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Tsetse Genetics: Contributions to Biology, Systematics, and Control of Tsetse Flies

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Abstract

Tsetse flies (Diptera: Glossinidae) constitute a small, ancient taxon of exclusively hematophagous insects that reproduce slowly and viviparously. Because tsetse flies are the only vectors of pathogenic African trypanosomes, they are a potent and constant threat to humans and livestock over much of sub-Saharan Africa. Despite their low fecundity, tsetse flies demonstrate great resilience, which makes population suppression expensive, transient, and beyond the capacities of private and public sectors to accomplish, except over small areas. Nevertheless, control measures that include genetic methods are under consideration at national and supranational levels. There is a pressing need for sufficient laboratory cultures of tsetse flies and financial support to carry out genetic research. Here we review tsetse genetics from organismal and population points of view and identify some research needs.

Keywords

sex determination; population genetics; cytoplasmic incompatibility; sterile insect technique; chromosomal polymorphism

INTRODUCTORY REMARKS

Distribution and Importance of Tsetse Flies

There are 33 species and subspecies of tsetse flies (*Glossina*, Glossinidae), and all but 2 are restricted to sub-Saharan Africa (20,47,62). Tsetse flies are distributed discontinuously throughout their range, and each taxon is restricted to a relatively specific habitat (62). Adult tsetse flies are strictly hematophagous. All species of tsetse flies are probably capable of transmitting pathogenic trypanosomes. However, only a few species (such as *Glossina morsitans morsitans*, *G. m. centralis*, *G. pallidipes*, *G. palpalis palpalis*, *G. fuscipes fuscipes*, and *G. tachinoides*) are major vectors of trypanosomes that affect humans (causing African sleeping sickness) and domestic animals (causing a lethal wasting disease, nagana).

Trypanosomiasis had a profound effect on the development of sub-Saharan Africa (69) and continues to do so. Current losses due to trypanosomiasis in cattle are about 4.5 billion dollars (U.S.) per year, with about 3 million cattle deaths (8); major epidemics of human trypanosomiasis continue to affect people from Angola to Sudan (81).

Tsetse Life Cycle and Population Biology: Implications for Genetic Studies

Under ideal conditions adult tsetse flies may live several months, and during this time they feed approximately every other day. Tsetse flies are limited to short flights (<5 min) and fly only about 15 to 30 min day^{-1}, and a population that inhabits savanna can advance into suitable

habitat at approximately 7 km year^{-1}. All tsetse flies reproduce by adenotrophic viviparity, i.e., the fertilized egg embryonates and hatches in the female's reproductive tract and the larva feeds on material ("milk") produced by the female's reproductive accessory glands until it completes its development. The first mature offspring is produced when the female is about 16 to 17 days old, and subsequent progeny are produced maximally at approximately 9-day intervals. Mature larvae do not feed after parturition but simply burrow into the ground and pupariate; adults emerge about 30 days later. This method of reproduction severely limits the fecundity of tsetse flies, and although female flies have an upper limit of about 20 offspring, females average only 6 to 8 offspring in a well-managed laboratory colony and probably only about 2 reproducing offspring (averaged over all seasons) in nature. (For a recent summary of tsetse reproductive biology, see Reference 36.) The low fecundity of tsetse flies is said to make them ideal targets for genetic and other methods of control that target reproduction, although this has been questioned (80).

Each tsetse species harbors from one to three prokaryotic symbiont species, and these symbionts may provide opportunities to reduce the vector competence of tsetse flies (1). The most important symbiont, *Wigglesworthia glossinidia*, resides in a special bacteriome in the anterior part of the midgut and probably was a symbiont in the ancestor of all extant tsetse species; it likely produces one or more substances that are essential for tsetse reproduction. *Sodalis glossinidius*, a secondary symbiont not known to be essential for any tsetse species, is found in the midgut and other tissues of several tsetse species. *Wolbachia* is found in the gonads of some tsetse species and is probably inherited through a strictly maternal lineage. Its effects on tsetse flies have not been established, although in other insects *Wolbachia* has a variety of effects on their hosts, including inducing cytoplasmic incompatibility (19,93). Tsetse flies are not ideal subjects for traditional genetic studies because maintenance of laboratory colonies is labor intensive and requires a reliable source of antibiotic-free blood. At this writing, only one laboratory in North America, three in Europe, and four in Africa maintain tsetse flies.

The low fecundity of female tsetse flies, combined with a reproductive period spread over several months, greatly prolongs the time required to establish lines that are homozygous at the loci of interest, and has, to date, precluded the establishment of both isofemale and recombinant inbred lines. On the other hand, the reproductive biology of tsetse flies generally requires that the offspring from a large portion of the individuals in each generation be retained to establish the next generation, thus minimizing genetic drift within even relatively small colonies (30). Indeed, allozyme, mitochondrial, and microsatellite surveys of extant cultures show abundant diversities. Notwithstanding their low fecundities, tsetse populations are highly resilient principally through the operation of density-dependent factors (76–78).

Status of Tsetse Genetics and Objectives of this Review

Tsetse flies have been subjected to intense study because of their importance as vectors of pathogenic trypanosomes; the results of much of this work have been summarized by Leak (62). Most early work on tsetse genetics was reviewed by Gooding (25), but subsequent reviews were limited to the following specific topics: hybrid sterility (31), quality control in tsetse colonies (30), tsetse fly–trypanosome interactions (92), use of paratransgenics to suppress vector competence (1,5), and genetics of natural populations in relation to dispersal (51,54). Much of the recent literature has not been covered in these reviews, and no recent comprehensive review of tsetse genetics has been published. It seems appropriate to do this now for several reasons. There is a lack of suitable prophylactic drugs and vaccines against trypanosomiasis, and chemotherapeutic agents remain too expensive and dangerous to use for most people in endemic areas, thus vector control remains the most viable method for large-scale control of trypanosomiasis. However, "nearly 100 years of control efforts have failed to curb the distribution of tsetse fly infestations or the resulting incidence of trypanosomiasis in

Africa …" (72a). The control efforts used against tsetse flies include use of insecticides, habitat modification (including elimination of the flies' hosts), and eradication by release of sterile males. Currently, tsetse fly suppression is conducted in ~128,000 km^2, i.e., about 1.5% of the tsetse-infested area (2).

The failure to reduce significantly the area adversely affected by tsetse flies has made two things obvious. First, if the currently used methods are to be successful, additional information (such as the genetic structures and natural boundaries of target populations) is needed before tsetse flies can be effectively suppressed or eradicated. Second, it may be more effective to employ genetic methods to suppress tsetse populations or to prevent tsetse flies from serving as vectors of the pathogenic trypanosomes. Several methods have been suggested, and tsetse flies were the first insects against which genetic methods were employed (88), albeit on a limited scale. Given the current tsetse fly/trypanosomiasis problem in Africa and the interest in genetic approaches to vector control, this is an opportune time to present an analytical review of current knowledge of tsetse genetics and the prospects for applying this knowledge to ameliorate the tsetse fly/trypanosomiasis problem in Africa. However, space constraints force us to ignore much exciting work on the genetics of tsetse symbionts and tsetse fly–trypanosome interactions.

GENETIC VARIATION

Classical genetic variation includes the morphological and chromosomal. Morphological mutants, numerous in *Drosophila*, are rare in tsetse flies if for no other reason than low reproduction rates provide relatively few flies to screen. Moreover, their usefulness is limited to laboratory work because they usually confer a loss of fitness. Chromosomal variation includes inversions and these are most readily detected by cytological examination of polytene chromosomes that are transiently present in the trichogen cells of the thorax of pharate adults. However, relatively few preparations give satisfactory spreads. Mitotic and meiotic chromosomes also are useful and more easily prepared than polytene chromosomes.

Chromosome diversity is surprisingly abundant within and among tsetse species. Members of the subgenera *Glossina s. str*. and *Machadomyia* have karyotypes of 2N = 4 + XY plus heterochromatic, telocentric supernumerary chromosomes that vary in number within and among the taxa (85). Supernumerary chromosomes also occur in *G*. (*Austenina*) *brevipalpis*, which is also polymorphic for the number of chromosomes (two or three) that have rDNA loci, demonstrated by fluorescence in situ hybridization (94). Inversions occur in tsetse polytene chromosomes (23,48) and sex chromosome aneuploidy occurs in both natural and laboratory populations of *G. p. palpalis* (67,85).

Allozymes and isozymes in representatives of all tsetse subgenera have been examined (21, 37,39–41,57). These codominant genomic markers allow a reproducible means to study linkage, sex determination, and population genetics. There are two problems with using allozyme loci. First, freshly killed or frozen tissues are required in order to preserve enzyme activity. Second, population genetics studies assume that the genetic markers employed are selectively neutral, and evidence has accumulated that balancing selection acts upon some allozyme loci (64), including those in *G. pallidipes* (52). Allozyme diversity (i.e., heterozygosity) is the probability that a randomly chosen insect is heterozygous averaged over loci. Tsetse flies show low levels of diversity, averaging 6%, compared with other dipterans such as house flies, *Drosophila*, or stable flies, among which diversities vary from 10% to 19%. Heterozygosities at only polymorphic loci, however, average 27% in field-collected specimens of *G. morsitans s. l*. and 19.5% among colonies of *G. p. palpalis* and *G. p. gambiensis*. Expected heterozygosities are directly related to the effective (i.e., reproductive) population sizes and the mutation rates. Mutation rates at biochemical loci in animals are approximately 10^{-6}

gamete^{-1} generation^{-1}. Thus the rather low allozyme diversities in tsetse flies are best explained by their small population sizes. Nevertheless, sufficient allozyme diversity exists to allow research on the breeding structure of tsetse populations (58).

Microsatellites form a class of simple sequence repeats of various motifs, commonly in units of two different nucleotides, for example CA. Microsatellite loci are usually selectively neutral, well distributed in the nuclear genome, and highly polymorphic. Codominant alleles have different numbers of repeat units and can be distinguished by size, after amplification by the polymerase chain reaction. Amplification requires primers that anneal to conserved regions flanking the repeated sequence. Acrylamide gel electrophoresis separates the alleles, and the process can be automated and genotypes scored by using proprietary software. Microsatellite loci in the *palpalis* (65,82,84) and *morsitans* groups (4,72) have been characterized.

Abundant variation occurs at mitochondrial loci in *morsitans* group flies (56,59,60,96). Rather less variation was found in *G. palpalis gambiensis* populations (J.G. Marquez & E.S. Krafsur, unpublished data). Variants in mitochondrial DNA do not recombine and, with rare exceptions, are inherited matrilineally. Such diversity is particularly useful for examining maternal lineages and testing for bottlenecks in population size.

FORMAL GENETICS

Linkage Groups

Linkage groups, genes that are on the same chromosome, are established through standard "three-point-cross" experiments, and the number of linkage groups in a taxon equals the number of chromosomes that have functional genes. Cytogenetic studies have been carried out on at least some taxa in each subgenus of *Glossina* (2 of 15 *Austenina*, 4 of 9 *Nemorhina*, 1 of 2 *Machadomyia*, and 6 of 7 *Glossina s. str*.) (25). In all subgenera, except *Austenina*, sex chromosomes are structurally different, i.e., heteromorphic; females are XX and males XY. The basic number of chromosomes appears to be a pair of sex chromosomes plus two pairs of autosomes, a situation found in *Nemorhina*. In members of other subgenera the number of supernumerary or B chromosomes varies (0 to 8 in *Glossina s. str*., 8 to 12 in *Machadomyia*, and 12 to 22 in *Austenina*) (25,94). Functional genes on B chromosomes have not been demonstrated and thus the demonstration of only three linkage groups in *G*. (*N*.) *p. palpalis* (41), *G*. (*G*.) *m. morsitans* (40), and *G*. (*G*.) *m. submorsitans* (37) is consistent with the cytological information.

To establish linkage groups and map loci using standard genetic techniques, one must establish laboratory colonies that have different alleles at the variable loci. Few variable loci have been identified and brought to fixation in tsetse colonies, but these led to mapping 16 loci in *G. m. morsitans* (40), 10 loci in *G. p. palpalis* (41; R.H. Gooding, P. Solano & S. Ravel, unpublished data), and 8 loci in *G. m. submorsitans* (37). Where enzyme loci have been mapped in two or more tsetse species, the loci occur in the same linkage groups in each taxon. *Pgm* is in linkage group I (X chromosome) of all taxa; *Mdh* and *Pgi* are in linkage group III of all taxa; *Odh* is in linkage group II of *G. p. palpalis* and *G. m. morsitans*; and *Ao* and *Est-1* are in linkage group II of *G. m. morsitans* and *G. m. submorsitans*. Establishing linkage groups having few loci that are separated by nearly 50% recombination (specifically linkage group III) has been aided by the fact that, as is typical of the higher Diptera, there is little or no intrachromosomal recombination in male tsetse flies (40,41). The arrangement of biochemical marker loci indicates that the linkage groups presently in *Nemorhina* and *Glossina s. str*. were probably inherited intact from a common ancestor. However, those biochemical loci shared with other higher flies indicate that (at least some) tsetse flies have linkages that are different from those found in other higher flies (41).

Mapping loci on the X chromosome of *G. m. submorsitans* has been complicated by a complex of six inversions (23) that suppresses recombination in a region of approximately 24% recombination that is believed to have four loci (37). Inversions occur in X chromosomes and autosomes of several species of tsetse flies (summarized in Reference 23), but none have been associated unequivocally with variations in biological characters in any tsetse species.

At least 40 microsatellite loci have been identified in tsetse flies (4,65,72,84), but only 2 of these have been mapped, both in *G. p. palpalis* (R.H. Gooding, P. Solano & S. Ravel, unpublished data). It is unfortunate that more microsatellite loci have not been mapped in tsetse flies, as they would provide an excellent opportunity to compare the linkage groups in various tsetse species and thus contribute to our understanding of tsetse evolution. Such work is unlikely, however, because few laboratories maintain tsetse flies.

An additional shortcoming of our knowledge of tsetse linkage groups is that linkage groups II and III have not been associated with specific polytene chromosomes (86), nor with those identified by Giemsa C-banding patterns (85,94), nor with locations of rDNA loci (94). The latter study is the only one to establish a physical map for tsetse flies, and thus may be useful in future attempts to associate genetic linkage groups with specific chromosomes. The 28S rDNA loci are on the long arm of autosome L_2 in six representatives of the subgenera *Nemorhina*, *Machadomyia*, and *Glossina s. str*. However, in *G*. (*Austenina*) *brevipalpis* all individuals have two 28S rDNA loci located near secondary constrictions on long chromosomes. A few individuals have a third 28S rDNA locus on a long chromosome, but not on the chromosome's shorter homolog (94). The Y chromosomes of *G. austeni* and *G. pallidipes* have additional sites that bind probes for ribosomal genes, but whether these represent functional genes is not known.

A major limitation to linkage mapping in tsetse flies has been a dearth of marker genes. Use of expressed sequence tags has identified nearly 9000 genes in *G. m. morsitans*, of which about 4000 have been tentatively assigned functions, by comparison with data from *Drosophila* (63). This study may well lay the foundations for a tsetse genome project.

Sex Determination

Little is known about the sex determination mechanism in tsetse flies. In all subgenera, except *Austenina*, the sex chromosomes are structurally different. Sex chromosome aneuploidy occurs in tsetse flies; females may be XX, XXY, or XXXY, and males may be XY, XYY, or XO (67,85). The Y chromosome appears to be required only for production of motile sperm. This situation suggests that sexual phenotype is based on the ratio of X to autosomes, as in *Drosophila*. This possibility is supported by the occurrence, in laboratory colonies, of bilateral gynandromorphs and mosaic females (a likely outcome of mitotic nondisjunction early in embryogenesis).

Approximately equal numbers of males and females occur in most tsetse colonies. One or more X-linked factors cause sex ratio distortion in colonies of *G. m. submorsitans* from Nigeria and Burkina Faso (25–27,74). The factor has been treated as a single gene, *Sr*, (37), although it may be due to several genes located on one of two types of X chromosome found in *G. m. submorsitans*. The two types of X chromosome are distinguished from each other by three inversions in each arm (23). Males with the sex ratio distorter allele (Sr^d) sire few male offspring (37); those that are produced are usually sterile (23). There is no known effect of Sr^d in females. One would expect Sr^d to become increasingly frequent in a closed population (43), with the result that the proportion of males in the population may become so low that the population could become extinct. This has not happened in closed *G. m. submorsitans* colonies. Although the mechanism of preventing Sr^d from approaching fixation has not been established, there is evidence that genes on the Y chromosome within a population modulate or suppress

the expression of Sr^d from that population, but not from another population of *G. m. submorsitans* (23). This appears to be similar to the situation in *Drosophila simulans* (70).

Female tsetse flies have twice as many X chromosomes as males and dosage compensation occurs in *G. m. morsitans* and *G. p. palpalis* (38). There are three possible mechanisms for dosage compensation (inactivation of genes on one X in females, downregulation of transcription of both X chromosomes in females, or doubling the rate of transcription of X chromosome genes in males), but it is not known which mechanism functions in tsetse flies.

POPULATION GENETICS

Populations are dynamic. One objective of population genetics research is to obtain a snapshot of the population breeding structure. The Hardy-Weinberg rule, assuming no dynamism at all, states that gene frequencies will be homogeneous among sampled populations if matings are random, the genetic variation examined selectively is neutral, the mutation rate is negligible, and the sampled populations are infinitely large. Most deviations from these initial assumptions are caused by departures from random matings within and among populations and population sizes that are not large. Thus, we can test hypotheses about gene flow and population sizes by sampling a series of populations and estimating the amounts and spatial components of gene diversity.

With free exchange of reproducing flies and random mating, gene frequencies in populations approach homogeneity, and among-population variance in gene frequencies is small. Variance in gene frequencies increases with greater genetic divergence. The standardized variance in gene frequencies among subpopulations is termed F_{ST}, and this parameter is also defined as the correlation between two randomly chosen gametes in a subpopulation, relative to the correlation in the population as a whole. F_{ST} represents the departure from random mating among subpopulations. The departure from random mating within subpopulations is F_{IS} and this statistic is normally close to zero. An F_{IS} estimate significantly greater than zero, when averaged over loci, can indicate the sample contained individuals from two or more subpopulations that differ in their allelic frequencies—the Wahlund effect. F_{ST} can be related to migration and dispersal by various theoretical models. According to Wright's island model, the mean number of breeding migrants, *Nem*, in a generation is related to F_{ST}, thus $F_{ST} = (1 + 4N_em)^{-1}$. In principle, numerically little gene flow among populations prevents genetic differentiation by drift. Moreover, the amount of gene flow is virtually independent of the sizes of the populations and thus the "critical" level of gene flow is about one reproducing fly per generation. Below this number genetic differentiation increases by random drift to fixation, and above it further differentiation, as a result of drift alone, does not occur (97).

Ecological work on tsetse flies indicates a great capacity for dispersal and mixing of populations (7,75), so F_{ST} estimates near zero were expected. It was therefore surprising that the spatial patterns of allozyme diversities in 11 *G. pallidipes* populations from Kenya, Zambia, Zimbabwe, and Mozambique indicated a high level of population structuring (mean $F_{ST} = 0.25$) and a correspondingly low average rate of gene flow (*Nem* = 0.75). Moreover, mating was random within populations (58). The F_{ST} estimate of 0.25 was supported by further allozyme work on Kenyan and Ethiopian *G. pallidipes* samples (E.S. Krafsur, unpublished data) and by diversities at microsatellite loci (54). A study of mitochondrial variation in *G. pallidipes* disclosed an even greater degree of genetic differentiation among 18 populations from Ethiopia, Kenya, Zambia, Zimbabwe, and Mozambique. Particularly large differences between East and southern African *G. pallidipes* populations were observed (60). Mean diversity in the East African populations was 0.55 ± 0.25, and that in southern African populations was 0.15 ± 0.19. If Mozambique is excluded, diversity becomes only 0.09 ± 0.13, averaged over the Zambia and Zimbabwe samples. High levels of genetic differentiation at

mitochondrial and microsatellite loci also characterized *G. morsitans s. l.* in East, West, and southern Africa, and diversities of southernmost populations were reduced (55,56,59,96).

The north-south contrast in *G. pallidipes* is different at allozyme loci. Allozyme diversity among southern populations was 0.21 ± 0.08 compared with 0.16 ± 0.08 in the northern populations, providing empirical evidence for balancing selection favoring heterozygotes. Balancing selection cannot occur with mitochondrial loci because mitochondrial variation is single copy and maternally inherited. Thus, mitochondrial variation is more subject to loss than are alleles at diploid loci when populations become small. The bottlenecks detected in southern *G. m. centralis* and *G. pallidipes* populations are consistent with the historical record that indicates a great loss of mammalian hosts to a rinderpest epizootic that began in 1887 (22).

In West Africa, two X-linked microsatellite loci in *G. p. gambiensis*, sampled in Senegal and Burkina Faso, indicated genetic differentiation on macrogeographic and microgeographic scales (82,83). *G. p. gambiensis* populations from Mali and Senegal were highly differentiated from each other (F_{ST} analog $G_{ST} = 0.81$) at mitochondrial loci, but Senegal populations were monomorphic and diversity in Mali was only 0.28 (J.G. Marquez & E.S. Krafsur, unpublished data). *G. p. gambiensis* is riverine and experiences seasonal expansions and contractions. *G. m. submorsitans* in The Gambia showed much less diversity at mitochondrial loci than populations in Ethiopia. In this species, $F_{ST} = 0.35$ (59) and was later confirmed by using microsatellite loci (55). The paucity of genetic variation in Senegal and The Gambia can be attributed to earlier tsetse fly control projects.

Work Needed on Population Genetics

Genetic evidence indicates that most sampled *morsitans* group and *palpalis* group populations are highly differentiated and exchange reproductives at surprisingly low rates. Chromosomal and sex-determining polymorphisms likely provide isolating mechanisms. Additional lines of evidence consistent with the hypothesis of low effective dispersal rates were briefly reviewed (54).

There is substantial ecological evidence that tsetse flies, particularly the savanna group, are highly vagile. An estimated root mean square displacement of 200 m day^{-1} suggests a rate of advance of about 7 km $year^{-1}$ (75,95). Other estimates based on mark-release-recapture experiments suggest daily mean square displacement rates in *G. pallidipes* of up to 1.1 km (7,45). These ecological estimates of vagility appear to contradict the indirect estimates of gene flow obtained from population genetics. However, genetically derived estimates of N_em do not necessarily contradict the ecological estimates because the two indices measure different things. N_em is the mean number of reproducing flies exchanged among idealized subpopulations (i.e., at mutation-drift equilibrium) of equal and constant size, averaged over many generations. Moreover, N_em is a nonlinear function of F_{ST}, so that its average value is not very useful where gene flow differs greatly among the various subpopulations. Indeed, N_em is not an instantaneous measure of dispersal. What does it really tell us?

The answer to this question lies in the fact that genetic differentiation at selectively neutral loci occurs largely because genetic drift within subpopulations is greater than gene flow among them. The effects are cumulative over time. Dispersal without a high rate of per capita reproduction will have no detectable genetic effects and the genetic differentiation of subpopulations via drift will be unimpeded. Thus, the seeming disparity between high rates of dispersal measured ecologically and indirect measures based on gene frequencies can be the result of dispersal without reproduction. Reproductive failure of dispersed flies may have many causes, one of which may be natural selection in which immigrants (or their progeny) are at a selective disadvantage.

Thus, we need to learn in some detail the relative strengths of selection, drift, and dispersal in establishing the gene frequency patterns observed among tsetse populations. Chromosomal polymorphisms, tsetse vector–trypanosome interactions, and polymorphisms in sex determination may be important mechanisms that isolate conspecific tsetse populations. More extensive geographic sampling of the principal species is also required.

SYSTEMATICS

All tsetse flies are members of the genus *Glossina*, which has been considered a member of the family Muscidae but is now recognized as the only genus in the Glossinidae. This family is grouped with three other families that reproduce by adenotrophic viviparity (Hippoboscidae, Streblidae, and Nycteribiidae) in the superfamily Hippoboscoidea (68). McAlpine's classification (68) of the higher flies is based largely upon morphology and is supported by DNA sequence data for the 28S rRNA gene (90).

There are a number of unresolved problems and anomalies in the currently accepted arrangement of the taxa within *Glossina* (47). The problems include the placement of *G. austeni* in an appropriate subgenus, the evolutionary relationships among the subgenera (or species groups), the taxonomic status of several nominal species and at least one "variation" of a subspecies, and the (perennial) question of whether there are cryptic species of tsetse flies. Upper Oligocene *Glossina* fossils from Florissant CO were shown to be a closely related sister group to extant *Glossina*; they are, therefore, irrelevant to the biogeography of extant tsetse taxa (42) now confined to Africa and the southernmost Arabian peninsula. Here we consider only those systematics problems for which there is some genetic information.

On the basis of the structure of male and female genitalia, as well as ecological information, extant tsetse flies are usually divided into three species groups or their corresponding subgenera: *fusca* (*Austenina* Townsend), *palpalis* (*Nemorhina* Robineau-Desvoidy), and *morsitans* (*Glossina s. str*. Wiedemann) (47). This classification has one major anomaly: *G. austeni*, which is traditionally placed in the *morsitans* group, has characteristics of *Glossina s. str*. (male genitalia), *Austenina* (female genitalia), and *Nemorhina* (habitat preferences and some external structures of adults) (18). This problem was resolved by Dias (18), who erected the subgenus *Machadomyia* Dias to include two subspecies of *G. austeni*. The first genetic evidence indicating that *G. austeni* was not a member of *Glossina s. str*. was found before the formal erection of the subgenus *Machadomyia*. Allele frequencies (at 12 enzyme loci determined in laboratory colonies of *G. austeni*, four other taxa in *Glossina s. str*., and four taxa of *Nemorhina*) placed *G. austeni* either within the subgenus *Nemorhina* or as the sister group of that subgenus (24). The analysis was later extended (using 10 enzyme loci) to include one additional member of *Glossina s. str*. and two species of *Austenina* (39), with the result that *G*. (*M*.) *austeni* was placed as the sister group of *Glossina s. str*. DNA sequence data (based on a 126-bp sequence of a conserved region of rDNA in two subspecies of *G. morsitans s. l*., *G. austeni*, and two closely related *Nemorhina* spp.) indicated that *G. austeni* is closer to the *G. morsitans* subsp. than to the *Nemorhina* spp. (12). However, the limited number of taxa studied makes it difficult to know whether this work contributes much to answering the question of the validity of the status of the subgenus *Machadomyia*. The position of *G. austeni* was subsequently addressed using the DNA sequences of internal transcribed spacer-2 (ITS-2) of tsetse flies and the evolutionary relationships of the primary symbiont of tsetse flies, *Wigglesworthia glossinidia*, based on the 16S rDNA sequences of the symbiont (10). The genetic evidence is consistent with Dias' placement of *G. austeni* (18) in a distinct subgenus within the genus *Glossina s. l*. However, the available genetic evidence does not provide guidance on where to draw the boundaries between subgenera of *Glossina s. l*., and the question of whether to accept the subgenus *Machadomyia* hinges on this point. The main advantage of placing *G. austeni* in *Machadomyia* is that this eliminates the necessity of pointing out, in each

discussion of flies in the *morsitans* group (which traditionally includes *G. austeni*), that *G. austeni* is an "aberrant" species within the group.

The failure to identify the sister group of *Glossina* has meant that the hypothesized ancestral state of each character used to define the subgenera is not stated unequivocally and thus polarization of characters is not established. Identifying the sister group of *Glossina* is difficult because the reproductive biology of the Hippoboscoidea is markedly different from that of the other higher flies, and because Glossinidae is the most generalized family within the Hippoboscoidea. The result is that there are several conflicting theories, summarized by Potts (73) and Jordan (47), about the relationships among the subgenera. The differing views arise from assumptions about the habitat occupied by the ancestors of tsetse flies (lowland rain forest or wooded savanna), and whether complex genitalia are indications of advanced evolution or the age of a taxon. It seems to us that the most objective assumption that can be made is that the genetic differences among species will be greatest within the oldest subgenus, if genetic changes occur at approximately the same rate within all tsetse species. The greatest differences, among taxa within the same subgenus, were between *G.* (*A.*) *longipennis* and *G.* (*A.*) *brevipalpis*, both for allele frequencies (39) and DNA sequences in ITS-2 (10). This conclusion is supported by the observation that interspecific mating and hybridization do not occur among the *Austenina*, but do occur among species and subspecies of *Nemorhina* and *Glossina s. str*. (summarized in Reference 31). (Hybridization of the two subspecies of *G.* (*M.*) *austeni* has not been attempted.) The studies by Gooding et al. (39) and Chen et al. (10) differ with regard to the order in which the subgenera *Nemorhina*, *Machadomyia*, and *Glossina s. str*. evolved. DNA sequences (ITS-2 from the flies and 16S rDNA from their symbionts) are undoubtedly more reliable than allele frequencies for determining relationships among taxa, so the strongest evidence indicates that the two most closely related subgenera are *Machadomyia* and *Glossina s. str*. (10). The foregoing conclusions must be tempered by the fact that the data were obtained from laboratory colonies and only 60% of the taxa from three subgenera and only 13% of the species and subspecies of *Austenina* have been examined.

The status of several tsetse taxa has been questioned (reviewed in Reference 47), but genetic and hybridization studies have addressed only one major question, namely the relationships among the subspecies of *G. morsitans s. l.* and whether *G. swynnertoni* is a subspecies of *G. morsitans s. l.* The relative fecundity of hybrid females (relative to that of females from colonies of *G. m. morsitans*, *G. m. centralis*, and *G. m. submorsitans ugandensis*) indicated that *G. m. centralis* is more closely related to *G. m. submorsitans ugandensis* than to *G. m. morsitans* (15). This conclusion is at variance with the conclusion based upon allozyme allele frequencies (24) and restriction mapping of an approximately 2.93-kb fragment of mtDNA (87). The latter studies used *G. m. submorsitans* from West Africa and group *G. m. morsitans* and *G. m. centralis*. The discrepancy likely arose from using *G. m. submorsitans* from different regions; the results lend credence to the suggestion (66) that there has been introgression of genes from *G. m. centralis* into East African *G. m. submorsitans*, resulting in a distinct variant: *G. m. submorsitans ugandensis*.

A more extensive hybridization study, using two colonies of *G. m. morsitans* (from Tanzania and Zimbabwe), one colony of *G. m. centralis* (from Tanzania), and two colonies of *G. m. submorsitans* (from Nigeria and Burkina Faso), produced five phenograms illustrating the relationships among the populations studied. One phenogram was based on allele frequencies at 12 biochemical loci, and the others were based on hybridization indices (using proportion of females fertilized in intercolony crosses, puparia produced per female, F_1 adults produced per female, and the proportion of hybrid females fertilized in backcrosses). There were no statistical differences between the phenograms, all of which grouped *G. m. morsitans* and *G. m. centralis* as sister taxa (26).

In a series of studies in which flies, with genetic markers on each chromosome, from subspecies of *G. morsitans s. l.* were crossed and backcrossed, it was established that the major cause of hybrid male sterility was an apparent incompatibility between sex chromosomes from two taxa (28,29,33). There were asymmetries in the success of reciprocal crosses. The *G. m. morsitans* × *G. m. centralis* cross was more productive than the reciprocal cross, and almost all *G. m. submorsitans* males were unable to fertilize *G. m. morsitans* or *G. m. centralis* females. There was evidence that a maternally inherited sterility factor (MISF) was associated with hybrid male sterility, at least in the backcross males in experiments that originated with *G. m. morsitans* × *G. m. centralis* (28). It was hypothesized that MISF occurred in the ancestor of *G. morsitans s. l.* and that MISF underwent a series of mutations. Furthermore, it was assumed that females with the mutant form of MISF can be fertilized by males that have either ancestral or mutant MISF, but females with ancestral MISF can be fertilized only by males with the ancestral form. The model provides a mechanism for rapid spread of the maternally inherited factor. [It is not known whether MISF is *Wolbachia*, but this prokaryote occurs in *G. m. morsitans* and *G. m. centralis*, but not in *G. m. submorsitans* (71).] A hypothesis, to explain the evolution of the sex chromosomes in *G. morsitans s. l.* (28), assumed that in the derived population there were two mutations on the X chromosome ($X^1 \rightarrow X^2 \rightarrow X^3$) and one mutation on the Y chromosome ($Y^1 \rightarrow Y^2$). Furthermore, it was assumed that for relatively short periods a population could be polymorphic for either sex chromosome and that only two combinations of sex chromosomes (X^1/Y^2 and X^3/Y^1) were sterile. On the basis of available data, both hypotheses place *G. m. centralis* as the least derived subspecies and *G. m. submorsitans* as the most derived (28,29,31).

The question of the status of *G. swynnertoni* arises from the finding that, under laboratory conditions, *G. swynnertoni* hybridizes as readily with *G. morsitans s. l.* as the subspecies of *G. morsitans* hybridize among themselves (88,89). Furthermore, *G. swynnertoni* apparently mates readily with *G. m. centralis* under field conditions (46). Phenograms, based on allele frequencies at 10 enzyme loci (39), the presence of 40 cuticular alkanes in adult tsetse flies (9), and sequence data for ITS-2 (10), placed *G. swynnertoni* within *G. morsitans s. l.* However, there was no consensus on exactly where *G. swynnertoni* should be placed. Hybridization experiments suggest that *G. swynnertoni* is most closely related to *G. m. centralis*, since reciprocal crosses between these taxa yield offspring, whereas *G. swynnertoni* females cannot be fertilized by *G. m. morsitans* or *G. m. submorsitans* (34,35). One major difference between the results of hybridization of *G. swynnertoni* with the subspecies of *G. morsitans s. l.* and hybridization among the subspecies of *G. morsitans s. l.* is that in experiments involving *G. swynnertoni* sterile males and females persist at high frequencies, through several backcross generations.

Because of the importance of some tsetse species as vectors of pathogenic trypanosomes, the patchy distribution of sleeping sickness foci, and the discontinuous distribution of tsetse species, the question of whether there are cryptic species of tsetse flies must be considered. There are two approaches to this problem; both involve extensive sampling over most, if not all, of the geographic range of the species of interest. The first approach relies on population genetics analyses and the second on hybridization experiments; each has its advantages and drawbacks. The most productive approach would likely be to use the results of extensive population genetics surveys to identify those populations that have significant genetic differences and then to conduct hybridization experiments to determine whether the genetic differences are chance events or indicative of intrinsic reproductive isolation.

To date, most sampling for both population genetics studies and establishment of colonies has been largely opportunistic. Nonetheless, the genetic variation found at allozyme, microsatellite, and mitochondrial loci indicates restricted gene flow in *G. pallidipes* sampled from Ethiopia south to Mozambique and Zimbabwe (52,58,60); *G. m. morsitans* in Mozambique, Zimbabwe,

Zambia, and Tanzania (96); *G. m. centralis* from Zambia, Angola, and Botswana (56); *G. m. submorsitans* from The Gambia and Ethiopia (59); and *G. p. gambiensis* in Senegal, Mali, and Burkina Faso (66a,82,83).

Three attempts (31,48,61) to uncover reproductive isolation between tsetse flies (*G. m. centralis*, *G. m. morsitans*, and *G. pallidipes*) that have been raised in culture from different geographic areas failed to find evidence of cryptic species. However, significant bionomic differences between tsetse flies from different regions occur in *G. m. morsitans* (48) and *G. pallidipes* (61), and sex ratios and hybridization patterns differ between *G. m. submorsitans* populations from East and West Africa (32,33). Recently, during linkage mapping experiments that utilized *G. p. palpalis* from colonies originating in Nigeria and Zaire, sterile males were found among the F_1 and backcross generations, raising the possibility that one of the two populations came from a cryptic taxon (R.H. Gooding, P. Solano & S. Ravel, unpublished data). Overall, the literature indicates sufficient genetic variation throughout a species' range to justify a systematic search for cryptic species, particularly in taxa such as *G. m. submorsitans*, which exists as discontinuous populations over a wide geographic range, and *G. palpalis s. l.*, which tends to be associated with river systems.

GENETIC METHODS OF POPULATION SUPPRESSION

Tsetse flies have long been thought to be susceptible to genetic control methods because of their relatively low reproductive rates (50). A female must live at least 26 days to produce two mature larvae. The generation time for most savanna species is about 45 days (22). Under ideal conditions, tsetse flies can increase at a per capita rate of 2% per day (95), which leads to a population doubling in 36 days.

Methods for genetic control of tsetse flies include the introduction of sterile males into a population, cytoplasmic incompatibility, and the use of semisterility that arises from certain chromosome rearrangements. The object of the sterile insect technique (SIT) is to reduce the fertility of target populations to the extent that they cannot replace themselves, i.e., birth control. Cytoplasmic incompatibility can be used, in theory, to introduce high genetic loads into a natural population and replace it with insects deemed less noxious. The object of using semisterile mutants is also twofold: to introduce desirable genes into natural populations while inducing a high degree of sterility (14).

The suggestion of a genetic control method for tsetse flies predates Knipling. Unknown to Knipling, Vanderplank (88) suggested the use of sterile hybrids that obtain when reciprocally crossing two related species or subspecies. Hybrid inviability or sterility commonly results when crossing closely related taxa, and they are most clearly expressed in the heterogametic sex. Sterility among tsetse hybrid males is most often associated with the presence of sex chromosomes from two taxa; occasionally autosomes are involved and maternally inherited factors (possibly *Wolbachia*) may be involved in hybrid male sterility in the *morsitans* group (31,33,35). Vanderplank (88) and colleagues carried out an experiment, in 1943, in which *G. swynnertoni* was eliminated from a region of Tanzania that had experienced a human trypanosomiasis epidemic. *G. swynnertoni* is allopatric to the closely related *G. morsitans* and they freely mate with each other. Vanderplank released into an isolated *G. swynnertoni* population field-collected *G. m. centralis* puparia. Hybrid females were semisterile and the males were completely so. At the end of the trial, *G. swynnertoni* had been replaced by *G. m. centralis*, but *G. m. centralis* could not survive long in the region because of its aridity. The region then became essentially tsetse fly–free and was reinhabited by the people who were driven out by the earlier trypanosomiasis epidemic. This was the first field trial of a genetic control method. No further fieldwork on hybrid sterility in tsetse flies has been reported.

Sterility can be induced by exposure to ionizing radiation that causes dominant lethal mutations in their gametes. SIT field trials require the mass production, sterilization, and release of radiosterilized puparia or adult male tsetse flies and have been carried out in Zimbabwe, Burkina Faso, Nigeria, Tanzania, and, most recently, on Unguja island, Zanzibar, where eradication of *G. austeni* from Zanzibar was achieved (91). These have largely been "proof of principle" projects, designed to develop methods and to establish whether target wild populations could be eliminated.

There are, however, more conceptually sophisticated genetic tools for tsetse fly control than for sterile male release. Reciprocal interchanges between nonhomologous chromosomes cause semisterility in heterozygotes. About one half of the normal fertility is observed in simple interchanges between two linkage groups because heterozygotes produce duplication-deficient gametes. Semisterility is heritable: One half of the progeny of a heterozygous by wild-type arrangement or homozygous rearrangement are interchange heterozygotes. Higher levels of sterility are achieved when more than two linkage groups are involved. Interchange homozygotes, on the other hand, should be normally fertile. Curtis (13) suggested that the release of strains of tsetse flies made homozygous for one or more interchanges could be used to induce high levels of sterility in target populations. Because interchange heterozygotes are less fertile than either homozygous arrangement, an unstable equilibrium will obtain between the two, leading to a frequency-dependent increase in the favored karyotype. Genetic recombination between karyotypes is reduced, and thus Curtis (14) wrote that interchange homozygotes could be used as a transport mechanism to drive and fix desirable genotypes into a target population. Such desirable genes in tsetse flies might include conditional lethal traits and refractoriness to trypanosome infection. Curtis produced numerous chromosome interchanges in *G. austeni*, but no viable homozygotes were obtained. It seems that most radiation-induced rearrangements are lethal or semilethal when homozygous.

Cytoplasmic incompatibility (CI) is another theoretical method to enhance SIT and drive desirable genotypes into a target population (16). CI is caused by *Wolbachia*, intracellular prokaryotes that typically reside in the ovaries, testes, and other tissues of infected insects. The organisms are maternally transmitted. Infected females usually are fertile and fecund, but uninfected females inseminated by an infected male produce eggs that do not undergo embryogenesis. This asymmetry in fertility causes the proportion of *Wolbachia*-infected insects to increase in a population, given adequate fidelity of vertical transmission and strength of the CI. Therefore, a novel *Wolbachia*-infected strain carrying a maternally inherited factor such as one or more antiparasitic genes could be driven into a natural population, diminishing its vector potential. Symbiotic gut bacteria characteristic of tsetse flies can in principle be engineered to express antiparasitic substances (1,6). Wolbachia has been detected in G. m. morsitans, G. m. centralis, G. swynnertoni, G. brevipalpis, and G. austeni, but not in G. m. submorsitans, G. p. palpalis, G. p. gambiensis, G. longipennis, G. pallidipes, G. fuscipes, or G. tachinoides (11,71).

Critique and Comparative Evaluation of Genetic Control Measures

Space does not allow adequate treatment of feasibility of genetic control methods for tsetse flies. We can acknowledge, however, the contentious debate set off by the publication of the Pan African Tsetse and Trypanosomiasis Eradication Campaign by the Organization of African Unity and African Heads of State in 2000 (17,45,49). The campaign would incorporate traps, targets, judicious use of insecticides, and SIT.

Unlike CI and chromosomal rearrangements, SIT technology is available for use today even while undergoing progressive refinements. Unfortunately, experience with SIT has not taught us as much as it should have because most field trials confounded treatments and failed to measure important variables. The Zanzibar data (91), however, allow analysis. Ratios of 10

sterile flies to 1 fertile fly were required to achieve downward trends in target population density. Because of their low reproduction rate, mass production of sterile tsetse flies is limiting. Competitiveness of sterile, released flies is therefore an issue and needs to be improved if eradication is to proceed on an area-wide basis. Even though tsetse populations are highly resilient (79) and can recover from as few as 16 fertile females (45), they are indeed susceptible to high genetic loads. Hargrove's analysis (45) of the Zanzibar data shows that a target tsetse population declines when sterile mating rates of 20% to 58% are achieved, depending on its assumed daily mortality rate. A more hypothetical sterile mating rate threshold, ζ, at which a downward trend in population occurs can be estimated by $\zeta \approx 1 - R_0^{-1}$, where R_0 is the net reproductive rate (53). For a thriving tsetse population in which the mean daily mortality rate is 1.5% to 2%, the mean age at first reproduction is 15–16 days and the frequency of subsequent reproductions thereafter occur at 9- or 10-day intervals, ζ varies from 50% to 70%. A sterile mating rate of 25% would cause a population to decline for which the daily mortality rate is 3%. Achieving the foregoing levels of sterile matings is not insurmountable (91), and sterile mating much in excess of the threshold only weakly increases the rate of decline in a target population (53). It seems to us, therefore, that two principal issues must be addressed in using SIT or other genetic methods to help eradicate a tsetse species from a large area. The first issue in achieving tsetse fly eradication by a genetic control method is to produce enough competitive flies for release against natural populations, and the second is contending with the likelihood of recolonization from unchallenged populations.

Competitiveness is adversely affected by radiosterilization and can be avoided, in principle, by using CI and engineered symbionts. CI, however, still requires a great deal of laboratory research and development. For now, ways must be found to improve competitiveness of radiosterilized tsetse flies. The question of density dependence in competitiveness requires investigation after it was shown that it seemed to vary inversely with sterile male abundance in *G. p. gambiensis* (77). It is important to learn how competitiveness varies with target population density, thus calling for field research without the confounding effects of insecticidal treatments.

Reinvasion of areas from which tsetse flies have been eradicated is a serious prospect (44, 45). Indeed, it may be amplified by density-dependent factors (78). Tsetse fly dispersal is the subject of a rich literature of observational, experimental, and analytical components. On the other hand, inferences based on gene flow estimates in *G. pallidipes*, *G. morsitans*, and *G. palpalis* suggest that many or most tsetse populations are fairly localized. This could in principle be explained by genetically based isolating mechanisms or adaptation to local conditions. If natural selection were to account in large measure for the low rates of gene flow, the question of reinvasion may be less acute than ecological studies indicate.

CONCLUSIONS

Genetic studies on tsetse flies have helped clarify systematic relationships among taxa and have provided important data on gene flow among natural populations. Genetic maps have been prepared for the major species. New questions have been raised about isolating mechanisms. Tsetse flies are cytogenetically favorable, thereby offering opportunities to learn more about isolating mechanisms among taxa and populations. Molecular tools are available to study more definitively the mechanisms of sex determination, vector-trypanosome adaptations, and adaptation of tsetse flies to their various environments. Genetic knowledge, however, greatly lags about this small, aberrant, but medically and economically extremely important group of insects. Much more is known about the pathogenic trypanosomes that are transmitted exclusively by tsetse flies, and unfortunately this knowledge is largely without reference to their vectors. Two reasons come to mind: the difficulty and expense of establishing and maintaining tsetse cultures and the grossly inadequate financial support for tsetse research.

The number of tsetse workers continues to decline even as the incidence of human sleeping sickness increases. In North America, only the Yale University School of Medicine now maintains tsetse flies. Required are geographically systematic sampling of natural populations and their trypanosomes, and more workers in the vineyard.

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