TSETSE GENETICS: A REVIEW

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ABSTRACT

About 140 papers were reviewed and the following aspects of tsetse genetics discussed: cytogenetics, sex determination, visible traits, biochemical and molecular genetics, vectoring ability, behavioural genetics, linkage groups, population genetics, genetic aspects of radiation and chemosterilants, and genetic aspects of reproductive strategies (such as multiple matings, sperm precedence, interspecific mating and hybridization). Genetic information is most extensive for Glossina morsitans morsitans *Westwood and substantial amounts of information exist for other members of the* morsitans *group and for members of the* palpalis *group. Information on genetics of members of the* fusca *group is restricted to cytological observations on two species. Of three species groups recognized on the basis of structural and ecological features, two are supported by available genetic information. Genetic data are insufficient to determine if the* fusca *group can be defined on the basis of such features.*

RÉSUMÉ

L'auteur passe en revue environ 140 articles donnant des informations sur la génétique des mouches tsétsés et discute des aspects suivants: la cytogénétique, la détermination du sexe, les traits visibles, la génétique moléculaire et biochimique, la capacité vectorielle, la génétique du comportement, les groupes de liaison, la génétique des populations, les aspects génétiques de l'irradiation et des chimiostérilisants, et les aspects génétiques des stratégies reproductrices (comme l'accouplement multiple, la préséance du sperme, l'accouplement interspécifique et l'hybridation). Glossina morsitans morsitans *est l'espèce dont la génétique est la mieux connue, mais il existe aussi des informations substantielles concernant les autres membres du groupe* morsitans *et ceux du groupe* palpalis. *Concernant le groupe* fusca, *il n'existe que quelques observations cytologiques sur deux espèces. Les données génétiques disponibles supportent l'établissement de deux des trois groupes d'espèces définis à partir de traits morphologiques et écologiques. Les données sont insuffisantes pour établir si le groupe* fusca *peut aussi être défini à partir de tels traits génétiques.*

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INTRODUCTION

There are about 30 species and subspecies of tsetse flies (Jordan 1977; Potts 1973), all of which belong to the genus *Glossina* Wiedemann. Within the genus species are placed in three species groups or subgenera (Newstead *et al.*. 1924; Glasgow 1970; Potts 1973; Jordan 1977): subgenus *Austenina* Townsend = *fusca* group (14 taxa); subgenus *Nemorhina* Robineau-Desvoidy = *palpalis* group (9 taxa); subgenus *Glossina s. str.* = *morsitans* group (7 taxa). These divisions are based largely upon structure.

Males and females of all tsetse species are haematophagous and feed fairly often. Females of most species mate when fairly young and apparently can store viable sperm for the rest of their lives. A single egg is matured at a time and fertilization is internal. At the end of embryogenesis the first instar larva hatches and it and the subsequent two instars are nourished by secretions from the "milk glands" of the female. When a female is approximately 16 days old she deposits her first offspring. Ovulation takes place shortly after larviposition and females are able to produce a larva about every nine days. Although females are long lived, only a modest number of offspring (probably no more than 6 or 8 in a well managed colony and considerably fewer under field conditions) are produced per female. Tsetse flies contain symbiotic bacteroids in a mycetome located in the anterior part of the midgut, as well as rickettsia-like organisms demonstrable in the nurse cells and developing oocyte. These symbionts undoubtably play a role in the reproduction of the fly and may have the potential to influence the inheritance of certain traits. (For recent reviews of tsetse physiology see Langley (1977) and Tobe and Langley (1978).)

Tsetse flies are confined to Africa between 5°N latitude and 20°S latitude (Potts 1973) and their importance as vectors of African trypanosomiases is well known. Largely because of their vectoring capacity, tsetses have been intensively studied and there exists a large body of information on their biology, physiology, ecology and medical/veterinary importance. However, for a variety of reasons (including their low rate of reproduction, and until fairly recently, difficulties in maintaining colonies), comparatively little work has been done on their genetics. For example, as late as 1963 intra-taxon variations known to occur in nature were all attributed, in whole or in large part, to environmental effects (Glasgow 1963).

With the current interest in non-chemical control of pests, the situation has been redressed somewhat, and research on tsetse genetics is proceeding in several laboratories. I therefore feel that this is an opportune moment to summarize the literature on tsetse genetics, to evaluate its contribution to our understanding of these flies, and to indicate areas where further work is needed.

CYTOGENETICS

Cytogenetic studies of tsetse flies began with the (unpublished) demonstration by Slizynski (cited by Vanderplank 1948) that *G. m. centralis* has 3 pairs of chromosomes. Since then karyotypes have been determined for about half the taxa, meiosis has been described in detail for both males and females of several taxa, and detailed comparisons of some taxa using Giemsa C-banding and polytene chromosomes have been carried out. In the present section I

Table 1. Chromosome formulae for *Glossina* species.

Species	Formula[1] (2n=)[2]	Refs.[3]
Group I		
fusca congolensis Newstead & Evans	18L +2M_{10} +(XX/XY)	A B C
brevipalpis Newstead	16	D
Group II		
palpalis palpalis (Robineau-Desvoidy)	2L_1 +2L_2 +(XX/XY)	E F G H
palpalis gambiensis Vanderplank	2L_1 +2L_2 +(XX/XY)	H I
tachinoides Westwood	2L_1 +2L_2 +(XX/XY)	B H J K L
fuscipes fuscipes Newstead	2L_1 +2L_2 +(XX/XY)	B D H K L M N
Group III		
longipalpis Wiedemann	4L +2M +2Sh(=2S?)	E
morsitans morsitans Westwood	2L_1 +2L_2 +(XX/XY) +2-7S	B H J K L O P Q R
morsitans submorsitans Newstead	2L_1 +2L_2 +(XX/XY) +2-7S	H Q
morsitans centralis Machado	2L_1 +2L_2 +(XX/XY) +1-3S	D H Q
pallidipes Austen	2L_1 +2L_2 +(XX/XY) +0-2S	D H O S T
austeni Newstead	2L_1 +2L_2 +(XX/XY) +8-12S	B K L O P
swynnertoni Austen	8	D H Q U

[1] As far as possible the most recent terminology is used: A=autosome; L, L_1, L_2=long autosomes; M=medium autosome; Sh=short autosome; S=supernumerary or B chromosomes.

[2] Aneuploidy and polymorphisms are discussed in text sections on sex determination and on population genetics.

[3] References: A=Itard 1971b; B=Itard 1973b; C=Itard 1971c; D=Maudlin 1970; E=Baldry 1970; F=Maudlin 1979; G=Riordan 1968; H=Southern 1980; I=Itard 1974; J=Itard 1966; K=Itard 1970a; L=Itard 1971a; M=Maudlin 1968; N=Pell and Southern 1976; 0=Amos and Dover 1981; P=Craig-Cameron *et al.* 1973b; Q=Pell *et al.* 1973; R=Southern *et al.* 1972b; S=Hulley 1968; T=Southern and Pell 1981; U=Southern *et al.* 1972a.

shall summarize much of this information but the reader is referred, for additional details, to two other reviews of cytogenetics (Itard 1973b; Southern 1980).

Chromosome number

Of the 30 taxa in the genus *Glossina*, chromosome formulae have been determined for 13 (two from the *fusca* group, four from the *palpalis* group, and seven from the *morsitans* group). This subject has been reviewed twice (Itard 1973b; Southern 1980) but is presented here briefly (Table 1) for the sake of completeness. Where sex chromosomes have been identified, females are homogametic (XX) and males are heterogametic (XY). Members of the *fusca* group have the largest number of chromosomes. The simplest chromosome formula (2n = 4 autosomes + [XX or XY]) occurs among members of the *palpalis* group. Flies of the *morsitans* group are characterized by having, in addition to the basic complement of chromosomes found in the *palpalis* group, a variable number of small, univalent supernumerary (=B) chromosomes which lack (at least during male meiosis) pairing mates. Although there is an obvious need to obtain additional information on the chromosome number in more taxa (notably those of the *fusca* group), the pattern, with regard to chromosome numbers, which has thus far emerged is consistent with the generally accepted arrangement of the species.

Chromosome structure

In *G. f. fuscipes* the sex chromosomes and four other pairs are metacentric while six pairs are acrocentric (Itard 1971c). Within the *palpalis* and *morsitans* groups three of the chromosomes are similar in form. The longest chromosome, L_1, is always submetacentric and has a secondary constriction (the nucleolar organizer region) on the long arm (Southern *et al.* 1972a; Southern and Pell 1973; Itard 1973b; Pell *et al.* 1973). L_2 and X are metacentric (or nearly so) and, except for the X of *G. pallidipes* which has a prominent secondary constriction (Southern and Pell 1981), these chromosomes lack secondary constrictions (Southern *et al.* 1972a; Southern and Pell 1973; Itard 1973b; Pell *et al.* 1973). The heterochromatic Y chromosome is acrocentric in *G. f. fuscipes* and *G. m. morsitans* (Itard 1973b; Pell *et al.* 1973), submetacentic in *G. austeni*, *G. m. submorsitans*, and *G. pallidipes* (Itard 1973b; Pell *et al.* 1973; Southern and Pell 1981) and metacentric in *G. tachinoides*, *G. p. palpalis* and *G. m. centralis* (Itard 1970a, 1973b, 1974; Southern and Pell 1973). Polymorphisms have been observed and are discussed in the section on population genetics. The supernumerary chromosomes are heterochromatic and very short in *G. pallidipes* and *G. m. submorsitans*, longer (and of variable length) in *G. m. morsitans* and *G. austeni*. The supernumeraries of *G. m. centralis* differ from all the others by being metacentric and (as pointed out by Southern 1980) it is interesting to note that the length of each arm is approximately the same as the lengths of the supernumerary chromosomes of *G. pallidipes* and *G. m. submorsitans*. However this may not indicate an evolutionary connection between B chromosomes since studies of satellite DNA indicate that B chromosomes arose from A chromosomes within each taxon (Amos and Dover 1981; see section on biochemical and molecular genetics.)

Giemsa C-banding patterns

Giemsa C-banding of the L_1, L_2, and X chromosomes conforms to a basic pattern (found in *G. austeni*) with variations occurring mainly among members of the *morsitans* group. Chromosomes in members of the *palpalis* group are remarkably similar to the corresponding

chromosomes in *G. austeni* (Davies and Southern 1976). *G. tachinoides* is unusual in that the banding pattern of the X chromosome is identical to that of chromosome L_1 and that both are remarkably similar to L_1 from *G. p. gambiensis* and *G. p. palpalis* (from Zaire) (Southern 1980). The L_2 and X chromosomes from *G. f. fuscipes* are nearly identical to their homologs in *G. p. palpalis* (from Nigeria) (Southern 1980). These similarities and the amount of intra-taxon variation were used by Southern (1980) to indicate the limited usefulness of the Giemsa C-banding technique for phylogenetic studies. The Y chromosome of *G. m. morsitans* and *G. tachinoides* is uniformly stained, in *G. austeni* it shows a banding pattern, and in *G. f. fuscipes*, *G. p. gambiensis*, and *G. p. palpalis* the Y chromosomes have only one band, uniquely positioned in each species. Supernumeraries found in the *morsitans* group have a Giemsa C-banding pattern similar to the Y chromosome in each taxon (Davies and Southern 1976). More recent work has shown a significant amount of polymorphism in the Giemsa C-banding patterns (Jordan *et al.* 1977; Southern 1980; Southern and Pell 1981) and has revealed that *G. m. centralis* and *G. m. submorsitans* have small non-staining zones in the Y chromosome while in *G. pallidipes* one arm of the Y does not stain (Southern and Pell 1981).

Polytene chromosomes

Polytene chromosomes have been reported in a number of tissues in larvae, "pupae", and pharate adults within the puparia of several species (Burchard and Baldry 1970; Riordan 1970; Southern *et al.* 1973a, 1973c; Southern and Pell 1973, 1974, 1981; Pell and Southern 1976). The most detailed studies have been by Southern and his colleagues who published photos, diagrams and verbal descriptions of polytene chromosomes found in the trichogen and tormogen cells associated with the macrochaetae on the thoraces of *G. m. morsitans* (Southern *et al.* 1973a, 1973c), *G. austeni* (Southern and Pell 1974), *G. f. fuscipes* (Pell and Southern 1976) and *G. pallidipes* (Southern and Pell 1981). Polytenes have also been studied in other taxa and in some hybrids (Southern and Pell 1973; Pell and Southern 1976) but detailed accounts have not yet been published.

In tsetse flies only L_1, L_2, and X chromosomes form polytenes. Polytene nuclei in trichogen and tormogen cells lack all traces of Y and supernumerary chromosomes, and also lack chromocentres. In describing the polytene chromosomes Southern and his colleagues have divided the six polytene arms in each species into a total of 100 units, each of which was subdivided into two or three divisions. The nucleolar organizer is found at approximately the same location (position 51B to 53B) in L_1R in each of the species. The region of the X chromosome which associates with the Y chromosome during meiosis is represented by a fibrillar mass.

In these studies *G. austeni* was chosen as a reference species and the percentages of the bands in each species which are represented in *G. austeni* were calculated. Of the three species compared to *G. austeni*, *G. m. morsitans* was the most similar, having from 47.5% (for XL) to 100% (for L_1L) of its bands represented in *G. austeni* (Southern and Pell 1974). The corresponding figures for *G. pallidipes* are 23.7% (for XL) and 66.4% (for L_1L) (Southern and Pell 1981) and the figures for *G. f. fuscipes* are 12.9% (for XL) and 69.4% (for L_1L)(Pell and Southern 1976). The data indicate that chromosome L_1 has undergone the fewest evolutionary changes, and the X chromosome the greatest number of changes. The results also present limited support for placing *G. austeni* in the *morsitans* group rather than in the *palpalis* group but polytene analyses of more taxa are needed, as are detailed comparisons between each of the possible pairs of taxa, before a firm conclusion can be drawn from polytene chromosome data.

Supernumerary or B chromosomes

The small (usually telocentric) chromosomes found in members of the *morsitans* group were first recognized to be supernumerary or B chromosomes by Itard (1970a). They do not occur in members of the *palpalis* group and too few members of the *fusca* group have been examined to warrant comment on their distribution in that group. The number of supernumeraries varies from individual to individual and, although their numbers usually vary within fixed limits, some populations appear to lack them (Southern 1980). Southern (1980) pointed out that most studies have been done on flies from established colonies and that few localities were sampled to establish these colonies, thus the full extent of variation in the numbers of supernumeraries may not yet be realized. He also pointed out that the consistently pycnotic appearance of the supernumeraries indicates that they are not the site for RNA production but these cytogenetic studies have been done on flies maintained under fairly uniform laboratory conditions and it is possible that supernumeraries have an important role under certain conditions encountered in the field. In support of this Southern (1980) points out that "there is some evidence the individuals of *G. m. morsitans* with six or seven supernumeraries emerge as adults significantly later than those with just two or three."

An analogous situation occurs with Y chromosomes in *G. p. palpalis* (Southern 1980; see also section on sex determination.). Since *G. p. palpalis* lack supernumeraries I wonder if these apparently unrelated situations may not have a similar selective value under natural conditions. There is as yet no satifactory explanation for the sex chromosome polymorphism observed in natural populations of members of the *palpalis* group (Maudlin 1979). The suggestion (Davies and Southern 1976; Amos and Dover 1981) that the supernumerary chromosomes may have arisen from the Y chromosome may be pertinent to the above. The association of Y chromosomes with the supernumeraries during meiosis in *G. austeni* (Southern and Pell 1973) and the similar Giemsa C-banding patterns of Y chromosomes and supernumerary chromosomes (discussed above) are also consistent with the suggestion that the B chromosomes arose from the Y chromosomes. However the demonstration that Y and B chromosomes do not have extensive satellite DNA similarity (Amos and Dover 1981) does not support the suggestion of an evolutionary relationship between Y and B chromosomes and required a two step hypothesis to explain the evolution of the supernumeraries from the A (probably Y) chromosomes (Amos and Dover 1981).

Meiosis

Meiosis in tsetse flies has been summarized by Southern (1980) and the reader should consult that review for details. However several aspects of meiosis which pertain to other aspects of tsetse genetics covered in this review will be discussed briefly here.

Spermatocyte nuclei in *G. austeni* contain a large vesicle containing extra-chromosomal DNA (Southern and Pell 1973) which is apparently responsible for synthesis of an RNA which remains within the nucleus and is ultimately reorganized into fibres (Craig-Cameron *et al.* 1974). The vesicle is absent from *G. m. submorsitans* and *G. m. centralis* (Southern and Pell 1973). A small vesicle was found in *G. m. morsitans* recently isolated from Africa and from a colony maintained at Langford England, but it suddenly disappeared from the latter suggesting some environmental influence upon its expression (Southern and Pell 1973; Craig-Cameron *et al.* 1974).

In the *morsitans* group, pairing between the X and Y chromosomes during meiosis always involves a segment of the X chromosome adjacent to the centromere but the segment of the Y

chromosome involved in the pairing varies from species to species (Southern *et al.* 1972a, 1972b; Southern and Pell 1973; Southern 1980). Since pairing between X and Y chromosomes of *G. pallidipes* apparently does not occur (Southern 1980; Southern and Pell 1981) it is possible that the pairing segment is missing from one of the chromosomes (probably the Y chromosome). Pairing between the X and Y chromosomes of *G. m. morsitans* involves two heterochromatic segments and is achiasmate (Southern *et al.* 1972b; Southern and Pell 1973).

Meiosis in male and female tsetse flies differs with regard to three phenomena: formation of chiasmata, behaviour of the B chromosomes, and timing. Chiasmata are found during female meiosis (Davies and Southern 1977) and only rarely during male meiosis in *G. m. morsitans* (Craig-Cameron *et al.* 1973a, 1973b; Southern and Pell 1973; Southern 1980) suggesting that genetical recombination is more frequent in females than in males. (See section on linkage groups.) Chiasmata occur during female meiosis (Davies and Southern 1977) but not during male meiosis in *G. austeni* (Craig-Cameron *et al.* 1973a, 1973b). Chiasmata were reported in about 1% of male *G. f. fuscipes* and such males often showed "at least three apparent chiasmata per nucleus in L_1 and L_2 bivalents" (Pell and Southern 1976). Perhaps there is a locus controlling genetical recombination in tsetse flies with the population of *G. f. fuscipes* examined having a rare allele which permits the process in males. During male meiosis the supernumerary (or B) chromosomes behave as univalents and are distributed randomly to the poles while in females they appear to form true bivalents which segregate at anaphase I (Davies and Southern 1977). Male meiosis is completed within a few hours nine to ten days after larviposition (Southern *et al.* 1972b) while in females meiosis occurs throughout adult life and metaphase I of meiosis may last from a few hours (during the first reproductive cycle) to six or seven days (during subsequent cycles)(Davies and Southern 1977).

Meiosis in *morsitans* group hybrid males proceeds normally (Southern and Pell 1973; Southern *et al.* 1973b), but in hybrid males in the *palpalis* group the chromosomes tend to fragment during meiosis (Southern 1980). (This latter observation does not seem consistent with Vanderplank's (1948) observation that hybrid males in the *palpalis* group are fertile if they can successfully transfer sperm. See section on paternal aspects of hybridization.) In both groups hybrid females are (to varying degrees) fertile. The above observations, combined with the extensively similar polytene banding patterns observed in closely related taxa, suggests not only that genetic material may be passed from one taxon to another, but that genetical recombination might produce completely new chromosomes and thus novel combinations in the descendants of the hybrids. However, in the only experiment designed to search for genetical recombination in female hybrids (produced by crossing *G. m. morsitans* x *G. m. centralis*), no recombination was found between two X chromosome loci (*ocra* and *salmon*) which, in *G. m. morsitans*, are separated by about 37 map units (Gooding 1982b).

SEX DETERMINATION

In all tsetse species studied which have sex chromosomes, males are heterogametic (i.e. males are XY and females are XX). Aneuploidy involving the Y chromosome is wide-spread among tsetse species (Southern 1980) but sex chromosome aneuploidism is most easily studied, and has been most extensively studied, in members of the *palpalis* group since these flies lack supernumerary chromosomes. Sex chromosome aneuploidy occurs in both field populations (Maudlin 1979) and laboratory colonies (Southern 1980) of *G. p. palpalis*. The number of Y chromosomes has no effect upon the sex phenotype of the fly (Maudlin 1979; Southern 1980)

and it appears that sex phenotype is determined by a balance between the number of autosomes and the number of X chromosomes: females may be XX, XXY or XXXY; males may be XY, XYY or XO (Maudlin 1979; Southern 1980). The finding that the Y chromosome does not influence the apparent sex of the adult is consistent with an earlier observation in which a mutant *G. m. morsitans* created by γ-irradiation, had a portion of the Y chromosome inserted into one autosome, and had lost at least two-thirds of the long arm of the Y chromosome, yet the males appeared normal (Southern and Pell 1973). The Y chromosome is required for production of motile sperm (Southern 1980) and there is a curious correlation between the number of Y chromosomes in *G. p. palpalis* and the time spent in the puparium. Those flies lacking a Y chromosome (XX or XO) emerge 24-36 hours before flies having one Y chromosome (XXY or XY); and those with two or three Y chromosomes (XXYY, XYY, or XYYY) do not emerge for another 12 hours.

Further evidence to support the hypothesis that sex phenotype is determined by the balance between autosomes and number of X chromosomes could be found by searching for gynandromorphs or for mosaics having male and female characters. The apical bristles on the scutellum in some species show a marked sexual dimorphism (see Buxton 1955, pp. 6-7). Female *G. m. morsitans* have bristles which are much shorter than those of males and we have observed a female having one long and one short bristle. This mosaic is consistent with the hypothesized mechanism for determining sex phenotype but the situation may be more complicated since in one line of *G. m. morsitans* the length of the scutellar apical bristles in females is much longer than normal (unpublished work in my laboratory).

Maudlin (1979) has pointed out that sex ratio distorting genes may exist in tsetse, and in fact significant sex ratio distortion is a feature of the two *G. m. submorsitans* colonies (one from Upper Volta and one from Nigeria) which I maintain in my laboratory. In both colonies there is a significant excess of females and this excess has remained relatively stable over several years and therefore can not be due simply to lethal recessives on the X chromosome. No explanation of this permanent excess of females is yet available.

VISIBLE TRAITS

Most taxonomic and zoogeographic papers on tsetse refer to variations in structure or colouration of adults, but the extent to which these are under genetic control has not been determined. Despite the large numbers of flies observed during field and laboratory studies each year, few reports have been published describing distinctive intra-population variations in structure or colour. Variations have been found in the colour of *G. m. morsitans* (Shircore 1913) and of *G. brevipalpis* (Burtt 1944) but no attempt was made to establish their genetic basis. A brief list of naturally occurring colour variants is provided by Vanderplank (1948) who pointed out that these variants are of no taxonomic significance but may be useful in studying the genetics of body colouration. More recently, mutations controlling body colour (Bolland *et al.* 1974; Vloedt 1980) and eye colour (Gooding 1979) have been found in *G. m. morsitans*, and the genetics of these traits has been described.

G. m. morsitans adults have dark brown bodies with brownish-black transverse bands on abdominal tergites and similarly coloured spots on the nota. Two mutant strains having yellowish bodies and yellowish-brown bands or spots have been established. The first, (designated *ocra* = *oc*) was found in a laboratory colony which descended from flies collected near Kariba, Zimbabwe (Bolland *et al.* 1974); the second (designated *oT*) descended from a

male collected near Tanga, Tanzania (Vloedt 1980). The locus for *oc* and *oT* is on the differential part of the X chromosome (Bolland *et al.* 1974; Vloedt 1980; see also section on linkage groups), and these alleles are completely recessive to the wild type allele. Since reciprocal crosses involving females homozygous for either *oc* or *oT*, with males having the other allele, produced offspring having ocra bodies (Vloedt 1980), both alleles must involve the same biochemical or physiological processes. Wild type and ocra males are equally competitive in mating experiments conducted under laboratory conditions (Kawooya 1977; Vloedt 1980). Some females with ocra bodies will mate more than once and, although some females use sperm from two matings, or only from the second mating, most females use only sperm from the first mating. The latter two phenomena are not unique to either *ocra* (studies by Kawooya 1977; Vloedt 1980) or *oT* (studies by Vloedt 1980) (see sections on multiple mating by females and use of sperm by multiply mated females). With respect to most criteria used to measure success of a tsetse colony, *oc* and *oT* were as good as, or better than, wild type flies from Tanzania, although about 10% of the ocra flies tend to lose their wings (Vloedt 1980; Langley, commenting on Vloedt's paper, gave this figure as 30% for his *ocra* colony). However, the success of the ocra flies, under laboratory conditions, is not translated into success in the field. Recapture rate of laboratory reared ocra flies in Tanzania was less than 20% of that of laboratory reared wild type flies (Dame in discussion of Vloedt 1980), indicating fairly strong selection against this phenotype in the field.

Compound eyes of wild type *G. m. morsitans* are dark brown and only one variant (designated *salmon* = *sal* because of the eye colour) has been found (Gooding 1979). The allele *sal* has an X chromosome locus and, at least as regards eye colour, it is completely recessive to the wild type allele (Gooding 1978, 1979). This allele is pleiotropic, affecting a variety of morphological and physiological traits in hemizygous males, and in females homozygous for *sal*: compound eyes and ocelli are salmon and testes are very pale, but the spermathecae are normal (Gooding 1979); heads of salmon flies have less xanthommatin than do those of wild type flies, and salmon flies excrete tryptophan while wild type flies excrete kynurenine (Gooding and Rolseth 1984); adult longevity is shorter, fewer offspring are produced, there is a lower pregnancy rate in females, and mating competitiveness of males is about half that of wild type males (Gooding 1982a); light is detected at a lower intensity, and light adaptation is faster, and occurs at lower light intensity (Davis and Gooding 1983). Although salmon and wild type males differ neither in timing of their spontaneous activities, nor in total number of activity periods, salmon males become active slightly sooner after "lights on", have activity periods of shorter duration and are more responsive to moving images (Gooding 1983b). Unlike the situation with *ocra*, there is evidence for assortative mating (Gooding 1982a; see section on behavioural genetics). Susceptibility to infection with *Trypanosoma brucei brucei* Plummer and Bradford (M'Pondi *et al.*, *in prep.*) and *Trypanosoma congolense* Broden (Distelmans *et al.*, *in prep.*) is greater in salmon males than in wild type males. The biochemical lesion caused by the allele *salmon* is a lack of tryptophan oxygenase activity and this accounts for much of the pleiotropic nature of this allele (Gooding and Rolseth 1984).

Most of the impetus for studying *salmon* comes from the lethal or semi-lethal nature of this maternally influenced, genetically rescuable allele. When *sal/sal* females are mated with hemizygous *sal* males, about 80% of the offspring produced die in the puparia, while adults which do emerge have very pale eyes, and most die within a few days (Gooding 1978, 1979). When *sal/sal* females are mated with wild type males they produce the expected number of

phenotypically wild type females and these have normal viability, but the pale eyed male offspring die, either in the puparia or as young adults (Gooding 1978, 1979). Lethality of *salmon* has been demonstrated at 23°C and 25°C, and in two genetic backgrounds (Gooding 1982a). The possibility of using *salmon* as a genetic control agent has been investigated both theoretically (Gooding 1978, and with an unpublished model which includes provision for density dependent effects) and in experimental laboratory populations (Gooding 1982a). Computer models and laboratory experiments indicate *salmon* may be effective as a genetic control agent if salmon flies behave the same as wild type flies in the field. However, the greater susceptibility of salmon flies to at least two species of trypanosomes makes it unlikely that releases of this fly, into any locality where trypanosomiasis is endemic, could be justified.

Size of tsetse flies may also be considered as a "visible trait". No genetic studies on size *per se* have been undertaken, but heritability (h^2) of teneral adult weight has been estimated to be between 0.09 and 0.16 in *G. m. morsitans* (Gooding and Hollebone 1976). The selective pressures for maintaining fly size within fairly narrow limits in each species have not been determined but extremes of size tend to be eliminated from tsetse populations in nature (Glasgow 1963; Phelps and Clarke 1974).

BIOCHEMICAL AND MOLECULAR GENETICS

Our knowledge of biochemical genetics of tsetse consists mainly of information on the electrophoretic mobility and banding patterns of several enzymes from whole flies examined on starch gel (Geest and Kawooya 1975; Geest *et al.* 1978; Etten 1982c) or from thoraces, midguts, or testes examined on polyacrylamide gel (Rolseth and Gooding 1978; Gooding and Rolseth 1978, 1979, 1982; Gooding 1981a, 1982b). It is important to realize that not all variation in electrophoretic mobility is due to genetic factors, thus variation in mobility is not proof of genetic variation. For some enzymes the variation in mobility has been shown, by breeding experiments, to be under genetic control; for others the only evidence is that the banding patterns correspond to patterns known to be under genetic control in other species, or the data are consistent with what one would find in a population in Hardy-Weinberg equilibrium.

On starch gel electrophoresis, the following enzymes are monomorphic (i.e. only one allele has been demonstrated) in laboratory colonies of *G. m. morsitans*. The designation of the locus is given, in italics: lactic dehydrogenase (*ldh*), malic dehydrogenase (*mdh*, Geest and Kawooya 1975; but see also Table 2); NADP-dependent malic dehydrogenase (*mdh-t*, Geest and Kawooya 1975; but information on this enzyme was later withdrawn by Geest *et al.* 1978 as being "in error".); an esterase (est_5 Geest *et al.* 1978; see also Table 2); adenylate kinase (*Ak*), catalase (*Cat*), isocitrate dehydrogenase (*Idh*), phosphoglucoisomerase (*Pgi*), peroxidase (*Po*, Geest *et al.*. 1978); glucose-6-phosphate dehydrogenase (*G-6-pd*, Geest *et al.* 1978; but see also Table 2). Variation in mobility of xanthine dehydrogenase (*Xdh*) was believed by Geest and Kawooya (1975) to be due to non-genetic factors.

Variations seen in electrophoretic banding patterns of alkaline phosphatase (*alph*) and a leucine aminopeptidase (lap_2) in *G. m. morsitans* (Geest and Kawooya 1975) may or may not be under genetic control. For alkaline phosphatase there were 140 flies with a double band pattern and two flies which had an additional double band, and for leucine aminopeptidase all 480 flies had a double banded pattern but for one fly the migration of these bands was less than in the other flies. No genetic model was offered for either *alph* or lap_2 and the frequency of the

Table 2. Genetics of molecular variation in *G. m. morsitans*.

ENZYMES	LOCUS	NO. ALLELES	HETERO. BANDING[1]	EVID.[2]	REF[3]
aldehyde oxidase	*Ao*[4]	3	2	2[5]	A
aldehyde oxidase	*Ao*[4]	3	3	2,3	B
alkaline phosphatase	*Alkph*	2	2	2,3	C
arginine phosphokinase	*Apk*	2	2	2,3	C
esterase	Est_1	2[6]	1[6]		A
esterase	Est_2	2[6]	1[6]		A
esterase	Est_3	2[6]	1[6]		A
esterase	Est_4	2[6]	1[6]		A
esterase	*Est.1*	2		3[7]	E
esterase	*Est.2*	4		3[7]	E
glucose 6-P deH	*G6pd*	2		3[7]	E
α-glycero-P deH	*Gpd*	2	2	2	A
α-glycero-P deH	*Gpd.2*	2		3[7]	E
leucine aminopeptidase	lap_3	3	2	1[8]	F
leucine aminopeptidase	lap_3	4		3	A
malic deH	*Mdh.1*	2		3[7]	E
malic enzyme	*Me*	4	2	1[9]	F
octanol deH	*Odh*	3	3	2,3	D
xanthine oxidase	*Xo*	2	3	2,3	B

[1]Two bands in heterozygotes are interpreted as indicating that the active enzyme is a monomer, or that heterodimers are inactive. A pattern in which heterozygotes have three bands indicates that the active enzyme is a dimer; the band having the intermediate electrophoretic mobility being the heterodimer.

[2]Evidence abbreviated as follows: 1=apparent agreement between observed and expected phenotype frequencies; 2=population tested was in Hardy-Weinberg equilibrium; 3=established by breeding experiments using two or more of the alleles.

[3]References abbreviated as follows: A=Geest *et al.* 1978; B=Rolseth and Gooding 1978; C=Gooding and Rolseth 1978; D=Gooding and Rolseth 1979; E=Gooding and Rolseth 1982; F=Geest and Kawooya 1975.

[4]Term *Ao* used independently by Geest *et al.* (1978) and by Rolseth and Gooding (1978), may not refer to the same locus; note difference in number of bands observed in heterozygotes.

[5]Although Geest *et al.* (1978) claim the population was in Hardy-Weinberg Equilibrium, the data they published do not support this claim.

[6]Flies had one band or no bands for these esterases and the existance of null-alleles was assumed by Geest *et al.* (1978). No analyses of the data were possible, nor were breeding experiments performed, to provide evidence for the genetic interpretation offered by Geest *et al.* (1978).

[7]Breeding data were not presented by Gooding and Rolseth (1982).

[8]Analysis of data published by Geest and Kawooya (1975) shows that criterion 3 (see footnote 1, above) has been met.

[9]Data published by Geest and Kawooya (1975) indicate the population was not in Hardy-Weinberg equilibrium.

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rare allele was too low to permit one to determine whether the observed frequencies of phenotypes agreed with those predicted for a population in Hardy-Weinberg equilibrium. Similarly for another leucine aminopeptidase (lap_1, Geest and Kawooya 1975) a single fly was found having two bands, while 479 flies had one band. The data do not permit testing of a genetic model. The lap_2 data were subsequently re-interpreted and it was proposed that the zone of staining represents enzymes controlled by two loci, one of which is monomorphic and the other has two alleles, one of which is extremely rare (Geest *et al.* 1978).

Electrophoretic variation has been found in at least 14 enzymes from *G. m. morsitans* and the data are summarized in Table 2. The exact number of enzymes, for which the genetics has been established, is in doubt because of difficulties in comparing work done by van der Geest and his co-workers, using starch gel, with work done in my laboratory, where polyacrylamide is used. We have both reported upon genetics of an aldehyde oxidase but, with our technique heterozygotes have three bands, while only two were found using starch gel. Thus these may not be the same aldehyde oxidase. Similarly it is not possible to determine whether the loci we have designated *Est.1* and *Est.2* (Gooding and Rolseth 1982) correspond to any of the esterase loci studied by Geest *et al.* (1978).

On starch gel electrophoresis 12 monomorphic loci and three polymorphic loci were found in *G. pallidipes* collected from natural populations at eight localities in Kenya (Etten 1982c). Monomorphic loci were found for the following enzymes (the designations for the loci are given in italics): two non-specific esterases (*est-2*, *est-3*) two leucine aminopeptidases (*lap-1*, *lap-2*); malic enzyme (*me*); alkaline phosphatase (*alph*); xanthine dehydrogenase (*xdh*); octanol dehydrogenase (*odh*); lactate dehydrogenase (*ldh*); malic acid dehydrogenase (*mdh*); isocitrate dehydrogenase (*idh*) and α-glycerophosphate dehydrogenase (α-gpd). Polymorphic loci occur for an esterase (*est-1*, 2 alleles), aldehyde oxidase (*ao*, 3 alleles), and a leucine aminopeptidase (*lap-3*, 4 alleles). Unfortunately the banding pattern in heterozygotes, and the existance of heterozygous males were not described by Etten (1982c).

As indicated above most of the information available on the genetics of electrophoretic mobility of enzymes comes from studies of *G. m. morsitans* but there have also been some comparative studies involving other taxa and most of these are summarized in Table 3. The banding patterns for most of these enzymes are the same as those found in the homologous enzymes in *G. m. morsitans* where genetic control of the enzyme mobility has been established by breeding experiments, suggesting that electrophoretic mobility of these enzymes is under genetic control in all the taxa studied.

Satellite DNA (=highly repetitive sequences) makes up about 8% of the total DNA in *G. m. morsitans* pupae and about 20% of the total in *G. austeni* (Dover 1980; Amos and Dover 1981). The figures for other species are: 16% for *G. pallidipes*, 9.6% for *G. f. fuscipes* and 14.8% for *G. tachinoides* (Dover 1980). Two bouyant density classes (1.678 g/cm^3 and 1.685 g/cm^3) of satellite DNA occur in tsetse flies, the latter occurs in all taxa studied (i.e. five from *morsitans* group, four from *palpalis* group, and one from *fusca* group) while the former class of DNA occurs in all taxa except *G. austeni* (Dover 1980; Amos and Dover 1981). Experiments,

Table 3. Banding patterns found in various species by polyacrylamide gel electrophoresis.

Locus	No. bands in heterozygotes	Number of alleles in each taxon[1]								
		Gmc	Gmm	Gms	Gp	Ga	Gt	Gff	Gpg	Gpp
Mdh.2	U[2]	1	1[6]	1	1	1	1	1	1	1
To	U[2]	1	1[6]	1	1	1	1	1	1	1
Apk	2	1	2	1[7]	1	1	1[7]	1	1[7]	1
G6pd	2	2	2	1	1	1	1	1	1	1
Est.t	N[3]	1	1[6]	1	1	2	1	2	2	2
Alkph	2	2	2	1[7]	3	2	3[8]	1	3[8]	1
α-Gpd.2	3	1	2[5]	2[9]	1	1	3[8]	2	3[8]	1
Mdh.1	3	1	2	3[8]	1	1	3[8]	1	3[8]	2
Est.1	3[4]	1	2[5]	1	2	3	2	1	1	2
Xo	3	1	2	2	2	2	2	1	2	1
Ao	3	3	3	2	1	2	2	1	3	2
Odh	3	2	3	3[8]	2	4	2[8]	2	3[8]	2

[1]Most of the data are from Gooding (1982b) and where indicated the data are supplemented with, or confirmed by, data from other publications. Names of the taxa are fully spelled out in Table 1.

[2]Unknown since in these monomorphic loci no heterozygotes have been found.

[3]Heterozygotes are non-existent since the locus *Est.1* in on the X chromosome. (See section on linkage groups.)

[4]Three bands occur but the fastest migrating homodimer stains only very faintly under normal conditions (Gooding 1984).

[5]A rare allele occurs at each of these loci in the Handeni line of *G. m. morsitans* (Gooding and Rolseth 1982).

[6]See also Gooding and Rolseth (1982).

[7]This situation was also found in flies from natural populations in Upper Volta (Gooding 1981a).

[8]Natural populations in Upper Volta had three alleles at each of these loci (Gooding 1981a).

[9]Two alleles occur in natural populations in Upper Volta (Gooding 1981a).

in which isotopically tagged satellite DNA was homologously hybridized to metaphase chromosomes, demonstrated that hybridization occured mainly with B (=supernumerary) chromosomes but also with centromeres of autosome L_1 and X chromosome in *G. austeni*, all autosomes and both sex chromosomes in *G. m. morsitans*, and autosomes and X chromosomes (but possibly not the Y chromosome) in *G. pallidipes*. Tagged satellite DNA from *G. pallidipes* hybridized with autosomes and X chromosome of *G. m. morsitans* but not with either the Y chromosomes or B chromosomes. There was no appreciable hybridization between *G. austeni* satellite DNA and chromosomes of *G. m. morsitans*. The results indicate a closer

relationship between *G. m. morsitans* and *G. pallidipes* than between *G. m. morsitans* and *G. austeni*. These results also indicate that B chromosomes have arisen from A chromosomes within each species and that A and B chromosomes have evolved separately within a species, just as interspecific differences have arisen (Amos and Dover 1981).

VECTORING ABILITY

The ability of tsetse flies to transmit trypanosomes is influenced by a number of factors (for reviews see Jordan 1974; Maudlin 1980), and there is little direct experimental evidence for genetic control of vectorial capacity. Arguing by analogy with the mosquito/*Plasmodium* and mosquito/filaria models, Jordan (1974) suggested that individual variation in susceptibility to trypanosomes may exist and that the most rewarding studies may involve transmission of *Trypanosoma congolense* group and *Trypanosoma brucei* group.

In each of several natural populations of four tsetse species, there was a higher prevalence of *Trypanosoma congolense* among males than among females. The reverse was found in one population of *G. pallidipes* (Clarke 1969). In three tsetse species, given the opportunity to become infected with *T. rhodesiense* under laboratory conditions, the prevalence of mature infections was higher in males than in females (Harley 1971). Although differences in susceptibility of males and females have been previously noted, no explanatory model has been proposed. The simplest explanation is that the difference in infection is attributable to many biochemical and physiological differences between male and female flies, and is not due to any one gene or small number of genes. Unfortunately such an explanation is difficult to test and unlikely to stimulate work on the subject.

The simplest genetic model to explain the sex difference in vectoring capacity is that it is due to an X chromosome locus. However this explanation is not quantitatively consistant with the data. If the allele confering resistance were a recessive, all the available data sets (8 from Clarke 1969; 3 from Harley 1971) have a great excess of infected females. If susceptibility were due to a dominant allele, the same data sets, with one exception, are deficient in infected females. (The exception was *G. pallidipes* studied by Clark (1969), in which there was an excess of infected females.) The same discrepencies occur if one postulates involvement of two loci on the X chromosome.

A maternally influenced inheritance pattern for vectoring capacity, with apparently little or no dependence upon parental genotype, has been demonstrated in a laboratory colony of *G. m. morsitans* fed upon procyclic forms of *T. congolense* (Maudlin 1982). Males from the parental colony were slightly more susceptible to trypansomes than were females, but the difference was not statistically significant. The nature of the maternal influence was not determined. In Maudlin's experiments 26.5% of the F_1 flies developed mature infections compared to 17.5% in the parental generation. The proportion of infective and non-infective females producing offspring, and number of offspring produced by each type of female do not explain the increase in mature infections in the F_1. However this increase is consistent with the experimental design in which some F_1 flies were given more opportunities to become infected than were the parental generation. The effect of this experimental design upon the inheritance pattern is not descernable from the data.

Wild type *G. m. morsitans* males do not develop mature infections of *Trypanosoma brucei brucei* (M'Pondi *et al. in prep*) or of *Trypanosoma congolense* (Distelmans *et al. in prep.*) as readily as do salmon *G. m. morsitans*. Although these results demonstrate a genetic influence

upon vectoring ability, it is not known whether this is a direct, specific effect on the trypanosomes, or a more general effect of the pleiotropic allele *salmon*. (See section on visible traits.)

BEHAVIOURAL GENETICS

Tsetse flies present many opportunities for studying the genetics of behavioural phenomena (such as phototropism, circadian rhythms, habitat selection, host seeking, feeding and mating) which may have profound implications for control of these insects. However there have been few such studies.

Incursion of *G. tachinoides* into what are generally regarded as "atypical" habitats in Nigeria has been interpreted as indicating that this species may be more versatile, with respect to habitat selection, than had been previously suspected, and invasion of "atypical" habitats may have been due to (or may have resulted in) small genetic changes in the populations concerned (Baldry 1969). Unfortunately firm data on these points are lacking. (See section on interpopulation comparisons for information on genetics of natural populations of *G. tachinoides*.)

A comparison of various biological and metabolic parameters in *G. pallidipes* from Nkruman and Mwalewa, Kenya indicated that females in these populations feed at different frequencies (Etten 1982a). This difference was confirmed using females from the second generation of laboratory colonies (Etten 1982a) indicating that feeding frequencies are under genetic control. Similarly, the spontaneous activity of male *G. pallidipes* colonized from these two localities was different at both 24°C and at 30°C. In the field, activity of males during the early afternoon at Mwalewa was negatively correlated with temperature while at Nkruman there was no correlation between temperature and activity of males during the early afternoon. The results indicate that activity patterns and behavioural responses to temperature are under genetic control in *G. pallidipes* (Etten 1982b).

Duration of copulation is different in *G. pallidipes* from Kibwezi and Lambwe, Kenya (Jaenson 1978) and most of the difference is due to differences in the duration of the pre-transmission stage of copulation (Jaenson 1979a). Copulation duration in parental lines, in F_1, in F_2, and in backcrosses, indicates that this aspect of behaviour is controlled by genes on autosomes and on the X chromosome (with no evidence for involvement of the Y chromosome) and is mediated through the male (Jaenson 1978). Females also influence duration of copulation (Jaenson 1979b), but no genetic studies have established the number or location of the genes involved. In presenting data on polygenic control of copulation duration, Jaenson (1978) noted that the results were anomolous in that variance among F_2 males was equal to that among F_1 males, rather than the former being larger than the latter. Jaenson suggested that this "may be due to low heritability of the trait, interaction between genotype and environment and lack of homogeneity in the parental strains." Although this may be true, similar values for variances in F_1 and F_2 copulation time could also have been due to the rather small sample sizes (6 to 50 for F_1, 25 to 57 for F_2) since, even if there were no recombination in the F_1, 18 different combinations of chromosomes are possible in F_2 males, with the commonest type accounting for 12.5% of the sample.

Until recently the lack of suitable genetic markers prevented study of assortative mating within any *Glossina* taxon. (Assortative mating involving two taxa is reviewed in section on interspecific mating.) Even now only *G. m. morsitans* has genetic markers for such studies; the

two most convenient marker genes being *ocra* (body colour) and *salmon* (eye colour). The only published experiment indicated that assortative mating occurred in laboratory populations of salmon and wild type *G. m. morsitans* (Gooding 1982a). However, in this experiment the composition of the mating pairs was not determined by direct observation but rather by counting the number of each phenotype among F_1 females. Another interpretation of the results is that, following multiple mating, there was preferential use of contypic sperm or differential mortality of larvae *in utero* which was influenced by the compatibility of maternal and progeny genotypes. Assortative mating is thus another aspect of behavioural genetics which remains to be investigated in tsetse.

LINKAGE GROUPS

Although linkage groups are usually established through reciprocal crosses and/or the three-point-cross, electrophoretic techniques permit, under certain circumstances, assignment of a locus to either the X chromosome or the autosomes, without employing breeding experiments. This is possible since the structural genes on each chromosome, which are ultimately responsible for production of peptide chains, usually have co-dominant alleles. Thus, if a gene has electrophoretically detectable alleles, heterozygous males could occur only if the locus were on an autosome, but heterozygous females could occur if the locus were on either an autosome or the X chromosome. The criterion of heterozygotes in females, but not in males, was used to assign *Apk* (arginine phosphokinase, Gooding and Rolseth 1979) and *G6pd* (glucose 6-phosphate dehydrogenase, Gooding and Rolseth 1982) to the X chromosome of *G. m. morsitans*. Breeding experiments have confirmed the location of these loci. The existence of heterozygous males indicates that *lap-3* (the locus for leucine aminopeptidase-3) is on an autosome of *G. m. morsitans* (Geest *et al.* 1978).

On the basis of occurrence of heterozygotes in females but not in males, three loci have been assigned an X chromosome linkage and seven loci an autosomal linkage in several taxa (summarized in Table 4). For each locus, which has been assigned to either the X or the autosomes, the assignment has been the same in all taxa.

Breeding experiments established that *ocra* (body colour, Bolland *et al.* 1974; Gooding 1979) and *salmon* (eye colour, Gooding 1979) are located on the differential part of the X chromosome. Similarly, breeding experiments established that loci involved in determining the duration of copulation in *G. pallidipes* are spread among the autosomes and the X chromosome, but the number of loci and their exact location is unknown (Jaenson 1978; see section on behavioural genetics).

For *G. m. morsitans* four loci have been mapped in linkage group I (= X chromosome), seven have been mapped in linkage group II (an autosome), and one locus has been found in linkage group III (Gooding 1981b, 1983a, 1984). The linkage maps may be summarized as follows:

I(=X): *G6pd* <37.1 m.u.> *oc* <36.7 m.u.> *sal* <38.6 m.u.> *Apk*
II: α-Gpd <45.0 m.u.> (*Xo/Alkph*) <45.7 m.u.> (*Ao/Odh*) <8 m.u.> [*Est.1/Est.2*]
III: *Mdh*

The region of the X chromosome which has been mapped does not involve the large paracentric inversion found in the "Handeni" line. In linkage group II the loci which are grouped together in parentheses ( ) are so close together that they have not yet been separated by genetical

Table 4. Assignment of loci to X chromosome or to autosomes based upon banding patterns.

Locus	Loci on X chromosome (X) or autosomes (A) in each taxon[1]								
	Gmc	Gmm	Gms	Gp	Ga	Gt	Gff	Gpg	Gpp
Mdh.2									
To									
Apk		X[2]							
G6pd	X	X[3]							
Est.t					X		X	X	X
Alkph	A	A[4]		A	A	A[6]		A[6]	
α-Gpd.2			A[6]			A[6]	A	A[6]	
Mdh.1		A[3]	A[6]			A[6]		A[6]	A
Est.1		A[3]		A	A	A			A
Xo		A[5]	A	A	A	A		A	
Ao	A	A[5]	A		A	A		A	A
Odh	A	A[2]	A[6]	A	A	A[6]	A	A[6]	A

[1]Most of the data are from Gooding (1982b) and where indicated the data are supplemented with, or confirmed by, data from other publications. A blank in the table means that the enzyme is monomorphic in that taxon and thus can not be assigned a linkage.
[2]Gooding and Rolseth (1979).
[3]Gooding and Rolseth (1982).
[4]Gooding and Rolseth (1978).
[5]Rolseth and Gooding (1978).
[6]Gooding (1981a).

recombination. The esterase loci [*Est.1*/*Est.2*] were located 5 to 10 m.u. to the right of *Ao* in two different experiments but are so close to each other, and the variances of the distances from *Ao* are so large, that the order of these loci remains in doubt.

During mapping experiments, no evidence was found for genetical recombination in males (Gooding 1981b, 1983a, 1984). However, since "chiasma-like configurations" occur at a low frequency during meiosis in *G. m. morsitans* (Craig-Cameron *et al.* 1973b) and chiasma occur in about 1% of male *G. f. fuscipes* (Pell and Southern 1976), genetical recombination may occur at very low frequencies in males of these species.

POPULATION GENETICS

Population genetics may be considered as having two broad objectives: description of the genetics of a population or species; and an explanation of mechanisms responsible for maintenance of genetic variability within a population or species. The first objective may be subdivided into three more limited objectives: description of genotypes within a population; quantitative estimates of genetic variation within a population; and interpopulation comparisons of genetically determined traits. For practical purposes, each of these limited

objectives generally includes the previous objective and the literature reviewed will be treated accordingly. The second broad objective may be expanded to include explanations of evolutionary events and prediction of future events.

In reviewing the literature I have included some papers in which field observations suggest areas of interest to the study of population genetics, even though the observations themselves were not intended as contributions to population genetics.

Non-quantitative descriptions of single populations or species

Polytene chromosome analysis has demonstrated inversions in the L_1L arm in some individuals in a laboratory colony of *G. pallidipes* (Southern and Pell 1981). Two forms of the Y chromosome have been demonstrated by Giemsa C-banding in *G. m. morsitans* and *G. m. submorsitans* (Southern and Pell 1981).

Three colour or pattern variations in the abdominal markings of *G. p. palpalis* have been associated with the habitat and/or geographic location in which the flies were found in Nigeria (Nash 1937). The general trend observed was for abdominal markings to become lighter as the vegetation becomes thinner. However it has not been established whether this is the result of natural selection or a direct environmental influence upon a polygenic trait or a trait with low heritability.

On the basis of variations in types of habitats occupied by *G. tachinoides* in Nigeria, Baldry (1969) suggested that this species is extremely versatile and that in certain localities it invaded "man-made environments" and became adapted to these. Baldry's proposal that there are many sub-populations of *G. tachinoides*, which ought to be amenable to analysis by the methods of population genetics, gains some support from Bursell's study of *G. morsitans*. In the latter species there are significant size differences in flies collected at sites that are only a few miles apart, suggesting that there is little movement by these flies (Bursell 1966). Body size in *G. m. morsitans* is influenced by both environmental and genetic factors (see section on visible traits) and there is significant selection against small males in natural populations in Zimbabwe (Phelps and Clarke 1974). The effects of this selection upon genetics of flies in natural populations might be worth investigating as it may relate to the problem of maintenance of polymorphisms in nature.

Estimates of genetic variation within a single population

The first attempt to describe the amount of genetic variation within a species of tsetse was made by Geest *et al.* (1978) using starch gel electrophoretic techniques with *G. m. morsitans*. Twenty-three gene-enzyme systems were examined using flies from a colony whose ancestors came from Binga and Kariba districts in Zimbabwe. There was variation in mobility of 12 of the enzymes produced by the 23 to 28 loci examined, i.e. 43 to 52% of the loci were "polymorphic". (The exact number of loci is uncertain because of difficulties in interpreting the number of loci involved in producing double-banded, but non-varying, patterns for some enzymes.) Unfortunately, in this study most enzymes were reported only as varying or non-varying and allele frequencies were given for only three loci (*Ao*, α-Gpd, and lap_3). However it was concluded that mean heterozygosity was low "since in nearly all polymorphic loci, the most common allele occurs at a very high frequency" (Geest *et al.* 1978).

Interpopulation comparisons

It is assumed, for the purposes of this discussion, that each self-sustaining laboratory colony is a separate population. In studies of field collected flies (or their F_1 progeny) it is assumed that flies from each collection site are from a separate population. The latter is probably an oversimplification which may not be justified, despite the generally held view that tsetse flies do not move very far. (See for example Bursell 1966.)

Three laboratory populations (whose ancestors came from Handeni Tanzania, Kariba Zimbabwe, and Kariba-Binga Zimbabwe and involving the *ocra* mutation) were found to have a normal amount of variation among 14 loci examined by polyacrylamide gel electrophoresis (Gooding and Rolseth 1982). (This conclusion, documented below, is in contrast to the conclusion of Geest *et al.* (1978), that heterozygosity is low in colonies of *G. m. morsitans*.) The colonies did not differ significantly with regard to the number of polymorphic loci: 5-7 of 14 had a common allele with a frequency less than 99%; 4-6 of 14 had a common allele with a frequency less than 95%. Similarly the number of effective alleles per locus (1.43 ± 0.62 to 1.79 ± 0.94) did not differ significantly among the colonies. However, the mean heterozygosity per locus (H) was lower in flies from the Handeni colony ($7.3 \pm 2.7\%$) than it was in flies from the other two colonies ($16.7 \pm 5.7\%$ for Kariba, $16.0 \pm 6.5\%$ for *ocra* colony). Female fecundity and longevity, and pupal weight are higher in the Kariba colony than in the Handeni colony (Jordan *et al.* 1977) while the performance of the *ocra* colony is reportedly as good as that of the Kariba line (Vloedt 1980). On the basis of allele frequency data (i.e. calculation of mean genetic identity, Nei 1972) it appears that the Kariba and ocra colonies are more closely related than either is to the Handeni colony (Gooding and Rolseth 1982). Thus the Handeni colony and the Kariba colony differ in mean heterozygosity and in frequency of various alleles. Cytogenetic differences between these strains had previously been demonstrated in regard to structure of the Y chromosome, presence of B chromosomes, Giemsa C-banding and an inversion on the X chromosome (Jordan *et al.* 1977). These genetic differences are consistant with the proposal by Jordan *et al.* (1977) that the reproductive differences between the colonies may be related to the genetically diverse nature of the two colonies but the genetic basis for differences in laboratory performance of the colonies has not been established.

Variations in Giemsa C-banding between three laboratory colonies (designated simply as colonies A, B, and C) of *G. m. morsitans* have been demonstrated (Southern and Pell 1981). The banding patterns in these colonies differ from those illustrated for the Kariba and Handeni lines (Jordan *et al.* 1977), thus there appear to be at least five (laboratory) populations of *G. m. morsitans* with regard to Giemsa C-banding patterns. The same technique has demonstrated two types of Y chromosomes in *G. m. morsitans* and *G. m. submorsitans* (Southern and Pell 1981) but the frequencies of each type within various laboratory populations has not been reported. Other cytogenetic differences between populations include variations in chromosome numbers for *G. pallidipes* from Lugala, Uganda ($2n=6$) and from Kariba, Zimbabwe ($2n=8$) (Maudlin 1970). The difference is probably due to the presence of B chromosomes in the Kariba population.

Sex chromosome aneuploidy was found in *G. p. palpalis* at five sites along the Niger and Kaduna rivers (Maudlin 1979; see section on sex determination.). No significant differences were found among flies from different sites (overall 21 of 249, or 8.4%, of females collected were shown to be XXY). However, at another site on the Zogruma Reserve, 200 km west of the study area mentioned above, only 2.4% of the female *G. p. palpalis* were XXY (Maudlin 1980). The frequency of sex chromosome aneuploidy in the populations studied is high

compared to the (expected) rate of spontaneous primary non-disjunction of X chromosomes in females, and this led Maudlin (1979) to conclude that aneuploidy is "maintained in the population as a polymorphism" by some as yet unknown mechanism.

Five enzyme systems have been studied by polyacrylamide gel electrophoresis in two natural populations of *G. m. submorsitans*, seven natural populations and two laboratory colonies of *G. p. gambiensis*, and four natural populations and one laboratory colony of *G. tachinoides* (Gooding 1981a). All the natural populations were from within 150 km of Bobo-Dioulasso, Upper Volta. All three species showed the same pattern for all five loci studied: at each locus there was a common allele, usually with a frequency greater than 93%, and each population had no more than two other alleles, and the frequency of the second commonest allele was always less than 6.5%. For each species, two of the five loci were polymorphic (i.e. frequency of commonest allele was less than 99%) while either zero or one (for *G. m. submorsitans*) of the five loci had a common allele with a frequency of less than 95%. For natural populations H values were low (3.49 ± 2% for *G. m. submorsitans*, 2.45 ± 1.26% for *G. p. gambiensis*, and 2.33 ± 0.76% for *G. tachinoides*. The low values for H are not because the loci choosen have intrinsically little variation in tsetse flies since in laboratory colonies of *G. m. morsitans* the H values for these loci (*Apk Odh Mdh* α-Gpd and *Alkph*) vary from 9.4 to 20.4% (Gooding and Rolseth 1982). The reason for low heterozygosity among natural populations is unknown, but speculation has included both neutralist and selectionist interpretations of this polymorphism (Gooding 1981a).

Three of 15 enzyme loci were polymorphic in eight natural populations of *G. pallidipes* in Kenya (Etten 1982c). Mean heterozygosity per locus within these populations (calculated from data published by Etten) was rather low (2.4% to 5.7%) when compared to the value (12.2 ± 5.4%) obtained in a laboratory colony originating from Uganda (Gooding 1982b), but was comparable to values found in natural populations of tsetse in Upper Volta (Gooding 1981a). The discrepency between the heterozygosity in the laboratory and the natural populations of *G. pallidipes* probably is due to the lower resolving power of starch gel electrophoresis (used by Etten 1982c) when compared with polyacrylamide gel electrophoresis (used by Gooding 1982b). With one exception, the genotype frequencies in each population of *G. pallidipes* in Kenya differed from that of the neighbouring populations indicating restricted gene flow (Etten 1982c). Analysis (in my laboratory) of the allele frequency data, published by Etten (1982c), by a cluster analysis of the Nei's mean genetic identity values, showed that the grouping of populations, with two exceptions, did not correspond to the proximity of the populations to each other. This supports Etten's (1982c) conclusions but may be an indication of the hazards of making a comparison based upon few loci.

Comparisons limited to one or two traits or loci have limited value in comparing populations. However, for the sake of completeness a number of such studies will be mentioned here. *G. pallidipes* from the Lambwe Valley and from Kibwezi Forest Kenya, and flies in colonies established from these locations, differ in the duration of copulation (Jaenson 1978, 1979a). Similarly female *G. pallidipes* from Nkruman and Mwalewa Kenya feed at different frequencies (Etten 1982a; see section on behavioural genetics). Despite separation in the laboratory for about 25 generations (in each colony) *G. m. morsitans* maintained in the Department of Entomology, University of Alberta, were not significantly different from the (parental) colony at the Tsetse Research Laboratory, University of Bristol, when the frequency of genotypes were determined at the loci *Xo*, *Ao*, (Rolseth and Gooding 1978) and *Alkph* (Gooding and Rolseth 1978; see sections on biochemical and molecular genetics and on linkage

groups.). Similarly three laboratory colonies and a field colony examined at the Lap_3 locus were found to be similar (Geest *et al.* 1978). Although limited in scope, this study is interesting since two laboratory colonies and a field population from Zimbabwe were nearly identical, while the Handeni colony showed less genetic variation and had one less rare allele. (See similar comparison by Gooding and Rolseth (1982), cited above.)

Intertaxon comparisons

Allele frequencies at 12 enzyme loci have been determined in colonies of nine taxa using polyacrylamide gel electrophoresis (Gooding 1982b). In each taxon in the *moristans* group four to eight of the loci were polymorphic, except in *G. m. submorsitans* where only two polymorphic loci were found. Within the *palpalis* group four or five of the loci were polymorphic in each taxon. Mean heterozygosity per locus was much lower in the *palpalis* group taxa (5.0 to 7.0%) than it was in most of the *morsitans* group taxa (11.7 to 21.0%). The exceptional subspecies in the later group was *G. m. submorsitans* which had a mean heterozygosity per locus of 2.4%. A phenogram based upon the allele frequencies in the colonies was, with two major exceptions, in agreement with the generally accepted arrangement of the taxa. The first exception was that *G. austeni* was clustered with members of the *palpalis* group rather than with the *morsitans* group. The second exception was that *G. m. submorsitans* (originating from Upper Volta) was less similar to *G. m. morsitans* and *G. m. centralis* than was *G. pallidipes*. (Other information derived from this comparative study is covered in sections on biochemical and molecular genetics and on linkage groups.)

Population genetics and tsetse colonization

Much of the impetus for studying tsetse flies has come from the need to colonize these flies for use in control projects. This aspect of tsetse population genetics, and related matters, will be reviewed here. Because of the need for producing males which are competitive with field males, much of the work has been concerned with effects of prolonged colonization, inbreeding and/or adaptation to laboratory conditions. After two years of colonization (i.e. approximately 12 generations), *G. m. morsitans* released into the field had the same survival, dispersal, and rate of recapture as did field flies, and under laboratory conditions laboratory reared males were competitive with field males (Dame *et al.* 1975). A previously conducted laboratory evaluation of longevity, age specific fecundity, and puparial weights using this same species showed that females from a laboratory colony (a mixed population colonized for approximately 6 to 18 generations) were slightly superior to females emerging from field collected puparia (Jordan *et al.* 1970) but this difference may have been partly due to effects of shipping puparia. These experiments offer some assurances that colonization of *G. m. morsitans* for moderate lengths of time does not result in significant genetic drift or selection in medium to large colonies.

The possible consequences of intensive inbreeding have been studied using *G. austeni* (Jordan 1970) and *G. m. morsitans* (Jordan 1980). An inbred colony of the former species (consisting of 10 males and 10 females per generation) died out after 16 generations, but this was probably due to husbandry problems not related to inbreeding. The intensively inbred colony of *G. m. morsitans* lasted 40 generations without showing significant changes in female longevity, female fecundity, puparial weight, emergence rate or sex ratio (Jordan 1980). This colony began with a female mated to one male, and breeding stock for subsequent generations generally consisted of 10 females mated with three males. (Due to husbandry difficulties, not related to the inbreeding experiment, generation 14 consisted of only one female and her mate.)

By generation 40 the inbreeding coefficient was 0.9347, compared with 0.0303 for the parental colony. No morphological changes were found in the inbred colony (Jordan 1980) but by generation 26, flies were homozygous for malic enzyme and alkaline phosphatase, and 31 of 32 flies were homozygous for leucine aminopeptidase (Geest, in personal communication cited by Jordan 1980). By generation 40, xanthine oxidase, aldehyde oxidase (Rolseth and Gooding 1978) and alkaline phosphatase (which is probably different from that referred to above, Gooding and Rolseth 1978) were monomorphic. The frequencies of the alleles which became fixed rose from 0.36, 0.89 and 0.35 for *Ao*, *Xo*, and *Alkph* respectively. The results of the electrophoretic studies provide independent confirmation of the high value of the inbreeding coefficient calculated by Jordan (1980). The full significance of this inbreeding experiment is difficult to assess since it was not replicated and the female used to begin the experiment lived much longer and was much more productive than the average female.

Despite concerns about the effects of inbreeding, adaptation to the laboratory, genetic drift etc., little or no effort has been made to monitor genetic changes within tsetse colonies. Colony performance is usually gauged by puparial weights, female longevity and fecundity, emergence rates, and sex ratio at emergence. These are probably all polygenic characters, closely associated with fitness and probably with low heritability (h^2). The use of electrophoretic techniques to monitor changes in tsetse colonies has been proposed, and techniques which permit examination of up to 12 loci from a single male have been developed (Gooding and Rolseth 1982). However, as far as I am aware, genetic monitoring of large colonies is not practiced and I doubt that such monitoring is likely in the absence of firm evidence that flies being produced within a colony differ significantly from field flies, or in the absence of a failure of laboratory reared flies to perform adequately under field conditions. *A propos*, a laboratory population of *G. p. gambiensis*, used to provide sterile males for an eradication project in Upper Volta, had less heterozygosity than, but was otherwise not significantly different from, a natural population adjacent to the site where a sterile male release program had been successfully carried out (Gooding 1981a). Nevertheless, further genetic studies on laboratory and field populations ought to be carried out to investigate what changes occur upon colonization of tsetse flies and the consequences of occasional introduction of field collected flies (or their offspring) into well-established colonies.

GENETIC ASPECTS OF RADIATION AND CHEMOSTERILANTS

General Aspects of Radiation Genetics

Exposing living organisms to X-irradiation or to γ-irradiation may cause somatic damage as well as a variety of genetic changes such as point mutations, chromosome rearrangements, and induced sterility through creation of dominant lethals. In this section I review the tsetse literature dealing with only the last three effects but do not cover the considerable amount of material which has been published on the use of the sterile male technique for control of tsetse flies.

In other organisms X- and γ-irradiation have been used to create mutants for genetic study, to create various chromosome aberrations as an aid to mapping loci, and to study the time of chromosome pairing and duplication. However, as far as I can determine from the literature, the first two approaches have not been attempted with tsetse flies.

The type of chromosome aberration induced by γ-irradiation depends upon the stage of meiosis at which irradiation is administered. For *G. m. morsitans* this has been established by irradiating females (with 700 rads using ^{60}Co) at various times during the second larviposition

cycle (Southern *et al.* 1975). (Under the conditions used, embryogenesis takes 96 to 120 hours, the first stadium lasts 26 h, the second stadium lasts 48 h, and larviposition takes place on the ninth or tenth day of the cycle.) Cytogenetic analysis of male progeny, nine to ten days after larviposition, established the following. The frequency with which translocations were created by radiation rose during the first 72 hours then declined to near zero by hour 120 of the larviposition cycle. The frequency of creating chromosome gaps and breaks (apparently induced in single-stranded chromosomes) rose in males irradiated during hours 48 to 120 then declined to near zero by hour 168 of the larviposition cycle. Chromatid aberrations begin to appear in males irradiated between hour 96 and 120; in flies irradiated between hours 144 and 168 these aberrations are the main, if not the only ones found. It was concluded by Southern *et al.* (1975) that by hour 144 chromosome duplication is advanced or even completed.

Chromosome translocations

Curtis (1968a) outlined methods for creating chromosome translocations by treating post-teneral male tsetse flies with less than sterilizing doses of irradiation, and described methods for identifying lines carrying translocations by determining reduced fertility in progeny of outcrossed individuals. (He also discussed the practical uses of chromosome translocations as they might be applied to tsetse control. See also Curtis and Hill 1971, and Curtis and Robinson 1971.) Translocations were produced in *G. austeni* by exposing nine day old males to 5 to 7 krad ^{60}Co γ-irradiation and were identified, as indicated above, on the basis of inherited semi-sterility (Curtis 1969a). The semi-sterility occured at a high frequency (ca. 34%) and in some lines was passed through males and females (and thus involved the autosomes) while in other families the inheritance pattern was holandric (indicating translocations involving the Y chromosome) (Curtis 1969a, 1969b, 1970b, 1971). Lines homozygous for translocation(s) (T/T) were believed to have been established (Curtis 1970b, 1971) but flies in at least some of these lines were less viable than wild type flies (Curtis 1971; Curtis *et al.* 1972). (The reduced viability was associated with reduced ability of T/T females to maintain normal pregnancies.) In two translocation lines the initial inheritance pattern indicated that the translocations involved autosomes, but after several generations there was a switch to a holandric inheritance pattern (Curtis 1971). Although several explanations were advanced, the nature of the switch-over was not established even though it was shown that after the switch-over the translocation involved the Y chromosome (Curtis *et al.* 1972). By examining some flies cytogenetically and their siblings by breeding experiments it was established (Curtis *et al.* 1972) that a translocation (in at least one line) caused partial sterility. Other lines with inherited partial sterility were also shown, by cytogenetic analysis, to have translocations (Curtis *et al.* 1972). In the cytogenetic studies cited above, and in work on *G. m. morsitans* reported by Curtis *et al.* (1973), the most common translocations involved exchange of segments from the long arms of L_1 and L_2. Other translocations involved exchanges between supernumeraries and L_1, Y or other supernumeraries.

Induction of Sterility by Irradiation

Most work on effects of radiation on tsetse flies has been directed towards induction of sterility in males by γ-irradiation and determination of somatic effects (notably effects on longevity and mating competitiveness) of this radiation. The work has been largely directed toward use of sterile males as control agents and the subject has been reviewed from this perspective several times (Dame 1970; Dame and Schmidt 1970; Jordan 1974, 1977, 1978;

Davidson 1978; Cuisance *et al.* 1980; Dame *et al.* 1980; Curtis and Langley 1982).

The preliminary work on radiation induced sterility used field collected puparia transported to laboratories in England (Potts 1958) or Zimbabwe (Dean *et al.* 1968; Dean and Wortham 1968; Dean and Clements 1969). Radiation induced sterility was first demonstrated in *G. morsitans* (probably *G. m. centralis* since the puparia were collected at Singida, Tanzania) by Potts (1958). These preliminary experiments were conducted under conditions which did not permit maintenance of self-sustaining colonies. The results indicated that about 65% of the males were sterilized by 5,760 rad when irradiated with γ-radiation from a ^{60}Co source at some time during the last two thirds of the flies' life in the puparium. A decade later it was shown that greater than 95% sterility could be produced in male *G. m. morsitans* by γ-irradiation of puparia within a week or two of emergence with 8 to 15 krad (Dean *et al.* 1968; Dean and Wortham 1968). Irradiation of younger puparia resulted in sterilization at doses as low as 4 krad but under these conditions more profound somatic effects were induced. Female *G. m. morsitans* are sterilized by as little as one or two krad applied to either puparia or to one day old adults (Dean and Wortham 1968). Similar results were obtained with *G. pallidipes*: females were completely sterilized by exposure of puparia, one to two days before adult eclosion, to 4 krad and approximately 90% sterility was induced in males exposed to 5 to 18 krad within 10 days prior to eclosion. The greatest effects were observed when younger puparia were exposed to γ-radiation (Dean and Clements 1969).

Following establishment of tsetse colonies in Europe it was possible to conduct more precise experiments using flies of known age under conditions which were more nearly ideal for maintenance of the flies. (Selected data on levels of sterilization induced by various doses of irradiation are presented in Table 5.) These studies were largely directed towards perfection of sterile male release techniques but they also provided an understanding of mechanisms by which sterilization was induced.

The effectiveness of irradiation in sterilizing male insects may be explained by either of two models. The first proposes that irradiation kills sperm or prevents their production. During mating dead sperm and/or accessory gland secretions are passed to females which are thus rendered "sterile" by one of two mechanisms. As a result of a single act of mating (even with a sterilized male) the female may become refractory and never mate with other males available to her. Alternatively the female that mates with a sterilized male may have her spermathecae, or spermathacal ducts, filled with dead sperm and/or accessory gland secretions from the sterilized male and be unable to accept and store viable sperm from a normal male. The second model proposes that radiation produces dominant lethal mutations in sperm of treated males, and that such sperm are able to compete with normal sperm and fertilize eggs but that the resulting embryos fail to complete development.

The evidence available clearly establishes the second of the above mechanisms as the explanation for radiation induced sterility in male tsetse flies. Sperm are motile in radiation sterilized *G. m. morsitans* (Dean and Wortham 1968), *G. pallidipes* (Dean and Clements 1969) and *G. p. palpalis* (Hamann and Iwannek 1981). Females mated with radiation sterilized male *G. austeni* (Custis 1968b, 1968c), *G. m. morsitans* and *G. tachinoides* (Itard 1970b, 1971a) will re-mate with normal males but produce few if any offspring. F_1 flies descended from irradiated (partially sterilized) male *G. tachinoides* (Itard 1973a) or *G. m. morsitans* (Curtis *et al.* 1973) were either sterile or semi-sterile indicating that there had been genetic damage to their fathers. Further evidence that dominant lethals are being created by irradiation is distortion of sex ratio resulting in an excess of males among progeny of partially

Table 5. Sterilization of laboratory reared male tsetse flies by irradiation.[1]

Species	Stage irradiated (age, days)	krad	percent sterility	reference[2]
austeni	adult, 10	5	70	A
		7	90	A
		12	98	A
m. morsitans	adult, 1	20-25	100	B
	adult, 3	10.1	67	C D
		14.6	92	C D
	adult, 4	20	96	C D
		25	100	C D
	puparium, ca 30[3]	7	72	E F
		10	92	E
		15	94	E F
		7 (in N)	60	E F
		10 (in N)	88	E F
		15 (in N)	94	E F
palpalis	adult, 4	11	94	G
		15 (in N)	94	G
p. palpalis	adult, 2	2[4]	84	H
		5[4]	94	H
		7.5-15[4]	100	H
tachinoides	adult, 1-9	6	68	B C D
		10	95	B C D
		15	98	B C D

[1]Unless otherwise indicated, all data pertain to flies exposed to γ-irradiation in air. The data in this table are not a complete summary but only a representative sample.

[2]References: A=Curtis 1968c; B, C, D=Itard 1968, 1970b, 1971a; E=Langley *et al.* 1974; F=Curtis and 1972; G=Curtis and Langley 1982; H=Hamann and Iwannek 1981.

[3]Puparia from which almost all females had emerged were stored for five days at 11°C then irradiated in either air or nitrogen.

[4]Irradiated with β-irradiation.

sterilized male *G. austeni* (Curtis 1968c), *G. tachinoides* (Itard 1973a) and *G. m. morsitans* (Curtis *et al.* 1973). The latter study was the most complete and it was suggested by the authors that sperm carrying an X chromosome were more likely to have had a dominant lethal

induced in them than were the sperm carrying a Y chromosome. Calculations of the number of dominant lethals in male and female zygotes, and calculations of the relative lengths of the chromosomes most likely to be susceptible to induction of lethal mutations (i.e. $L_1 + L_2 + X$ in female determining sperm and $L_1 + L_2$ in male determining sperm) were in general agreement with the above interpretation (Curtis *et al.* 1973). Female *G. austeni* which mated twice (once with a normal male and once with a male sterilized by exposure to 12 krad of γ-radiation) used sperm from the first mating (regardless of whether this was with a normal or a sterilized male) for about 70% of the fertilizations. This clearly establishes that sperm from sterilized males were fully competitive with normal sperm (Curtis 1968b, 1968c). Female *G. p. palpalis* which mated with males sterilized by γ-radiation ovulated in a normal manner on the eighth or ninth day after emergence and histological examination showed that each egg was fertilized but that development usually ended at cleavage division (Matolin and Vloedt 1982). Only rarely did development proceed to gastrulation and development of a more or less fully formed embryo was even rarer.

Induction of Sterility by Chemosterilants

The effects of aziridine chemosterilants on tsetse have been studied for about 20 years and a recent paper (Curtis and Langley 1982) summarized much of the information as it applies to control of tsetse by the sterile male release technique. Topical application (either directly or by having flies contact a previously treated surface) of apholate and metepa results in various levels of sterility in male and female *G. m. centralis* (Chadwick 1964). Similar experiments on *G. m. morsitans* have established the chemosterilizing ability of apholate (Dame *et al.* 1964), tepa (Dame *et al.* 1964, 1975; Dame and Ford 1966, 1967), metapa (Dame *et al.* 1964; Bursell 1977; House 1982) and bisazir (Coates and Langley 1982). Sterilization of male and female *G. pallidipes* by metepa has been demonstrated by House (1982).

Male *G. m. morsitans* are permanently sterilized by exposure to tepa (Dame and Ford 1966) and female *G. m. morsitans* mated to bisazir treated males do not regain fecundity with the passage of time (Coates and Langley 1982). After treatment with either of these chemosterilants sperm remain motile (Dame and Ford 1966; Coates and Langley 1982). From 14% to 45% of the fertilizations of twice mated *G. m. morsitans* females used sperm from the second mating, regardless of whether this or the first mating was with tepa sterilized males (Dame and Ford 1967). This indicates that the sperm of chemosterilized males are competitive with those of normal males. Some chemosterilant treated males fathered offspring which died within the puparia indicating that some of the lethal mutations induced by apholate and metepa in *G. m. centralis* (Chadwick 1964) and tepa in *G. m. morsitans* (Dame and Ford 1966) have an effect late in the development of the fly.

Several antibiotic sulfonamides interfere with reproduction of tsetse flies. The phenomenon has been studied most extensively in *G. austeni* and *G. m. morsitans* and the subject has been reviewed recently by Southern (1980). The sulfonamides cause degeneration of bacteroids in the midgut mycetome after flies have fed upon these compounds for about 19 days. About six to ten days later fragmentation of chromatin in nurse cells occurs but Rickettsia-like symbionts found in nurse cells and oocytes are not affected. The sulfonamides appear to adversely affect production of folic acid which is, among other things, a precursor of purines and thymine. This deficiency has an adverse effect upon DNA synthesis in polyploid nurse cells in which L_1, L_2, and X chromosomes are replicating in the lampbrush state. The overall effect of this degeneration is that nurse and follicular cells are unable to synthesize and transfer to the oocyte

the ribosomes and transfer-RNA essential for embryogenesis and thus sterilization of the female tsetse results (Southern 1980).

SOME GENETIC ASPECTS OF REPRODUCTION

General Comments

Some aspects of mating behaviour and reproduction in tsetse flies appear to result from an attempt by each individual to increase the frequency of its genes in the next generation. Thus males will attempt to mate with as many females as possible and to induce monogamous behaviour in mated females. The major strategy of females is to protect and nourish their offspring until they have matured and are nearly ready to pupariate. Although females become monogamous, as a result of stimuli received from males, they do so gradually and thus retain, for some period of time, the ability to "hedge their bets" by mating with other males. One might consider that male and female tsetse flies are playing an evolutionary game with their partners: males divide their sperm production into aliquots of a certain size so as to maximize the number of potential mates, and females have spermathecae a little larger than necessary for storage of sperm from a single mating and are thus able to accept sperm from at least one additional male. Some consequences of this "evolutionary game" are considered below.

Multiple mating by males

Multiple mating is to be expected in males of any organism but in tsetse flies, where meiosis occurs in pharate adults in the puparia, males are restricted in the number of females which they can successfully inseminate. *G. austeni* males, for example, can inseminate a maximum of 9 to 15 females (Curtis 1968b). The average volume of sperm transferred by *G. m. submorsitans* is 40% to 75% of the volume of the spermathecae and the amount transferred at the eighth mating is not significantly less than what is transferred at earlier matings (Pinhão 1980). Not all matings result in sperm transfer and with *G. m. submorsitans* from 7 to 23% of matings fail to result in sperm transfer and occasionally congenitally sterile males (i.e. those who never transfer sperm, though they mate repeatedly) are found (Pinhão 1980). Copulation without sperm transfer has also been observed with *G. pallidipes* (Jaenson 1979b). Whether these phenomena occur in the field as well as in the laboratory is not known. Individually marked male *G. pallidipes*, in the presence of females and other males in an observation chamber, vary considerably in the frequency with which they mate; some mate as often as four times in five hours while others do not mate at all (Rogers 1973a). There is no information available on the genetics of such variation.

Multiple mating by females

Females which have mated are less receptive than are virgin females. The physiological basis for this is a combination of physical stimuli (stimulation of tactile receptors in female genitalia during mating and distension of the uterus by the developing larva) and chemical factors (from male accessory glands) transferred to the female at copulation (Gillott and Langley 1981). Nevertheless, females will mate more than once, especially if given an opportunity to do so within a day or two of the first mating. Multiple matings were first demonstrated under laboratory conditions in *G. p. palpalis* (Jordan 1958) and have subsequently been demonstrated in other species. About 40% of *G. pallidipes* females given an opportunity to mate every day for the first 13 days after eclosion, did so more than once, and such females were more fertile than were females that mated only once (Jaenson 1979b).

About 12% of wild *G. pallidipes* females which were observed *in copula* for 15 minutes at bait animals, and then forcibly separated from their mates, were found to be inseminated (presumably during a previous mating experience). This provides some evidence that multiple mating does occur in nature, in at least this species (Rogers 1973b).

Use of sperm by multiply mated females

Observing multiple mating by female tsetse flies is not proof that sperm are being transferred on each occasion, or that sperm from more than one mating can be effectively stored and used. Evidence for use of sperm from more than one mating requires marking sperm in some way. This has been accomplished by use of tepa sterilized male *G. m. morsitans* (Dame and Ford 1967), radiation sterilized male *G. austeni* (Curtis 1968b, 1968c, 1970a) and genetically marked (*ocra* vs. wild type) *G. m. morsitans* (Kawooya 1977; Vloedt 1980). These experiments established that sperm from both inseminations may be used but that at each pregnancy there is a greater probability of using sperm from the first mating than from the second. Almost all *G. austeni* females mated first to radiation sterilized males and then to normal males eventually became pregnant, indicating that virtually every female that mates twice has the capacity to use sperm from the second mating (Curtis 1968c). By scoring the offspring of individual females mated with two genetically different males it was established that some females used sperm from both matings (Kawooya 1977; Vloedt 1980; and unpublished work in my laboratory). Considering the frequency of multiple matings and the frequency of using sperm from the first mating, Kawooya (1977) estimated that, in populations where females have the opportunity for multiple mating, about 10 to 20% of the progeny will be from second matings.

Evidence for use of sperm from two matings in nature is limited to the single observation of a *G. m. centralis* female which was recaptured from *G. swynnertoni* habitat and which produced one male offspring having typical *morsitans*-type genitalia and another having genitalia typical of *morsitans/swynnertoni* hybrids (Vanderplank 1947). Since parthenogenesis does not occur in tsetse flies, this female must have mated with, and used sperm from, both *G. m. centralis* and *G. swynnertoni*. The extent to which use of sperm from two different matings occurs in nature might be resolved using electrophoretic techniques but the task would not be easy.

Interspecific mating

Results of hybridization experiments have indicated genetic similarities and taxonomic affinities among some of the taxa of tsetse flies, have demonstrated some of the mechanisms for preserving the genetic integrity of various taxa, and have begun to define the limits to incorporation of alien genes into the genomes of some species or subspecies.

Despite the fact that tsetse flies have sex recognition pheromones which appear to be species specific, intertaxon mating occurs rather extensively among tsetse flies under laboratory conditions (Vanderplank 1944, 1947, 1948; Curtis 1972; Huyton *et al.* 1980). In cages where flies had an opportunity to mate with their own or another species, *G. pallidipes* engaged only in conspecific matings while *G. m. centralis* and *G. swynnertoni* mated randomly resulting in a high insemination rate (92 to 96%) but in only 10 to 24% of females producing offspring (Vanderplank 1944, 1947). More surprising than this was the result of another experiment alluded to by Vanderplank (1947) in which an undisclosed number of male *G. swynnertoni* and *G. m. centralis* were individually identified and allowed to mate with females of either their

own or the other species. With one exception, each male engaging in a conspecific mating on the first occasion did so again on the second occasion, and each male engaging in an allospecific mating on the first occasion repeated this the second time. The exception was a *G. swynnertoni* male which changed from conspecific to allospecific mating. In the absence of a detailed description of the numbers of males and females used in the experiment it is difficult to speculate upon its significance. Nonetheless, this experiment raises the question of whether males, of these species, vary in their preference for mates, or, whether males mate randomly on the first occasion and learn from this an acceptable experience. The genetic aspects of either explanation may be well worth investigating.

There is sometimes a marked discrepancy between the tendency of males and females of a given species to engage in allospecific mating. *G. austeni* females are attractive to only *G. austeni* males and *G. tachinoides* females are attractive to only *G. tachinoides* and *G. austeni* males (Huyton *et al.* 1980). However, *G. austeni* males were attracted to, and attempted to mate with, at least some females from each of seven taxa with which the males were placed, and *G. tachinoides* males attempted to mate with *G. m. morsitans* and *G. p. palpalis* females as well as *G. tachinoides* females (Huyton *et al.* 1980).

The female behaviour mentioned above indicates one mechanism by which the genetic integrity of the species is preserved. Other prefertilization mechanisms known to occur in tsetse include the inability of *G. austeni* males to transfer sperm to *morsitans* group females because of the structure of the males' genitalia (Southern 1980). Similarly *palpalis* group males sometimes fail to transfer sperm during allospecific matings and those which transfer sperm usually puncture the abdomen of the females, with their claspers, causing death of the females (Vanderplank 1948).

Experiments on interspecific matings have also been carried out in the field. Jackson (1945) placed large numbers of *G. swynnertoni* puparia and *G. m. centralis* puparia in a *G. swynnertoni* habitat and later collected mating pairs within about 90 meters of the release site. The number of conspecific and allospecific pairs collected demonstrated that mating between these species was random. In a similar but less extensive experiment Vanderplank (1947) found a female *G. m. centralis* mating with a male *G. swynnertoni* after release of *G. m. centralis* into a *G. swynnertoni* habitat.

Hybridization

Hybrids of closely related tsetse flies have been produced in the laboratory (Potts 1944; Vanderplank 1944, 1947, 1948; Curtis 1972; Southern and Pell 1973; Southern *et al.* 1973b; Curtis *et al.* 1980; Gooding 1982b) and evidence for hybridization in the field has been presented by Vanderplank (1947, 1949). The most complete tabulation of intertaxon matings, including those crosses which do and those which do not produce hybrid offspring, was presented by Vanderplank (1948). Earlier work on the subject (dating from 1907 to 1947) has been reviewed by Vanderplank (1948) and some of the later work has been reviewed by Southern (1980) and Curtis and Langley (1982). Practical implications of the subject have been reviewed by Jordan (1974) and Maudlin (1980).

In many hybridizing taxa there is a marked asymmetry in the suitability of females (Vanderplank 1944, 1947, 1948; Curtis 1972). For example the mating of *G. swynnertoni* females with *G. m. centralis* males produces far fewer offspring per female than does the reciprocal cross (Vanderplank 1944, 1947); mating *G. f. martinii* females with *G. f. fuscipes* males results in half as many females becoming pregnant as does the reciprocal cross

(Vanderplank 1948); and using *G. m. morsitans* males to inseminate either *G. m. centralis* or *G. morsitans submorsitans ugandensis* Vanderplank is far less likely to produce offspring than are either of the reciprocal crosses (Curtis 1972). These and other examples reported by Vanderplank (1948) suggest an interaction between the pregnant female and the embryo or larva which she is carrying. This suggestion is supported for the *G. f. fuscipes* / *G. f. martinii* model when one considers that, regardless of which species is the sperm donor, 93 to 100% of the eggs of the other species are fertilized *in vitro* and will develop to hatching, but *in vivo* hybridization produces far lower pregnancy rates (Vanderplank 1948). *In vitro* fertilization of eggs was accomplished for several species and in general the fertilization rate was higher than found for *in vivo* hybridization pregnancies (Vanderplank 1948). *In vitro* fertilization, like *in vivo* hybridization, occurred between taxa within a species group but never between taxa from different species groups (Vanderplank 1948).

Maternal aspects of hybridization

Females mated to allospecific males have lower fertility than they would have had if they had mated with conspecific males (Vanderplank 1944, 1947, 1948; Curtis 1972; Curtis *et al.* 1980). F_1 hybrid females, backcrossed to either parental taxon, show a further decline in fertility (Vanderplank 1948; Curtis 1972; Curtis *et al.* 1980), but fertility in hybrid females of subsequent generations (produced by repeated backcrosses to one parental taxon) rises as the genetic composition of the females approaches that of the ancestral taxon (Curtis 1972). Decreased female fertility is not due to cytoplasmic or chromosomal factors but rather it appears to be due to several loci resulting in some sort of genetic incompatability between the mother and her offspring (Curtis 1972; Southern *et al.* 1973b). The nature of this incompatability has not been elucidated but it should be noted that at least two sets of maternal gene products are transferred to the offspring. The first set consists of m-RNA, t-RNA, ribosomes *et cetera* produced by nurse cells and transmitted to oocytes. The second set consists of proteins, from the milk glands, which are fed to the larva *in utero*. There are ample opportunities for imbalances resulting from maternal and/or progeny genomes but none has yet been demonstrated.

Paternal aspects of hybridization

F_1 hybrid males, regardless of their parentage, are unable to fertilize females (Vanderplank 1947, 1948; Curtis 1972). Hybrid males from the *palpalis* group are usually unable to successfully copulate because of spines on their claspers which kill their mates; if these spines are removed copulation can take place and the hybrid males are fertile (Vanderplank 1948). Vanderplank (1947, 1948) reported that F_1 hybrid males from the *morsitans* group are able to transfer motile sperm to their mates but are, nonetheless, sterile. However, Curtis (1972) and Southern *et al.* (1973b), working with *G. morsitans* hybrids (*G. m. morsitans* X (*G. m. centralis* or *G. m. submorsitans ugandensis*), reported that the F_1 males were not able to inseminate females although they did have sperm with sub-normal mobility. The discrepency, between the reports of Vanderplank (1947, 1948) and those of Curtis (1972) and Southern *et al.* (1973b), may be due to strain differences or to environmental differences during the experiments.

Meiosis in male hybrids of *G. morsitans* subspecies proceeds normally and there was pairing of L_1 and of L_2 chromosomes throughout their lengths. The pairing of the X and Y was characterized by the section of the Y, characteristic of the paternal taxon, associating with the

appropriate section of the X chromosome. F_1 male hybrid sterility clearly does not arise from errors at meiosis (Southern *et al.* 1973b, Southern and Pell 1973).

Hybrid males, produced by backcrossing F_1 females to a parental taxon, (i.e. B_1 males) may be classified as either sterile or fertile (Vanderplank 1948; Curtis 1972). The relative numbers of each of these types led Curtis (1972) to suggest that there is a single locus controlling male fertility (via sperm mobility) with each of the *G. morsitans* subspecies being characterized by a unique allele at that locus. The subject was explored further by using the X chromosome marker *ocra* (see section on visible traits) in *G. m. morsitans* which were crossed to *G. m. centralis*. B_1 males were scored for body colour and insemination ability. The results demonstrated involvement of the X chromosome in the ability of B_1 hybrid males to inseminate *G. m. morsitans* and *G. m. centralis* and suggested that for fertility there must be compatability between the X chromosome and the Y and/or the autosomes (Curtis *et al.* 1980). The results were not as clear cut as might have been hoped and B_1 males capable of inseminating were found among both ocra and wild type males. It was suggested that this may have come about by genetic recombination in F_1 females resulting in separation of the marker locus, *ocra*, and the locus controlling sperm motility. However, a single, and rather limited, experiment found no evidence of genetic recombination in the region occupied by *ocra* and *salmon* on the X chromosome in hybrid F_1 (*G. m. morsitans* X *G. m. centralis*) females (Gooding 1982b). A more complete analysis of the genetic basis of male hybrid sterility must await creation of genetic strains which are appropriately marked at loci on each of the chromosomes.

CONCLUDING REMARKS

Tsetse flies were among the first insects to be recognized as vectors of disease causing organisms (see review by Service 1978) and (according to Curtis and Langley, 1982) they were the first medically important insects against which genetic methods of control were directed. It is ironic therefore that genetic studies of tsetse flies have lagged so far behind those of other medically important insects. Reasons for this, and for its recent partial redress, were touched upon in the Introduction. During the past two decades considerable information has been acquired on the genetics of tsetse flies and the subject should no longer be considered as in its infancy. With the exception of the *fusca* group, genetic studies have passed beyond the purely descriptive stage and the search for markers, and they have now reached a point where they may be applied to answering fundamental questions about these flies.

Studies of tsetse genetics have been undertaken primarily because of the medical and veterinary importance of these insects. Such studies have already made contributions to the control of tsetse flies and it is to be hoped that further contributions will be forthcoming. There remains the question of whether genetic studies with tsetse flies will contribute anything unique to the field of genetics in general. If such contributions are to be made they are most likely to be in the areas of genetics of transmission of disease causing organisms, genetics of reproductive physiology, functions of the B chromosomes, and the relationship between tsetse flies and their symbionts. These seem to me to be potentially profitable areas of study for they are areas where tsetse flies are distinctly different from *Drosophila* species and from almost all other readily studied vector species.

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