the *Culicoides* from turf-mosses in the Hautes-Vosges. These turf-mosses are special biotopes: oligotrophic, acid ($3.5<pH<4$) and with low pO^2 and temperature. These parameters are stable all year round. The following species were collected in 1981: *Culicoides cubitalis* Edwards, *fascipennis* (Staeger), *grisescens* Edwards, *impunctatus* Goetghebuer, *obsoletus* (Meigen), *punctatus* (Meigen), *reconditus* Campbell and Pelham-Clinton, *stigma* (Meigen), *truncorum* Edwards. The following was found in cow-dung: *chiopterus* (Meigen).

Kettle in Scotland and Callot and Kremer in France and in Switzerland found in the same biotopes: *achrayi* Kettle and Lawson, *albicans* (Winnertz), *carjalaensis* Glukhova, *cubitalis* Edwards, *fascipennis* (Staeger), *grisescens* Edwards, *heliophilus* Edwards, *impunctatus* Goetghebuer, *odibilis* Austen, *pallidicornis* Kieffer, *pseudoheliophilus* Callot and Kremer, *pulicaris* (L.), *pumilus* (Winnertz), *punctatus* (Meigen), *reconditus* Campbell and Pelham Clinton, *segnis* Campbell and Pelham Clinton, *sphagnumensis* Williams, *stigma* (Meigen), *subfascipennis* Kieffer, and *truncorum* Edwards. In cow-dung: *Culicoides chiopterus* (Meigen), *dewulfi* Goetghebuer, and *scoticus* Downes and Kettle.

Culicoides albicans, carjalaensis, fascipennis, grisescens, heliophilus, impunctatus, and *sphagnumensis* are considered as specific turf-moss species. The differences between our report and the previous reports can be due to the small number of samples, the disappearing of a species, or a temporary eclipse of a species.

A GENETIC-SEXING STRAIN BASED ON MALATHION RESISTANCE FOR *CULEX TARSALIS*[1]

P. T. McDONALD[2] AND S. M. ASMAN[2, 3]

ABSTRACT. A genetic-sexing strain of *Culex tarsalis* that links malathion resistance and male-determining genes via a translocation was established. In this strain females are susceptible to malathion and males are malathion resistant. A discriminating dose of 0.1 ppm malathion applied to first as well as fourth instars eliminates over 99 percent of the females while permitting the males to survive.

In the last decade the possibility of using genetic methods as alternatives to pesticides for control has been explored for *Culex tarsalis* Coq. The SMR (sterile male release) approach has recently been under consideration, and its implementation requires efficient mass production procedures. The development of a reliable, safe and accurate method of eliminating females from the release population is also necessary for the establishment of an efficient program. Sex separation by genetic means is the most desirable approach where accurate and non-damaging mechanical methods are not available.

Sex separation by sex-linked conditional lethal systems has been developed for 5 species of mosquitoes (Baker et al. 1978, Baker et al. 1981, Curtis 1978, Curtis et al. 1976, Seawright et al. 1978). The conditional lethal for the *Culex*

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tritaeniorhynchus system was a temperature sensitive gene induced by ethyl methanesulfonate. In 4 anopheline species an insecticide resistance gene has been linked via a translocation to the male-determining gene. Crossing susceptible females to the translocated males produced susceptible female progeny which were killed by insecticide application while the resistant male progeny survived.

The genetic behavior of malathion susceptibility was used to develop a sex-sorting strain of *Cx. tarsalis* for the following reasons: the likelihood of finding alternative alleles for the gene in natural populations was high; previous investigations had documented that malathion resistance was a single gene system (Calman and Georghiou 1970, Matsumura and Brown 1961, Plapp et al. 1961), while other organophosphate compounds were associated with polyfactorial systems (Apperson and Georghiou 1975); malathion resistance was partially dominant, and this would allow for discrimination between heterozygous resistants and homozygous susceptibles (Matsumura and Brown 1961).

The release of males carrying malathion resistance into a field population is not a serious concern as widespread resistance to this insecticide in the field has already been documented (Zboray and Gutierrez 1979).

This paper reports the successful establishment of a sex-sorting strain that uses malathion susceptibility as a conditional lethal to eliminate females.

MATERIALS AND METHODS

The first objective was to select malathion susceptible and resistant lines. Malathion treatments were of 24 hour duration, and fourth instar larvae were treated unless otherwise specified. The malathion susceptible line was selected following treatment to samples of several families. The samples were challenged with 0.1 ppm malathion while remaining larvae of each family were untreated. If all larvae in the treated group died, the untreated siblings were saved for the malathion susceptible colony. The malathion resistant line was gradually selected over 3 generations by successive treatments at levels of 0.1, 1.0 and 5 ppm malathion (American Cyanamid Co.).

The genetic basis of malathion resistance was reconfirmed for the newly-selected strains to determine if the same genetic system was involved as described by Matsumura and Brown (1961). Crosses between susceptible females and resistant males were made. The backcross of susceptible females to F-1 males followed. For each line (parental, F-1, backcross) two or more samples of either 60 or 120 larvae were challenged with doses of malathion ranging from 0.003 to 20 ppm. The data on survivorship were used to construct dosage-mortality curves for each population.

The major objective was to isolate a genetic sexing strain in which malathion resistance could be linked with the male-determining gene. Sex determination in *Cx. tarsalis* has been shown to be controlled by a single gene with homozygous *m/m* females and heterozygous *M/m* males (Barr and Meyers 1966). Since the malathion gene is autosomal, a translocation linking it with the male-determining gene was sought.

To isolate such a translocation, susceptible females were mass-crossed with resistant males that had been irradiated at 2500 rads from a Co-60 source. The F-1 progeny were challenged as fourth instars with 0.1 ppm malathion to eliminate susceptibles and select resistant heterozygotes.

Surviving F-1 male progeny were backcrossed to the susceptible strain females, and individual rafts were examined to determine hatch. Egg rafts with a hatch between 30 and 70 percent, which could have resulted from a mating with a translocated male parent, were reared. The fourth instars were subjected to the 0.1 ppm malathion treatment to possibly eliminate females. When the resistant adults that ultimately emerged were

scored, families having greater than 75 percent male survivorship were saved.

Suspected translocation lines were examined cytologically by preparing spermatogonial metaphase cells in aceto-lactic-orcein stain.

When a sex-sorting strain was established, first instars were challenged to determine the lowest concentration of malathion capable of eliminating the females. Concentrations of 0.001, 0.01, 0.1 and 1.0 ppm malathion were administered to samples of 60 larvae. The survivors were reared to adulthood and sexed.

The extent to which sex-sorting properties would be maintained in the absence of selection was also evaluated to determine strains suitable for mass production. Two colonies of the sex-sorting strain were established by allowing 100 founding pairs in each of 3 generations to cycle without selection for resistant males. Thereafter samples of fourth instars of both colonies were challenged with 0.1 ppm malathion and surviving adults were scored. Since the sex-sorting strain was constructed for the sterile male release program, the competitiveness of sterilized males from this strain was evaluated under laboratory conditions. Four competition crosses were set. Two crosses simulated releases into a Br-81 population, a laboratory strain colonized for 6 months. The other 2 crosses were with the malathion susceptible strain. The first pair of crosses determined the competitiveness of irradiated Br-81 and irradiated sex-sorting males against unirradiated Br-81 males, and indirectly to each other. The second pair allowed the same type of comparison but with the MS (malathion susceptible) males.

RESULTS AND DISCUSSION

SELECTION OF MALATHION SUSCEPTIBLE AND RESISTANT STRAINS. The malathion susceptible strain was started from the Berkeley strain, a vigorous laboratory strain derived from a mixture of several geographically distinct populations. This strain proved to be uniformly susceptible at the 0.1 ppm level. The malathion resistant strain was selected from a laboratory population initiated in 1979 from collections at the Poso West field site outside Bakersfield, California.

In the development of a resistant strain the 2 goals were to achieve a vigorously reproductive strain and to have a uniformly high degree of resistance. Initial attempts to select a strain at the 5 ppm level caused such a high mortality that a stepwise increase in resistance level was implemented to conserve a higher degree of genetic variability than a harsh selection for the most highly resistant individuals would have allowed. A line was selected at 5 ppm and continued to require selection, as up to 50 percent of the test samples were susceptible at 5 ppm presumably because these were heterozygotes rather than homozygotes for resistance. Selection was continued to keep the line from reverting to a less resistant status while the focus of the research was shifted to the development of a sex-sorting strain.

GENETICS OF MALATHION RESISTANCE. Dosage mortality curves were constructed for the susceptible, resistant, F-1 and F-1 backcross to susceptible groups (Fig. 1).

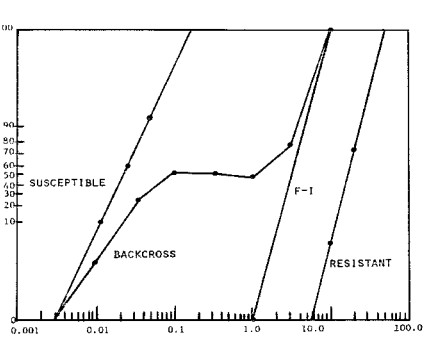

Fig. 1. Dosage mortality curves for populations of *Culex tarsalis:* malathion susceptible, malathion resistant, F-1 (susceptible females × resistant males) and backcross (susceptible females × F-1 males). Each point represents a test of at least 60 individuals.

The F-1 of susceptible females and resistant males were partially dominant in agreement with findings by Matsumura and Brown (1961). The F-1 backcross to susceptible females identified 50 percent of the progeny as homozygous susceptibles while another 50 percent were partially dominant heterozygotes. This confirmed that a single gene determined malathion resistance and was in accord with the earlier findings of Matsumura and Brown (1961).

In addition to the 120 backcross progeny treated at 0.1 ppm in the trial for constructing the dosage-mortality curve, another 280 were treated at the same dose. Of the total 400 larvae 53.8 percent survived treatment and 52.1 percent of those emerging were males. This indicated that the gene for malathion resistance was autosomal, and that a translocation linking that gene with the male-determining gene would be required for developing a sex-sorting strain.

While the extrapolated dosage-mortality line of the susceptible line showed a 99.9 percent mortality at 0.1 ppm, the susceptible line had in fact been selected at that dosage. Thus this concentration was selected as the appropriate dose for discriminating between susceptibles and heterozygotes.

SEX-SORTING STRAINS. When susceptible females were crossed to the F-1 males of irradiated malathion resistant males they produced 220 egg rafts, 38 of which had hatch rates between 30 and 70 percent. Each of these 38 families was challenged as fourth instars with 0.1 ppm malathion. From these, 2 lines had greater than 75 percent males (Table 1). This suggested that a translocation linking the malathion-resistant and male-determining genes had been induced.

Cytological analysis of spermatogonial metaphase cells indicated that in both strains the translocations involved the 2 longest chromosomes and not the shortest chromosome (Fig. 2). In this species the longest chromosome is sex-determining, carrying linkage group 1 while the median length and shortest chromosomes are autosomes carrying linkage groups 2 and 3 respectively (McDonald et al. 1978). Thus, these 2 translocations were designated T(1;2)22*A* and T(1;2)23*A*. Both strains were followed for 4 generations to characterize them for sex-sorting properties by successive challenges with a discriminating dose of malathion (Table 1). On testing the 4th generation both strains demonstrated the characteristic semisterility of translocated strains with 44.6 percent of 823 eggs hatching for T(1;2)22*A* and 43.7 percent of 2965 eggs hatching for T(1;2)23*A*.

The T(1;2)22*A* strain was discontinued after unsuccessfully inducing an inversion that would link the resistant and

Table 1. Larval survival after 0.1 ppm malathion treatment* and adult sex ratio of 2 translocated strains of *Culex tarsalis*.

		Larval survival		Adult survival		
Strain	Generation	No. larvae treated	% surviving	No. males	No. females	% males
T(1;2)22*A*	1	52	50.0	20	1	95.2
	2	2634	35.5	270	192	63.2
	3	222	57.2	76	38	66.7
	4	322	50.9	102	47	68.5
T(1;2)23*A*	1	58	58.6	14	0	100
	2	1670	38.3	195	0	100
	3	1639	58.7	771	15	98.1
	4	1213	52.1	593	1	99.8

* Treatment to 4th instars for 24 hr.

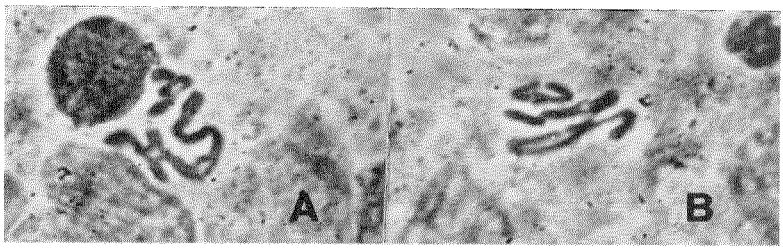

Fig. 2. Spermatogonial metaphase cytology of sex-sorting translocation strains. A - T(1;2)22*A*. B - T(1;2)23*A*.

male-determining genes more closely. Similar procedures have been developed for the *Anopheles albimanus* sex-sorting strains (Seawright et al. 1981). In the absence of such an inversion, the T(1;2)22*A* strain required more culling than would be practical.

In the T(1;2)23*A* strain some 15 females survived treatment in the third generation (Table 1). In the fourth generation individual families were reared and analyzed to determine whether additional resistant females would appear at random or all in one family. Among 594 progeny in 20 families only a single female survived the malathion treatment. This confirmed that the male-linked translocation system was intact, and that the recombination rate between sex and malathion genes was indeed very low. The presence of the females in the third generation may have resulted from incomplete application of malathion in that generation.

When the first instars were tested at doses from 0.001 to 1 ppm, there were 18 male survivors at 0.001, 24 males and 12 females at 0.01 ppm, 22 males at 0.1 ppm, and 11 males at 1.0 ppm. From these preliminary tests it appeared that 0.1 ppm was the appropriate discriminating dose for first instars.

One of the requirements of a sex-sorting strain is that it can be mass-produced over several generations without selection and significant loss of the sex-sorting properties. The suitability of such a strain for field use can be determined on the basis of the frequency of recombinant resistant females among the males surviving the insecticide challenge (Seawright et al. 1981).

In the case of the T(1;2)23*A* strain the feasibility of rearing in large numbers for a release was estimated by rearing the stock for 3 generations and subjecting the samples of progeny to malathion challenge. The survivors were 196 males only in one colony and 180 males only in the other. This suggested that this sex-sorting strain could successfully be mass produced and sorted for males in a sterile male release program.

The competitiveness value for irradiated males was determined for each of the 4 crosses (Table 2). The competitiveness value for irradiated (T;2)23*A* males was not statistically different from those of the irradiated or unirradiated Br-81 males. Likewise the competitiveness value for irradiated T(1;2)23A males was not statistically different from those of the irradiated or unirradiated MS males. The testing of the T(1;2)23*A* males against males from both laboratory strains was encouraging in that the T(1;2)23*A* strain males were at least as competitive as the Br-81 and MS males. Competition tests of the T(1;2)23*A* strain males against field-collected males for field-collected females are scheduled for the future.

The preliminary trials involving sex separation of first instars, maintenance of the strain without selection each generation and competitiveness in the laboratory

Table 2. Competitiveness of T(1;2)23*A* males separated by malathion, challenged at 6000 rads and tested with Br-81 and MS populations of *Culex tarsalis*.

Cross	Females Strain (No.)	U* Males Strain (No.)	I* Males Strain (No.)	Rafts from U males	Rafts from I males	Competitiveness** of I males against U males
I	Br-81(52)	Br-81(50)	T(1;2)23*A*(50)	4	12	3.0***
II	Br-81(52)	Br-81(50)	Br-81(50)	6	6	1.0
III	MS(55)	MS(50)	T(1;2)23*A*(50)	15	23	1.5
IV	MS(55)	MS(50)	MS(50)	22	16	0.7

* U = unirradiated, I = irradiated.

** Competitiveness = No. I rafts/no. U rafts).

*** Significantly >1.0 ($p < .05$). Other competitiveness values do not differ from 1.0. However, I and II do not differ significantly ($\chi^2 = 1.87$), and III and IV do not differ significantly ($\chi^2 = 2.58$).

all indicate that the (T1;2)23*A* sex-sorting strain deserves consideration for larger scale outdoor trials and possibly field releases in a SMR pilot study.

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