ARTICLE

Hybridization Asymmetries in Tsetse (Diptera: Glossinidae): Role of Maternally Inherited Factors and the Tsetse Genome

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ABSTRACT Among the *morsitans*-group of tsetse there are several pairs of taxa in which there is a marked hybridization asymmetry (HA), i.e., one cross produces significantly more offspring than does the reciprocal cross. To investigate the relative contribution of maternally inherited factors (MIF) and chromosomal factors to HA, three hybrid lines were established in which flies have MIF from one taxon and chromosomes from another. HA was then compared among crosses of the parental taxa and crosses of each parental taxon with the appropriate hybrid line. The results indicate that HA in reciprocal crosses of *Glossina morsitans morsitans* Westwood and *Glossina swynnertoni* Austin and in reciprocal crosses of *G. m. morsitans* and *Glossina morsitans centralis* Machado are caused by chromosomal factors, not MIF. Reciprocal crosses of *G. m. centralis* and *G. swynnertoni* do not display HA, and none developed as a result of a novel combination of MIF and tsetse chromosomes.

KEY WORDS *Glossina morsitans centralis*, *Glossina morsitans morsitans*, *Glossina swynnertoni*, tsetse, hybridization asymmetry

AMONG TSETSE, AND many other insects, there is often an asymmetry in the success of hybridization, with one cross being much more productive than the reciprocal cross (Werren 1997, and references therein). The first report of hybridization asymmetry (HA) among tsetse was that the cross *Glossina morsitans centralis* Machado × *Glossina swynnertoni* Austin resulted in a greater proportion of pregnant females than did in the reciprocal cross (Vanderplank 1947). (Throughout this article I cite the taxon of females first in each cross.) In an extensive series of hybridizations (Vanderplank 1948), only one other HA was found among *morsitans*-group tsetse: *Glossina morsitans morsitans* Westwood × *G. swynnertoni* was more likely to result in pregnant females than was the reciprocal cross. Additional HAs have been reported using one of two criteria: the average fecundity of females (number of offspring per female per ovulation cycle) (Curtis 1972, Curtis et al. 1980, Rawlings 1985), or the numbers of females that did or did not become pregnant within a specified period (Gooding 1985, 1993, 1997). Among the *morsitans*-group the following crosses are significantly more productive than the reciprocal crosses: *G. m. morsitans* × *G. m. submorsitans* variety *ugandensis* (Curtis 1972); *G. m. morsitans* × *G. m. centralis* (Curtis 1972, Curtis et al. 1980, Gooding 1985, Rawlings 1985); *G. m. submorsitans* × *G. m. morsitans* (Gooding 1985, Rawlings 1985) and *G. m. submorsitans* × *G. m. centralis* (Gooding 1985, Rawlings 1985) and *G. m. morsitans* × *G. swynnertoni* (Gooding 1993, 1997). Interestingly, HA observed by Vanderplank (1947) in the *G. m. centralis*/*G. swynnertoni* crosses was not confirmed in a recent study, using well established laboratory colonies (Gooding 1997).

Vanderplank (1948) suggested two possible causes of HA: "(a) lowered activity or viability of the sperm in an alien environment; (b) higher mortality of the larval stages of the interspecific crosses, because of genetic differences or to adverse effect of an alien environment in the uterus." The first proposed mechanism is consistent with the report that 10 of 11 *G. m. centralis* eggs placed with *G. swynnertoni* sperm were fertilized and completed embryonation when maintained at 27°C on sterile agar (Vanderplank 1948). Curtis (1972), in a study of hybridization of subspecies of *G. morsitans*, considered three possible explanations for HA in tsetse: (1) unfavorable interactions between the egg cytoplasm and that of the sperm; (2) unfavorable interactions between the egg cytoplasm and gene products of paternal origin; and (3) unfavorable interactions between maternal gene products and paternal gene products in the egg, zygote or developing larva, in utero. The data did not support the first two hypotheses, but fertility of females in each recurrent backcross was generally consistent with the third hypothesis, if it is further postulated that females of the species that were most fertile after hybridization had a higher "tolerance" for the foreign genes, than did the females of the less productive cross (Curtis 1972). Rawlings (1985), studying hybridization among the same taxa, listed the same possible explanations for hybridization asymmetry as were postulated by Curtis (1972), but did not conclude which was most likely.

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Experiments designed to elucidate the genetic basis of hybrid male sterility provided another perspective on HA in *G. m. morsitans*/*G. m. centralis* crosses (Gooding 1987). Some backcross males that had both sex chromosomes from the same subspecies and that descended from *G. m. centralis* were sterile but others were able to inseminate and to fertilize both *G. m. centralis* and *G. m. morsitans.* However, among the corresponding males that descended from *G. m. morsitans*, inseminators fertilized 19 of 19 *G. m. morsitans* but only 2 of 19 *G. m. centralis* ($\chi^2 = 27.249$). The offspring of the *G. m. centralis* females were deformed and either failed to pupariate or died as teneral adults. This asymmetry occurred even among males whose marker genes indicated that all their chromosomes were from *G. m. centralis.* Because the backcross males descending from *G. m. morsitans* females were assumed to carry maternally inherited factors (MIF) from *G. m. morsitans* (i.e., egg cytoplasm, mitochondria, and symbionts), the asymmetry was interpreted as being caused by the presence of a maternally inherited sterility factor (MISF). The model proposed for the evolution of this factor (Gooding 1987) was that females carrying MISF could be fertilized by males that do, and those that do not, carry MISF, but that females that lack MISF could only be fertilized by those that do not carry it. The identity of MISF was not hypothesized, but the discovery, in *morsitans*-group tsetse (O'Neill et al. 1993), of *Wolbachia*, a prokaryotic symbiont that infects the ovaries, and other tissues, in many species of insects, offered a possible explanation for the asymmetry because its influence on fertility is usually the same (Werren 1997, and references therein) as that hypothesized for MISF. An additional characteristic of the *Wolbachia*-induced HA is that it is usually stable over many generations of recurrent backcrossing to uninfected males (Werren 1997).

The discovery of fertility asymmetry among *G. m. morsitans*/*G. m. centralis* backcross males led to the suggestion that this asymmetry could be used to establish a colony to provide males for genetic control of *G. m. centralis*, if MISF were stable through many generations in the presence of *G. m. centralis* genes (Gooding 1987). However, MISF was not stable after recurrent backcrossing to *G. m. centralis*, and by the ninth backcross generation, >80% of the males fertilized *G. m. centralis* (Gooding 1990a). Nonetheless, intraspecific HA may play a useful role in tsetse control. Beard et al. (1993) proposed that *Wolbachia* be used to drive, into a tsetse population, *Sodalis glossinidius* Dale & Maudlin, a midgut symbiont that may be amenable to genetic engineering that could establish a strain of tsetse that cannot transmit trypanosomes.

In view of the possibilities of using intertaxon hybridization for genetic control of tsetse (Potts 1944, Vanderplank 1944, Curtis 1972, Rawlings 1985, Gooding 1987), and the proposed use of maternally inherited factors such as *Wolbachia* as a drive mechanism to spread antitrypanosomal factors in a tsetse population (Beard et al. 1993), it is desirable to have a thorough understanding of the basis and stability of HA. Currently, such studies with tsetse are only tractable through hybridization of closely related members of the *morsitans*-group. There are several indices available for comparing HA among tsetse (Gooding 1990b); here I use the number of teneral adults produced per female in the least productive cross divided by the corresponding number from the reciprocal cross (i.e., a variation of the index HSI′ of Gooding 1990b). The modified indices are 0.00 for *G. m. morsitans*/*G. swynnertoni* (Gooding 1997), 0.30 for *G. m. morsitans*/*G. m. centralis* (Gooding 1985), and 0.89 for *G. m. centralis*/*G. swynnertoni* (Gooding 1997). The rank order of these pairs of taxa, from highest HA to lowest HA is the same as when expressed in terms of the numbers of fertile and sterile matings in reciprocal crosses: *G. m. morsitans* × *G. swynnertoni*, 46 fertile, 26 sterile, compared with zero fertile and 20 sterile in the reciprocal cross ($\chi^2 = 29.704$) (Gooding 1997); *G. m. morsitans* × *G. m. centralis* (23 fertile, three sterile) c.f. *G. m. centralis* × *G. m. morsitans* (11 fertile, 19 sterile, $\chi^2 = 13.570$) (Gooding 1985); *G. m. centralis* × *G. swynnertoni* (25 fertile, 50 sterile) c.f. *G. swynnertoni* × *G. m. centralis* (18 fertile, 44 sterile, $\chi^2 = 0.126$) (Gooding 1997).

Two pairs of taxa, *G. m. morsitans*/*G. swynnertoni* and *G. m. morsitans*/*G. m. centralis*, show marked HA; and for these pairs of taxa the objective of the experiments was to determine the relative contributions to HA of MIF and chromosomal factors. For *G. m. centralis*/*G. swynnertoni* there is no HA, and experiments were conducted to determine whether a novel combination of MIF from one taxon and chromosomes from another taxon will produce HA.

Materials and Methods

Flies and Colony Maintenance. The origin and histories of the colonies of *G. m. centralis*, *G. m. morsitans*, and *G. swynnertoni* were reported previously (Gooding 1987, 1993). Hybrid lines, carrying MIF from one taxon and at least 99% of their chromosomal genes from another, were established as follows. The taxa of interest were crossed and F_1 females, and those in at least six subsequent generations, were mated to males from the paternal taxon. Each hybrid line was then maintained as a closed colony of 60–90 females that had mated to an equal number of males. I established the following hybrid lines: line 88–45, *G. m. morsitans*/*G. m. centralis;* line 93–48, *G. m. morsitans*/*G. swynnertoni;* line 94–40 *G. m. centralis*/*G. swynnertoni.* These lines permit crosses in which the flies from the hybrid line differed from the other line in either MIF (assuming MIF is stably inherited) or chromosomes, but not both.

Throughout the experiments, flies were maintained at 24.5°C and ≈60% RH, by feeding on rabbits every other day, using a protocol that conformed to the guidelines of the Canadian Council on Animal Care (University of Alberta Biosciences Animal Care Committee, SOP RP5109).

Hybridization Procedure. For each comparison, males from the lines being compared were mated for the first time when they were 1–2 wk old, and were

Table 1. Results of hybridizing various tsetse taxa and hybrid lines that carry novel combinations of maternally inherited factors and tsetse genomes

Experimental comparison no.	Most fertile cross[a]	Most fertile cross no.		Reciprocal cross no.		
		Fertile	Sterile	Fertile	Sterile	χ^2
1.1	G.m.m. × G.swynn.	16	7	2	20	14.707[b]
1.2	G.m.m. × 93-48	24	6	0	27	34.099
1.3	G.swynn. × 93-48	41	2	40	2	0.238
2.1	(G.m.m. × G.m.c.	23	3	11	19	13.570)[c]
2.2	88-45 × G.m.c.	29	1	27	1	0.449
2.3	G.m.m. × 88-45	29	0	12	17	21.303
3.1	G.m.c. × G.swynn.	17	10	13	15	0.922
3.2	G.m.m. × G.swynn.	27	9	0	38	41.694
3.3	88-45 × G.swynn.	18	14	9	20	2.965
4.1	G.m.c. × G.swynn.	14	11	7	14	1.538
4.2	94-40 × G.swynn.	39	0	36	0	NC[d]
4.3	G.m.c. × 94-40	20	10	13	9	0.726

Numbers in body of table are the numbers of females that produced offspring within 28 d of mating (fertile), or that failed to do so (sterile, but had sperm in the spermathecae, at dissection).

[a] Taxa and lines used: G.m.c., *G. m. centralis*; G.m.m., *G. m. morsitans*; G.swynn., *G. swynnertoni*; 88-45, a hybrid line with MIF from *G. m. morsitans* and chromosomes from *G. m. centralis*; 93-48, a hybrid line with MIF from *G. m. morsitans* and chromosomes from *G. swynnertoni*, 94-40, a hybrid line with MIF from *G. m. centralis* and chromosomes from *G. swynnertoni*.

[b] Chi-square values (with 1 df) were calculated with Yates correction; critical values are 3.841, $P = 0.05$; 5.024, $P = 0.025$; 6.635, $P = 0.01$; 7.879, $P = 0.005$ and 10.828, $P = 0.001$.

[c] Data from Gooding (1985).

[d] NC, not calculated.

used two or three times with at least 48 h and at least one opportunity to feed between consecutive matings. Females were mated when ≈3 d old. After mating, each female was maintained until she deposited a larva or for 28 d, which is adequate time to have deposited two larvae. Females that did not deposit a larva were dissected to determine whether or not they had motile sperm in the spermathecae. Only data from inseminated females were used.

Experimental Crosses. The objective of experiment 1 was to determine whether HA for *G. m. morsitans* and *G. swynnertoni* is caused by MIF or to chromosomes. For each cross, 45–50 males from each of three lines, *G. m. morsitans*, *G. swynnertoni*, and line 93–48, were mated three times, each time to a female from a different line. The intracolony matings served to determine fertility within each colony.

Experiment 2 evaluated the basis for HA in *G. m. morsitans* and *G. m. centralis* crosses. Two sets of reciprocal crosses were carried out between flies from line 88–45, which carries MIF from *G. m. morsitans* and chromosomes from *G. m. centralis:* the first set of crosses was between flies from line 88–45 and *G. m. centralis*, the second set used flies from 88–45 and *G. m. morsitans.* There were 30 pairs of flies in each cross.

Experiment 3 investigated, indirectly, the role of MIF and chromosomes in HA for crosses of *G. m. morsitans* and *G. swynnertoni.* Three sets of reciprocal crosses were set up: *G. m. centralis*/*G. swynnertoni* (crosses with no HA); *G. m. morsitans*/*G. swynnertoni* (crosses with high HA); and *G. swynnertoni*/88–45, the line having MIF from *G. m. morsitans* and chromosomes from *G. m. centralis.* There were 45 pairs of flies in each cross.

Experiment 4 was run to determine whether MIF from *G. m. centralis* and chromosomes from *G. swynnertoni* would interact to cause HA that had not previously existed. The experiment was run in the same way as experiment 1, but using *G. m. centralis*, *G. swynnertoni* and 94–40, the line carrying MIF from *G. m. centralis* and chromosomes from *G. m. centralis.*

Data Analysis. The extent of HA was determined for each reciprocal cross, using chi-square with Yates correction.

Results and Discussion

In Table 1, experiments and reciprocal crosses are identified by a two-digit comparison number, with the first digit indicating the experiment and the second indicating the pair of taxa or colonies for which the comparison was made. In experiments 1 and 4, intracolony crosses were carried out to establish the fertility rates among inseminated females from each colony: *G. m. centralis* = 100%, *G. m. morsitans* = 95%, *G. swynnertoni* = 100%, line 93–48 = 98%, and line 94–40 = 98%. The fertility in line 88–45, determined at the time experiment 3 was conducted, was 96%.

The previously observed HA in the *G. m. morsitans*/*G. swynnertoni* crosses (Gooding 1993, 1997) was confirmed (Table 1, line 1.1). Reciprocal crosses of *G. m. morsitans* and line 93–48 showed a similar HA (Table 1, line 1.2), but the *G. swynnertoni*/93–48 crosses showed no HA (Table 1, line 1.3). Because line 93–48 has MIF from *G. m. morsitans* and chromosomes from *G. swynnertoni*, the results indicate that *G. m. morsitans*/*G. swynnertoni* HA is determined by chromosomes, rather than by MIF.

There was no HA when reciprocal crosses of line 88–45 and *G. m. centralis* were carried out (Table 1, line 2.2), but there was when *G. m. morsitans* and line 88–45 were crossed (Table 1, line 2.3). Because line 88–45 has MIF from *G. m. morsitans* and chromosomes

from *G. m. centralis*, the results indicate that HA was influenced more by chromosomes than by MIF.

Experiment 3 confirmed the lack of HA in crosses of *G. m. centralis* and *G. swynnertoni* (Table 1, line 3.1) and the marked HA in crosses of *G. m. morsitans* and *G. swynnertoni* (Table 1, line 3.2). There was no HA in reciprocal crosses of *G. swynnertoni* and line 88–45, which has MIF from *G. m. morsitans* and chromosomes from *G. m. centralis* (Table 1, line 3.3). This supports the conclusion drawn from experiment 1: HA observed in reciprocal crosses of *G. m. morsitans* and *G. swynnertoni* is caused by chromosomal factors, not by MIF.

The above results are consistent with the hypothesis that HA among *morsitans*-group tsetse is caused by chromosomal factors, not MIF. However, in the models tested in experiments 1 and 2, two novel combinations of MIF and chromosomal factors resulted in an increase in the magnitude of the asymmetry, in those crosses that displayed HA. Similarly, increased HA was observed by Gooding (1987) when backcross males with MIF from *G. m. morsitans* and chromosomes from *G. m. centralis* inseminated both *G. m. centralis* and *G. m. morsitans*, but fertilized only the latter. These findings suggested that a novel combination of MIF and genomic factors may produce HA in a model where one does not already exist. This was tested in experiment 4 using line 94–40, which has MIF from *G. m. centralis* and chromosomes from *G. swynnertoni.* The results (Table 1, lines 4.1–4.3) do not indicate any HA arising from a novel combination of MIF and chromosomal factors.

The tentative conclusion that HA in the *morsitans*-group is caused by chromosomal factors rests upon the assumption that there is no paternal leakage, i.e., transfer from the male to the offspring, of factors that are normally considered to be maternally inherited. Such transfers occur for *Wolbachia* in some *Drosophila* spp. (Hoffman and Turelli 1988), as do host genome/*Wolbachia* interactions (Turelli 1994, Werren 1997). If either occurs in *morsitans*-group tsetse, the above interpretation will need to be reevaluated. Similarly, if paternal leakage of *Wolbachia* occurs, or if *Wolbachia*/host genome interactions occurs among *morsitans*-group tsetse, the proposal to use *Wolbachia* as a mechanism to drive antitrypanosomal factors into tsetse (Beard et al. 1993) may need to be revised.

Because the results in Table 1 are for inseminated females, the analyses are consistent with the hypothesis that the chromosomal effects act primarily within the females upon: (1) some aspect of sperm transfer from the spermathecae to the uterus, (2) sperm penetration of the egg, (3) survival and functioning of the male's chromosomes within the egg, or (4) that gene products of male origin interact unfavorably with those of female origin in the embryo or developing larva, and that this results in abortion. The latter possibility is essentially the hypothesis proposed by Curtis (1972) that HA arises from unfavorable interactions between maternal gene products and paternal gene products in the egg, zygote or developing larva and that females of the hybridizing species differ in their tolerance of foreign gene products. There is, however, insufficient evidence to eliminate any of the above possibilities.

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