ON LETHALS AND THEIR SUPPRESSORS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA WILLISTONI*

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SUMMARY

Naturally occurring and radiation-induced lethals were studied in experimental populations maintained in population cages.

The populations were started with four lethals, two wild and two induced, each of them having frequency of 0.25. The lethals still had frequencies from 0.03 to 0.18 when the populations were 348 days old.

The analysis of the lethals showed that all of them were able to survive in homozygous condition. The survival of the flies homozygous for the lethals was due to the presence of recessive suppressors.

The proportion of the lethals accompanied by theirs own suppressors was measured in a sample taken when the populations were 866 days old and varied from 0 to 58.3%.

The implications of the presence of suppressors regarding the behaviour of lethals in populations are analysed and discussed.

INTRODUCTION

The maintenance of a large amount of lethal genes in the natural populations of cross-breeding organisms presents a problem of the greatest theoretical and practical importance. A part of the lethal genes is completely recessive or deleterious in their heterozygotes and maintained in the populations by recurrent mutations, while another part of the lethals is represented by genes which are overdominant and kept in equilibrium in the populations by selection in favour of their heterozygotes. In the first case the genetic load is a mutational load, while in the second, it is a balanced load. While no doubt exists about the occurrence of a mutational load as well as of a balanced load, much has been discussed about their relative importance[1,4,8–11,15,16]. The practical concern with the problem is due to the fact that the genetical effects of exposure to radiation would be much more serious if the main component of the total genetical load were mutational than if it were balanced.

This paper will present data and theoretical considerations regarding a factor,

the role of which has been ignored in the maintenance of the lethals in populations; the factor is the occurrence of genes which suppress the lethal effects.

MATERIAL AND METHODS

The work was made in experimental laboratory populations of *Drosophila willistoni* derived originally from the island of São João, Angra dos Reis. The chromosomes studied were lethal second chromosomes present in the natural population or induced by radiation. The lethal second chromosomes called n_1, n_2, n_3, and n_4 were isolated from flies caught in the natural population. Second chromosomes with normal viabilities were also isolated from flies of the same sample. Males which were homozygous for the normal chromosomes were irradiated with a dose of 800 R from a caesium-137 source of 50 C. The radiation-induced lethal second chromosomes were recovered and the ones called r_1, r_2, r_3, r_4, were used. The lethals r_1 and r_3 are alleles, while all the others are non-allelic.

All the experimental populations were started at July 26th, 1961, in population cages similar to those described by WRIGHT AND DOBZHANSKY[18]. Populations A and B were started with equal quantities of n_1/r_1, n_1/r_2, n_2/r_1 and n_2/r_2 flies in a total number of about 5000 individuals. Populations D and E were started with 5000 individuals with equal numbers of n_3/r_3, n_3/r_4, n_4/r_3 and n_4/r_4 flies. Therefore all the starting flies were heterozygous for two lethal second chromosomes, one wild and one induced and every lethal had an initial frequency of 0.25 in the experimental populations.

All the lethal chromosomes used in the experiments were kept in the laboratory, in balanced lethal system stocks. The flies in the stocks had one of the lethals in one of the second chromosomes and *St Hk 207 abb bw* in the other. *St* stands for Star, which is lethal when homozygous, *Hk* for Hook, *abb* for abbreviated and *bw* for brown. The number 207 designates a very long pericentric inversion which prevents recombination. To simplify the explanations *207* will be used as meaning the whole *St Hk 207 abb bw* chromosome. The balanced stocks will be therefore $r_1/207$, $r_2/207$, etc.

Samples were taken periodically to study the frequencies of the lethals in the populations. Two methods of analysis were used. In the first method[12] eggs were collected in the cages and put to develop in quarter bottles under optimal conditions. The males that hatched were crossed, in individual cultures, with flies having *Em* (emarginate) in one second chromosome and *abb bw* in the other. Among the F_1 flies of each cross, a single wild-type male, having therefore one second chromosome from the population and one with *abb bw*, was selected and crossed with a female *St Hk 207 abb bw/br*.

Among the offspring of this last cross, 5 virgin females having *St Hk* phenotype but non *abb bw*, being therefore *St Hk 207 abb bw*/wild, were crossed with 5 males having the same genotype. The offspring of this cross is the one analysed to detect the lethal chromosomes from the populations. No wild-type fly appears among the offspring when the wild second chromosome has a lethal one. All the flies will be *St Hk 207 abb bw*/lethal. Wild-type flies and *207* appear in the 1:2 proportion when the chromosome from the population is normal. It is necessary to identify the lethal one when the second chromosome is lethal. The identification is done by allelism test, crossing the $207/l_x$ flies with the balanced strains $207/r_1$, $207/r_2$ ...etc. The absence of wild-type flies in a cross $207/l_x \times 207/r_1$, for example, shows that l_x and r_1 are alleles

TABLE I

FREQUENCIES OF THE LETHAL CHROMOSOMES 348 DAYS AFTER THE ORIGIN OF THE POPULATIONS

At the start every lethal had a frequency of 0.250. The frequencies were determined by the second method and correspond to $q + t$.

Cage A			*Cage B*			*Cage D*			*Cage E*		
Lethal chromosome	*Frequency*	*Chromosomes studied*	*Lethal chromosome*	*Frequency*	*Chromosomes studied*	*Lethal chromosome*	*Frequency*	*Chromosomes studied*	*Lethal chromosome*	*Frequency*	*Chromosomes studied*
n_1	0.127	181	n_1	0.161	192	n_3	0.067	194	n_3	0.075	200
n_2	0.130	215	n_2	0.109	201	n_4	0.052	208	n_4	0.033	206
r_1	0.078	241	r_1	0.070	226	r_3	0.182	241	r_3	0.102	195
r_2	0.043	228	r_2	0.112	241	r_4	0.034	261	r_4	0.073	177

and most certainly identical, as the population cages were started with only four different non-allelic lethal second chromosomes. The method permits the estimation of the individual frequencies of the four lethals in each population.

The second method used in the analysis of the samples is also important regarding the data to be discussed. Males hatched from the eggs sampled were crossed with *207* females. The F_1 hybrids were then crossed with the balanced lethal stocks to see, by allelism whether, or not the chromosome from the population had lethals which were alleles to the experimental ones.

This second method always gave higher frequencies of lethals than the first method even when the sample studied by it was taken from the cage many days after the other, analysed by the first method. The explanation for the discrepancy will be given below.

We have considered as the normal viability that of flies which carry two second chromosomes taken at random from the population. The frequency of wild-type flies in the offspring of the crosses *207*/wild × *207*/wild, taking wild-type chromosomes at random, was found to be 35.5% and was taken as the normal viability in all the calculations.

RESULTS

Radiation-induced, and naturally occurring lethals are very quickly eliminated when introduced in large amounts into natural populations. A series of experiments was made in populations of isolated islands in which the same lethals as were used in the laboratory populations which were studied here were introduced in large quantities. The lethals n_1, n_2, r_1 and r_2 reached frequencies of 0.241, 0.231, 0.196, and 0.155, respectively, after the introduction into the island of São João, while in the island of Queimada Pequena, n_3, n_4, r_3 and r_4 reached respectively 0.162, 0.191, 0.137, 0.220. Eighty-six days after the introduction to São João the frequency of n_1 fell to 0.017, n_2 to 0.029, r_1 to 0.004 and r_2 to 0.009, while at Queimada Pequena, n_3 fell to 0.012, n_4 to 0.010, r_3 to 0.004 and r_4 to 0.005. The analysis of these experiments is now being done and a preliminary report has been published by DA CUNHA *et al.*[3].

The behaviour of the same lethals in the laboratory populations is at great variance with that observed in nature. The experimental populations, as stated above, were started with the lethals having frequencies of 0.250. The frequencies of the lethals 348 days from the beginning of the populations, may be seen in Table I.

TABLE II

FREQUENCIES OF WILD-TYPE FLIES HOMOZYGOUS FOR SEVERAL CAGE CHROMOSOMES IN THE TEST BY HOMOZYGOSIS AND IN THE TEST AGAINST *207*/LETHAL BALANCED STOCKS

Cage	*Age (days)*	*Chromosome number*	*Frequency homozygous, homozygosis test*	*Number studied*	*Allele to*	*Frequency homozygous, allelism test*	*Number studied*
A	75	107	0.038	103	n_1	0	112
	75	337	0.048	166	r_1	0	94
	191	804	0.254	208	n_1	0	98
B	191	546	0.096	103	r_2	0	156
	191	429	0.455	200	n_1	0	128
	191	449	0.109	146	n_1	0	104
	348	449	0.325	126	r_1	0	109
	348	29	0.361	166	n_1	0	102
	348	76	0.078	128	n_1	0	101
	348	84	0.047	106	n_1	0	99
	348	141	0.339	106	n_1	0	159
	348	152	0.144	97	r_2	0	115
D	191	703	0.571	112	r_3	0	98
	348	73	0.321	165	n_3	0	102
					n_4	0	156
	348	199	0.085	106	r_4	0	96
E	75	348	0.186	102	n_3	0	104
					r_3	0	95
	348	23	0.058	120	r_4	0	143

It is obvious that the decrease in the frequencies of the lethals is much slower in the cages than in the islands.

Indications of a factor of possible importance in the slow elimination of the lethals in the cages were obtained by analysis of the 2nd, 3rd, and 4th samples. As it was said above, the analysis of the frequencies of the lethals involves the production of flies homozygous for chromosomes from the cages. Wild-type and *207* flies appear in the proportion 1:2 when the chromosome from the cage is normal and no wild-type fly appears when a lethal is present in the chromosome tested. Several chromosomes, that should be lethal-free, by the criteria above, were used to make heterozygous flies having the *207* chromosome as their homologous one. These heterozygous flies were afterwards tested for allelism with the lethal-balanced stocks *207*/n_1, *207*/n_2 ... etc. No wild-type fly was produced in several crosses *207*/"lethal-free" × *207*/lethal. These results indicate that in the "lethal-free" chromosomes there were lethal genes, allele to the ones present in the balanced *207*/lethal stock. However the lethal gene in the chromosome from the cage does not produce its lethal effect when the chromosome is homozygous. The first explanation, which was confirmed later, was that recessive genes which suppress the lethal ones were present in the tested chromosome. The homozygotes for the tested chromosomes survive because they are homozygous for the lethals and also for the lethal suppressors. The tests with *207*/lethal produce, when the two lethals are alleles, flies which are homozygous for the lethals but heterozygous for the suppressors, which being recessive do not prevent the lethal effects. Table II shows the frequencies of wild-type homozygous flies for several chromosomes in the homozygosis tests and the absence of wild-type flies in allelism tests

TABLE III

TOTAL FREQUENCIES OF THE LETHALS AND THEIR FREQUENCIES WITH AND WITHOUT SUPPRESSORS. FREQUENCIES OF SUPPRESSORS AMONG THE LETHALS

Sample taken when the populations were 866 days old.

Cage	*Lethal chromosome*	*Chromosomes studied*	*Total frequency lethals*	*Frequency lethals with suppressors*	*Frequency lethals without suppressors*	*Frequency suppressors among lethals*
A	n_1	254	0.0433	0.0039	0.0394	0.0909
	n_2	255	0.0471	0.0274	0.0196	0.5833
	r_1	262	0.0305	0.0038	0.0267	0.1250
	r_2	255	0.0196	0.0039	0.0156	0.2000
D	n_3	193	0.0622	0.0103	0.0518	0.1666
	n_4	193	0.0155	0	0.0155	0
	r_3	204	0.0539	0.0049	0.0490	0.0909
	r_4	203	0.0049	0	0.0049	0

using the same chromosomes. It should be kept in mind that the normal viability for second chromosome homozygotes was taken as being, in the homozygosis tests, 35.5% of wild-type homozygous flies to 64.5% of heterozygous *207*/wild flies.

The presence of suppressors explains why analysing samples by crossing the flies directly to *207* and testing afterwards the hybrids against the balanced *207*/lethal strains gives more lethal chromosomes than by using the homozygosis method.

When the presence of the suppressors was detected it was too late, due to the long duration of the tests, to go back to the beginning and to analyse the frequencies in which they occur.

The frequencies of lethals together with the frequencies of their suppressors were analysed in a sample taken when the experimental populations were 866 days old. The chromosomes of these samples were made homozygous and after that, everyone, irrespective of its viability, was tested for allelism to the experimental lethals balanced with *207*. The results obtained are presented at Table III. The lethal chromosomes are separated into two classes, namely, lethal with suppressor and lethal without suppressor. The frequencies of suppressors among the lethals are shown in a special column. Regarding two lethals, n_4 and r_4, no suppressor was found in the cage D. However, suppressors for those two genes were found in previous samples from the same cage. Suppressors for all other lethals were found with frequencies among the

TABLE IV

VIABILITIES, AS PERCENTAGE OF THE NORMAL, OF SOME HOMOZYGOTES FOR CHROMOSOMES HAVING A LETHAL AND ITS SUPPRESSOR

Chromosome number	*Lethal chromosome*	*Viability (%)*	*Chromosome number*	*Lethal chromosome*	*Viability (%)*
334	n_1	28.17	205	n_2	92.96
7	n_2	59.15	355	r_1	115.50
11	n_2	84.51	319	r_2	84.51
23	n_2	19.72	108	r_3	14.08
33	n_2	59.15	135	n_3	129.58
35	n_2	14.08	203	n_3	39.44
584	n_2	59.15			

lethals, ranging from 9% to 58.3%. These frequencies show that the suppressors, relatively to the lethals, are very common in the experimental populations.

Table IV shows the viabilities of flies homozygous for second chromosomes which have at the same time a lethal and its suppressor. The homozygotes for the lethals and for their suppressors have viabilities ranging from 14% to 129.68% of the normal viability.

Suppressors also occur sporadically in the *207*/lethal stocks giving origin to wild-type flies. However the stocks are continuously watched and every wild-type fly which occurs is eliminated.

The analysis of the experiments carried out in nature, in the islands of Angra dos Reis, is showing that suppressors may also be involved.

DISCUSSION

The mathematical models in population genetics often rest on too simple assumptions. The biologist should not forget this fact and should recognize that the pools in the Mendelian populations are very highly organized. The gene-pools are not mosaics of independent pieces but an assembly of interdependent genes. The genes of a gene-pool are co-adapted through their interactions in the determination of the phenotype. The present paper brings new facts bearing on the co-adaptation of the genes in the gene-pool. The destiny of lethal genes in a natural population does not depend only on the effects of the genes themselves, but also of their interactions with the rest of the members of the gene-pool. Prominent in the interactions with the lethals are their suppressors, whose presence and role in the populations have not hitherto been studied.

The results here reported show that suppressors of lethals may be very common in populations and highly important in the determination of the frequencies of occurrence of the lethals. The data also show that the results so far obtained in the analysis of the frequencies of lethals in natural populations may be misleading. The tests used to determine the frequencies of lethals always involve the production of homozygous chromosomes. The frequencies obtained are therefore underestimated because chromosomes with lethals may survive as homozygotes thanks to the presence of suppressors.

The data presented at Table III show that 58.3% of the lethals n_2, 20% of r_2, 16.6% of n_3, 12.5% of r_1, 9% of n_1 and of r_3 would not be detected by tests by homozygosis. Determination of lethals by homozygosis only may give a gross underestimation of their frequencies.

The presence of suppressors has marked effects on the dynamics of the lethals as the following model will show. If, of two loci in the same chromosome, one mutates to produce a lethal *l* and the other produces a suppressor *su*, four different types of chromosomes would be produced. The four types with their frequencies are:

$$su^+ l^+ = p;\ su^+ l = q;\ su\ l^+ = r;\ su\ l = t.$$

The frequency of the lethal will be $q + t$ and that of the suppressor, $r + t$. The four types of chromosomes will give 10 different genotypes of which su^+l/su^+l and $su^+l/su\ l$ will be lethal combinations and will have frequencies of q^2 and $2qt$ respec-

tively. The lethal being completely recessive, all the other combinations including *su l*/*su l* will have survival equal to 1. Taking as mutation rates

$$l^+ \underset{v}{\overset{u}{\rightleftarrows}} l \text{ and } su^+ \underset{y}{\overset{x}{\rightleftarrows}} su,$$

the 12 possible combinations produced by mutation will appear with the rates shown in Fig. 1. Another factor to be considered, in the analysis of the equilibrium, is the rate of recombination D between the two loci. Only two genotypes will present recombinations which will affect the chromosomal frequencies, namely, $su^+ l^+/su\ l$ with frequency $2pt$ that will produce $su^+l = pt\ D$ and $su\ l^+ = pt\ D$, and $su^+l/su\ l^+$ with

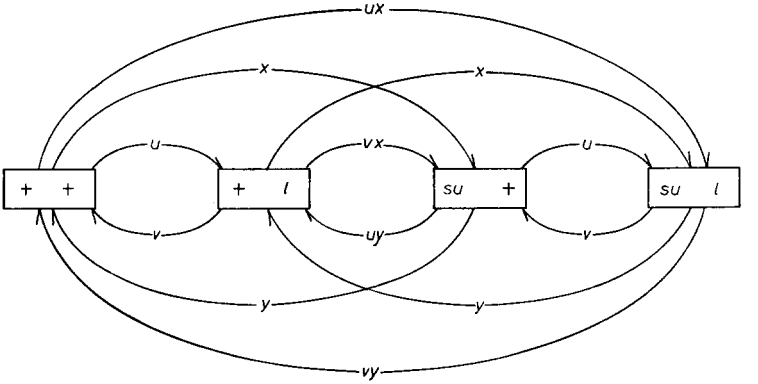

Fig. 1. Types of mutations and their rates of occurrence.

frequency $2qr$ that will produce $su^+l^+ = qr\ D$ and $su\ l = qr\ D$. These values should be added to their original frequencies and subtracted from those of the classes from which they originated.

The changes in the frequencies of the several types of chromosomes, considering the selection against the lethals, crossing-over and mutation, will be:

$$\Delta q = \frac{pq + ptD + qr - qrD}{\overline{w}} - q + up + uyr + yt - vq - vxq - xq \qquad (1)$$

$$\Delta r = \frac{pr + ptD + qr - qrD + r^2 + rt}{\overline{w}} - r + xp + vxq + vt - ur - uyr - yr \qquad (2)$$

$$\Delta t = \frac{pt - ptD + qrD + t^2 + rt}{\overline{w}} - t + uxp + xq + ur - vt - yt - vyt \qquad (3)$$

The mean adaptive value of the population $\overline{w}$ is equal to $1-q^2-2qt$.

Making Δq, Δr and Δt equal to zero, it is possible to calculate the values $\hat{q}$, $\hat{r}$ and t at equilibrium. However this system of 3 equations is very complex because it is a non-linear system. A numerical resolution is easier and it was solved by iteration using an IBM 1620 electronic computer. The programme was made using the mutation rates and the recombination values presented at Table V where are also shown the

TABLE V

THEORETICAL VALUES FOR THE FREQUENCIES OF THE SEVERAL CHROMOSOMAL TYPES IN POPULATIONS AT EQUILIBRIUM AND VALUES OF $s = t/(1-q)$ FOR THE SEVERAL EQUILIBRIA

	$u = v = x = y = 10^{-6}$ $D = 0.0$	$5 \cdot 10^{-6}$ $D = 0.0$	$u = v = x = y = 10^{-5}$				
			$D = 0.0$	$D = 0.0001$	$D = 0.001$	$D = 0.05$	$D = 0.10$
$su^+ l^+ = \hat{p}$	0.52157	0.49392	0.49389	0.47398	0.43990	0.38606	0.38408
$su^+ l = \hat{q}$	0.00022	0.00296	0.00045	0.00066	0.00109	0.00152	0.00153
$su\ l^+ = \hat{r}$	0.47608	0.49490	0.49482	0.51800	0.55500	0.60990	0.61190
$su\ l = \hat{t}$	0.00213	0.00822	0.01084	0.00736	0.00401	0.00252	0.00249
Total	1.00000	1.000	1.00000	1.0000	1.00000	1.0000	1.00000
$s = t/(1-q)$	0.00213	0.00824	0.01084	0.00736	0.00401	0.00252	0.00252

values found for $\hat{q}$, $\hat{t}$ and $\hat{r}$ at equilibrium (twenty copies of the programme are deposited at the Editorial Office of *Mutation Research*).

The values of $\hat{q}$ at equilibrium obtained in the calculations are very close to 0.0006, found experimentally by DA CUNHA *et al.*[2], and are lower than the values estimated for recessive lethals in the absence of suppressors. The results obtained in the programme show that the equilibrium values for recessive lethals found in nature are lower than the values expected on the assumption of complete recessivity and may be explained by the presence of suppressors rather than by deleterious effects in heterozygous condition. According to DOBZHANSKY AND WRIGHT[5] it is possible, knowing the mutation rate u, to estimate the elimination of lethals in heterozygous condition from the deviations expected on the assumption of complete recessivity.

TABLE VI

CORRESPONDENCE BETWEEN THE MODEL USING ONE GENE PAIR l^+ AND l, AND THE MODEL WITH TWO GENE PAIRS, THE LETHAL, l^+ AND l, AND THE SUPPRESSOR, su^+ AND su

Single pair model			*Lethal plus suppressor model*		
Chromosome	*Type*	*Frequency*	*Chromosome*	*Type*	*Frequency*
Non-lethal	l^+	$(1-q)$	Non-lethal	$su^+ l^+$	p
				$su\ l^+$	r
				$su\ l$	t
Lethal	l	q	Lethal	$su^+ l$	q
	Total	$(1-q)+q=1$			$p+r+t=(1-q)$

Viability	*Frequency*	*Genotype*	*Genotype*	*Frequency*	*Viability*
1	$(1-q)^2$	l^+/l^+	$su^+ l^+/su^+ l^+$	p^2	1
			$su^+ l^+/su\ l^+$	$2pr$	1
			$su^+ l^+/su\ l$	$2pt$	1
			$su\ l^+/su\ l^+$	r^2	1
			$su\ l^+/su\ l$	$2rt$	1
			$su\ l/su\ l$	t^2	1
	Sub-total	$(1-q)^2$		Sub-total	$(p+r+t)^2=(1-q)^2$
$(1-s)$	$2(1-q)q$	l^+/l	$su^+ l^+/su^+ l$	$2pq$	1
			$su\ l^+/su^+ l$	$2qr$	1
			$su\ l/su^+ l$	$2tq$	0
	Sub-total	$2q(1-q)(1-s)$		Sub-total	$2q(p+r)$
0	q^2	l/l	$su^+ l/su^+ l$	q^2	0
	Sub-total	0		Sub-total	0
$\bar{w} = (1-q)^2 + 2q(1-q)(1-s)$			$w = (1-q)^2 + 2q(p+r)$		

PAVAN AND KNAPP[13] have estimated the selection against the heterozygotes for lethals as $s = 0.0226$ and PROUT[14] as $s = 0.020$.

Making the comparison between WRIGHT'S[17] single pair of genes model and the lethal plus suppressor one, as shown at Table VI, it may be seen that elimination of heterozygotes for lethals calculated on the basis of WRIGHT'S model, may be apparent but not real. What is apparently elimination of heterozygotes in WRIGHT'S model may be death of the homozygotes for lethal but heterozygous for the suppressors, which are not distinguished from the heterozygotes for lethals. It should be kept in mind that the estimations so far made for the elimination of heterozygotes are based on the frequencies of lethals which are underestimated due to the ignorance of the suppressors. The frequencies of lethals expected from WRIGHT'S model are correct but the observed ones are misleading due to the class *su l/su⁺l*. The usual tests by homozygosis do not distinguish the chromosome *su l* from the lethal free ones. Therefore, *su l/su⁺l* with viability o is included among the lethal heterozygotes decreasing the whole viability of the heterozygous class. While in WRIGHT'S model the elimination of lethals is $q^2 + 2qs(1 - q)$ in the suppressor model it is $q^2 + 2qt$.

The fitness of the population in the first model, $(1 - q)^2 + 2q(1 - q)(1 - s)$ being equal to the fitness in the second $(1 - q)^2 + 2q(p + r)$ we have $2q(1 - q)(1 - s) = 2q(p + r)$. The solution of the equation gives $s = t/(1 - q)$.

This result explains why, even without selection against heterozygotes for lethals a value of selection against the heterozygotes may be obtained if suppressors are ignored. The value obtained is a function of the relation between the frequency of chromosomes *su l* and the frequency of *su⁺l*.

The occurrence and the unexpectedly high frequencies of recessive suppressors in population makes necessary the re-evaluation of several aspects of the research on population genetics. It was already pointed out above that the evaluations of the frequencies of lethals in natural populations, as it is usually done, may be significantly underestimated. It was also pointed out that calculated elimination of lethal heterozygotes may be a deception caused by suppressors. The occurrence of a lethal in high frequency and during many generations is another observation that may be due to the presence of suppressors. The explanation of this fact as due to the heterozygotic effects of the lethal was always dubious, while suppressors are very probably responsible for it. Special attention to suppressors is also advisable in the work on synthetic lethals. Many synthetic lethals could be due to recombinations between *su l* and *su⁺l⁺* chromosomes, which are both normal in homozygosis tests but could give rise to lethal *su⁺l* chromosomes.

The present paper extends to the recessive lethals the observations of GLASS[7] and of GARDNER *et al.*[6] on the widespread occurrence of suppressor genes for erupt-eye and for tumorous-head. It also supplies new facts in favour of GLASS'S ideas on the important role of genes acting as suppressors in the organization of the gene-pools of Mendelian populations.

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