GENETIC VARIATION IN THE CLOVER LEAFHOPPER'S ABILITY TO TRANSMIT POTATO YELLOW-DWARF VIRUS

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INTRODUCTION

STOREY (1932) discovered that individuals of *Cicadulina mbila* (Naude) varied in ability to transmit maize-streak virus, *Marmor maidis* var. *typicum* H. (HOLMES 1939). He was able to breed two races, an active race that could transmit the virus and an inactive race that was unable to do so. His experiments indicated that ability to transmit was controlled by a single sex-linked dominant gene. Later, BENNETT and WALLACE (1938) showed that families of *Eutettix tenellus* (Baker) vary in their ability to transmit curly-top virus (*Chlorogenus eutetticola* H.).

It is the purpose of this paper to deal with the hereditary variation in transmission of potato yellow-dwarf virus, *Marmor vastans* H. var. *vulgare* Black (BLACK 1940) by individual leafhoppers of the species *Aceratagallia sanguinolenta* (Prov.). The different expressions of this genetic variability are in part elucidated, and it is shown that by selective breeding, races may be obtained that differ greatly in their infective ability. An abstract of this work has already appeared (BLACK 1941).

MATERIALS AND METHODS

Materials and methods of general application in the present work will be described here; any deviations in individual experiments will be dealt with under such experiments. Crimson clover (*Trifolium incarnatum* L.), reported earlier (BLACK 1938) as susceptible to the virus, was selected as a test plant. It shows distinctive symptoms when infected with yellow dwarf and has certain advantages over potato (*Solanum tuberosum* L.) as a test plant in insect transmission studies. It is more readily grown from seed to obtain virus-free seedlings; the plants are better hosts for the leafhopper; the seedlings are readily accommodated in smaller cages and for a longer period. The crimson clover seed was sown in sand and the young seedlings transplanted individually to soil in 2½-inch pots shortly after expansion of the unifoliate leaf. Sowings were timed so that seedlings with the first trifoliate leaf just beginning to unfold were available when required for testing the infectivity of insects. Each seedling was covered by a celluloid cylinder having a cheesecloth top and a small hole cut in one side near the upper end. The insect to be tested was introduced through the hole and the aperture plugged with nonabsorbent cotton. The insects were in the nymphal stage when placed on their first test plants, and since they were tested individually, they were unable to mate and reproduce when they reached the adult stage. Subsequent transfers were made by tilting the pot and cage at about a 45° angle toward a good source of light, inducing the insect to hop to the upper part of the cage, and substituting a new pot and seedling at the bottom. With practice, transfers to fresh plants could be made rapidly in this manner. After the removal of the insect, each test plant was held for observation in the greenhouse for five weeks or longer.

The statistical significance of the data obtained was determined by extensive use of the fourfold table to ascertain the chi-square values. Yates' correction was employed in all cases. From these values the probabilities were determined by referring to tables in FISHER (1936) or YULE (1922). Throughout the paper the expression "odds x:1" indicates the probability that any difference is not due to chance alone.

ACTIVE AND INACTIVE RACES

Sixty clover leafhopper nymphs that had fed for 12 days on infected crimson clover were tested individually for their ability to transmit. Some infected several plants, while others infected none. This result suggested the possibility of obtaining active and inactive races of the clover leafhopper corresponding to those secured by STOREY for *Cicadulina mbila*. Insects transmitting the virus were bred among themselves in an attempt to obtain an active race, while those failing to transmit were also bred among themselves in an attempt to obtain an inactive race. Because of the life history of the insect and the latent period of infection in the plant, it was necessary to make the matings when about half of the results of the infectivity tests were available. Therefore, about four times as many matings were made as were actually needed, and each impregnated female was caged separately on a crimson clover plant in the acute stage of yellow dwarf. By the time the progeny was ready to be tested, all the results of the infectivity tests on the parents were available and it was then possible to select and test the best families and so to continue each line. In general, among the individuals of the active race the male infecting most plants was mated with the corresponding female. Some males were used to impregnate as many as three females. Many brother to sister matings were made in producing the third, fourth, and fifth generations. However, debility due to inbreeding manifested itself in slower growth, infertility, early death, and lack of vigor. Such matings were therefore avoided in later generations. Nevertheless, debility due to inbreeding was a serious handicap, especially in the active race. When the two races were crossed, the increase in the number, vigor, longevity, and growth rate of the progeny was striking.

The breeding program was carried through nine generations, not counting the original parental generation. In each generation an attempt was made to test 105 individuals in each race, 15 individuals in each of seven families. The test of the active and inactive race in each generation was carried out at the same time and under the same conditions, except that each family of insects was fed on a different plant. These plants, however, were selected for uniformity. An endeavor was made to transfer insects that were being tested for infectivity to a fresh plant every week. This was not always possible, but on the average each insect was tested on five plants over a period of six weeks. The number of cast skins, the deaths, and the sex of newly emerged adults were recorded at each transfer so as to furnish a complete record of each insect. The incubation period in each infected clover plant was also recorded. Examples of the data sheets for a family of the active race and for one of the inactive race are presented in figure 1. Presentation and analysis of some of the data on ecdyses and deaths of the insects and incubation period in the plant are reserved for another paper. The data analyzed in the present paper are restricted to those insects which survived at least the period during which they were confined to the first test plant. The data are also restricted to those plants from which the insects were removed alive. For comparisons involving sex it was necessary to eliminate also all data appertaining to insects that died as nymphs.

Active race, generation 9, family 23.

SEX→		♀	♀	♀	♂	♀	♂	?	♀	♀	♀	♂	♂	♀	♂	♂
INSECT NO.→		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dates	6-29-39	^{a}	^{c}	^{a}	3^{a}	^{a}	4^{o}	d	3^{a}	^{a}	2^{a}	3^{o}	^{a}	^{a}	^{a}	2^{a}
insects	7- 6-39	d	^{a}	d	3	4	^{a}			5	3	^{a}	pd	d		3
placed on	7-13-39				3				3	3	2		3			2
successive	7-20-39		2		4	2	5		3	2	3		3			2
test plants	7-27-39		2		2	2	1		3	3	1	2	4		2	d

Inactive race, generation 9, family 2.

SEX→		♂	♀	♂	♂	♂	♀	♂	♀	♂	♂	♂	♂	♂	♀	?
INSECT NO.→		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dates	6-29-39	^{a}	3^{a}	^{a}	^{a}	^{a}	^{o}	^{a}	^{a}	^{a}	^{o}	^{a}	^{a}	^{a}	^{a}	^{o}
insects	7- 6-39		3				^{a}				^{a}					d
placed on	7-13-39		2													
successive	7-20-39		2_{d}													
test plants	7-27-39			d	d	d										

FIGURE 1.—Examples of the records kept on the performance of each family of insects tested. Each number indicates the week in which the crimson clover test plant showed the first symptoms of yellow dwarf. (Weeks were counted from the day the insect was removed from the plant.) Superscript ^{o}=no cast skin present when insect removed from plant; superscript ^{c}=a cast skin found, insect still in nymphal stage; superscript ^{a}=cast skin found, insect now an adult. Subscript $_{d}$=insect dead when removed from plant; pd=plant died from causes other than yellow dwarf.

Members of the active selection fed on 3340 plants and infected 1845, or 55.24 percent, whereas members of the inactive race fed on 3762 and infected 204, or 5.42 percent. It is apparent from table 1 that in each generation there was a striking difference in the percentage of infective insects in the two races. In every case the odds against the difference being due to chance alone were greater than 100:1. In the ninth generation the active race contained 80 per-

TABLE 1

Genetic variation in the ability of the clover leafhopper to transmit potato yellow-dwarf virus.

GENERATION	ACTIVE RACE		INACTIVE RACE	
	$\frac{\text{INSECTS INFECTIVE}}{\text{INSECTS TESTED}}$	PERCENTAGE OF INFECTIVE INSECTS	$\frac{\text{INSECTS INFECTIVE}}{\text{INSECTS TESTED}}$	PERCENTAGE OF INFECTIVE INSECTS
1	$\frac{53}{60}$	88.3	$\frac{27}{60}$	45.0
2	$\frac{16}{39}$	41.0	$\frac{4}{44}$	9.1
3	$\frac{78}{101}$	77.2	$\frac{20}{103}$	19.4
4	$\frac{67}{103}$	65.0	$\frac{1}{97}$	1.0
5	$\frac{87}{92}$	94.6	$\frac{6}{99}$	6.1
6	$\frac{72}{91}$	79.1	$\frac{4}{102}$	3.9
7	$\frac{84}{92}$	91.3	$\frac{17}{103}$	16.5
8	$\frac{71}{94}$	75.5	$\frac{2}{104}$	2.0
9	$\frac{78}{98}$	79.6	$\frac{2}{104}$	2.0
Total	$\frac{606}{760}$	79.7	$\frac{83}{816}$	10.2

cent of infective insects, while the inactive race contained only 2 percent.[1] It is of interest to note that after this program of selective breeding the inactive race should still contain active individuals, for in the nine preceding generations all their direct ancestors had failed to transmit the virus. Moreover, beginning with the breeding of the seventh generation it was possible to continue the inactive selection by mating only individuals from families, none of whose 15 members transmitted the virus. In this connection it is worth noting that by the second generation the inactive selection was confined to the descendants of only four of the original insects. If multiple factors are involved in the ability to transmit, it might be that the breeding program as carried out produced the least active race possible from the recombination, without

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[1] In spite of the failure to isolate a race none of whose members would transmit, it is thought best to retain STOREY's terminology "active" and "inactive" to designate the races.

mutation, of genes present in these four insects. Inclusion of additional insects might make possible the selection of an even less active race. Similar considerations may be involved in the failure to get 100 percent of the members of the active race to transmit in spite of the fact that for nine generations all the ancestors of the race had transmitted the virus.

HYBRIDS BETWEEN THE TWO RACES

Hybrids between the two races were obtained by mating individuals belonging to the seventh and eighth generations. Tests of these hybrids were carried out at the same time and under the same conditions as tests of the eighth and ninth generations, respectively. In the eighth generation, out of 104 hybrids tested, 22, or 21.2 percent, were infective, and in the ninth generation, out of 102 hybrids tested, 31, or 30.4 percent, were infective. In each generation the odds are greater than 100:1 that the difference between the hybrids and either race is not due to chance alone. It is clear that the hybrids are intermediate in their ability to transmit. The fact that the vigor of the hybrids was noticeably greater than that of either race is considered good evidence that the differences in transmission by the active and inactive races and by the hybrids are not correlated with vigor.

SEX-LINKAGE

In table 2, the data on the infectivity of the hybrids of the eighth and ninth generations are presented in detail. The outstanding feature of the table is the high proportion of infective males to noninfective males (26:33) resulting from crosses between inactive males and active females. This proportion is significantly different (odds greater than 100:1) from the proportions in groups a or b or a+b+d (table 2). However, the odds are only 16:1 that the differ-

TABLE 2

Active and inactive progeny of crosses between the two races of clover leafhoppers.

CULTURE	PARENTS: ACTIVE ♂ × INACTIVE ♀				CULTURE	PARENTS: INACTIVE ♂ × ACTIVE ♀			
	MALES (GROUP A)		FEMALES (GROUP B)			MALES (GROUP C)		FEMALES (GROUP D)	
	ACTIVE	INACTIVE	ACTIVE	INACTIVE		ACTIVE	INACTIVE	ACTIVE	INACTIVE
G8-19	1	3	4	7	G8-25	3	9	0	1
G8-20	0	8	0	7	G8-27	2	5	0	7
G8-22	1	9	1	4	G8-28	5	3	3	3
G9-34	1	7	0	7	G8-29	2	5	0	9
G9-36	1	6	1	7	G9-39	7	3	4	1
G9-37	4	2	3	5	G9-40	6	2	2	5
G9-38	0	7	0	7	G9-41	1	6	1	6
Total	8	42	9	44	Total	26	33	10	32

ence between the proportions in groups c and d is not due to chance and group d is the closest control on group c, since the insects in c and d were from the same families and within each family fed on the same source plants. Differences between a, b, and d are not significant (odds 1:1 or less).

If we consider the data on numbers of plants infected by the hybrids (table 3), we find that in group c the proportion of plants infected to plants healthy is significantly different (odds greater than 100:1) from the proportions in groups a, b, or d. Differences between a, b, and d are not significant (odds less than 1:1). In view of these various comparisons, there seems to be good reason for concluding that males produced by mating inactive males and active females are significantly different, in regard to ability to transmit, from the females produced by such crosses and from both males and females resulting from the reciprocal crosses. These results suggest that sex-linkage is involved in the ability to transmit or that an autosomal gene (or genes) with a maternal (plasmatic) effect has a different degree of dominance in the two sexes. These two hypotheses will be discussed later.

TABLE 3

Condition of plants inoculated by progeny of crosses between the two races.

	PARENTS: ACTIVE ♂ × INACTIVE ♀					PARENTS: INACTIVE ♂ × ACTIVE ♀			
CULTURE	MALES (GROUP A)		FEMALES (GROUP B)		CULTURE	MALES (GROUP C)		FEMALES (GROUP D)	
	PLANTS INFECTED	PLANTS HEALTHY	PLANTS INFECTED	PLANTS HEALTHY		PLANTS INFECTED	PLANTS HEALTHY	PLANTS INFECTED	PLANTS HEALTHY
G8-19	1	15	7	36	G8-25	7	41	0	4
G8-20	0	32	0	27	G8-27	2	26	0	28
G8-22	2	38	2	18	G8-28	6	26	4	20
G9-34	3	37	0	35	G8-29	1	25	0	36
G9-36	3	32	3	37	G9-39	30	20	10	13
G9-37	14	16	13	26	G9-40	23	17	2	33
G9-38	0	35	0	35	G9-41	4	30	1	31
Total	23	205	25	214	Total	73	185	17	165

PERCENTAGE OF INFECTIVE MALES AND FEMALES

In table 4, data on the infective males and females in different population groups are presented. In both races and in the hybrids between them there is a higher percentage of infective males than females. In the active selection and in the population as a whole, the odds are greater than 20:1 that this difference is not due to chance alone. Since these populations are of mixed genetic constitution, the greater transmission by the males probably has the same basis as the results obtained with the hybrids alone.

TABLE 4

Percentage of male and female leafhoppers transmitting the potato yellow-dwarf virus.

INSECT GROUP	SEX	TOTAL INSECTS	INFECTIVE INSECTS	PERCENTAGE OF INFECTIVE INSECTS	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE
Active	Male	352	285	81.0	25:1
	Female	373	277	74.3	
Hybrids	Male	109	34	31.2	10:1
	Female	95	19	20.0	
Inactive	Male	379	45	11.9	7:1
	Female	397	33	8.3	
All insects	Male	840	364	43.3	32:1
	Female	865	329	38.0	

EFFICIENCY OF TRANSMISSION

The number of plants infected divided by the number of plants on which infective insects were tested has been employed as a measure of the efficiency of transmission. For convenience, the resulting fraction has been termed the efficiency index. It should be emphasized here that noninfective insects and the plants on which they were tested do not enter into the calculation of the efficiency indices. For example, most of the members of the inactive race and the plants on which they were tested are not considered because most of the insects were noninfective. The efficiency index for the inactive race was derived from only the small fraction of insects in this race that were infective and the plants on which these infective insects were tested.

TABLE 5

Efficiency of transmission of potato yellow-dwarf virus by male and female leafhoppers.

INSECT GROUP	SEX	PLANTS INOCULATED	PLANTS INFECTED	EFFICIENCY INDEX	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE
Active race	Male	1285	901	0.70	13:1
	Female	1256	838	0.67	
Hybrids	Male	152	96	0.63	9:1
	Female	82	42	0.51	
Inactive race	Male	221	130	0.59	97:1
	Female	156	70	0.45	
All insects	Male	1658	1127	0.68	92:1
	Female	1494	950	0.64	

The efficiency indices for infective males and females (table 5) in the different population groups are consistently higher for the males. The difference is slight and is significant only in the inactive race and in the total population. In table 6 the data are rearranged to show the differences in the efficiency among the infective members of the active and inactive races and the hybrids. Insects in the active race were significantly more efficient than insects of the same sex in the inactive race. In both sexes the efficiency of the hybrids fell between that of the two races. However, the only significant differences were those between female hybrids and female members of the active race and between the totals for the active race and the hybrids. In general, it appears that greater efficiency and a higher proportion of infective insects are correlated whether the latter proportion is associated with the race or the sex.

TABLE 6

Efficiency of transmission of potato yellow-dwarf virus in the two races of leafhoppers and their hybrids.

SEX	INSECT GROUP	PLANTS INOCULATED	PLANTS INFECTED	EFFICIENCY INDEX	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE	
Male	Active	1285	901	0.70	10:1	>100:1
	Hybrid	152	96	0.63	1:1	
	Inactive	221	130	0.59		
Female	Active	1256	838	0.67	>100:1	>100:1
	Hybrid	82	42	0.51	1:1	
	Inactive	156	70	0.45		
Both sexes	Active	2541	1739	0.68	>100:1	>100:1
	Hybrid	234	138	0.59	5:1	
	Inactive	377	200	0.53		

> = greater than.

DISCUSSION

The genetics of the ability of the clover leafhopper to transmit potato yellow-dwarf virus is complicated by the fact that even under favorable experimental conditions one cannot be certain that an insect which possesses the ability to transmit will do so. That insects having this ability failed to transmit is indicated by the fluctuations in the percentage of infective insects from generation to generation (table 1). A small percentage of infective insects did not infect any but the last plant on which they were tested. This suggests that, had the insects been transferred to additional plants, a few more might have been added to the infective class.

It is possible that an autosomal gene or genes in the homozygous condition might reduce the chances of an insect becoming infective to the low level existing in the inactive race, might reduce the efficiency of the few infective insects in this race, and, in the heterozygous condition, might exert an intermediate action both on the probability of an insect becoming infective and on efficiency. If in addition the gene, or genes, produced a maternal (plasmatic) effect on the egg cytoplasm with a different degree of dominance in the two sexes, all the data might be accounted for.

A simpler explanation must also be considered—namely, that the sex mechanism is the same as in Drosophila and that a single incompletely dominant gene affecting the tendency of an insect to transmit and the efficiency of transmission is located on the sex chromosome. Such an arrangement accounts for the proportion of infective insects among the hybrids of groups a, b, and d of table 2 being lower than that of group c. However, it does not account for the percentage (44.1 percent) of infective insects in group c being significantly less (odds greater than 100:1) than the percentage (77.6 percent) of infective insects in the active race during the eighth and ninth generations. If the hypothesis under consideration were true, there should be no difference between the insects in group c and those of the eighth and ninth generations of the active race. It is suggested that the difference may be due to other inhibiting genes present on other chromosomes.

It is worth pointing out here that the active and inactive groups in hybrids between the active and inactive races of *Aceratagallia sanguinolenta* are different from the corresponding groups in *Cicadulina mbila*. In *A. sanguinolenta* group c (table 2) might be considered active and groups a, b, and d inactive. In *C. mbila* (STOREY 1932) the group corresponding to a is inactive and the groups corresponding to b, c, and d are active.

SUMMARY

By selective breeding through ten generations, two races of clover leafhoppers have been obtained, one "active," the other "inactive." In the last generation, 80 percent of the active race, 2 percent of the inactive race, and 30 percent of the hybrids proved infective when tested under the same conditions. Infective individuals appeared in every generation in the inactive race, even though all ancestors in preceding generations had failed to transmit the virus. Conversely, in every generation some individuals in the active race failed to transmit the virus, although all ancestors in preceding generations had transmitted.

A slightly but significantly higher percentage of males (43 percent) than of females (38 percent) transmitted the virus. Although a difference between males and females was demonstrated to be significant only in the total population and in the active race, the difference was in the same direction in the inactive race and in the hybrids.

The male infective insects were more efficient vectors than the female infective insects in both races and in the hybrids. However, such differences in efficiency were demonstrated to be significant only for the whole population and for the inactive race. Infective members of the active race were significantly more efficient vectors than infective individuals of the same sex belonging to the inactive race. The hybrids were intermediate in efficiency, but in most cases their position was not demonstrated to be significant.

Evidence was obtained indicating that the males resulting from crosses between inactive males and active females were more active than the females derived from such crosses and more active than the males and females resulting from reciprocal crosses. Two genetic hypotheses explaining these differences are discussed.

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