PERSISTENCE OF A MUTANT GENE IN DROSOPHILA POPULATIONS OF DIFFERENT GENETIC BACKGROUNDS[1]

YONG JAI CHUNG[2]

Genetics Laboratory, University of Wisconsin, Madison, Wisconsin 53706

Received September 14, 1967

THE existence of polymorphisms in many populations is a well established fact, but the ways in which they get started are not understood. This study was undertaken to throw some light on the subject by observations on a mutant which, while presumably not polymorphic in the population wherein it arose, nevertheless tended to persist in laboratory cultures.

The mutant gene was studied in a variety of genetic backgrounds: (1) a "familiar" background of genes with which it had long been associated, (2) a background of genes from an unrelated inbred line, (3) a background of a mixture of unfamiliar genotypes, and (4) a background half familiar and half unfamiliar.

The mutant, Sb^W (Stubble bristles) has been studied in detail by FRYDENBERG (1963, 1964), who showed that the apparent overdominance for fitness was "associative," depending on coupling linkage to an inversion. The results reported here substantiate his conclusion that the mutant persists in the population only when associated with the inversion. Furthermore, persistence of the mutant depends almost entirely on the adjacent chromosome region being heterozygous in $Sb^W/+$ relative to $+/+$ and hardly at all on the heterozygosity of the rest of the genome or the length of association of the mutant with its genetic background.

MATERIALS AND METHODS

The Stubble mutant used in these experiments was descended from a single mutant *D. melanogaster* female caught in a wholesale fruit store in Madison, Wisconsin. Several strains were established and the mutant, despite being lethal when homozygous, tended to persist in some stocks without artificial selection.

FRYDENBERG (1963) showed the mutant to be located on chromosome 3 between curled wings (*cu*, 50.0) and ebony body (*e*, 70.7). This is the region where the standard mutant Stubble bristles (*Sb*, 58.2) is located (BRIDGES and BREHME 1944). FRYDENBERG demonstrated the functional allelism of the new mutant and standard *Sb* by the criterion of lethality of mutant/*Sb* heterozygotes and designated it Sb^W (Stubble Wisconsin). He also showed that the homozygote is invariably lethal and that death occurs in the larval stage. His stocks frequently carried one or both of two inversions, apparently the same as *In(3R)Mo* and *In(3R)C* (BRIDGES and BREHME 1944).

In my experiments two strains of Sb^W were used. Sb^W-A had been maintained for several years in a small mass culture and was therefore somewhat inbred. Sb^W-B was of the same origin, but for about a year prior to the beginning of these experiments had been maintained in a Bennett type population cage (FRYDENBERG 1963) with a population number of about 1000.

[1] Paper Number 1073 from the Genetics Laboratory. This work was supported by the Public Health Service (GM-7666).
[2] Present address: Department of Science, Ewha Women's College, Seoul, Korea.

Throughout the rest of this paper, the superscript will be dropped and *Sb* is understood to mean Sb^W.

All experiments were started with a population of 100 males and 100 females. Two initial *Sb* gene frequencies, 0.5 and 0.1, were used. Censuses were made each week by collecting a sample of 200 flies, which were scored and returned to the cage. The populations were maintained in transparent plastic cages of dimensions 13 × 11 × 8 cm. Each cage had nine holes in the bottom which were threaded to receive 2 × 8 cm screw-top vials. One hole was used for sampling and the remaining eight were used to provide food. On alternate days the oldest vial was replaced by a fresh one with slanted food, thus providing a food turnover every 16 days. The medium was a standard cornmeal, molasses, yeast, agar type sprayed with live yeast and containing 0.5% propionic acid as a mold inhibitor. The temperature was roughly 25°C throughout the experiments.

The C series of experiments involve populations directly from *Sb*-A and *Sb*-B. In both of these cases the *Sb* gene was in a "familiar" background of genes with which it had been associated for more than eight years. Each strain was replicated four times.

In the E series the *Sb* gene was introduced into an inbred line by four generations of backcrossing *Sb* females to males from the inbred line. Thus the mutant gene was in a background that, except for chromosome regions linked to *Sb*, was largely alien and homozygous. The four inbred lines were: (1) an inbred line marked by the mutant cinnabar eye color (*cn*, 2—57.5), (2) an inbred line marked by *cn* and claret eye color (*ca*, 3—100.7), (3) an inbred line marked with ebony body color (*e*, 3—70.7), and (4) an inbred line marked with brown eye (*bw*, 2—104.5), ebony, and the fourth chromosome mutants cubitus interruptus (*ci*) and eyeless (ey^R). All lines had been sib mated or half-sib mated for at least two years. The mutants were not scored during the experiments; only *Sb* was recorded.

After the completion of these experiments four others were started. All these utilized a Stubble strain that had been examined cytologically and shown to carry an inversion, presumably *In(3R)C*. Experiment F was done in the same way as the C series. In Experiment G the *Sb* females were backcrossed for four generations to Canton-S, a strain that had been maintained for several years as a laboratory stock in a small mass culture. In Experiment H the *Sb* females were backcrossed in large numbers to a heterogeneous population derived from a large sample

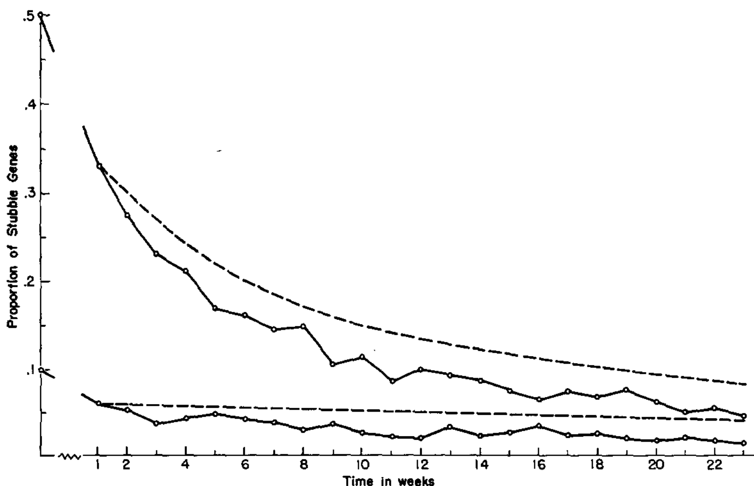

FIGURE 1.—Change in frequency of the *Sb* gene with time. *Sb* is from small mass culture; background genotype from the same stock; initial frequencies 0.5 and 0.1 (CAI and CAII). Each line is the average of four replications. Dotted line shows expected change for a completely recessive lethal.

of flies collected in nature and kept for several weeks in a population cage. Finally, Experiment I started with F_1 hybrids between the Stubble strain and Canton-S. In these experiments all cages were started with a *Sb* gene frequency of 0.5, that is 100% Stubble heterozygotes.

RESULTS

The changes in *Sb* frequency in the C series are shown in Figures 1 and 2. Each of the two strains, A and B, was started at two initial gene frequencies, 0.5 and 0.1, with four replications of each combination. The averages of the four replications are shown in the Figures. In the A series (Figure 1) *Sb* is from the stock culture; in the B series (Figure 2) *Sb* is from the population cage. It is clear that there is no significant difference in the two series.

The first count was made after the progeny had emerged and from this time on the points on the graph represent weekly enumerations. The gaps in the graphs indicate that the time before the first count was more than one week.

The dashed line in the graphs shows the expected change for a completely recessive lethal. This is based on an effective generation length of 2.5 weeks as estimated for these cages by CROW and CHUNG (1967). This estimate is not very precise, but even if the generation length estimate is badly in error, there is too rapid a decrease to be consistent with complete recessivity; so there must be some selection against the *Sb*/+ heterozygote.

The amount of selection against the heterozygotes required to reduce the mutant gene frequency at this rate is estimated roughly as:

CAI (Figure 1, upper): 0.16
CAII (Figure 1, lower): 0.13
CBI (Figure 2, upper): 0.14
CBII (Figure 2, lower): 0.17

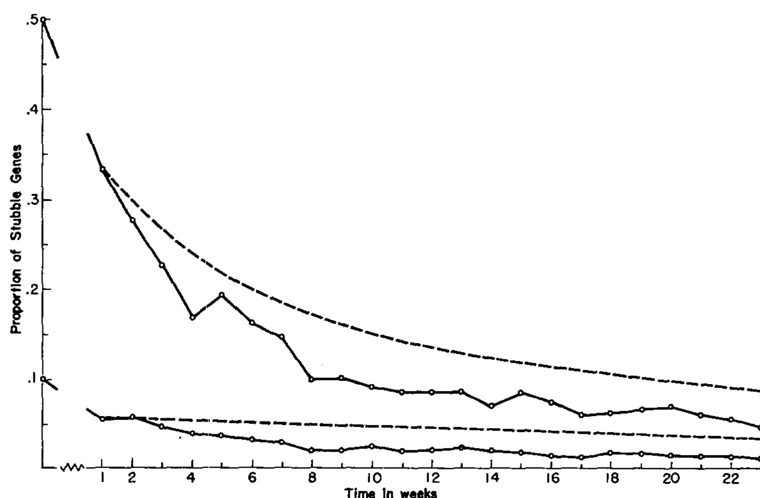

FIGURE 2.—Same as Figure 1, except that *Sb* is from a population cage (CBI and CBII).

These estimates were made by DR. J. F. CROW as follows:

Genotype	$+/+$	$+/Sb$	Sb/Sb
Fitness	1	$1-h$	0
Frequency (zygotes)	p^2	$2pq$	q^2

The change in mutant gene frequency in one generation is

$$\Delta q = \frac{pq(1-h)}{p^2+2pq(1-h)} - q = -\frac{q(q+h-2qh)}{1+q-2qh} \qquad (1)$$

Since the population is actually changing continuously we treat Δq as approximately equivalent to dq/dt. Integration leads to

$$\frac{1-h}{h}\ln\frac{h+q_t(1-2h)}{h+q_0(1-2h)} + \frac{1}{h}\ln\frac{q_0}{q_t} = t \qquad (2)$$

where t is the number of generations.

For example, in the CA experiment the frequency of the mutant gene changed from 0.33 to 0.05 in 20 weeks or 8 generations. Letting $q_0 = .33$, $q_t = .05$, and $t = 8$ in equation (2) leads to $h = .16$.

The inversion, apparently *In(3R)C*, was found in most of the cultures at the end of the experiment, so loss of the inversion from the population is not the explanation of the mutant's nonpersistence. It is rather that the population was in approximate linkage equilibrium for the inversion and the mutant gene, since the inversion, when it was present, was found about as often in nonmutant as mutant flies.

Thus in populations where the mutant gene has had time to come to linkage equilibrium there is no overdominance, but instead a consistent selection against the heterozygotes of about 15%. This is in agreement with the conclusions of FRYDENBERG (1964).

The contrasting results in the E series are illustrated in Figures 3, 4, 5, and 6. Figures 3 and 4 show the results when the *Sb* mutant from the stock culture is put into a background that is both unfamiliar and inbred. The population reaches equilibrium rather quickly and the same equilibrium is approached from above as from below. Inbred strains 3 and 4 both had the mutant ebony on the third chromosome and the higher *Sb* frequency at equilibrium reflects the low fitness of the *e* homozygotes. The heterosis is clear in each case.

These cultures also showed the presence of the inversion at the end of the experiment, mainly in the *Sb* flies. The linkage of the inversion to the mutant gene persisted through the experiment, long enough to maintain the polymorphism through roughly ten generations.

Figures 5 and 6 show two different starting mutant frequencies with the same inbred lines as in Figures 3 and 4. In this case the *Sb* came from a population cage. The results are similar in that there is clear heterosis. However, there is not the sharp difference between lines 1 and 2 and lines 3 and 4 that was found in the *Sb* from the small mass culture.

The dotted lines indicate periods when the cultures had infestations of mites and counts could not be made. The most striking instance is line 3 in Figure 5. In this case following a mite infestation during which the population number became very small, the frequency of the *Sb* gene started to decrease very rapidly.

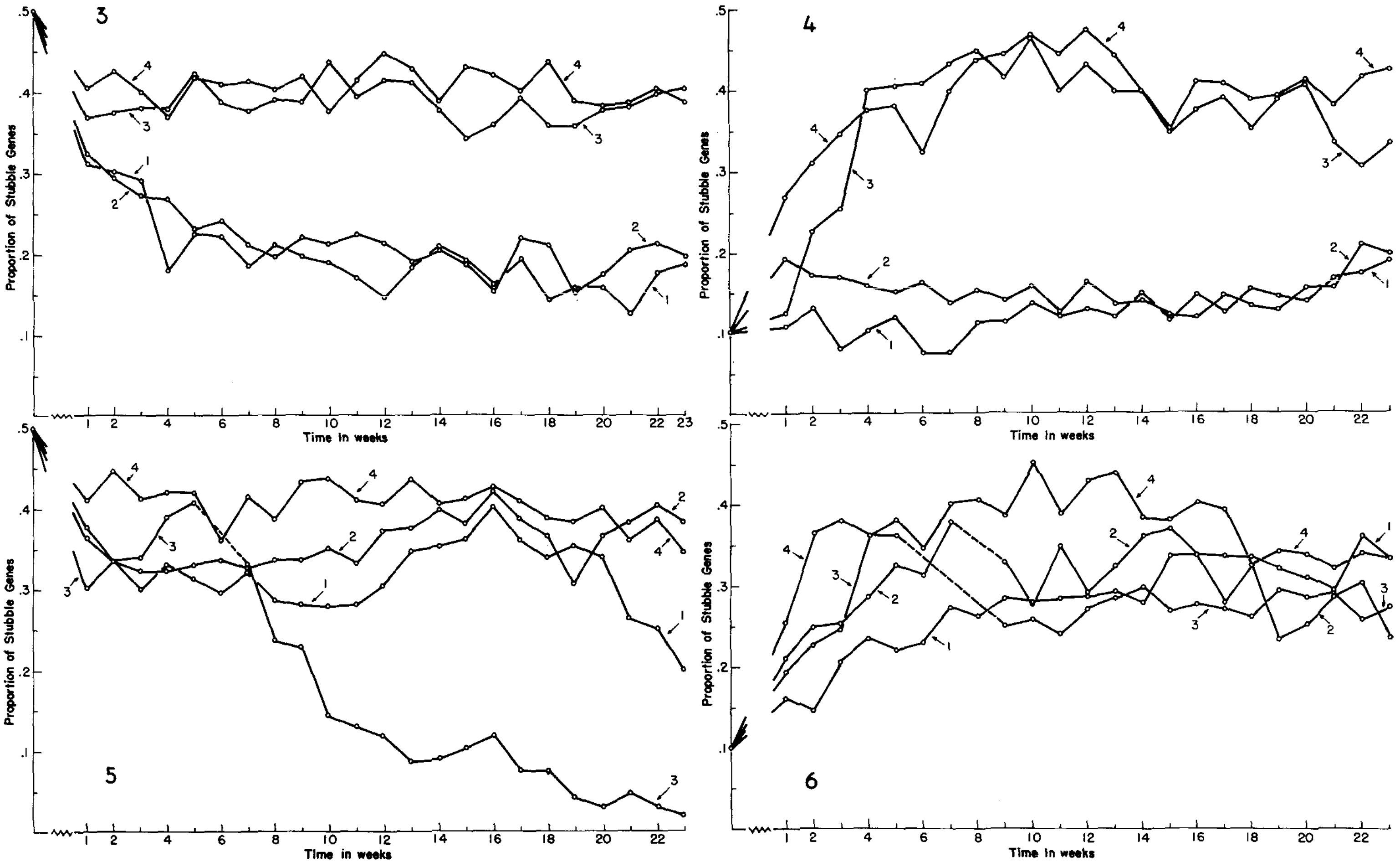

FIGURE 3.—*Sb* from small mass culture in background of four inbred lines. Initial frequency 0.5 (EAI). Numbers refer to the four lines. FIGURE 4.—Same as Figure 3, except that initial frequency is 0.1 (EAII). FIGURE 5.—*Sb* from population cage; same inbred lines as Figure 3. Initial frequency 0.5 (EBI). FIGURE 6.—Same as Figure 5, except initial frequency is 0.1 (EBII)

The most probable explanation is that the mutant happened to become separated from the inversion at about this time, and therefore had the same selective disadvantage as was found in the C series of experiments.

After these experiments were completed another set (F, G, H, and I) was started. These all utilized a *Sb* culture in which the inversion was still present (CA1-2). The procedures were as before, except that all the experiments started with a *Sb* gene frequency of 0.5 (100% Stubble heterozygotes).

The curves in the F series (Table 1 and the two lower curves in Figure 7) show a rate of decrease of *Sb* in the early generations that is consistent with a slight heterosis. This is shown by the fact that the observed frequencies are con-

TABLE 1

Frequency of the gene Sb *with inversion in each of four backgrounds*

Weeks	Background*							
	F Familiar		G Canton-S		H Heterogeneous		I F_1 Hybrid	
0	.500	.500	.500	.500	.500	.500	.500	.500
1	.435	.388	.440	.425	.410	.445	.395	.385
2	.375	.355	.423	.390	.378	.365	.385	.385
3	.335	.345	.418	.388	.345	.330	.338	.333
4	.348	.323	.413	.408	.305	.318	.343	.343
5	.310	.290	.398	.435	.285	.268	.358	.300
6	.298	.273	.408	.438	.285	.258	.328	.315
7	.263	.238	.430	.418	.258	.253	.330	.333
8	.293	.163	.445	.418	.240	.183	.345	.350
9	.260	.180	.438	.400	.250	.158	.258	.325
10	.285	.218	.403	.415	.225	.110	.293	.265
11	.313	.165	.418	.423	.203	.140	.300	.318
12	.273	.190	.425	.405	.205	.125	.288	.283
13	.225	.198	.413	.433	.175	.108	.273	.270
14	.165	.170	.423	.423	.168	.095	.283	.273
15	.233	.128	.423	.453	.155	.073	.308	.260
16	.183	.168	.395	.413	.150	.048	.280	.255
17	.210	.133	.430	.418	.138	.055	.295	.270
18	.163	.145	.423	.393	.140	.060	.293	.263
19	.128	.120	.358	.343	.130	.040	.313	.303
20	.100	.130	.370	.323	.125	.015	.308	.290
21	.135	.123	.388	.363	.135	.033	.323	.263
22	.103	.085	.370	.360	.088	.038	.323	.280
23	.123	.093	.363	.308	.113	.015	.350	.270
24	.103	.113	.350	.345	.143	.025	...	...
25	.095	.128	.368	.343	.143	.020	...	...
26	.098	.085	.318	.345	.110	.010	...	...
27	.055	.088	.355	.343	.110	.013	...	...
28	.078	.085	.378	.318	.125	.010	...	...
29	.055	.088	.353	.350	.130	.005	...	...
30	.050	.085	.330	.343	.100	.025	...	...

* (F) Familiar background from the same stock; (G) Canton-S background, (H) heterogeneous background from wild population, and (I) F_1 hybrid between *Sb* and Canton-S. Two replications of each.

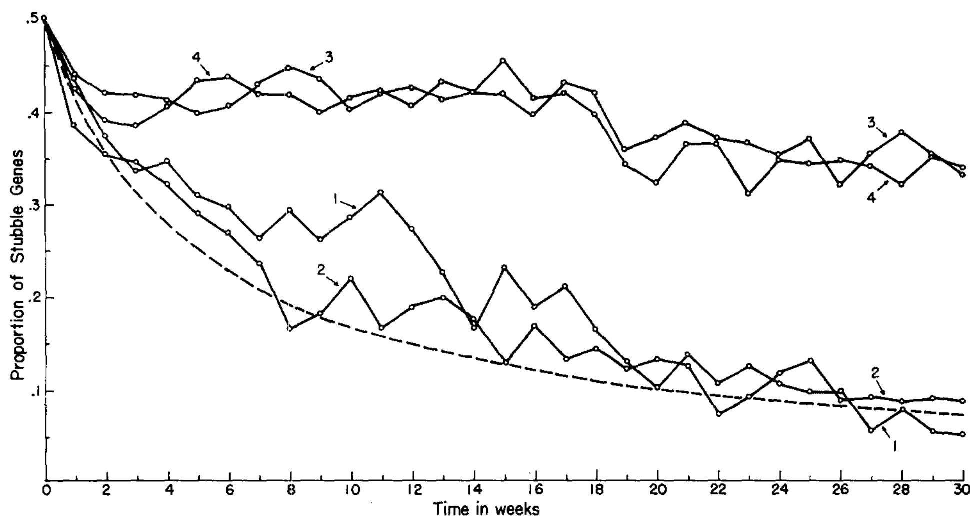

FIGURE 7.—*Sb* carrying inversion from CAI-2. Curves 1 and 2 are two replications with background genotype from the same stock (F). Curves 3 and 4 are with Canton-S background (G).

sistently higher than the dashed line, which gives the expectation for a neutral heterozygote. Later the data points shift somewhat and approach the theoretical curve.

The difference between the lower curves in Figure 7 and the C series in Figures 1 and 2 can be accounted for by the linkage relations of the mutant and the inversion. The early experiments started with populations in approximate linkage equilibrium. The populations in Figure 7 started with an excess of chromosomes in which the *Sb* gene and the inversion were in coupling.

This was confirmed by direct cytological analysis. The data are shown in Table 2. Each number in the table represents the proportion of inversions found among ten F_1 larvae when males from the cage were crossed to an inversion-free strain. Such counts were made for each of the last 14 weeks. As can be seen the average proportion of inverted chromosomes transmitted by *Sb* flies during this period was 0.33 whereas from the non-*Sb* was only 0.15. In the replicate cage the corresponding values were .40 and .22. The intrinsic disadvantage of the *Sb*

TABLE 2

Frequency of the inversion In(3R)C *in experiments F, G, and H*

	Background genotype					
	Familiar		Canton-S		Heterogeneous	
Father's genotype	F-1	F-2	G-1	G-2	H-1	H-2
Sb/+	.33	.40	.45	.46	.27	.06
+/+	.15	.22	.04	.00	.00	.03

The data represent the proportion of F_1 larvae showing the inversion when *Sb*/+ and +/+ males were mated to females from a stock with normal gene sequence. Ten larvae were examined from each class of males for each of the weeks from the 17th through the 30th.

mutant is approximately balanced by the excess of mutants in coupling with the inversion, so that the mutant is approximately neutral when averaged over the various genotypes in which it occurs. The fact that the curves at first are above the theoretical value and later begin to approach it, if the trend is real, is probably due to the population changing toward linkage equilibrium. Once again, the results are in complete agreement with FRYDENBERG's conclusions that when the mutant persists it is because of "associative overdominance" caused by linkage to the inversion.

The upper curves in Figure 7 show the results of two replicates of the G experiment in which the *Sb* mutant is in a background of Canton-S chromosomes. The Canton-S stock had also been kept in the laboratory for a number of years in small mass culture. Clearly in this case the mutant gene persists. In these cultures the mutant remained in coupling for the most part. As can be seen in Table 2, the inversion was almost always found in the *Sb* flies rather than the non-*Sb* flies.

The H series, shown in Table 1 and illustrated by the lower curves in Figure 8, represent still another background. This time the *Sb* gene was backcrossed for four generations into a population derived from wild flies. This background was as heterozygous and heterogeneous as a population in nature. In this case, as the curves show, the mutant gene tended to decrease in frequency at about the same rate as a completely recessive lethal. It might be expected from its higher frequencies that the first replicate had a higher fraction of chromosomes in which Stubble was linked to the inversion. This is borne out in Table 2. All of the inversions in replicate I were found in the progeny of mutant flies. Even so, in the heterogeneous environment the inversion did not appear to confer enough extra heterozygosity to give it any great advantage. The second replicate showed the

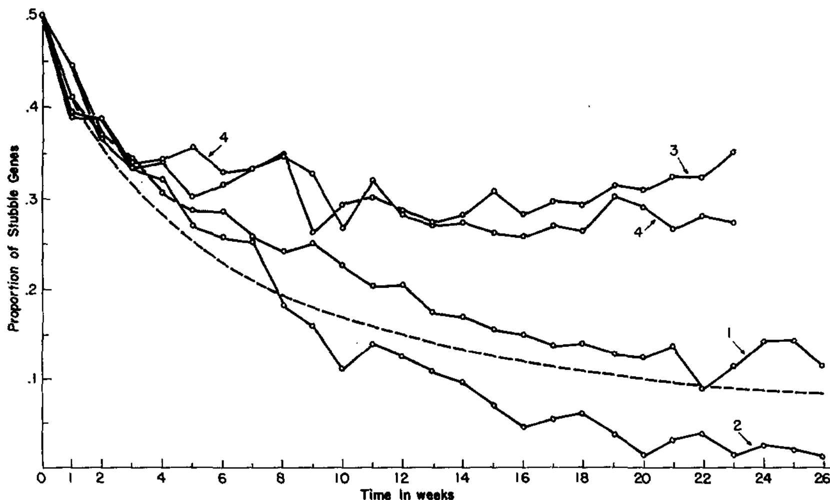

FIGURE 8.—*Sb* carrying inversion from CAI-2. Curves 1 and 2 are two replications with background genotype from a heterogeneous wild population (H). Lilnes 3 and 4 started with F_1 hybrid between *Sb* and Canton-S (I).

Sb mutant being eliminated more rapidly than if it were neutral in heterozygotes. It might be suspected that the inversion either was in linkage equilibrium with the mutant or had become largely lost from the population. As can be seen from the last column in Table 2, the inversion was almost eliminated and in the few cases where it was found, was both in coupling and repulsion with *Sb*.

Finally, the two upper curves in Figure 8 and the last two columns in Table 1 show the results of the I experiment. In this case the starting population was a group of F_1 hybrids between the Stubble strain and wild type Canton-S. Here, as can be seen, there was clear heterosis. For regions linked to *Sb* and the inversion the flies are relatively heterozygous in *Sb* flies and homozygous in non-*Sb*. On the other hand the background genotype is a heterogeneous mixture of genes from the *Sb* and Canton-S strains.

DISCUSSION

The first experiment clearly supports the view of FRYDENBERG (1964) that the *Sb* mutant is intrinsically harmful and that when it persists in a population it is because of "associative" overdominance caused by linkage with the inversion. That the inversion itself shows effective overdominance in this population is shown by its clear persistence even when the *Sb* gene was being rapidly eliminated. The *Sb* gene, being somewhere near linkage equilibrium with the inversion, could be eliminated while the inversion persisted.

On the other hand, when this chromosome region was introduced into an inbred line there was sufficient heterozygosity in this region, compared to the nonmutant types which are homozygous in this region, for the inversion and the associated mutant to persist at high frequencies.

The last four experiments can be very roughly summarized this way:

Experiment	*Sb*/+ region	+/+ region	Background genes	Result†
G	heterozygous	homozygous	homozygous	persistence
I	heterozygous	homozygous	heterozygous	persistence
H	heterozygous	heterozygous	heterozygous	elimination
F	homozygous	homozygous	homozygous	elimination

Comparison of G and I shows that as long as genes in the relevant region are more heterozygous in the inversion heterozygotes than in the flies homozygous for the normal sequence, the inversion and the associated mutant persist. The background genotype is unimportant in determining the outcome, at least relative to the heterozygosity of the immediate region.

Experiment H shows that the heterozygosity for the *Sb* — *In(3R)C* region is not advantageous unless the nonmutant chromosomes are relatively homozygous. In this experiment where the nonmutant chromosomes were highly heterogenous and heterozygous, the mutant as well as the inversion tended to be eliminated. In replication I where the inversion remained in coupling with the mutant, both are at higher frequencies and there may possibly be at least a temporary equilibrium. In the other replicate the inversion apparently got separated from the mutant and both seem to be disappearing rapidly.

The tendency for the inversion to be eliminated in the H experiment whereas it persisted in the F experiments argues that the persistence of the inversion depends mainly on the inversion heterozygotes having more heterozygous loci than inversion homozygotes. This could easily be true in the small population from which the *Sb* culture was derived, but when the structurally homozygous chromosomes were also genetically heterozygous, as in the G experiment, the inversion tends to disappear. It might be that the lower fitness of *Sb* in the H experiment was because of selection among the flies introduced from nature for genotypes that competed most effectively with *Sb*. However, unless such genotypes were very common, there would not have been time for their selection. More likely the general level of heterozygosity in this population was approximately the same in Inversion/+ as in +/+ flies; hence the nonpersistence of the inversion, as if it, too, were intrinsically disadvantageous.

Despite the long period for possible coadaptation in the *Sb* strain, which had been kept in the laboratory for over eight years in a background of chromosomes from the same population, this does not seem to be a major factor. Being in a "familiar" background is far less important than the relative heterozygosity of the region in determining persistence.

FRYDENBERG (1963, 1964) ascribed the heterosis of *Sb* to the overdominance associated with an inversion rather than to intrinsic superiority of the Stubble heterozygote. The inversions involved in his strain were *In(3R)Mo* and *In(3R)C*. His strain was originally derived from this laboratory, as were the strains used in these experiments. However, *In(3R)Mo* was not found in these studies, only *In(3R)C*. Perhaps the original Stubble population carried both inversions but subsequently *In(3R)Mo* was lost from the strains used in my experiments.

Others have also suggested that simple heterosis without coadaptation may contribute to a balanced polymorphism. CARSON (1958) and SUSMAN and CARSON (1958) reported that no prior coadaptation is required in some instances. CANNON (1963) concluded that the fitness of populations, when wild genomes are introduced into mutant populations as observed by CARSON and myself, is due to "captured luxuriance". It is rapidly produced, sustained over a period of several generations, and consequently does not involve any period of evolutionary coadaptation. The results reported here are also in agreement with the behavior of the standard Stubble mutant reported by POLIVANOV (1964); his gene showed temporary heterotic effects in monochromosomal populations. MERRELL (1965) showed that in the absence of inversions all dominant mutants tested were rapidly replaced when a single wild-type fly was introduced.

It should be emphasized that these experiments offer no evidence as to whether the homozygous disadvantage is caused by recessive genes or by homozygosity for loci which are overdominant. The results are equally compatible with either situation, and of course with a mixture of both.

Finally, these experiments possibly offer some insight into the origin of polymorphisms. Experiment I shows that when two populations, differing by an inversion, hybridize there may be a long period during which the two gene sequences are both maintained. This is especially true if the two populations are

both small and relatively homozygous. This suggests that hybrids between small populations may be a starting point for inversion polymorphisms. If the populations remain polymorphic for many generations by this mechanism various modifiers can occur that may stabilize the polymorphism. The failure of a polymorphism to be established in H shows the necessity for the two populations both to be small or, for other reasons relatively homozygous, for this system to work effectively.

Experiment G (and the C series) show the possibility of an inversion polymorphism getting started through introgressive hybridization (ANDERSON 1949). If the recurrent parent strain is relatively homozygous an introduced inversion could persist for many generations, again possibly constituting a starting point for polymorphism.

SUMMARY

A dominant mutant, Sb^{W} (Stubble bristles), was found in a natural population of *Drosophila melanogaster* and tended to persist in some laboratory cultures, despite being lethal when homozygous. It has been shown to be associated with, but separable from, an inversion.—In populations where *Sb* is linked to the inversion and heterozygosity is greater in inversion heterozygotes than in inversion homozygotes the mutant persists and reaches an equilibrium frequency. In other cases the mutant is eliminated as if the selection against the mutant heterozygotes were about 15%. The background genotype and the coadaptation are relatively unimportant in determining the persistence of the mutant.

LITERATURE CITED

ANDERSON, E., 1949 *Introgressive Hybridization.* Wiley, New York.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster.* Carnegie Inst. Wash. Publ. **532**.

CANNON, G. G., 1963 The effect of heterozygosity and recombination on the relative fitness of experimental populations of *Drosophila melanogaster.* Genetics **48**: 919–942.

CARSON, H. L., 1958 Increase in fitness in experimental populations resulting from heterosis. Proc. Natl. Acad. Sci. U.S. **44**: 1136–1141.

CROW, J. F., and Y. J. CHUNG, 1967 Measurement of effective generation length in Drosophila population cages. Genetics **57**: 951–955.

FRYDENBERG, O., 1963 Population studies of a lethal mutant in *Drosophila melanogaster.* I. Behaviour in populations with discrete generations. Hereditas **50**: 89–116. —— 1964 Population studies of a lethal mutant in *Drosophila melanogaster.* II. Behaviour in populations with overlapping generations. Hereditas **51**: 31–66.

MERRELL, D. J., 1965 Competition involving dominant mutants in experimental populations of *Drosophila melanogaster.* Genetics **52**: 165–189.

POLIVANOV, S., 1964 Selection in experimental populations of *Drosophila melanogaster* with different genetic background. Genetics **50**: 81–100.

SUSMAN, M., and H. L. CARSON, 1958 Development of balanced polymorphism in laboratory populations of *Drosophila melanogaster.* Am. Naturalist **92**: 359–364.