

**Morphology and protein pattern of the accessory reproductive glands of male
Blattella germanica (Dictyoptera: Blattellidae)**

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Accessory reproductive glands, cockroach, *Blattella germanica*, uricose glands, utricles, conglobate gland

Abstract. The basic function of the accessory reproductive glands (ARG) of male insects is to provide materials for the production of the spermatophore. The morphology of the ARG of *Blattella germanica*, including the ejaculatory pouch and duct, the uricose glands, the utricles of the mushroom-shaped body, and the conglobate gland are described. Special attention is given to the fine discrimination of the different tubules associated with the ARG, particularly in the mushroom-shaped body, where up to 12 types of tubule have been recognized. The analysis considered not only morphology and biometry, but also protein pattern. The remarkable diversity found reveals a fascinating complexity, which can be explained if the roles played by the spermatophore they produce are considered.

INTRODUCTION

The basic function of the accessory reproductive glands (ARG) of male insects is to provide materials for the production of the spermatophore. In some cases, ARG secretions are also involved in the modulation of female behaviour (Leopold, 1976; Guillott & Friedel, 1977). ARG have been reviewed by a number of authors from the perspectives of morphology (Adiyodi & Adiyodi, 1975; Happ, 1984), biochemistry (Chen, 1984) and physiology (Guillott, 1988; Happ, 1992). A general monograph on accessory reproductive structures in insects has been published by Kaulenas (1992), and the structure and production of spermatophores was discussed by Mann (1984).

Previous studies of *Blattella germanica* (L.) (Dictyoptera: Blattellidae) considered the role of juvenile hormone in endocrine regulation of ARG maturation (Bellés & Piulachs, 1992; Piulachs et al., 1992; Vilaplana et al., in press). Studies on the formation of the spermatophore, the endocrine events accompanying this process, and the physiological roles of this structure are in progress. However, a thorough study of the morphology of the ARG is necessary to analyse the entire phenomenon, and the purpose of this paper is to report this morphological study. A number of authors have examined the morphology of the ARG of *B. germanica*, including Brehm (1880), Snodgrass (1937), Khalifa (1950), Ballan-Dufrançais (1968), Bellés & Piulachs (1992) and Piulachs et al. (1992). The present study concentrates on the fine discrimination of the different tubules associated with the ARG, especially in the mushroom-shaped body, in terms of morphology, biometry and protein pattern.

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MATERIAL AND METHODS

Insects

Virgin males of *B. germanica* (L.) were from a colony fed on Panlab dog chow and water, and reared in the dark at $30 \pm 1^\circ\text{C}$ and 60–70% r.h. Unless stated otherwise, the studies were performed on 10-day-old virgin males, in which maturation of the ARG was complete (Piulachs et al., 1992).

Morphological observations and measurements

Dissections of ARG were carried out under Ringer's solution on specimens anaesthetised with CO_2 . Measurements of tubules were performed with an ocular micrometer adapted to a binocular stereomicroscope. Depending on the type, between 13 and 107 tubules (n) were measured, and glands from 5 to 10 specimens were used for these purposes. For general descriptions the ARG was placed in ventral view, thus right and left orientations refer to this view unless stated otherwise. To achieve a better contrast between the different types of tubule, they were stained with methylene blue (2% in aqueous solution) for 30 sec.

Electrophoretic studies

ARG were explanted and processed as described elsewhere (Bellés & Piulachs, 1992; Piulachs et al., 1992; Vilaplana et al., in press). SDS-PAGE of ARG proteins was performed using the method of Laemmli (1970) with 15% polyacrylamide slab gels. Electrophoresis was run at constant voltage (150 V) for 45 min, and gels were stained with Coomassie blue. Molecular markers were from Sigma, Madrid (Sigma-Marker, Low Range: MW 6.5–66 kDa, and Phosphorylase b from rabbit muscle: MW 97 kDa). To reveal the main protein band(s) of the different types of tubule studied, an appropriate number of them were loaded on the corresponding lane, as indicated. Since the number of tubules of each type present in the whole ARG is also stated, this system of quantification allows comparisons in anatomical terms (i.e., gland-equivalents for each type of tubule). At least 3 replicates of each electrophoretic study were performed.

RESULTS

General description

The internal reproductive organs of male *B. germanica* (Fig. 1) consist of a pair of testes with their corresponding vas deferens, which terminate in a pair of seminal vesicles. The seminal vesicles are inserted in the ejaculatory pouch, and are surrounded by a mass of tubules arranged in two main groups: the uricose glands, placed in the apical part of the mass, and a large mushroom-shaped body formed by a multiplicity of tubules or utricles. The ejaculatory pouch connects with the ejaculatory duct, which terminates in the gonopore. The conglobate gland lies close to the distal part of the ejaculatory duct, and opens separately into the genital pouch.

The ejaculatory pouch shows two distinct lobes (Figs 2–3): one to the right and the other to the left. The seminal vesicles, the uricose glands and all the utricles are inserted on the apical part of the right lobe. In addition, a small protuberance (the seminal lobe: Fig. 2) close to the seminal vesicles is also observed in the apical part. The left lobe shows only superficial varicosities, and connects laterally with the right lobe and with the ejaculatory duct.

Uricose glands

These form a group of 4–6 tubules, united at the base to form a single point of insertion, which connects with the apical part of the right lobe of the ejaculatory pouch (Figs 1, 4). The tubules are long (3.5 mm, on average, in mature specimens), show multiple constrictions (more than 40 each) and seldom dichotomize. They contain a very evident opaque white secretion, which stains poorly with methylene blue.

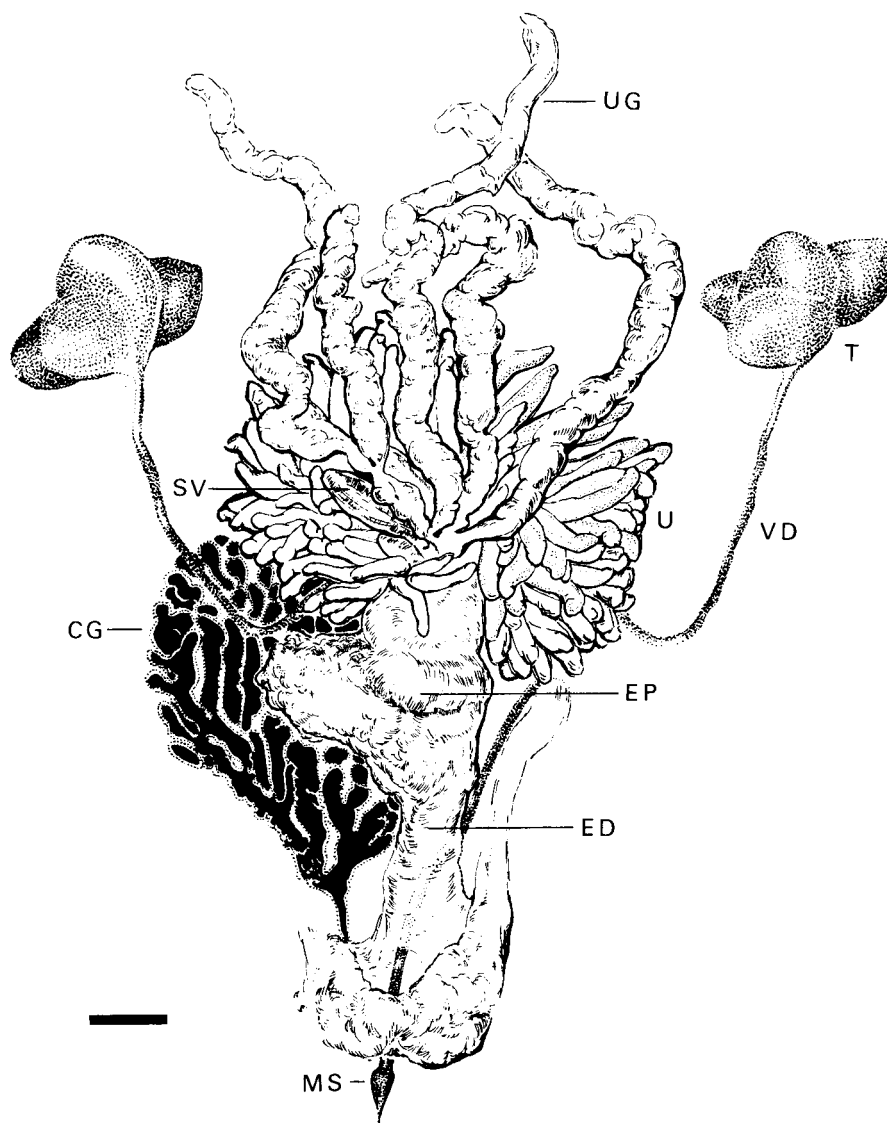
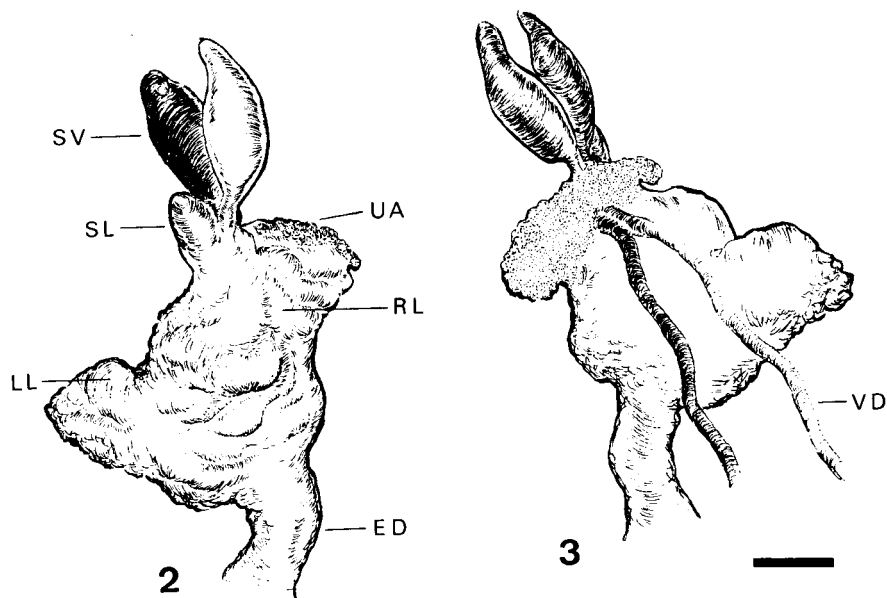


Fig. 1. Ventral view of the internal reproductive organs of male *B. germanica*. CG – conglobate gland, ED – ejaculatory duct, EP – ejaculatory pouch, MS – median sclerite, SV – seminal vesicles, T – testis, U – utricles, UG – uricose glands, VD – vas deferens. The conglobate gland has been represented in black for clarity. Scale bar: 0.5 mm.



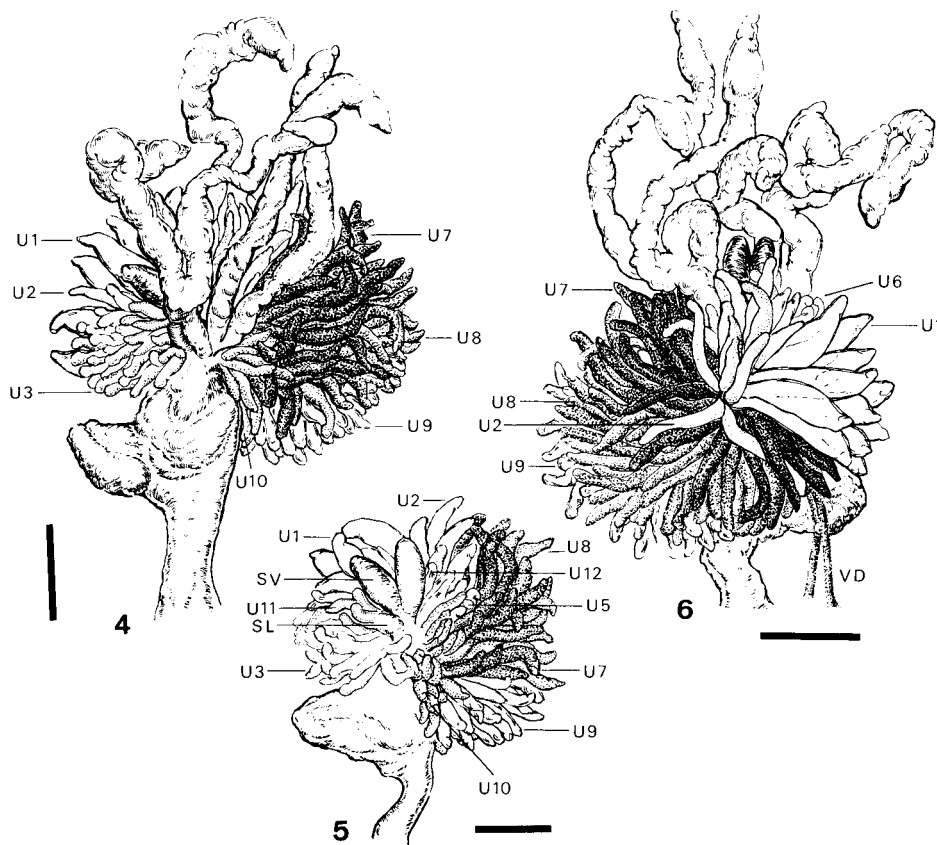
Figs 2–3. Ventral (2) and dorsal (3) view of the ejaculatory pouch of *B. germanica* with the uricose glands and utricles removed. ED – ejaculatory duct, LL – left lobe, RL – right lobe, SL – seminal lobe, SV – seminal vesicles, UA – utricular area, VD – vas deferens. Scale bar: 0.2 mm.

Utricles of the mushroom-shaped mass

All are inserted in a limited area in the apical part of the right lobe of the ejaculatory pouch. There are between 200 and 220 utricles, and they are distinctly heterogeneous. According to morphological features, up to 12 types may be recognized (from U1 to U12). To nominate them we have used a system combining features of size (long, medium, short), colour (transparent, translucent, white) and location (apical, dorsal, ventral, dorsolateral, ventrolateral, right or left).

Long and transparent, dorsolateral left (U1). Group formed by 10–14 tubules, of notable size (0.63 ± 0.16 mm long \times 0.18 ± 0.04 mm wide, $n = 32$). Their morphology is homogeneous and the diameter is quite constant along the tubule, except at the apex, where it becomes sharply pointed (Figs 4–8, 10). Their secretion is transparent during maturation, with no granules apparent, and stains poorly with methylene blue. They are situated close to the apical zone of the ejaculatory pouch, in a dorsolateral left position, forming irregular crowns. Close to them are types U3 to the left, and U7 and U8 to the right (dorsal view).

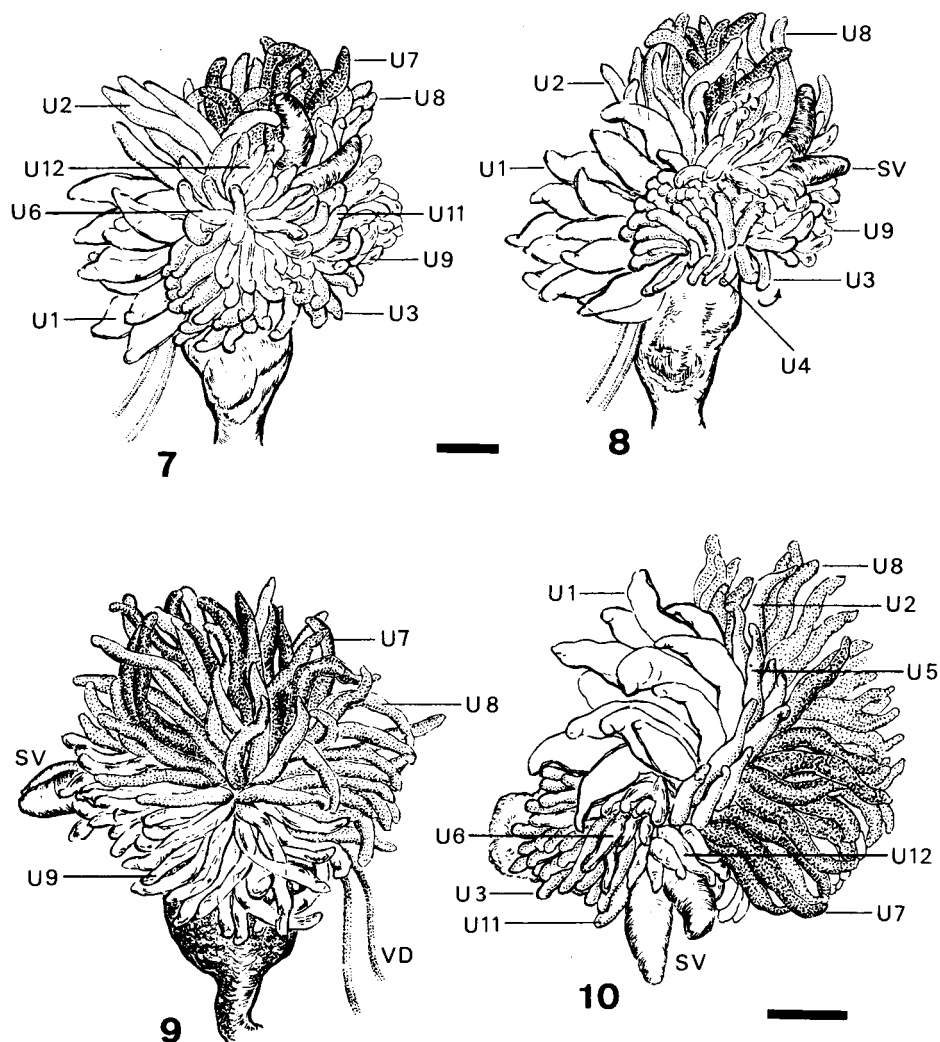
Long and translucent, dorsal (U2). This group comprises 7–9 long, thin tubules (0.83 ± 0.19 mm long \times 0.14 ± 0.02 mm wide, $n = 25$). Some heterogeneity was observed in length. The diameter is quite constant, and the tubules are moderately pointed apically (Figs 4–8, 10). Their secretion is translucent, but changes to opaque white when immersed in Ringer's solution. There are no granules apparent, and it stains poorly with methylene blue. The tubules are spread along the basal-apical axis on the dorsal side of the ejaculatory pouch. Close to them, and from dorsal view, are types U8 and U5 to the right, U1, U3 and U6 to the left, and U5 and U12 in the apical part.



Figs 4–6. Accessory reproductive glands of *B. germanica* in ventral view (4), ventral view with the uricose glands removed (5) and dorsal view (6), showing different types of utricles (U1, U2, U3, U5, U6, U7, U8, U9, U10, U11, U12). SL – seminal lobe, SV – seminal vesicles, VD – vas deferens. Scale bars: 0.5 mm.

Medium length and white, ventrolateral left (U3). This group is formed by 31–34 medium tubules (0.51 ± 0.12 mm long \times 0.07 ± 0.02 mm wide, $n = 98$). Their morphology is homogeneous; they show some constrictions, and the apex is broad and round (Figs 4, 5, 7, 8, 10). However, some heterogeneity is observed in the secretion. That from the tubules situated in the most apical part of the ensemble maintains its transparency when placed in Ringer's solution, but that from those more basal becomes white. Both secretions stain poorly with methylene blue. The tubules are spread to the left on the ventrolateral side of the ejaculatory pouch. Close to them are types U2, U6 and U11 in the apical part.

Short and transparent, ventrolateral left (U4). This group is composed of 6–9 small tubules (0.22 ± 0.09 mm long \times 0.09 ± 0.06 mm wide, $n = 22$). Some heterogeneity is observed in size. The diameter is quite constant along each tubule and the apex is rounded (Fig. 8). The secretion is transparent and does not change when immersed in Ringer's solution. There are no granules apparent and it stains poorly with methylene blue. The



Figs 7–10. Mass of utricles (mushroom-shaped body) of the accessory reproductive glands of *B. germanica* in lateral left view (7), lateral left view with the utricles U3 partially removed (8), lateral right view (9), and apical view (10), showing different categories of utricles (U1, U2, U3, U4, U5, U6, U7, U8, U9, U11, U12). SV – seminal vesicles, VD – vas deferens. Scale bars: 0.5 mm.

tubules are situated to the left of the ejaculatory pouch, close to the base of the utricular mass, and they are partially hidden by U3.

Medium length and transparent, apical (U5). This group comprises 5–6 medium tubules (0.47 ± 0.08 mm long \times 0.10 ± 0.02 mm wide, $n = 22$). Their morphology is quite constant; they are cylindrical and pointed at the apex (Figs 5, 10). Their secretion is transparent and homogeneous, but becomes translucent in Ringer's solution. It stains poorly with methylene blue. These tubules are situated to the right of the ejaculatory pouch, in the

apex of the utricular mass, and are spread perpendicularly to the seminal vesicles. They are surrounded by types U2, U7 and U12.

Short and translucent, apical left (U6). This group consists of 5–6 quite narrow tubules, but showing two categories of length. Half of them are long and relatively wide (0.60 ± 0.05 mm long \times 0.12 ± 0.02 mm wide, $n = 13$), whereas the others are shorter and thinner (0.41 ± 0.05 mm long \times 0.10 ± 0.01 mm wide, $n = 12$). All are cylindrical, with no constrictions, and apically rounded (Figs 6, 7, 10). The secretion shows small granules, and is translucent, although it becomes white in Ringer's solution. It stains intensely with methylene blue. These tubules are spread to the left in the apical part of the ejaculatory pouch, and are surrounded by types U1, U2, U3, U11 and U12.

Long and translucent, lateral right (U7). This group comprises some 25 long, thin tubules (0.73 ± 0.20 mm long \times 0.12 ± 0.03 mm wide, $n = 76$). They are sinuous and the apex is sharply pointed (Figs 4–7, 9–10). The secretion is translucent and homogeneous and stains intensely with methylene blue. The tubules are spread to the right of the ejaculatory pouch, close to the apical part, and are surrounded by types U5, U8, U9 and U12.

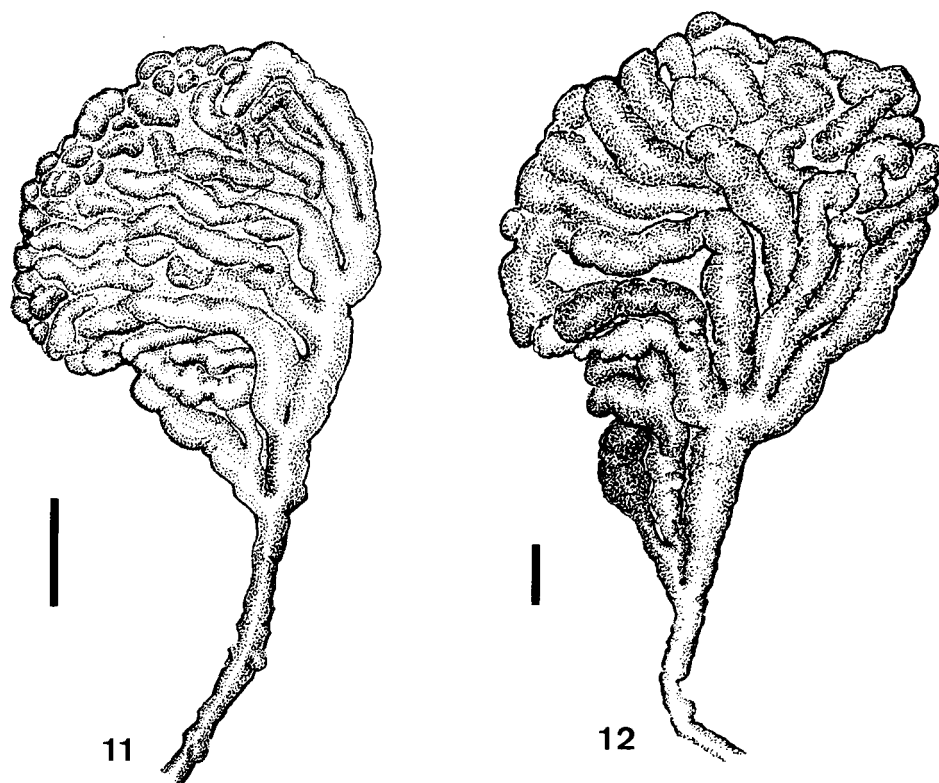
Long and semi-transparent, lateral right (U8). This group consists of some 35 long tubules (0.68 ± 0.17 mm long \times 0.11 ± 0.03 mm wide, $n = 97$). They are cylindrical and pointed at the apex (Figs 4–10). Some heterogeneity is observed in their secretion, which ranges from semi-transparent to translucent. There are no granules apparent and the secretion stains poorly with methylene blue. The tubules are spread to the right of the ejaculatory pouch, close to the apical part, and are surrounded by U7 and U9.

Medium length and transparent, lateral right (U9). This group is composed by 31–40 medium tubules (0.54 ± 0.11 mm long \times 0.10 ± 0.02 mm wide, $n = 107$). They are constant in their morphology. The diameter shows some constriction and they are rounded apically (Figs 4–9). The secretion is transparent and homogeneous and it stains intensely with methylene blue. The tubules are spread to the right of the ejaculatory pouch, close to the basal part, and are surrounded by types U3, U8, U10 and U11.

Short and translucent, lateral right (U10). This group consists of 23–31 thin tubules (0.45 ± 0.12 mm long \times 0.07 ± 0.02 mm wide, $n = 72$). They are constant in their morphology, cylindrical and rounded apically (Figs 4–5). The secretion is translucent and homogeneous and it stains poorly with methylene blue. They lie on the right side of the ejaculatory pouch, in the basal part, below the insertion point of the uricose glands. Close to them are types U9 and U7 to the right, and U5 in the apical part.

Short and translucent, ventrolateral left (U11). This group comprises 4 small tubules (0.34 ± 0.08 mm long \times 0.10 ± 0.02 mm wide, $n = 16$). They are cylindrical and apically rounded (Figs 5, 7, 10). The secretion is translucent and contains small granules; it stains poorly with methylene blue. The tubules connect with the left side of the ejaculatory pouch, close to the apical part and beside the seminal lobe. Close to them are types U3 to the left, and U12 in the apical part.

Short and transparent, apical (U12). This group is composed by some 8 small tubules (0.40 ± 0.11 mm long \times 0.10 ± 0.03 mm wide, $n = 25$). They are cylindrical and sharply pointed at the apex (Figs 5, 7, 10). The secretion is transparent and homogeneous, but becomes slightly translucent with Ringer's solution; it stains poorly with methylene blue. These tubules lie in the apical part of the ejaculatory pouch, just behind the seminal vesicles, and are surrounded by types U5, U6, U7 and U11.



Figs 11–12. Ventral view of the conglobate gland of *B. germanica* from a freshly emerged adult (11) and from a 10-day-old adult. Scale bars: 0.2 mm.

Conglobate gland

The conglobate gland is a triangular, rather flat structure formed by coiled tubules (Figs 11–12) which derive from a single ductule which opens separately into the genital pouch. Up to 50 ramifications can be counted from this single connecting ductule. The gland grows from adult emergence (1.10 ± 0.15 mm long and 0.87 ± 0.19 mm wide, $n = 26$, in freshly emerged males) to maturity (1.78 ± 0.15 mm long and 1.36 ± 0.13 mm wide, $n = 15$, in 10-day-old adult males), due to the accumulation of an opaque white secretion. Under methylene blue, the secretion accumulated in the connecting ductule and the first basal ramifications becomes intensely stained, whereas the stain is less apparent at the distal region of the tubules.

Protein patterns

In order to determine whether morphological diversity is reflected at the level of protein pattern, an electrophoretic analysis (SDS-PAGE) was performed on the entire ARG, the uricose glands, different types of utricles and the conglobate gland (Fig. 13). The pattern corresponding to the entire ARG shows 4 main bands (A to D in Fig. 13) close to 60, 45, 17 and 13 kDa of molecular weight, respectively, and a less apparent band close to 20 kDa

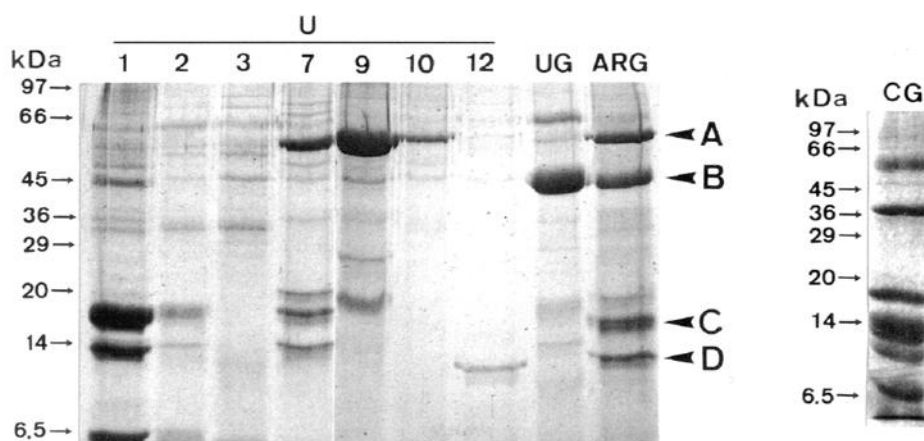


Fig. 13. Protein pattern (15% SDS-PAGE) of the accessory reproductive glands of male *B. germanica*. Lanes: ARG – whole glands (uricose glands + utricles + ejaculatory pouch) (0.2 gland equivalent loaded), CG – conglobate gland (1 gland equivalent loaded), UG – uricose gland (4 tubules loaded), U1 – utricule type 1 (10 tubules loaded), U2 – utricule type 2 (12 tubules loaded), U3 – utricule type 3 (80 tubules loaded), U7 – utricule type 7 (20 tubules loaded), U9 – utricule type 9 (40 tubules loaded), U10 – utricule type 10 (54 tubules loaded), U12 – utricule type 12 (82 tubules loaded). The arrows to the left indicate the molecular weight markers, and the four main bands of ARG, labelled A to D, are indicated to the right of the corresponding lane.

(not labelled in Fig. 13). Band B appears to correspond with the uricose glands, according to the pattern shown by the corresponding lane (UG, Fig. 13). With reference to the utricles studied (U1, U2, U3, U7, U9, U10 and U12), since a different number of tubules were loaded for each type, only a qualitative comparison is possible. A main band with a similar molecular weight to A appears in U9, and also in U7 and U10. Bands with a mobility similar to C and D appear in U1, and also in U2 and U7. However, the banding patterns differ in all the utricles studied, which confirms their peculiarity at the level of protein composition, in addition to morphological features. It is evident that the pattern of the conglobate gland (CG, Fig. 13) is different from that of ARG. Here, 6 main bands appear with molecular weights close to 50, 36, 18, 14, 10 and 7 kDa.

DISCUSSION

Morphological study of the ARG of *B. germanica* reveals a remarkable diversity of tubular structures. This diversity has been elucidated by several authors, although using different systems of nomenclature. Therefore, it will be useful to find correspondence between names used by previous authors and those used herein. The first author to describe the ARG of *B. germanica* was Brehm (1880), who distinguished two groups of tubules: the utriculi majores (which correspond to our uricose glands), and the utriculi breviores (our utricles). In 1937, Snodgrass described the tubules of the ARG by using colour and length features, and followed a nomenclature similar to that of Brehm (utriculi majores, and utriculi minores instead of breviores). Later, Khalifa (1950) renamed the utriculi majores as long tubules, whereas he distinguished two categories within the

utricles: “dorsal short tubules” and “intermediate tubules”. Roth & Dateo (1964) (see also Roth, 1967) were the first to recognize the occurrence of uric acid in the utriculi majores (Brehm, Snodgrass) or “long tubules” (Khalifa) and renamed them as uricose glands. These authors used the name utriculi breviores for the remaining tubules of the ARG.

The most complete description of the ARG of *B. germanica* is that of Ballan-Dufrançais (1968). In addition to identifying the utriculi majores, this author distinguished up to 7 types of tubule in the mass of utricles: dorsal odd right (which would correspond to our U1), median even (our U2), lateral even (our U3), anterior odd right (possibly our U5 or U12), posterior odd right (our U7 + U8 + U9) and ventral odd left and anterior odd left (possibly our U6, U11 or U12). Our types U4 and U10 have no clear correspondence with any described by Ballan-Dufrançais (1968).

The conglobate gland of *B. germanica* was described briefly by Bellés & Piulachs (1992). Morphological and cytological observations on *Periplaneta americana* were published by Beams et al. (1962) and Vijayalekshmi & Adiyodi (1973). Although the name conglobate gland is used currently, some authors (Happ, 1984, for example) use phallic gland as a synonym.

The morphological diversity of ARG tubules is paralleled at the level of protein pattern, as shown by electrophoretic studies. The general pattern of the ARG (with 4 main bands, A to D, corresponding to secretory proteins: Piulachs & Bellés, 1992; Vilaplana et al., in press) and conglobate gland (with 7 main bands also corresponding to secretory proteins: Vilaplana, Piulachs & Bellés, unpubl.) are clearly different. That of uricose glands is also typical, showing only one main band which is easily recognisable as band B of the whole pattern of the ARG. Due to technical constraints not all the types of utricles were included in our electrophoretic studies, but those used (U1, U2, U3, U7, U9, U10 and U12) showed a diversity of protein patterns, with differences not only quantitative but also qualitative in most cases.

Taken together, these results suggest a remarkable complexity of the ensemble of ARG, which can be explained if the complex roles played by the spermatophore they produce are taken into account. Studies conducted to define these roles, not only in connexion with sperm transfer, but also related to the energetic supply that it provides to the female, and also to its function in avoiding sperm competition, are now in progress. These studies will assist in the understanding of the fascinating complexity of these spermatophore-producing glands, from an evolutionary point of view.

ACKNOWLEDGEMENTS. Financial support from the DGICYT, Spain (project No PB92-0026: Studies on the reproductive biology in cockroaches) is gratefully acknowledged.

REFERENCES

- ADIYODI K.G. & ADIYODI R.G. 1975: Morphology and cytology of the accessory sex glands in invertebrates. *Int. J. Cytol.* **43**: 353–397.
- BALLAN-DUFRAÎÇAIS C. 1968: Données morphologiques et histologiques sur les glandes annexes mâles et le spermatophore au cours de la vie imaginale chez *Blattella germanica*. *Bull. Soc. Zool. Fr.* **93**: 401–421.
- BEAMS H.W., ANDERSON E. & KESSEL R. 1962: Electron microscope observations on the phallic (conglobate) gland of the cockroach, *Periplaneta americana*. *J. R. Microsc. Soc.* **81**: 85–89.

- BELLÉS X. & PIULACHS M.D. 1992: Juvenile hormone and maturation of male accessory reproductive glands in the cockroach *Blattella germanica* (L.). In Mauchamp B., Couillaud F. & Baehr J.C. (eds): *Insect Juvenile Hormone Research. Fundamental and Applied Aspects*. INRA, Paris, pp. 101–109.
- BREHM S. 1880: Recherches comparatives des organes génitaux de *Blatta germanica* et *Periplaneta orientalis*. *Horae Soc. Entomol. Rossicae* **11**: 177–181 (in Russian).
- CHEN P.S. 1984: The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annu. Rev. Entomol.* **29**: 233–255.
- GUILLOTT C. 1988: Arthropoda-Insecta. In Adiyodi K.G. & Adiyodi R.G. (eds): *Reproductive Biology of Invertebrates. Vol. 3*. Wiley, New York, pp. 319–471.
- GUILLOTT C. & FRIEDEL T. 1977: Fecundity-enhancing and receptivity-inhibiting substances produced by male insects: a review. In Adiyodi K.G. & Adiyodi R.G. (eds): *Advances in Invertebrate Reproduction. Vol 1*. Peralam-Kenoth, India, pp. 199–218.
- HAPP G.M. 1984: Structure and development of male accessory glands in insects. In King R.C. & Akai H. (eds): *Insect Ultrastructure. Vol 2*. Plenum, New York, pp. 365–395.
- HAPP G.M. 1992: Maturation of the male reproductive system and its endocrine regulation. *Annu. Rev. Entomol.* **37**: 303–320.
- KAULENAS M.S. 1992: *Insect Accessory Reproductive Structures*. Springer, Berlin, 224 pp.
- KHALIFA A. 1950: Spermatophore production in *Blattella germanica* L. (Orthoptera: Blattellidae). *Proc. R. Entomol. Soc. London* **25**: 53–61.
- LAEMMLI U.K. 1970: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685.
- LEOPOLD R.A. 1976: The role of male accessory glands in insect reproduction. *Annu. Rev. Entomol.* **21**: 199–221.
- MANN T. 1984: *Spermatophores. Development, Structure, Biochemical Attributes and Role in Transfer of Spermatozoa*. Springer, Berlin, 217 pp.
- PIULACHS M.D., MAESTRO J.L. & BELLÉS X. 1992: Juvenile hormone production and accessory reproductive gland development during sexual maturation of male *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *Comp. Biochem. Physiol. (A)* **102**: 477–480.
- ROTH L.M. 1967: Uricose glands in the accessory sex gland complex of male Blattaria. *Ann. Entomol. Soc. Am.* **60**: 1203–1211.
- ROTH L.M. & DATEO G.P., JR. 1964: Uric acid in the reproductive system of males of the cockroach *Blattella germanica*. *Science* **146**: 782–784.
- SNODGRASS R.E. 1937: The male genitalia of orthopteroid insects. *Smithson. Misc. Coll.* **96**: 1–107.
- VIJAYALEKSHMI V.R. & ADIYODI K.G. 1973: Accessory sex glands of male *Periplaneta americana* (L.): Part III – Histochemistry of the mushroom-shaped & conglobate glands. *Indian J. Exp. Biol.* **11**: 521–524.
- VILAPLANA L., PIULACHS M.D. & BELLÉS X. (in press): Inhibition of juvenile hormone during the formation of the spermatophore in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *Arch. Insect Biochem. Physiol.*

Received June 16, 1995; accepted October 27, 1995