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Genetics of tsetse fly, (part of a coordinated programme on sterile insect techniques for tsetse fly control or eradication)

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AUTHOR(S)

W. Helle

INSTITUTE

University of Amsterdam
Laboratory of Entomology
Amsterdam The Netherlands

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Institute	Laboratory of Experimental Entomology, University of Amsterdam, the Netherlands.
Chief scientific investigator	Dr. W. Helle
Scientist	Dr. L.P.S. van der Geest
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Introduction

Knowledge about the basic genetics of the tsetse fly and about genetic variation in tsetse fly populations is still very scarce. This is mainly due to the unattractiveness of these insect species for genetical studies, because of its rearing difficulties especially before 1960, and its low reproduction capacity.
The investigation reported here, was started in order to collect information about genetic variation in tsetse fly populations. It also aimed at the comparative examination of populations of tsetse flies, especially of Glossina m. morsitans by the analysis of genetic variation. This was done by studying the isoenzyme polymorphism of populations.
A large number of enzyme systems were studied and their genetic isoenzyme polymorphism was determined. As it was intended to show that the method could be used for field collected material, several factors which may affect the analyses were studied, such as age of the flies, pregnancy, presence of blood meals, starvation, storage at low temperatures, etc.

Material and Methods

For the experiments several different strains of G. m. morsitans were at our disposal: laboratory colonies from Maisons-Alfort, France; Langford, United Kingdom and Tanga, Tanzania, a field population from Rhodesia; a colour mutant (ocra) and an inbred line ("O"-line), both originating from the Maisons-Alfort colony. In addition, G. palpalis colonies from Vienna and Antwerp have been analysed for a few enzyme systems.

Most of the enzyme analyses were carried out on flies of the Maisons-Alfort colony. In a few instances, the other colonies have been used for comparison.

For the iso-enzyme determinations, horizontal starch gel electrophoresis was used, carried out on plates which were cooled at 0° C. For details is referred to Van der Geest and Kawooya (1975) and to Van der Geest et al. (in prep.). The following enzyme systems were included in the study: malic enzyme (me), leucine amino peptidase (lap), alkaline phosphatase (alph), xanthine dehydrogenase (xdh), lactate dehydrogenase (ldh), malate dehydrogenase (mdh), esterases (est), adenylate kinase (ak), catalase (cat), glucose-6-phosphate dehydrogenase (g-6-pd), isocitrate dehydrogenase (idh), phosphogluco isomerase (pgi), peroxidase (po), aldehyde oxidase (ao), and α-glycerophosphate dehydrogenase (α-gpd).

The genetic interpretation of observed enzyme polymorphism was in most instances merely based on the agreement of frequency of occurrence of the iso-enzyme variants with the Hardy-Weinberg equilibrium. Double bands on the zymograms were usually considered to be derived from heterozygous individuals, while single bands were assumed to belong to homozygotes. For lap-isozymes the mode of inheritance could be demonstrated by crossing experiments. Females of the "O"-line, homozygous for 1.00 lap_3 allele, were crossed

with males of the Maisons-Alfort colony. The fathers and the offspring were subsequently analysed for their genotype.

Results

Most of the results have been (Van der Geest and Kawooya 1975) or will be (Van der Geest et al. in prep.) published. For detailed information is therefore refered to these publications. Genetic polymorphism was observed for the following enzyme systems: Leucine amino peptidases, esterases, aldehyde oxidase, α-glycerophosphate dehydrogenase, malic enzyme, alkaline phosphatase and octanol dehydrogenase. For the other enzymes no genetic polymorphism could be detected. Three distinct zones on the zymograms possessed lap activity. The fastest zone, lap_1, shows very little activity and is usually not visible on the zymograms. The zone with medium mobility contains one band which doubles in all individuals, a few days after emergence. The slowest zone, lap_3, which is encoded by a single locus, is very polymorph: four bands with different mobilities have been observed, which occurs single in homozygous individuals and as double bands in heterozygotes. Frequency of occurrence of the different genotypes was in good agreement with expected values calculated with the aid of the Hardy-Weinberg equilibrium. The inheritance was studied by analysing the lap_3-genotype of the offspring of females of the "0"-line (homozygous for 1.00 lap_3) and males of the Maisons-Alfort stock. Females crossed with 1.00/1.00 males produced only 1.00/1.00 offspring, but when males with 1.00/1.05 males, gave rise to 1.00/1.05 and 1.00/1.00 pupae in a 1:1 ratio.
One female was crossed with a 1.05/1.05 male and produced only 1.00/1.05 offspring.
Malic enzyme, aldehyde oxidase and α-glycerophosphate dehydrogenase also show genetic polymorphism and each is encoded by a single locus. For me, 4 bands, for ao,

3 bands and for α-gpd, 2 bands were observed on the zymograms, occurring single in homozygous, and double in heterozygous flies. The frequency of occurrence of the genotypes was in agreement with the Hardy-Weinberg equilibrium.

Octanol dehydrogenase is a rather problematic enzyme system. Genetic polymorphism was found to occur, but the frequency of occurence of the different variants was not in agreement with the Hardy-Weinberg equilibrium. The zymograms of this enzyme system, however, tend to be rather vague and bands are not always discernable. The discrepancy of the observed frequency of occurring genotypes with expected values can very well be attributed to a wrong interpretation of some of the zymograms.

Alkaline phosphatase is also encoded by a single locus. Variation at this locus is very low (less than 1%).

Esterases are encoded by probably as many as six different loci. It was possible to charaterize the esterase bands on the zymograms chemically by the addition of specific inhibitors to the incubation media, by testing the temperature sensitivity and by studying the ability to hydrolyse specific substrates. For additional details concerning these procedures is referred to Van der Geest et al. (in prep.).

Est_1, the esterase with the highest electrophoretic mobility on the zymograms, belongs to the group of the arylesterases, just like est_2, but these two differ from each other with respect to their ability to hydrolyse naphthol-AS-acetate. Est_3 can probably be considered as an acetylesterase while est_4, est_5 and est_6 are clearly choline esterases (inhibition by organophosphates, eserine and heavy metals; low mobility).

No variation was observed for est_6, but for all other esterases variation occurs. Based on the frequency of occurrence of the different esterase genotypes, we must assume that for est_1, est_2, est_4 and est_5, null alleles

are involved, as otherwise no good explanation for observed frequencies of genotypes can be given. Est$_3$ was observed in only a few instances: insufficient data are obtained for this esterase to allow an evaluation of its mode of inheritance.
Five different populations were compared with respect to their variation at the lap$_3$- and alph-locus. For none of the populations, variation was observed for alph. The variation at this locus, mentioned in Van der Geest and Kawooya (1975), was for the Maisons-Alfort colony far less than 1%. Table 1 gives the variation at the lap$_3$-locus for the five populations.

TABLE 1

genotype	colony				
	ocra	Rhod.	M.-Al.	Langf.	Tanga
0.90/0.90	0.08	0.005	0.002	0.02	-
0.95/0.95	-	-	-	-	-
1.00/1.00	0.50	0.88	0.87	0.89	0.99
1.05/1.05	0.01	0.01	0.004	0.02	-
0.90/0.95	0.005	-	-	-	-
0.90/1.00	0.39	0.08	0.125	0.06	0.01
0.95/1.00	-	-	-	0.01	-
1.00/1.05	0.02	0.02	0.002	0.01	-

Frequencies of genotypes of variants at the lap$_3$-locus in five different populations of Glossina m. morsitans. The most common allele is designated as 1.00, the others are assigned arbitrary values, indicative of their respective mobilities.

The differencies between the Rhodesia and Tanga populations are very conspicuous. Both populations were recently collected in the field: the Tanga population has been kept shortly in the laboratory while the investigated Rhodesian sample consisted of field collected material, originating from the site were the

Maisons-Alfort colony was collected in 1965. This latter colony is very similar to the Rhodesian sample, despite its long history of laboratory breeding. The high degree of heterozygosity of the ocra colony is also striking. The difference between this colony and the Maisons-Alfort colony is not surprising: the ocra strain started as a single individual of the Maisons-Alfort colony. For the effect of factors such as age, blood meals and pregnancy is referred to Van der Geest and Kawooya (1975).

The Vienna and Antwerp colonies of Glossina palpalis were analysed for lap, and alph isozymes. Both colonies proved to be highly homozygous and showed only a low degree of variation for lap (in the order of 1%). No differencies were observed between both populations

Conclusions

The analysis of isoenzyme polymorphism is a valuable tool for the comparative examination of tsetse fly populations. Although a large number of enzyme systems yield reliable and reproducible zymograms, most results should be expected from the analyses of lap, ao, me and α-gpd isozymes. Esterases, although polymorph, produce often complicated zymograms and seem to be less suitable for such studies.

A further extension of the work to more enzyme systems may give additional suitable enzyme systems with a certain polymorph character. However, a species may show a high degree of geographic uniformity in both allele frequencies and level of genic heterozygosity. This is apparently not the case with the leucine amino peptidase isoenzymes, which are clearly different in some of the populations analysed in the present study. Several factors, such as pregnancy, presence of blood meals, age of the flies may have a negative effect on the quality of the zymograms. For this reason it is advisable, to collect pupae and to analyse the emerged flies at at least 24 hours after their first blood meals. Flies collected in the field can also be analysed, even after prolonged storage in liquid nitrogen, but may yield less reliable results due to e.g. pregnancy and the presence of - also unknown -

blood.

For sterile male technique projects, it seems opportune to determine the isoenzyme polymorphism of the colonies, which are to be released, and to compare this polymorphism with the variation of the population in the target area. In case of differences between these populations, it is wise to add more field material in the laboratory colony, or, rather, to start a new laboratory colony from the field population.

Publications

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