phase contrast microscope. When successful, the preparation was stained by adding a drop of acetic-orcein to one side of the coverglass and allowing it to penetrate. Preparations were made permanent by the deep freezing method.

Five pairs of metacentric and submetacentric chromosomes are common in all preparations (figs 1 and 2). The most common type is that with 13 chromosomes (figs 1c and 2c), occurring in 5 out of the 9 larvae showing at least 10 metaphases, which allowed unambiguous counting and consistency of chromosome number within preparations. Additionally to the five common pairs this type has a pair of very small acrocentric chromosomes (No. 6 in fig. 2c) plus a single slightly larger acrocentric one (No. 8 in fig. 2c). Probably the same pair of very small acrocentric chromosomes (No. 6 in fig. 2) occurs also in one of the preparations showing 14 chromosomes (figs 1b and 2b) and in that showing 15 chromosomes (figs 1a and 2a). In addition, these two preparations, together with that exhibiting 12 chromosomes (figs 1e and 2e), seem to have another small pair of acrocentric chromosomes in common (No. 7 in fig. 2) which in size lies in between the two types of small acrocentric chromosomes of the common cell type exhibiting 13 chromosomes. The preparation with 15 chromosomes has the single chromosomes (No. 8 in fig. 2) in common with the preparation with 13 chromosomes.

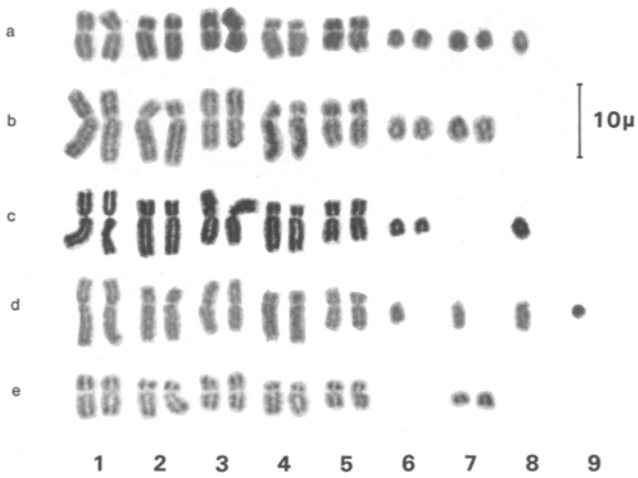

Figure 2. Karyograms of the different observed larval types. The five common pairs are numbered 1 to 5 and the different small acrocentric chromosomes are numbered 6 to 9.

One further deviating larva (figs 1d and 2d) has 14 chromosomes, but exhibits 4 single acrocentric chromosomes in addition to the five common pairs, three of them corresponding to the three small acrocentric types (Nos 6, 7, and 8 in fig. 2) seen in the other specimens, plus one which is even smaller (No. 9 in fig. 2).

The variation in chromosome number and type resembles that of B-chromosomes known from flowering plants and insects[2-4]. Due to the small sample size we cannot provide a picture of the variation in chromosome number and type on the population level at this moment, nor can we say anything on the segregation of the supernumerous chromosomes. The chromosomal pattern within local populations of the fly could be revealed if the sample size were increased.

Sex chromosomes have not been identified in the present study. In other Dipteran species females are often homogametic (XX) and males heterogametic (XY). With an X–Y sex determining mechanism the candidates for sex-chromosomes can be expected to be among the large submetacentric and metacentric chromosomes, here numbered 1 to 5. In all our preparations, however, only pairs of these chromosomes were found. A possible explanation of this observation could be that all of the 9 larvae studied were females. This is not very unlikely, as a very skewed sex-ratio is generally reported from field studies on *H. irritans*. For example, Bull[5] found only 2.84% males among 4704 individuals caught over the whole summer in north-east England and we found 10% males among 1402 flies from Denmark (unpubl. results). A further analysis of larval chromosomes could reveal the sex determining mechanism and thereby provide information on whether the observed skewed sex-ratios are representative for the *H. irritans* populations, and are not an artifact due to the sampling method, which could arise if only females approach vertebrates as they need protein for the development of the ovarioles.

Acknowledgments. We are grateful to Dr G. Thomas (Gronningen) for advice and sending larvae. Doth Andersen supported us by localizing metaphases, counting chromosomes and taking photos.

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0014-4754/87/040447-02$1.50 + 0.20/0

Biochemical and immunochemical evidence for the origin of the spermatophore material in *Glossina morsitans morsitans* Westwood

E. D. Kokwaro, B. M. Okot-Kotber, T. R. Odhiambo and J. K. Murithi

The International Centre of Insect Physiology and Ecology (ICIPE), P. O. Box 30772, Nairobi (Kenya), 24 July 1986

Summary. Protein patterns in secretions from fully differentiated male accessory reproductive glands (ARG), spermatophore (Sp) and testes (Te) of the tsetse, *Glossina morsitans morsitans* Westwood, were determined by isoelectrofocusing. Isoelectrofocusing patterns of total ARG proteins and those of Sp were remarkably similar. At least 27 bands were detected in ARG and Sp. Out of these, 13 were major protein bands and isoelectrofocused in the pI range of 4 and 6.55. About 10 of these 13 were found to be acidic. Ouchterlony immunodiffusion and straight line immunoelectrophoresis showed that male accessory reproductive gland secretory proteins and spermatophore share common immunological characteristics which are different from those of the testes.

Key words. Glossina morsitans; male accessory reproductive glands; spermatophore; testes; isoelectrofocusing; protein patterns; immunoelectrophoresis; double immunodiffusion.

In a number of insects, the spermatophore is a structure which serves as the vehicle for the transfer of sperms from male to female. Insect spermatophores seem to be elaborated from gelatinous protein secretions[1]. In the tsetse, *Glossina*, the spermatophore is directly placed within the female uterus. Once activated, sperms migrate through the opening in the spermatophore into the spermathecae, leaving behind the spermatophore wall. Recent ultrastructural study of the spermatophore and the significance of accessory reproductive gland material of *Glossina morsitans morsitans* Westwood showed similarities in the spermatophore and accessory reproductive gland secretions[2,3]. Histochemically, the outer and inner layers are carbohydrate and proteinaceous in nature. Biochemical studies of male accessory reproductive gland secretions and spermatophore of *G. morsitans* showed that spermatophore contains all the major proteins of accessory reproductive gland secretions[4]. The present study extended these findings and examined the immunological relationships of the tsetse accessory reproductive gland soluble components, spermatophore and testes.

Materials and methods. Tsetse, *Glossina morsitans morsitans* Westwood, were raised as previously described[2]. Adult male flies were used 7 days after emergence. Female flies used in mating for spermatophore formation were 3 days old. The accessory reproductive glands (ARG) and spermatophore (Sp) were carefully dissected out either in ice-cold distilled water or phosphate buffered saline (pH 7.2), and stored at −40 °C until used. When the required number had been obtained, they were thawed, pooled appropriately and 1 mM (final concentration) phenylmethsulphonyl fluoride added before vortexing for 1 or 2 min and centrifuging at 5000 rpm for 30 min at 0 °C to remove debris. The supernatants were then lyophilized and stored at −20 °C until used.

Immunization of rabbits. Secretions from ARG, Sp and extracts from testes (Te) were emulsified with an equal volume of Freund's complete adjuvant (FCA). These were injected into male rabbits i.m. in alternate thighs (0.2 ml/thigh) and also inoculated s.c. at multiple sites on the back. Doses for booster immunizations were similarly prepared in Freund's incomplete adjuvant (FIA), and inoculated into the rabbits as described above. Four booster immunizations were performed, the first three weeks after the primary dose, and thereafter at weekly intervals. While the immunizations were being carried out the rabbits were also bled from the marginal ear vein. Sera were separated, aliquoted and stored at −20 °C until required for experiments.

Isoelectrofocusing. Isoelectrofocusing of Te, Sp and ARG materials was performed in 1-mm-thick agarose-IEF plates with a broad range ampholyte, pH 3–10. The preparation of the gels was as described in Manufacturer's Manual (Pharmacia Fine Chemicals AB, Sweden). Briefly, 1-mm-thick 1% agarose-IEF gels containing sorbitol and pharmalyte, pH 3–10 were cast on to gelbond (114 × 225 mm). Electrode strips were soaked in 1 M NaOH (cathode) and 0.01 M H_2SO_4 (anode). Samples were applied by soaking pieces of filter paper (3 m) measuring about 3 × 5 mm in thawed extract and aligning them about 2 cm from the anode end. Running conditions were: Constant power of 10 W, maximum voltage of 1500 V and unlimited current. A volthour integrator VH-1 was used to standardize the running conditions. After the first 500 volthours (Vh), the runs were stopped and the applicators removed before resuming, until 1500 Vh had been achieved. After the runs, the gels were immediately fixed for 30 min in 5% sulphosalicylic acid and 10% trichloroacetic acid in distilled water. The gels were washed for 30 min in a

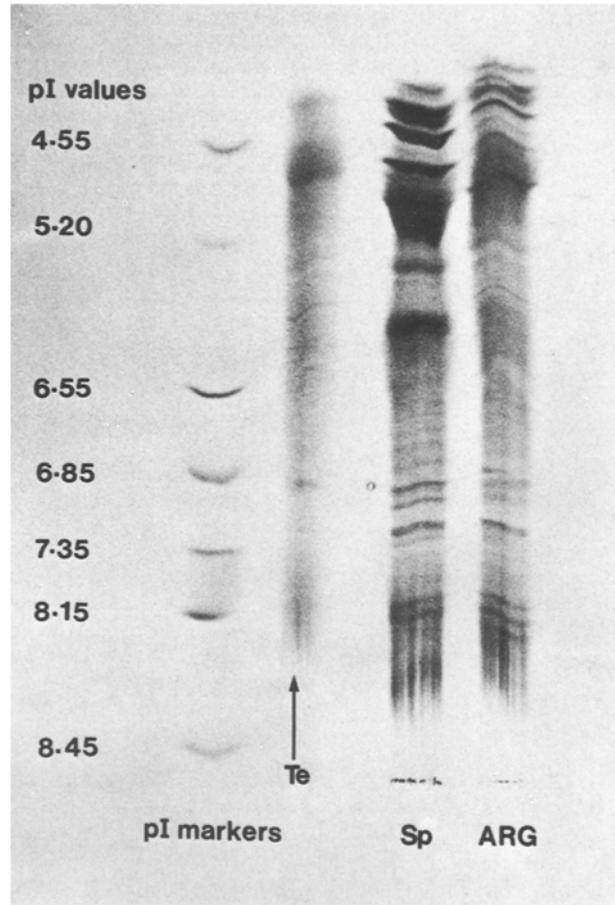

Figure 1. Isoelectrofocusing of general proteins from male accessory reproductive glands (ARG), spermatophore (Sp) and testes (Te) materials obtained from adult *Glossina morsitans*. Coomassie blue stain. Note that the Te extract gives rather weak bands rendering comparison difficult. Running conditions: Voltage: 1500 V; power: 10 W; current: Unlimited; volthour: 1500 Vh.

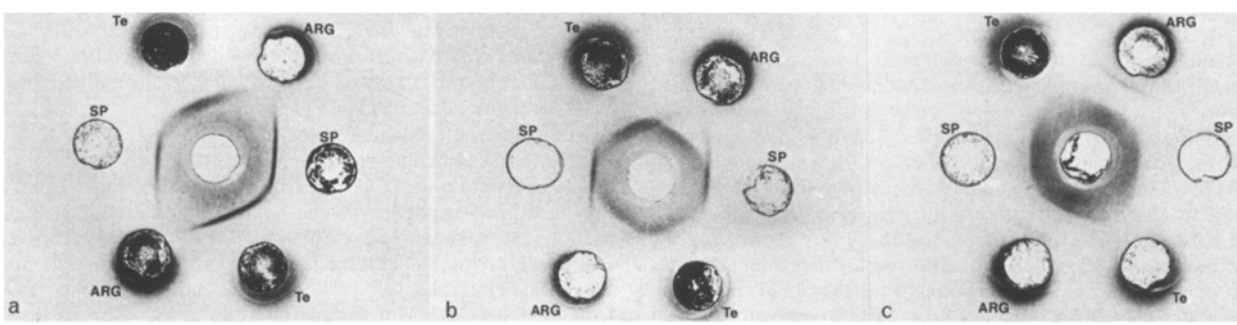

Figure 2. Ouchterlony immunodiffusion in agarose of immune rabbit sera (center wells) against antigens from male accessory reproductive glands (ARG), spermatophore (Sp) and testes (Te) of tsetse, *G. morsitans*. *a* center well contains anti-testes, *b* center well contains anti-spermatophore and *c* center well contains anti-accessory reproductive gland materials.

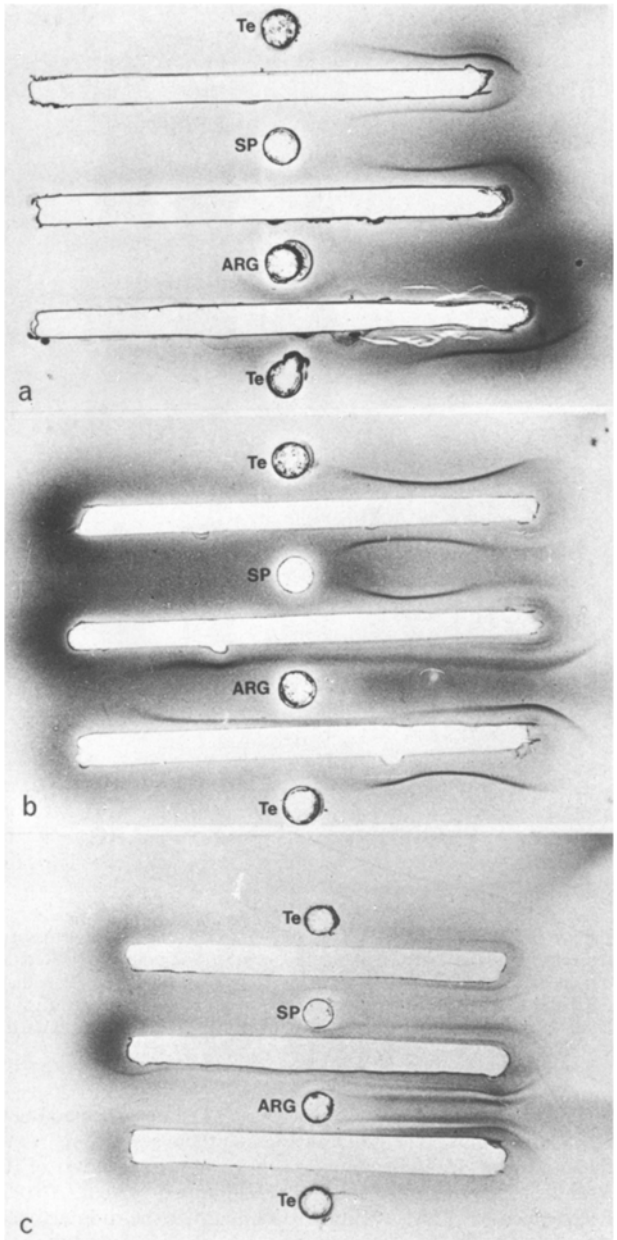

Figure 3. Immunoelectrophoretic analysis of antigens (circular wells) from (Te) testes, (Sp) spermatophore and (ARG) male accessory reproductive glands *a* against anti-Te, *b* against anti-Sp and *c* against anti-ARG. Running condition: Buffer pH 8.6 M 0.0065, current/plate 8 mA, duration 2 h, Coomassie blue stain.

destaining solution of 5 parts methanol, 1 part acetic acid in 5 parts distilled water. The gel was dried and stained as outlined above.

Double immunodiffusion. Immunodiffusion tests were conducted in 1% agarose using the method of Ouchterlony[5]. The ARG, Sp and (Te) materials inoculated into the peripheral wells were used as test antigens. The antisera was introduced into the central well and diffusion allowed to proceed for 18–24 h at room temperature in a humid chamber. After this period the immunoplates were washed in several changes of saline for over 12 h and dried. The plates were then stained with 0.25% Coomassie Brilliant Blue RR 250 prepared in 10% acetic acid and 50% methanol. Finally, the plates were destained in several changes of the destaining solution until the background was clear.

Immunoelectrophoresis. Immunoelectrophoresis plates, 8 × 14 cm, were layered with 1% molten agarose solution prepared in 0.0065 M barbital/HCl buffer, pH 8.6. The gels after solidifying were then used to electrophorese Te, Sp and ARG materials at 150 V with cooling for about 2 h. Each time, 3 plates were run, one for testing each of the antisera raised. Antisera against Te, Sp and ARG were added to the troughs cut in the plates and incubated at room temperature in a moist chamber for 24 h or until precipitation arcs were formed. The plates were then treated and stained as described for the double immunodiffusion experiment.

Results. Isoelectrofocusing of readily extractable material from ARG and Sp showed close similarity in their protein profiles (fig. 1). At least 27 protein bands were discernible from each profile and almost all the bands obtained from ARG corresponded to those of Sp. The bulk of these bands focused in the acidic zone (between PI 4 and 6.55) suggesting that these proteins are basically acidic in their molecular structure. About 13 major bands were detectable and most of them (about 10 bands) were found in the acidic region.

Repeated extracts and runs showed that the number and relative quantities of the bands were consistent. Testes extracts gave only weakly stainable protein bands rendering comparison difficult (fig. 1).

By double immunodiffusion, antiserum raised against extractable material from testes recognized materials from the testes and spermatophore, and hardly any material from ARG (fig. 2a). Anti-Te antiserum detected also some materials from the testes which is not found in the spermatophore. Anti-Sp antiserum recognized materials from Sp, ARG and Te (fig. 2b). The precipitation lines between Sp and Te joined in a smooth curve and differed from that existing between Sp and ARG which demonstrates a spur. Anti-ARG antiserum recognized, exclusively, antigenic materials from ARG and Sp, whereby the precipitin lines joined smoothly (fig. 2c). However, an additional precipitation line was formed only with material from ARG.

The same extracts were subjected to immunoelectrophoresis and tested against various antisera. Using anti-testes sera, defined precipitin lines were observed with materials from Te and Sp and no precipitation lines with ARG (fig. 3a). Anti-Sp antiserum could detect materials from Te but more strongly those from Sp and ARG with more precipitation lines observed here (fig. 3b). On the other hand, anti-ARG antiserum recognized only material from ARG and Sp and hardly detected any material from Te (fig. 3c).

Discussion. Isoelectrofocusing of extracted soluble proteins from Sp and ARG of *G. morsitans* has supported an earlier finding that Sp and ARG have a number of proteins with common physical properties. Samaranayaka-Ramasamy[4] showed that six major protein extracts of Sp could be resolved by SDS – polyacrylamide gel electrophoresis and these had the same molecular weights as those of ARG.

By isoelectrofocusing we were able to detect about 13 major bands in both Sp and ARG extracts. It is likely that more bands could have been detected if the extraction procedure involved harsher treatment of tissue e. g. through homogenization. We avoided this since we were more interested in the secretion (glandular lumen content), which is transferrable during mating, than in whole tissue extracts.

Immunochemical procedures showed similarities between Te and Sp, on the one hand, and Sp and ARG on the other. It is however apparent that the similarities detected between Te and Sp could have resulted from the obvious common contents of these tissues, namely the sperm, which could not be removed easily without loss of the extractable components. This could not be verified since clean sperm mass could not be obtained free from contamination by the surrounding tissue. Nevertheless, anti-testes serum could only detect materials from Te and Sp but not from ARG. This suggests that Te shares some common antigens with Sp but not with ARG. Since it is known that

sperms are not found in ARG, this could suggest that the precipitation arcs formed with Te and Sp immunogens could have been of sperm origin. Anti-Te serum, however, detected an additional arc which could be attributed solely to testicular contents. Anti-Sp and anti-ARG sera gave stronger and broader precipitation arcs with Sp and ARG and the type of precipitation lines were similar irrespective of the antiserum used, suggesting the presence of a sizeable number of common components in the two extracts. It is likely, therefore, that the material which goes into making the spermatophore originates in the ARG, as the ultrastructural studies of these two tissues showed common structural elements[2]. It is also not surprising that materials obtained from Sp and ARG show similar biochemical and immunological patterns. Recent studies on *Tenebrio molitor* L. extracts from Sp and ARG[6] have shown that they are immunologically similar, an observation which would further support the findings discussed in these investigations.

Acknowledgments. The authors thank Drs G. Happ and B.C. Njau for useful comments on the manuscript. The technical assistance of Mr P. Lisamula is acknowledged. The study received generous financial support from UNDP.

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0014-4754/87/040448-04$1.50 + 0.20/0

Sublethal virus infection depresses cytochrome P-450 in an insect

L. B. Brattsten[1]

E.I. Du Pont de Nemours & Co. Inc., Experimental Station, Bldg 402, Agricultural Products Department, Wilmington (Delaware 19898, USA), 20 May 1985

Summary. Insect-specific cytoplasmic polyhedrosis virus infections, endemic in many species of insects, cause the gut tissue to assume on opaque, milky-white appearance through virus multiplication and formation of polyhedral protein inclusion bodies. Electron microscopy shows that the endoplasmic reticulum membrane is severely reduced and fragmented in infected midgut cells. Metabolism of foreign, lipophilic compounds, catalyzed by the membrane-bound cytochrome P-450, is significantly depressed, and resistance to insecticides disappears. In the absence of toxicants, most insects in this condition survive with somewhat impaired fitness.
Key words. Insect cytoplasmic polyhedrosis virus; insecticide resistance, cytochrome P-450; *Heliothis virescens; Manduca sexta.*

Insecticide toxicity testing and biochemical toxicology studies depend on a supply of insects that are healthy, of uniform instar and age within the instar, and have constant and known exposure to temperature, relative humidity, crowding, and nutrients. It is also important to know the natural feeding habits of the species and the prehistory of insecticide exposure of the population. Insecticide toxicity is often directly related to the activities of several foreign compound-metabolizing enzymes including cytochrome P-450, glutathione transferases, and carboxylesterases, all of which are sensitive to the physiological condition of the insects and external factors to which they are exposed[2–4].

A laboratory colony of the tobacco budworm, *Heliothis virescens* (Fabr.) was established from individuals collected in 1980 from an insecticide-resistant population in Texas. The colony was reared at constant temperature, 26 °C, relative humidity, 40–60%, and photoperiod, 16/8 h of light/darkness; the larvae were fed an agar-wheatgerm-soy flour diet, containing chloramphenicol, sorbic acid and methyl paraben as preservatives[5]. During the course of a study undertaken to characterize the stable resistance mechanism(s) in the colony, abnormal, milky-looking guts were observed with increasing frequency. This observation coincided with a marked decrease in specific cytochrome P-450 content in the larval midguts (table 1). The sudden decrease in cytochrome P-450 content between October 3 and October 18 could not be attributed to experimental error, any changes in the nutritional or physical rearing conditions of the colony, or with its obvious developmental or reproductive behavior.

Cytochrome P-450 was quantified as the reduced carbon monoxide difference spectrum[6]. NADPH cytochrome c (P-450) reductase activity was measured with cytochrome c as substrate[7]. N-Demethylation of p-chloro N-methylaniline and epoxidation of aldrin were measured as previously described[8,9]. Glutathione transferase activity was measured with 1-chloro-2,4-dinitrobenzene as substrate[10]. Microsomal and soluble esterase activities were measured with l-naphthyl acetate as substrate[11]. Microsomes containing the cytochrome P-450 and associated activities and membrane-bound esterases and post-microsomal supernatants containing glutathione transferase and soluble esterase activities were prepared from midgut tissues as described earlier[12].

There was also an increase in toxicity of the carbamate insecticide methomyl; spraying third instar larvae, placed in petri dishes, with an acetone/water solution (75/25% v/v) containing known concentrations of the insecticide yielded the following data: in May, 1983 the 48-h LD_{50} was 412 ppm and in March of 1984 it was 119 ppm.

Table 1. Specific cytochrome P-450 content in midgut microsomes from 3-day-old fifth instar tobacco budworm larvae

Date of experiment	Cytochrome P-450 (nmole/mg protein)
August 12	0.401
August 15	0.531
September 15	0.470
October 3	0.643
October 18	0.148
October 24	0.154
October 25	0.208
October 27[1]	0.236
October 28	0.159
November 15	0.165
November 17	0.127
December 28[2]	0.060

The data are averages of duplicate determinations. The experiments were done in 1983. [1] 35% of the guts were milky; [2] 95% of the guts were milky.