and testes were studied in males 0, 3, 6, and 9 d after emergence. We chose these age categories to allow comparison with the honeybee drones investigated by Moors et al. (2005). To obtain the different ages, newly emerged males were paint-marked on the thorax and placed back in the colonies, and kept under laboratory conditions until they attained the appropriate age. They were then taken from the nest, the accessory glands and testes were dissected, and fixed in cold 2% glutaraldehyde for ~24 h, buffered at pH 7.3 with 50 mmol L⁻¹ Na-cacodylate and 150 mmol L⁻¹ saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer for 1 h. After dehydration in a graded acetone series (50%, 70%, 90%, and 100%), the glands were embedded in Araldite.

Microscopy

Semithin sections (1 µm) for light microscopy were made with a Reichert OmU2 microtome (Reichert, Austria) and stained with methylene blue–thionin (1%–1%). The sections were observed with an Olympus BX-51 microscope (Olympus, Hamburg, Germany). Ultrathin sections (70 nm) were made with a Reichert Ultracut E and manually double stained with uranyl acetate and lead citrate. Sections were examined under a Zeiss EM900 electron microscope (Zeiss, Oberkochen, Germany).

Measurements

Measurements of the accessory glands and testes were performed with the Olympus DP-Soft 3.2 soft-

ware on digital photographs of histological sections. We measured the width of the muscle layer, the thickness of the glandular epithelium, and the lumen diameter of the different ages. For the testis, we measured the diameter. To work randomly, a hexagon frame was placed on the image of both accessory glands and testis, so that the hexagon lines could serve as landmarks for measuring. All data are presented as arithmetic means $\pm$ SD, performed by the statistical program Statistica.

Results

Accessory glands

The accessory glands in *Vespula vulgaris* are paired and are situated in the posteroventral region of the male abdomen. They have a banana-like appearance and show an abundant supply of tracheae. The width of the accessory gland varies from $\sim 470 \mu\text{m}$ in just-emerged males to $\sim 650 \mu\text{m}$ in mature males. The length of the gland varies from $\sim 1975 \mu\text{m}$ in young males to $\sim 2200 \mu\text{m}$ in mature males. The accessory glands open into the seminal vesicles (Fig. 1) and consist of three different parts: the muscle layer, the glandular epithelium, and the lumen. Histological sections revealed considerable differences with respect to age. These differences are noticeable in all three parts. During the first days of adult life, the thickness of the muscle layer and the glandular epithelium decreases. However, the degree of decrease is more pronounced in the glandular epithelium than in the muscle layer. The lumen, on the other hand, increases at the onset of adult life and reaches a maximum diameter of $\sim 540 \mu\text{m}$ at 9 d (Figs. 2, 3A).

Fig. 1. Dissection image of the male genital system of a 3-d-old male. ag, accessory gland; f, forceps that were used to hold the sample for taking pictures; p, penis; vsem, vesicula seminalis.

There are no histological differences between the distal and the proximal part of the glands.

In adult males, the apical cell membrane of the epithelial cells is differentiated into an irregular mi-

Fig. 2. Measurements of the layers of accessory glands in relation to age ($n = 7$ for 0, 3, 6 d, $n = 5$ for 9 d). **A.** Muscle layer. **B.** Gland epithelium. **C.** Lumen.

Fig. 3. A. Semithin cross sections of the accessory glands of a 0-, 3-, and 6-d-old male. B, C. Electron micrographs of 3- and 6-d-old males, respectively. bm, basement membrane; de, desmosomes; ge, glandular epithelium; l, lumen; m, mitochondria; ml, muscle layer; mv, microvilli; n, nucleus; rer, granular endoplasmic reticulum; s, secretion.

crovillar border, which becomes less prominent with increasing age. At the apical region of the epithelium, numerous desmosomes were found. No cuticle is observed between the gland cells and the lumen. The lumen is filled with dark-staining secretion as a result of high secretory activity in the epithelial cells. The cytoplasm of the cells contains numerous mitochondria and a very extensive tubular granular endoplasmic reticulum regardless of age. This RER is distributed throughout the cytoplasm. We could not find Golgi complexes in the different ages. The thickness of the basement membrane ranges 0.7–1 μm (Fig. 3B).

Testes

The testes are also paired and connected to the seminal vesicles by the vas deferens. The width and the length of the testes differ between males between the ages 0 and 9 d. The width of the testes varies from ~1610 μm in recently emerged males to ~1290 μm in mature ones, and the length ranges ~2095 μm in just-emerged males to ~855 μm in 9-d-old males (Fig. 4).

The testes display degeneration during sexual maturation of the males. Their maximum diameter is reached at emergence, whereas the minimum is more or less reached at the age of 9 d (Fig. 5). In freshly eclosed males, the testes follicles have a dia-

meter of ~485 μm, and follicle cells with different developmental stages of sperm can be observed. At the lateral sides of each follicle, nuclei can be observed that may belong to the nutritive cells. In the testes of recently emerged drones (0 d), the assumed nutritive cells (follicle cells) display dark-staining strands, extending toward the developing sperm cells. In males aged 3 d, the follicle diameter is similar to that in newly eclosed males. All spermatogenic stages are still visible but in smaller quantities. In general, we can clearly see mature spermatozoa. In 6-d-old males, mature sperm migrates into the vas deferens. In testes of the oldest investigated males, it is no longer possible to distinguish well-defined cystic structures; the follicles are indistinguishable from each other. In each investigated testis, tracheae were observed (Fig. 6).

Discussion

Our measurements show that the three layers of the accessory glands degenerate with respect to age. In ants of *Formica* spp. and honeybee drones, different regions in the accessory glands were distinguished based on the composition of the secretions and histological sections (Jeantet 1972; Cruz-Landim & Dallocqua 2005; Moors et al. 2005). In *Vespa vulgaris*,

Fig. 4. Light micrographs of testis degeneration. **A.** 0 d. **B.** 3 d. **C.** 6 d. **D.** 9 d. Scale for A–C as in D. Insets, dissection images of the respective ages; scale bars = 1 mm. Arrows indicate vas deferens.

the epithelial thickness decreases during sexual maturation, but RER remains present in considerable quantity. The thick epithelium in the accessory

Fig. 5. Diameter of testes with respect to age ($n = 6$ for 0, 3, and 6 d, and $n = 5$ for 9 d).

glands of young males, together with the ultrastructural features of the cells (omnipresent RER), clearly indicates a high-secretory activity. The accumulation of secretion in the lumen more or less starts at the onset of adult life. Also at emergence of the male, the thickness of the epithelium starts to diminish as the gland cells have fulfilled their secretory function and start to degenerate. The presence of a thick and active epithelium surrounding a reduced lumen at emergence, followed by a filling up of the lumen and a simultaneous reduction in the epithelial thickness, is known for glands with an ephemeral secretory activity. Examples include the Dufour gland of queens of the ant *Polyergus rufescens* (Billen et al. 2001) and the mucus gland of the honeybee (Moors et al. 2005). The changes of the muscle layer, the lumen, and the glandular epithelium are comparable with these found in honeybees (Bishop 1920; Moors et al. 2005).

Honeybee males have the required amount of mucus by the time they reach sexual maturity, which they need during sperm transfer and for the formation of the mating sign after copulation (Koeniger 1986). As in honeybees, we assume that the males of *V. vulgaris* will have the required amount of secretion at the age of sexual maturity. Marchini et al. (2003) found a large amount of RER in the cytoplasm of the male accessory glands of the medfly *Ceratitis capitata*, which is in accordance with the presence of proteins in the secretions. Our ultrastructural observations demonstrate that the accessory glands of the male wasp have a high activity in the early life of the male. Also, their large relative size and rich tracheal supply emphasize the quick maturation of the glands.

Zander (1916) and Bishop (1920) reported that the testes of honeybee males undergo a gradual shrinking

Fig. 6. Light microscopic pictures of the testicular follicles and the vas deferens. **A, C, E, G.** Testicular follicles of 0-, 3-, 6-, and 9-d-old male, respectively; scales as in **G**. **B, D, F, H.** Vas deferens of 0-, 3-, 6-, and 9-d-old male, respectively; scales as in **G**. fcl, follicle cells; sg, spermatogonia; st, spermatids; sz, spermatozoa; t, trachea; vd, vas deferens.

as sperm is discharged and stored in the seminal vesicle during sexual maturation. Forbes (1954), Hölldobler (1966), and Ball & Vinson (1984) showed testes degeneration during sexual maturation in ants. Machida (1934) reported that the maturation of sperm starts in larval stages in *Polistes* wasps.

The presence of tracheae in the testes is also observed in the ant *Camponotus pennsylvanicus* (Forbes 1954). Romoser & Stoffolano (1998) reported the presence of an apical cell but also a complex of cells that apparently serve a trophic function, providing nutrients for the developing germ cells. As mentioned, the testicular follicles in *V. vulgaris* show a group of nuclei at their lateral sides. We assume that those nuclei belong to the trophic cells of the testes. Although no descriptions of such cells are available for social wasps, an analogy exists with the follicle cells mentioned by Snodgrass (1956) for the honey-

bee *Apis mellifera*. As stated in “Results”, darkly staining strands were found in between the nutritive cells and the developing sperm cells. Possibly similar structures are known for the milkweed bug (Bonhag & Wick 1953). Cruz-Landim & Dallacqua (2002) describe the ultrastructural features of the degenerating sperm cells and cyst cells.

We conclude that the elaboration and the accumulation of the accessory gland secretions in *V. vulgaris* are similar to the mucus gland secretions in *A. mellifera* during the first days of adult life. However, the glands cells are already degenerated at the age of 9 d in the wasp, which is earlier than in the honeybee.

High paternity seems to have evolved more than once: one time in the *Dolichovespula norwegica* group, a second time in the *Vespula* group (Foster et al. 1999). Species with the highest paternity values within the *Dolichovespula* group have large testes and

large male accessory glands (E. Schoeters, unpubl. data). In representatives of the *Vespula* group, large male accessory glands also occur. In addition, males of *V. vulgaris* have a special aedeagus, provided with two sharp hooks to anchor itself into the female (functioning either in prolonged copulation or increasing chances of paternity). In males of *Vespula germanica*, these hooks are lacking; in *Vespula rufa*, the aedeagus is more spatulate, possibly acting as a more forceful intromittent organ (E. Schoeters, unpubl. data).

The males of *V. vulgaris* have testes developing earlier in the pupa than is the case in *Dolichovespula* spp. In addition, the pupal testis mass is huge. Further development results in all sperm being present in the vesicula seminalis in young males, such as those of *V. vulgaris*, an observation contrasting with what is found in males of *Dolichovespula*. Males of *V. vulgaris* have very long sperm, ~360 µm (compare meliponine bees having sperm lengths ranging 80–300 µm [Zama et al. 2004]). Hosken (1998) pointed out, for bats, that larger testes are an adaptation for sperm competition. In the context of wasps, this can be translated into population size and thus competition among males to fertilize the eggs of the female. A way in which any given male can increase its reproductive success is by producing larger ejaculates or by having functionally mature testes at the time of emergence.

Apparently different biological routes have been followed to achieve higher paternity rates; the way in which males of *Vespula* achieve this is substantially different from the ways in which drones of *Apis* evolved a specialized endophallus. Various aspects of mating biology in males of social Hymenoptera certainly merit further attention.

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References

- Adiyodi KG & Adiyodi RG 1975. Morphology and cytology of the accessory sex glands in invertebrates. *Int. Rev. Cytol.* 47: 253–398.
- Ball DE & Vinson SB 1984. Anatomy and histology of the male reproductive system of the fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Int. J. Insect. Morphol. Embryol.* 13: 283–294.
- Billen J & Morgan ED 1998. Pheromone communication in social insects sources and secretions. In: *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*. Vander Meer RK, Breed MD, Winston ML, & Espelie KE, eds., pp. 3–33. Westview Press, Boulder, CO, USA.
- Billen J, Grasso DA, Mori A, & Le Moli F 2001. Structural and functional changes of the Dufour gland in gynes of the amazon ant *Polyergus rufescens* (Hymenoptera, Formicidae). *Zoomorphology* 121: 55–61.
- Bishop GH 1920. Fertilization in the honeybee: the male sexual organs, their histological structure and physiological functioning. *J. Exp. Zool.* 31: 225–267.
- Bonhag PF & Wick JR 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *J. Morphol.* 93: 177–283.
- Chapman RF 1998. *The Insects: Structure and Function*, 4th ed. Cambridge University Press, Cambridge, UK.
- Chen PS 1984. The functional morphology and biochemistry of insect male accessory glands and their secretion. *Annu. Rev. Entomol.* 29: 233–255.
- Colonello NA & Hartfelder K 2003. Protein content and pattern during mucus gland maturation and its ecdysteroid control in honey bee drones. *Apidologie* 34: 257–267.
- 2005. She's my girl—male accessory gland products and their function in the reproductive biology of social bees. *Apidologie* 36: 231–244.
- Cruz-Landim C & Dallacqua RP 2002. Testicular reabsorption in adult males of *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apidae, Meliponini). *Cytologia* 67: 145–151.
- 2005. Morphology and protein patterns of honey bee drone accessory glands. *Genet. Mol. Res.* 4: 473–481.
- Dallacqua RP & Cruz-Landim C 2003. Ultrastructure of the ducts of the reproductive tract of males of *Melipona bicolor bicolor* Lepeletier (Hymenopter, Apinae, Meliponini). *Anat. Histol. Embryol.* 32: 276–281.
- Davey KG 1985. The male reproductive tract. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. I. Kerkut GA & Gilbert LI, eds., pp. 1–14. Pergamon Press, Oxford, UK.
- Donisthorpe HJK 1917. Attitudes of wasps and psocids in copulation. *Entomol. Rec.* 29: 231–232.
- El-Deeb AA, Hammad SM, Nour, & El-Din IT 1966. The digestive and reproductive systems of *Vespa orientalis aegyptiaca* F. (Hymenoptera: Vespidae). *Bull. Soc. Entomol. Egypte* 50: 265–268.
- Ferreira A, Abdalla FC, Kerr WE, & Cruz-Landim C 2004. Comparative anatomy of the male reproductive internal organs of 51 species of bees. *Neotrop. Entomol.* 33: 569–576.
- Forbes J 1954. The anatomy and histology of the male reproductive system of *Camponotus pennsylvanicus* DeGeer (Formicidae, Hymenoptera). *J. Morphol.* 95: 523–555.
- Foster KR, Seppa P, Ratnieks FLW, & Thorén PA 1999. Low paternity in the hornet *Vespa crabro* indicates that

- multiple mating by queens is derived in vespine wasps. *Behav. Ecol. Sociobiol.* 46: 252–257.
- Grassé DG 1982. Ejaculate esterase-6 and initial sperm use by female *Drosophila melanogaster*. *J. Insect Physiol.* 27: 641–650.
- Hölldobler B 1966. Futterverteilung durch Männchen im Ameisenstaat. *Z. Vgl. Physiol.* 52: 430–455.
- Hölldobler B & Maschwitz U 1965. Der Hochzeitschwarm der Rossameise *Camponotus herculeanus* (Hymenoptera, Formicidae). *Z. Vgl. Physiol.* 50: 551–568.
- Hosken DJ 1998. Testes mass in megachiropteran bats varies in accordance with sperm competition theory. *Behav. Ecol. Sociobiol.* 44: 169–177.
- Jeantet AY 1972. Recherches histophysiologiques sur le développement post embryonnaire et le cycle annuel de *Formica* (Hyménoptère). 3. Données cytophysiologiques sur les glandes annexes males de *Formica polyctena* Foerst. *Ann. Sci. Nat.* 14: 285–303.
- Kluge MHE 1895. Das männliche Geschlechtsorgan von *Vespa germanica*. *Arch. Naturgesch* 61: 159–198.
- Koeniger G 1986. Mating sign and multiple mating in the honeybee. *Bee World* 67: 141–150.
- 1991. Diversity in *Apis* mating systems. Westview Press, Boulder, CO, USA. pp. 199–211.
- Koeniger G & Hänel H 1996. The bulbus gland of drones (*Apis mellifera* L.). *Pszczelnicze. Zeszyty. Naukowe.* 40: 45–54.
- Koeniger G, Hänel H, Wissel M, & Herth W 1996. Cornual gland in the honeybee drone (*Apis mellifera* L.): structure and secretion. *Apidologie* 27: 145–156.
- Koeniger N, Koeniger G, & Wongsiri S 1989. Mating and sperm transfer in *Apis florea*. *Apidologie* 21: 413–418.
- Kullenberg B, Bergström G, & Stållberg-Stenhagen S 1970. Volatile components of the cephalic marking secretion of male bumble bees. *Acta Chem. Scand.* 24: 1481–1483.
- Leopold RA 1976. The role of male accessory glands in insect reproduction. *Annu. Rev. Entomol.* 21: 199–221.
- Machida J 1934. Spermatogenesis of three species of *Polistes*. *Proc. Imp. Acad. Jpn.* 10: 515–518.
- Marchini D, Del Bene G, Cappelli L, & Dallai R 2003. Ultrastructure of the male reproductive accessory glands in the medfly *Ceratitis capitata* (Diptera: Tephritidae) and preliminary characterization of their secretions. *Arthropod. Struct. Dev.* 31: 313–327.
- Moors L, Spaas O, Koeniger G, & Billen J 2005. Morphological and ultrastructural changes in the mucus glands of *Apis mellifera* drones during pupal development and sexual maturation. *Apidologie* 36: 245–254.
- Noirot C & Quennedey A 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.* 19: 61–80.
- 1991. Glands, gland cells, glandular units: some comment on terminology and classification. *Ann. Soc. Entomol. Fr. (NS)* 27: 123–128.
- Parker GA 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm Competition and Sexual Selection*. Birkhead TR & Møller AP, eds., pp. 3–54. Academic Press, London, UK.
- Post DC 1980. Observations on male behaviour of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *Entomol. News* 91: 113–116.
- Romoser WS & Stoffolano JG 1998. *The Science of Entomology*, 4th ed. WCB McGraw-Hill, Boston, MA, USA.
- Ross KG 1983. Laboratory studies of the mating biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 56: 523–537.
- Schremmer F 1962. Wespen und Hornissen. Die Neue Brehm-Bücherei, Heft 298, Ziemsen, Wittenburg.
- Simmons LW 2001. *Sperm Competition and its Evolutionary Consequences in the Insects—Monographs in Behavior and Ecology*. Princeton University Press, Princeton, NJ, USA. 434 pp.
- Snodgrass RE 1956. *Anatomy of the Honey Bee*, 1st ed. Comstock Publication Association Cornell University Press, Ithaca, NY, USA.
- Thomas CG 1960. The European wasp (*Vespula germanica* Fab.) in New Zealand. *NZ. Dep. Sci. Ind. Res. Inf. Ser.* 27: 1–74.
- Woyke J 1958. The histological structure of the reproductive organs of the drone. *Proznán Soc. Friends Art Sci. Publ. Sect. Agric. Sylvic.* 19: 38–50.
- Woyke J & Ruttner F 1958. An anatomical study of the mating process in the honeybee. *Bee World* 39: 3–18.
- Zama U, Lino-Neto J, & Dolder H 2004. Structure and ultrastructure of spermatozoa in Meliponini (stingless bees) (Hymenoptera: Apidae). *Tissue Cell.* 36: 29–41.
- Zander E 1916. Die postembryonale Entwicklung des Geschlechtsapparates der Drohne (*Apis mellifica* L.). *Z. Angew. Ent.* 3: 7–20.