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Laboratory and Field Studies with a Compound Chromosome Strain of *Drosophila melanogaster*[1]

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Summary. A compound third chromosome strain of *D. melanogaster* was evaluated for population control potential in the laboratory and field. In the laboratory, the compound strain can replace a wild-type strain at release ratios above 4 compounds: 1 wild-type, but the compound strain proved to be ineffective in the field because of an inability to utilize tomatoes for oviposition sites.

The idea of using chromosomally altered strains to manipulate insect pest populations was originally suggested by Serebrovskii in 1940 and revived and extended by Curtis (1968); Whitten (1971); and Waterhouse *et al.* (1973). In addition to the pest control potential, the introduction of a genetically altered strain into a field population can also provide worthwhile information concerning the dynamics of a field population.

The organism of choice for testing possible genetic mechanisms for population control is *Drosophila melanogaster* because years of study have provided a number of systems suitable for field testing this species, which is the major pest of the processing tomato industry (Mason and Dorst, 1962; Stoner and Mason, 1969). The subject of this paper is the behaviour of a compound chromosome strain in the laboratory and the field. Foster *et al.* (1972) suggested that compound chromosomes have potential for suppressing or manipulating native populations.

A compound chromosome has two homologous arms attached to a single centromere (Fig. 1a). If disjunction of the compounds is regular at meiosis in both the male and female, the maximum fertility of the strain is 50% (half the zygotes formed are aneuploid and therefore lethal). Segregation in female *D. melanogaster* is fairly regular; the left and right compounds move to opposite poles. However, non-disjunctional gametes are frequently produced in the male (Fig. 1b; Baldwin and Chovnick, 1967) so the fertility of a strain of this type should be between 25 and 50%, depending on the frequency of this non-disjunction. Since a mating between a compound adult and a chromosomally normal adult is sterile (due to aneuploidy), there is complete selection against the heterozygote in a population consisting of a mixture of the two types. The population will thus move away from an unstable equilibrium point towards fixation of one type or the other, and, theoretically, a single release of compound flies should be sufficient to completely replace the native population. Such population replacement has two possible advantages: (1) the lower biotic potential of the compound strain might prevent the population from expanding to pest status in a single season, and (2) the compound strain can be used to introduce new conditional lethal genes such as susceptibility to an insecticide or to a temperature (Foster *et al.* 1972).

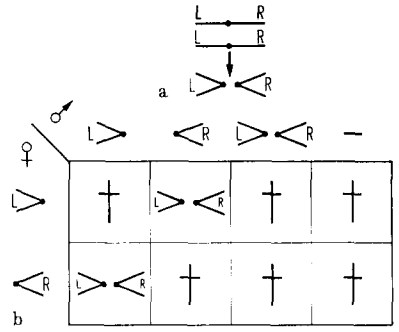

Fig. 1a. Formation of compound chromosomes from normal chromosomes

Fig. 1b. Zygotes produced from a compound chromosome strain of *D. melanogaster*

The location of the unstable equilibrium point depends on the fitness of the compound strain relative to the normal strain. At a fitness of 0.25 relative to normal, the compound strain should replace the normal strain at any release ratio above 1 normal : 4 compounds. Competition experiments at various release ratios of the Tokyo wild-type strain of *D. melanogaster* and a compound second chromosome strain indicated that theoretical expectations were closely approximated (Childress, 1972).

We were therefore encouraged to attempt laboratory and field releases of *D. melanogaster* with a compound third chromosome strain against wild-type and native populations.

[1] Mention of commercial product does not constitute recommendation or endorsement by the USDA.

Materials and Methods

Strains. The compound third chromosome strain used in the studies was marked with *sepia* (*se*), a mutant that changes the eye color from clear red to dark brown. This strain was checked for the presence of compounds by outcrossing to other compound strains and by examination of the mitotic chromosomes found in neural ganglia. The laboratory releases of *sepia* were made against two wildtype strains, Tokyo, collected in Tokyo, Japan, and maintained in the laboratory for many years; and a New Jersey strain collected in a New Jersey tomato field in 1969.

Laboratory Studies

The fitness of the *sepia* compound strain was first evaluated by placing the flies in competition with the Tokyo strain (ratios of 1:1, 1:3, 1:5, and 1:9) or with the New Jersey strain (ratios of 1:1, 1:5, 1:9, 1:15, and 1:30). All flies were isolated as virgins, held several days to insure sexual maturity, and transferred to the cages without etherization. Samples of the larval diet medium were then withdrawn every generation (10 days at 25 °C) from cages stocked with the Tokyo and *sepia* strains or every week from cages stocked with the New Jersey and *sepia* strains, and the emerging flies were scored.

Also, the New Jersey strain and the *sepia* compound strain were compared for egg production, viability, and adult survival by holding 25 pairs of each strain in separate cages, and introducing fresh medium twice a day for five weeks. The number of eggs laid and adults emerging were recorded, and the daily mortality was observed until all adults in both groups had died.

It is highly probable that most native females in a wild population are mated. To determine what effect this would have on the capability of a compound strain to replace a native strain in the field, the effect of remating with a compound male on the fertility of a wild strain was evaluated in the third laboratory test. One group of New Jersey females was mated twice with New Jersey males and a second group mated first with New Jersey males and then a day later with *sepia* compound males. All males were removed immediately after mating, and the females were brooded daily so we could determine the production of fertilized eggs over time.

A fourth laboratory test was made to determine if the female flies of each strain mated more frequently with one strain. Several jars containing 1 *se* or NJ female and 1 *se* and 1 NJ male were used and the type of offspring noted.

Field Studies

The first field tests were made in four quarter-acre plots in a woods and farmland area at the USDA Agricultural Research Center in Beltsville, Maryland. The four plots, at Woods, Beaver Dam, Telegraph, and Airport, were separated by at least one mile and were also isolated from the surrounding urban community. All four were planted with tomatoes of the Heinz 1409 cultivar (ca. 1170 per plot) to serve as hosts to populations of *Drosophila*. The buildup of populations of *D. melanogaster* in the Beltsville area is correlated with the ripening of the tomato crop (Wave, 1962). Density is low while the fruits are developing, but a two- to eight-fold increase oucurs weekly as the fruit ripens. The decline then coincides with the decay of the fruit and a decrease in temperature so populations are generally maximum in mid-September. Other species of *Drosophila* such as *D. tripunctata* and *D. busckii* and members of the *affinis* group are also found in the area, but *D. melanogaster* constitutes over 97% of the fly population at the time of peak population From Sept. 3, 1972 for 10 days (a period of relatively low density of the native flies), releases of *sepia* compound strain were made at the Woods and Beaver Dam sites; the Telegraph and Airport sites served as controls where we could measure the relative size of the native population and any migration of the released flies. Since we wished to maintain a ratio in the plots of 100 *se* compound to 1 native for one generation (10 days), we assessed the populations at the two release sites by trapping and adjusted the size of the releases accordingly at the two sites. Some 140,000 of these flies were marked with fluorescent dye so we could determine the extent of dispersion and longevity of the released flies by examining recaptured flies under UV light; in addition, 575,000 similarly marked flies were released at a site between the four plots.

The trapping to assess the daily ratio of released to native flies in each test plot was done with six pint-jar bait traps containing sugar, yeast, vinegar and lindane (Mason, 1963; Mason *et al.*, 1963). After the releases were ended, flies in the traps were monitored only three times a week. All captured flies were removed and returned to the laboratory for scoring. Previously, the relative attraction to bait traps of the two strains was determined from a series of controlled releases of New Jersey and *sepia* in small greenhouses. The traps caught about 25% more New Jersey than *sepia* strain. The collection data were not adjusted for this difference.

A check on the trap data was provided by slitting 10 ripe tomatoes and placing them in the release plots for 24 hours twice a week. Then the tomatoes were held for two weeks, and the number and phenotype of the emerging flies were recorded.

In the second test, we eliminated the complicating factor of immigration of large numbers of native flies by using three outdoor cages (4 × 4 × 2 m). Ten thousand *se* compound and 100 New Jersey wild flies were introduced into cage 1; cages 2 and 3 were populated with 1000 *se* compound and 1000 New Jersey wild flies, respectively. All cages were provided with tomatoes in excess of the amount required to support the population. Since the purpose of the test was to measure the increase in population of the individual strains when they were and were not in competition with the other strain, the population buildup was monitored three times a week over a 10-week period by using bait traps without lindane. The trapped flies were returned to the cages after scoring.

The *se* compound strain for the field tests was reared in 0.10 × .35 × .50 m wooden boxes lined with plastic sheets and filled with the standard cornmeal *Drosophila* medium. After eclosion, the flies were collected with a modified vacuum cleaner, etherized, counted, and taken to the release site where they were allowed to leave the carrying vessels as they recovered from the ether. The New Jersey wild strain was handled in a similar manner but was reared in flasks.

Results and Discussion

Laboratory Tests

Table 1 reports the results of the tests of the *sepia* compound and the Tokyo strains made to determine relative fitnesses. The data indicate that the unstable equilibrium point for a mixed population of Tokyo wild and *sepia* compound flies is probably near ratios of 1:3—1:5. We calculated the approximate fitness of the compound strain to be about 0.25 relative to the Tokyo strain from the rate of replacement of the wild strain by the compound strain.

The results of the first laboratory test thus met the theoretical expectations for the replacement of a wild-type strain by a compound strain and were similar to

results obtained earlier with a compound second chromosome strain (Childress, 1972). However, the concurrent tests with the more recently collected wild-type strain from New Jersey indicated that both the Tokyo and *sepia* strains had been changed by being maintained in the laboratory for many years (Table 2). For example, the New Jersey strain was noticeably

Table 1. *Competition between the sepia compound strain (se) and the Tokyo wild strain (Tok) in the laboratory (Samples of eggs were removed every generation, i.e. 10 days)*

Generation	% Tokyo ± s.e. in replicates	
	1	2
	1:1—20 pr Tok: 20 pr *se*	
1	87 ± 1.7	57 ± 3.1
2	81 ± 3.0	93 ± 2.3
3	93 ± 2.4	94 ± 1.4
4	94 ± 1.5	81 ± 2.5
	1:3—10 pr Tok: 30 pr *se*	
1	25 ± 2.9	16 ± 2.8
2	30 ± 2.4	45 ± 4.4
3	51 ± 3.4	7 ± 0.7
4	28 ± 2.3	4 ± 1.6
	1:5—7 pr Tok: 35 pr *se*	
1	23 ± 2.9	16 ± 2.9
2	11 ± 1.8	6 ± 2.1
3	5 ± 1.9	6 ± 1.8
4	4 ± 1.5	6 ± 1.5
	1:9—4 pr Tok: 36 pr *se*	
1	1 ± 0.8	3 ± 1.9
2	0	9 ± 2.1
3	7 ± 1.9	3 ± 1.2
4	1 ± 0.6	3 ± 1.1

more vigorous than the Tokyo strain. Even at the higher release ratios, New Jersey rapidly replaced the *sepia* strain, and the fitness of the *sepia* strain with respect to the New Jersey strain was roughly 0.03. In other words, the replacement of the New Jersey strain by the *sepia* strain should occur at release ratios greater than 33 *se*: 1+. Since the fitness of the New Jersey strain undoubtedly approximates that of a native population more closely than the Tokyo strain, the New Jersey strain was used in all other evaluations of the compound strain.

Table 3 reports the data for egg production and recovery of adults from 25 pairs of *sepia* and New Jersey strains. Over a 5-week period, the *sepia* strain produced about half as many eggs and a tenth as many adults as the New Jersey strain. Also the average and maximum survival times of the parents of the *sepia* strain were 8 days less (27.9 days for *se* vs. 35.9 days for NJ) and 31 days less (37 days for *se* vs. 68 days for NJ), respectively. Clearly, factors other than simple zygotic lethality (due to aberrant segregation of the compound chromosomes) were reducing the fitness of the compound strain.

In the third laboratory test, *sepia* females that mated originally with *sepia* males mated a second

Table 2. *Competition between the sepia compound strain (se) and the New Jersey wild strain (NJ) held at various ratios in the laboratory (Egg samples were removed every week)*

Week	% NJ ± s.e. in replicates			
	1	2	3	4
	1:1—10 pr NJ: 10 pr *se*			
1	98 ± 0.9	98 ± 0.9	99 ± 1.0	98 ± 0.7
2	99 ± 0.7	95 ± 1.2	100 ± 0.2	100
3	100	100 ± 0.3	100	96 ± 1.7
4	100	100	100	100
	1:5—10 pr NJ: 50 pr *se*			
1	63 ± 5.5	53 ± 2.9	71 ± 2.6	62 ± 2.5
2	80 ± 2.3	81 ± 2.2	72 ± 4.2	75 ± 5.3
3	100	89 ± 2.9	79 ± 2.6	60 ± 3.5
4	97 ± 1.0	93 ± 1.4	97 ± 0.9	86 ± 1.9
5	94 ± 1.6	94 ± 2.3	100	95 ± 1.6
6	100	100 ± 0.5	100	100
7	100	100	100	100
	1:9—10 pr NJ: 90 pr *se*			
1	22 ± 2.3	7 ± 1.7	0	55 ± 3.0
2	43 ± 2.1	57 ± 3.6	29 ± 2.9	58 ± 4.3
3	72 ± 4.8	50 ± 4.0	45 ± 5.9	25 ± 3.5
4	55 ± 3.6	24 ± 2.4	33 ± 2.7	69 ± 3.0
5	81 ± 2.3	74 ± 2.3	46 ± 1.8	99 ± 0.5
6	94 ± 1.5	99 ± 0.4	82 ± 1.9	96 ± 2.8
7	100	98 ± 1.1	86 ± 3.5	99 ± 1.2
8	100	99 ± 1.1	100	99 ± 0.6
9		100	100	100
	1:15—5 pr NJ: 75 pr *se*			
1	28 ± 6.0	3 ± 2.3	65 ± 8.2	0
2	57 ± 5.0	55 ± 6.8	44 ± 6.7	24 ± 5.7
3	71 ± 2.8	61 ± 2.8	6 ± 1.8	3 ± 1.2
4	91 ± 1.5	67 ± 2.4	22 ± 2.7	41 ± 2.3
5	99 ± 1.0	92 ± 2.2	55 ± 2.7	50 ± 2.5
6	100	98 ± 0.7	56 ± 2.4	64 ± 2.2
7	100	100	47 ± 2.6	86 ± 2.0
8	100	99 ± 0.6	95 ± 0.9	90 ± 1.3
9		100	97 ± 0.7	90 ± 1.6
10		100	100 ± 0.4	100
11		100	100	100
	1:30—5 pr NJ: 150 pr *se*			
1	13 ± 2.5	0		
2	42 ± 6.2	0		
3	30 ± 2.4	0		
4	79 ± 1.9	0		
5	95 ± 1.3	0		
6	100			

time with *sepia* males 46.3 hr after the initial mating (average of 10 females) and mated a second time with New Jersey males 52.4 hr after the initial mating (average of 11 females). New Jersey females that had previously mated with New Jersey males mated a second time with New Jersey males 45.3 hr after the initial mating (average of 9 females) and with *sepia* males 35.3 hr after the initial mating (average of 10 females). These differences in times were not significant. Results were similar when the first mating was with the different strain and the second with the same strain.

When females of the two strains were given a choice of mates, 25 of 45 *sepia* females (56%) mated with

Table 3. *Eggs and adult progeny from New Jersey wild and sepia compound chromosome strains (25 pairs parents were used.)*

Week	New Jersey wild			*Sepia compound*		
	Eggs	Adults	% Recovery ± s.e.	Eggs	Adults	% Recovery ± s.e.
1	2,861	2,556	89.3 ± .58	2,029	346	17.1 ± .84
2	4,279	3,473	81.2 ± .60	2,497	347	13.9 ± .69
3	2,386	2,216	92.9 ± .53	950	165	17.4 ± 1.23
4	1,754	1,593	90.8 ± .69	492	79	16.1 ± 1.66
5	180	166	92.2 ± 2.00	39	0	0
Total	11,460	10,004	87.3 ± .31	6,007	937	15.6 ± .47

sepia males and 24 of 41 NJ females (59%) mated with NJ males. These differences are not significant, so there appeared to be no preferential mating.

Fig. 2 shows the fertility of New Jersey females after mating once with New Jersey males (control; upper line) or after mating once with New Jersey males and a second time with *sepia* males (lower line). The percent emergence of adults is plotted against time (days) after the matings. The vertical lines at each point represent twice the standard error. A second mating with a compound male decreased fertility markedly within one day, even though the females were previouslyfertilized with wild-type sperm. These results are consistent with the work of Lefevre and Jonsson (1962), which indicates that sperm from a second mating will largely displace sperm from the initial mating. These investigators also found that fertility decreases with time, as in our test, which they attributed to an increasing inefficiency of fertilization as the sperm supply was depleted.

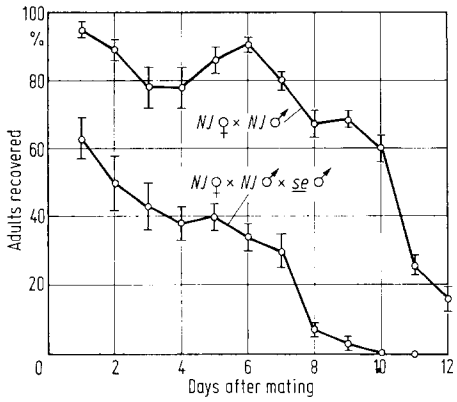

Fig. 2. Percent recovery of adults from daily broods of New Jersey females × New Jersey males (upper line) and New Jersey females × New Jersey males × *sepia* males (lower line). Vertical bars represent 2 × s.e.

Field Tests

From the laboratory cage studies, the initial concentration of the *sepia* strain must be greater than 97% to displace the native population. We decided, therefore, to aim at a release ratio of 100 *se* : 1+ (99% *se*) for the field release program. Although the releases were made while the density of native flies

Table 4. *Native (+) and released sepia compound (se) flies recovered from traps and tomatoes placed in 4 field plots. Releases were made at the Woods and Beaver Dam sites only (period indicated by the vertical line). A total of 1,222,500 flies were released at the two sites*

Week	No. recovered at Woods sites				No. recovered at Beaver Dam site				No. recovered at Airport site		No. recovered at Telegraph site	
	Traps		Fruit		Traps		Fruit		Traps		Traps	
	+	*se*	+	*se*	+	*se*	+	*se*	+	*se*	+	*se*
1*	0	2	—	—	1	3	—	—	0	7	2	8
2	13	58	—	—	5	5	—	—	5	5	1	8
3	3	6	—	—	2	6	—	—	0	11	2	51
4	73	5679	0	1	3	3	—	—	3	8	8	5
5	317	20118	17	2	105	5648	21	3	53	6	15	14
6	181	8387	49	4	78	8283	75	4	24	25	28	10
7	264	553	101	2	215	2930	101	1	114	78	51	8
8	605	230	12	1	356	166	31	5	219	63	203	11
9	797	194	59	3	296	69	122	1	214	48	157	22
10	251	35	107	0	124	8	26	0	54	2	12	12
11	206	25	—	—	29	8	—	—	31	12	36	2
12	28	1	—	—	37	2	—	—	21	0	11	0
13	41	2	—	—	6	0	—	—	6	1	5	1

* Week ending Aug. 12, 1972.

was still fairly low, the ratios actually achieved by the end of the release period were 62:1 at the Woods release site and 91:1 at the Beaver Dam release site.

From the trap collections (Table 4), the *sepia* strain was obviously unable to replace the native population. The preliminary tests indicated that the traps caught 25% fewer *sepia* than wild type flies, but even when this difference is taken into account, the *sepia* population clearly decreased in both the release and control plots after the initial period of release. (The presence of some *sepia* flies before the first releases, that is weeks 1–3, resulted from migration from an additional nearby release site.)

Only about 4% *sepia* were recovered from the slit tomatoes placed on the release sites though the trap data indicated that the percentage of *sepia* was considerably higher during the release period (Table 4). This failure of the *sepia* strain to oviposit on tomatoes is probably the major factor contributing to its decline after the releases. In the laboratory, the *sepia* strain had no difficulty ovipositing and completing development on tomatoes.

Dispersion of flies from the release plots was not a major factor in the lack of effectiveness. About 2% of the 140,000 flies marked with fluorescent dye and released in the plots were recaptured, and 99.7% of those were recaptured at the sites of release. The longevity of the *sepia* strain in the field was competitive with the longevity of the native population: 75% of the recaptures were made within the first three days after release, but marked flies were recaptured for 18 days. Also, marked flies were recaptured in traps more than 3.4 km from the release sites. The recaptures at the different distances indicated random dispersal.

The role of migration of native flies into the release sites was subsequently evaluated by monitoring the population of 10,000 *se*: 100 New Jersey in the outdoor cage (Table 5). Again, *sepia* strain was reduced by the presence of the second strain, indicating that the poor showing of *sepia* in the field was not necessarily the result of heavy migration of native flies from neighboring areas. The two other outdoor cages were populated with only *sepia* and only New Jersey flies. The population in the New Jersey cage increased rapidly; the *sepia* population dwindled to almost nothing in a few generations.

Table 5. *Number of Drosophila trapped in outdoor cages*

Weeks after original release	Cage 1*		Cage 2*	Cage 3*
	se	+	*se*	+
1**	651	17	77	154
2	141	15	17	398
3	127	157	10	412
4	26	103	5	1306
5	4	244	3	3253
6	0	1259	7	26,111
7	0	1200	5	14,494
8			32	15,577
9			0	26
10			0	12

* 10,000 *se* and 100 + released in Cage 1; 1,000 *se* released in cage 2; 1,000 + released in Cage 3.

** Week ending Aug. 30, 1972.

Our results did indicate that if the dynamics of a mixed population are known and the characteristics of the native strain and the strain to be released are thoroughly understood, then it should be possible to duplicate the results of the laboratory studies in the field. In the present case, we did not anticipate that the *sepia* strain would greatly reduce its oviposition on tomatoes in field conditions because it utilizes tomatoes efficiently in the laboratory. To make our future field studies more successful, we plan to replace the genetic background of a compound chromosome strain with that of a recently collected native strain, also to rear the flies at variable conditions (Long, 1970), and to incorporate tomatoes into the diet medium. These steps should overcome some of the major differences in viability between the two strains and will hopefully adapt the compound strain to field conditions.

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