Variation in chromosome number in the sheep headfly *Hydrotaea irritans* (Fallen) (Diptera: Muscidae)

V. Loeschcke and B. Christensen

Institute of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus C (Denmark), and Institute of Population Biology, University of Copenhagen, DK-2100 Copenhagen Ø (Denmark), 7 July 1986

Summary. Variation in the number of chromosomes was found in sheep headflies, ranging from 12 to 15 chromosomes. Of these, five pairs were common among all individuals. Among 9 larvae no males were identified.
Key words. Hydrotaea irritans; Muscidae; B-chromosomes; karyograms; sex ratio.

Hydrotaea irritans is ubiquitous in northern Europe. The fly visits animals to obtain proteinaceous food. It has been associated with the transmission of a harmful disease in cattle, summer mastitis.

Our interest in chromosomes of the sheep headfly arose from a study on the genetic differentiation among local populations. An analysis of the isozyme pattern in different Danish forest and pasture populations of *Hydrotaea irritans* and related species by starch-gel electrophoresis (Loeschcke et al., unpubl. results) revealed a peculiar banding pattern at four enzyme loci. The occurrence of certain heterozygous bands at one of these loci was linked to the occurrence of certain other heterozygous bands at the three other loci. Corresponding homozygotes of one type were never observed at any of the loci despite rather high frequencies of the 'heterotypes' in some local populations.

For the chromosome preparations third instar larvae were used, coming from hand netted flies caught around cattle during one day in the Staphorst area in the Netherlands[1]. Besides morphological characters an enzyme banding pattern revealed by starch-gel electrophoresis was used to ascertain the species determination (Loeschcke et al., in prep.). Larvae were kept at 15 °C in moist soil and fed with *Drosophila melanogaster* larvae. At an age between 6 and 8 weeks the larval neural ganglia were dissected out after decapitation and placed in a watch glass containing isotonic saline with 0.01 % colchemid for two hours. A hypotonic treatment was achieved by diluting with an equal amount of distilled water for the last 20 min. Each brain was then transferred to a small drop of 45% acetic acid on a slide for fixation and subsequent maceration. A coverglass was placed in position, and a slight pressure applied, before inspection with a

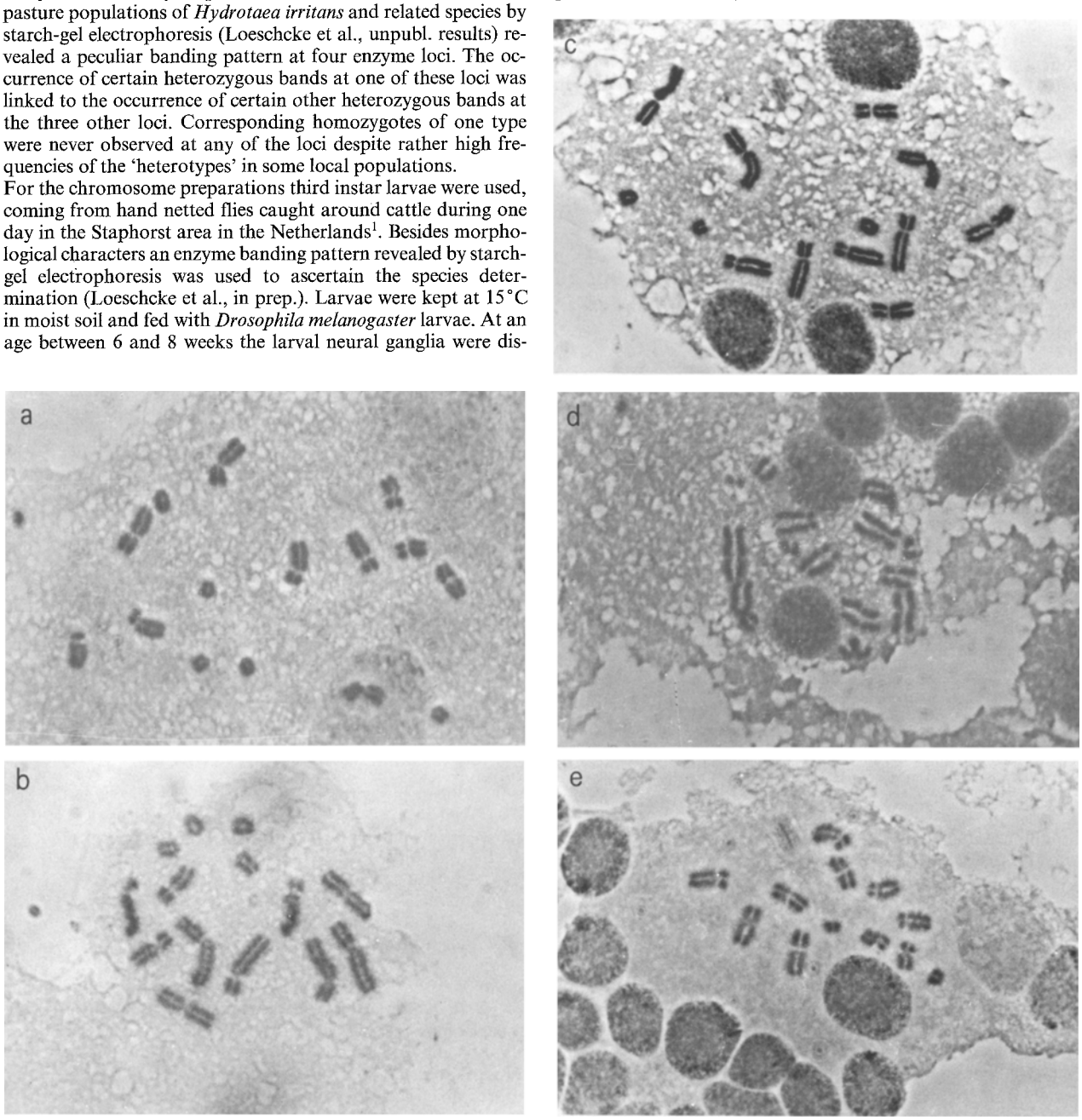

Figure 1. Metaphases exhibiting 15(a), 14(b), 13(c), 14(d), and 12(e) chromosomes.

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.

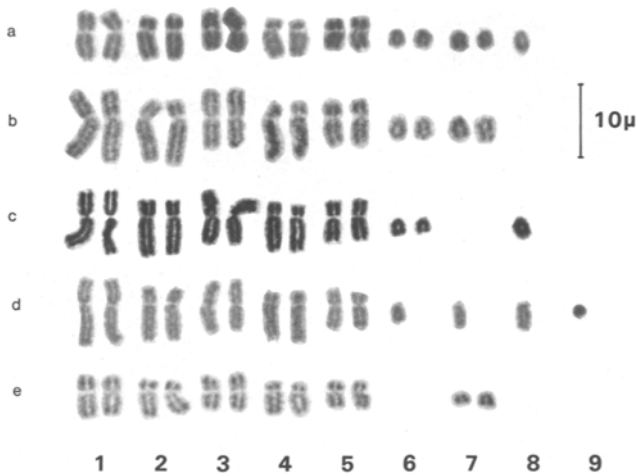

Figure 2. Karyograms of the different observed larval types. The five common pairs are numbered 1 to 5 and the different small acrocentric chromosomes are numbered 6 to 9.

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.

1 Flies and larvae were provided by Dr G. Thomas, Department of Animal Physiology, University of Gronningen, The Netherlands.
2 Rees, H., Sci. Prog., Oxford *61* (1974) 535.
3 Jones, R. N., Int. Rev. Cyt. *40* (1975) 1.
4 Jones, R. N., and Rees, H., B-Chromosomes. Academic Press, New York 1982.
5 Bull, S. G., Ecol. Ent. *9* (1984) 1.

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.