discussion. Hence the effect on the inclination of the grid lines due to rotation through the angle θ-ω as the result of unloading (sec. 7) can be neglected. But this means that the theory gives no appreciable rotation of grid lines in the plastic band for the compressible case from equation (22). For the incompressible case we find from equation (21) that the rotation is likewise so small as to be negligible in comparison with the experimental results.

We have here an example of a simple and mathematically correct theory which fails completely to explain the experimental facts. The discrepancies encountered with experiment could not have been predicted on purely theoretical grounds. It seems quite clear, however, from a consideration of the column headed $\Delta T/T$ in Table 1 that the primary difficulty lies in the value assigned by the theory to the quantity b for incompressible material and to the corresponding quantity c for compressible material.[1] This matter will be discussed in a forthcoming communication, and a modification will be proposed which gives results in satisfactory agreement with the data in Table 1.

[1] T. Y. Thomas, "On the Inclination of Plastic Slip Bands in Flat Bars in Tension Tests" and "The Effect of Compressibility on the Inclination of Plastic Slip Bands in Flat Bars," these PROCEEDINGS, **39**, 257–273 (1953).

[2] A nonpositive value of the band elongation may not be in agreement with the experimental fact. However, this situation is remedied by the modification of the theory proposed at the end of sec. 8.

[3] R. Hill, *The Mathematical Theory of Plasticity* (Oxford: Oxford University Press, 1950), p. 39.

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*ENVIRONMENTAL MODIFICATION OF HETEROSIS IN DROSOPHILA PSEUDOOBSCURA**

BY TH. DOBZHANSKY AND N. SPASSKY

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY, NEW YORK

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Recent work in population genetics has shown that the selective forces which operate in many natural populations are greater than classical evolutionists were inclined to assume. It is not surprising to find strong selection acting against deleterious mutants which produce hereditary diseases or malformations. However, permanent and "normal" constituents of some natural populations which display balanced polymorphism are likewise maintained by selection of the order of 0.1–0.8. Natural selection of this magnitude can be observed in nature and can be dealt with in laboratory experiments. Experiments of this sort have disclosed a further fact of interest: The adaptive values of some genetic components of natural populations are remarkably sensitive to environmental changes. A genetic variant which is deleterious under some conditions may become useful under only slightly altered conditions, and vice versa.

The chromosomal polymorphism in natural populations of *Drosophila*, particularly of *Drosophila pseudoobscura*, yields itself admirably to experimental study. Most experiments have been made with laboratory populations which contain chromosomes with ST (Standard) and CH (Chiricahua) gene arrangements kept at 25° C.

If the chromosomes are derived from the population of the same geographic locality, the ST/CH heterozygotes are usually heterotic, i.e., adaptively superior to the ST/ST homozygotes, and the latter are in turn superior to the CH/CH homozygotes. Because of this heterosis, the experimental populations reach a genetic equilibrium, at which ST chromosomes are more frequent than CH chromosomes. However, Wright and Dobzhansky[1] found that, while the above result is obtained at 25° C., the populations kept at 16° C. retain the frequencies of ST and CH present at the start of the experiment, generation after generation, with little or no change. It follows that, within the limits of experimental errors, the adaptive values of the karyotypes are similar at 16°. Spiess[2] has shown that in the related species, *D. persimilis*, certain structural heterozygotes exhibit heterosis at 16° but not at 25°. It is relevant in this connection that *D. persimilis* is characteristically an inhabitant of cooler climates than *D. pseudoobscura*.

Da Cunha[3] tested the effects of nutritional variables on heterosis in *D. pseudoobscura*. He worked with laboratory population cages containing ST and CH chromosomes, and he fed them on seven different species of yeasts and on two species of bacteria, in addition to the *Saccharomyces cerevisiae* (Fleischmann's yeast) with which most other experiments were done. He found that the adaptive values of the karyotypes varied considerably with the microörganism employed in the food. Most interesting is the fact that, with two of the yeasts and with the bacteria, the ST/CH heterozygotes no longer exhibit heterosis. The ST chromosomes tend to reach fixation, and CH chromosomes to be eliminated. The experiments of Da Cunha were preliminary in character, since he maintained his populations for periods of a few months only. The experiments to be described below represent an extension of the work of Da Cunha.

Material and Technique.—The initial material of our experimental populations consisted of F_1 hybrids between twelve strains homozygous for ST and twelve strains homozygous for CH. These strains were isolated from the population of Piñon Flats, Mount San Jacinto, California, by D. F. Mitchell and were kindly placed by him at our disposal. The same strains were used also in the experiments of Dobzhansky and Pavlovsky[4] and of Levene, Pavlovsky, and Dobzhansky.[5] The populations started, then, containing equal proportions, 50 per cent, of ST and CH chromosomes in their chromosome pool. The population cages used were of the type previously described.[1, 6] The cages were kept either in incubators at 25° and 21° C. or in a constant-temperature room at 16° C. The relative humidity in the incubators is lower than in the room, necessitating periodic irrigation of the cups with developing larvae by a yeast suspension. Such irrigation was practiced also at 16°, though at less frequent intervals.

Two species of yeasts were used, namely, *Zygosaccharomyces dobzhanskii* Shehata and *Kloeckera apiculata* (Lindner) Dvornik (Syn. *Kloeckera magna* [De Rossi] Janke). We are obligated to Professor H. Phaff, of the University of California, for the correct names of these yeasts. They were originally isolated from the contents of crops of *Drosophila* colleced in nature[3] and were maintained in our laboratory on slants. The technique of feeding the experimental populations described by Da Cunha[3] was followed in our experiments as precisely as possible. This technique does not guarantee that a single species of microörganism develops in the nutrient medium on which the flies feed, but it does insure that the desired microörganism will at all times be present in that medium.

The average length of a generation of *D. pseudoobscura* in the population cages is approximately 25 days at 25°, 30 days at 21°, and 37 days at 16° C. The samples were taken in all populations at intervals somewhat longer than a generation in the early stages and of about two or three generations later on. Each sample consisted of 300 chromosomes (150 larvae) taken in six subsamples on as many successive days.

Experiments at 16° C.—The population cages Nos. 99 and 101, fed on *Zygosaccharomyces*, and Nos. 100 and 102, fed on *Kloeckera*, were placed in the constant-temperature room at 16°. The initial populations contained, as stated above, equal numbers of ST and CH chromosomes (50 per cent of each). Samples taken 50 and 100 days after the start gave the results reported in the three upper rows of Table 1. No change in the frequencies of the chromosomes was observed. Testing the eight samples for homogeneity gives a chi-square of 8.31, which, for 7 degrees of freedom, corresponds to a probability of about 0.4. The mean frequency of ST chromosomes in the eight samples is 49.29 per cent, which is well within the expected range of deviations from the initial frequency of 50.00 per cent. It should be recalled that Wright and Dobzhansky[1] and Dobzhansky[7] observed no changes in the frequencies of ST and CH chromosomes in populations fed on *Saccharomyces cerevisiae* and kept at 16°. In these older experiments the results did not depend upon the initial frequencies of ST and CH in the populations, thus proving that the adaptive values of the three karyotypes were sufficiently close to equality to produce no appreciable alteration in the composition of the populations within the time intervals studied. Da Cunha[3] did observe some changes in two populations fed on *Zygosaccharomyces* at 16°. Our results are not necessarily contradictory to his, since the initial frequency of the ST chromosomes in our experiments may not be far removed from the equilibrium value in the experimental environment. It is nevertheless interesting that no changes were observed in our experimental populations at 16° for 100 days, which correspond to approximately three fly generations at that temperature. Such changes have occurred in similar populations at higher temperatures.

TABLE 1

FREQUENCIES (PER CENT) OF STANDARD CHROMOSOMES IN POPULATIONS STARTED AT 16° C. AND CONTINUED AT 21° C.

TIME (DAYS)	t (deg C.)	*Zygosaccharomyces* No. 99	*Zygosaccharomyces* No. 101	*Kloeckera* No. 100	*Kloeckera* No. 102
0	16	50.0	50.0	50.0	50.0
50	16	46.7	48.0	48.0	47.0
100	16	49.3	55.7	52.3	47.3
42	21	64.3	64.3	62.7	58.0
75	21	68.0	72.3	66.0	67.0
135	21	72.3	73.3	72.7	71.3
215	21	79.0	82.0	83.0	72.0
255	21	80.3	86.0	76.3	75.0
355	21	86.0	88.7	75.7	77.3
415	21	90.3	90.7	76.0	77.0
475	21	94.0	93.7	...	...
535	21	95.3	95.7	...	...

Experiments at 21° C.—Population Nos. 99–102 were transferred to an incubator at 21° after they had lived for 115 days at 16°. The chromosome frequencies found in the last sample (100 days; see Table 1) at the lower temperature may, then, be treated as the initial frequencies with which these populations started their life at

21°. Subsequent samples were taken at 42, 75, 135, 215, etc., days after the transfer to the higher temperature (Table 1).

The alteration of the temperature resulted in rapid genetic changes in all four populations. As shown in Table 1 and Figure 1, the frequencies of ST chromosomes rose from 50 per cent to about 70 per cent in 135 days (about four and a half generations). The subsequent fate of the populations fed on *Zygosaccharomyces* was, however, quite different from that of those fed on *Kloeckera*. In the former populations, the ST chromosomes continued an uninterrupted increase; a year after the transfer to the incubator they reached a level between 85 and 90 per cent, and they reached a level of about 95 per cent when the experiment was terminated, with samples being taken around the five hundred and thirty-fifth day. It appears that

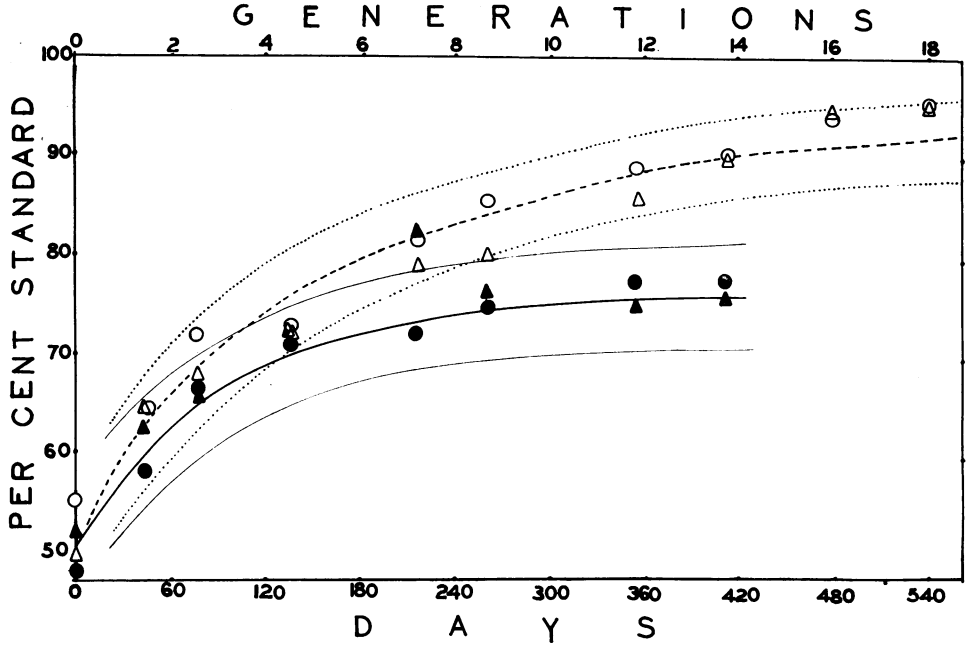

FIG. 1.—Frequencies of Standard chromosomes in populations kept at 21° C. *White symbols* = Fed on *Zygosaccharomyces; black symbols* = Fed on *Kloeckera; triangles* = Populations Nos. 99 and 100; *circles* = Populations Nos. 101 and 102.

in these populations the ST chromosomes were well on their way to fixation, and CH chromosomes on the way to elimination. The last stages of selection are, however, so slow that observing them is too laborious. The populations fed on *Kloeckera* (Nos. 100 and 102) reached the composition of about 75 per cent ST and 25 per cent CH chromosomes some 215 days after the start and failed to show appreciable further change for 200 more days. They were terminated when 415 days old. Here a genetic equilibrium has evidently become established.

Experiments at 25° C.—Four populations, two of which were fed on *Zygosaccharomyces* (Nos. 105 and 107) and two on *Kloeckera* (Nos. 106 and 108), were placed in an incubator at 25°. The initial frequencies of the ST and CH chromosomes were 50 per cent (the parental flies being F_1 heterozygotes). The results are reported in

Table 2 and Figure 2. For about three generations the ST chromosomes rapidly increased in frequencies. Later on, the increase became very slow, and equilibria became established some six to eight generations after the start. After the 240-day samples were taken, the populations were lost owing to an incubator accident.

TABLE 2

FREQUENCIES (PER CENT) OF STANDARD CHROMOSOMES IN POPULATIONS LIVING AT 25° C.

TIME (DAYS)	*Zygosaccharomyces* No. 105	*Zygosaccharomyces* No. 107	*Kloeckera* No. 166	*Kloeckera* No. 108
0	50.0	50.0	50.0	50.0
35	60.3	59.7	60.0	60.0
70	68.7	66.3	68.0	65.3
105	69.3	72.0	69.0	68.0
145	68.7	72.0	70.0	66.7
180	70.7	75.7	68.7	72.7
240	75.3	72.3	71.0	73.0

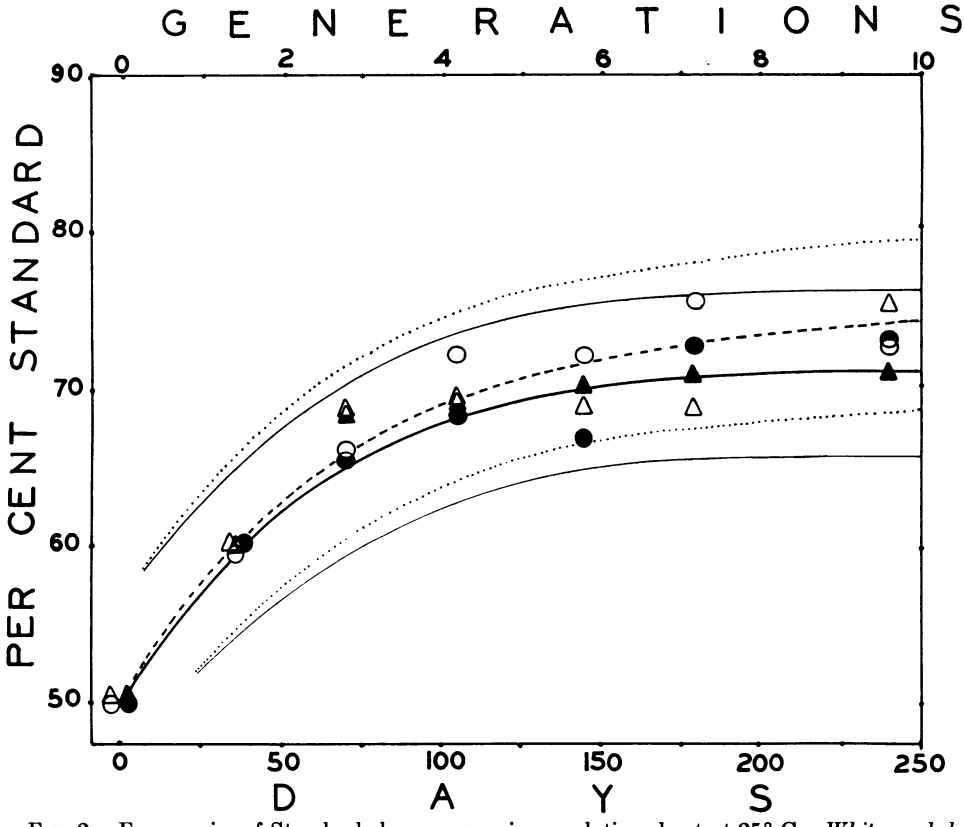

FIG. 2.—Frequencies of Standard chromosomes in populations kept at 25° C. *White symbols* = Fed on *Zrgosaccharomyces; black symbols* = Fed on *Kloekera; triangles* = Populations Nos. 105 and 106; *circles* = Populations Nos. 107 and 108.

As shown above, the populations fed on *Zygosaccharomyces* and on *Kloeckera* behaved very differently at 21°, fixation of ST taking place in the former and an equilibrium being established in the latter. At 25° the populations fed on these two yeasts behave similarly. The six pairs of samples, taken from the thirty-fifth to the two hundred and fortieth day at 25° (Table 2), show no influence of the

difference in nutrition; the chi-square turns out to be 2.81, which, for 5 degrees of freedom, has a probability of about 0.75.

Adaptive Values of the Karyotypes in Different Environments.—The changes observed in the populations at 21° and 25° permit estimation of the adaptive values of the three karyotypes ST/CH, ST/ST, and CH/CH. For this purpose the method of computation devised by Wright[1] can be used, there being no suggestion in the data that the adaptive values did not remain constant during the experiments (see, however, other situations[4, 5]). Taking the adaptive value of the heterozygotes, ST/CH, to be unity, we obtain the following estimates for the populations kept at 21°:

	ST/ST	ST/CH	CH/CH
Fed on *Zygosaccharomyces*	1.01	1.00	0.44
Fed on *Kloeckera*	0.79	1.00	0.39

When fed on *Zygosaccharomyces*, the adaptive value of ST/ST homozygotes is equal to or greater than that of the heterozygotes. Heterosis is, therefore, absent, and selection leads to eventual fixation of ST chromosomes in the population. When fed on *Kloeckera*, the heterozygotes are superior to both homozygotes. This heterosis causes the population to reach an equilibrium. For the populations kept at 25°, the estimates of the adaptive values are as follows:

	ST/ST	ST/CH	CH/CH
Fed on *Zygosaccharomyces*	0.77	1.00	0.30
Fed on *Kloeckera*	0.72	1.00	0.27
Fed on *Saccharomyces*	0.89	1.00	0.41

At 25° the heterozygotes show heterosis when fed on the two yeasts used in the present experiments and also when fed on Fleischmann's yeast (*Saccharomyces cerevisiae*). The estimates for the last-named food are from the work of Dobzhansky and Pavlovsky.[4] The figures suggest that the adaptive values of the homozygotes are relatively higher when Fleischmann's yeast is used than when *Zygosaccharomyces* or *Kloeckera* is fed, but the differences may or may not be significant.

Figures 1 and 2 show the course of selection in populations living at different temperatures and fed on different food. In these diagrams the continuous curves show the changes in the frequencies of ST chromosomes in populations fed on *Zygosaccharomyces* expected on the basis of the adaptive values of the three karyotypes given above. The dashed curves represent the expected course of selection in populations fed on *Kloeckera*. In either case, the thicker line indicates the expected values and the thinner ones show the limits of two standard errors above and below the expectation. It can be easily seen that the values actually observed (symbolized by triangles and circles) do not deviate from the theoretical ones more than was expected owing to sampling errors. The adaptive values of the karyotypes have remained reasonably constant during the course of the experiments; in particular, there is no indication in the data that the adaptive value of a karyotype depends upon the frequency in the population. It should be noted that changes in the adaptive values have been observed in experimental populations obtained by hybridization of geographic races,[4, 8] and interaction of karyotypes affecting their adaptive values occurs in some populations including six karyotypes.[5]

It is an oversimplification to believe that once a genotype is heterotic it should always be heterotic.[9] In reality the situation is more complex than this, and the

adaptive value of a genotype may vary greatly in different environments. The ST/CH heterozygotes are adaptively superior to the homozygotes at 25°; at 21° they are superior when fed on *Kloeckera* but not when fed on *Zygosaccharomyces;* at 16° the adaptive values became uniform on the foods tried (see, however, Da Cunha[3]). Furthermore, an over-all adaptive superiority of a genotype inferred from the behavior in populations under selection does not mean that the carriers of that genotype should be superior in all physiological characteristics, such as viability at different stages of the life-cycle, longevity, fecundity, sexual activity, etc. In a given environment a genotype is often superior to another genotype in some, but equal or inferior in other, characteristics. The adaptive value, or fitness, is a net result of interaction of all the characteristics which affect the transmission of the genes from one generation to the next; natural selection is concerned not with these characteristics separately but only with the over-all result of their interaction.

Reproducibility of Results in Experimental Populations.—Epling, Mitchell, and Mattoni[9] described seventeen experimental populations with different combinations of ST, CH, and Arrowhead chromosomes. In some of their populations equilibria were established, indicating that the structural heterozygotes were adaptively superior to the homozygotes; in others, one of the gene arrangements seemingly approached fixation; in still others, an equilibrium was reached and then lost. Examination of the data shows, however, that the competing chromosomes were of the same geographic origin in only three of the populations, while in fourteen populations hybridization of strains of different geographic origin was involved. Now it is known that in hybrids between populations of different geographic origin the genetic situation is so complex that the results of natural selection may be indeterminate. This has been shown to be the case even for populations of localities only some 10 miles apart.[5, 7, 8] To make a meaningful study of reproducibility of results of natural selection, experimental populations of uniform geographic origin must be compared. Adequate control of environmental variables is also essential. The four pairs of populations reported in Tables 1 and 2 represent replicate experiments that can be compared for this purpose. The results of the comparison are shown in the accompanying tabulation.

Populations	Chi-Square	Degrees of Freedom	Probability
99 and 101	7.38	8	0.5
100 and 102	13.00	6	0.04
105 and 107	4.46	5	0.5
106 and 108	2.74	5	0.7

The replicate experiments gave similar results, except that No. 100 gave one very high value for ST chromosomes on the two hundred and fifteenth day (Table 1). Dobzhansky and Pavlovsky[4] studied four replicate populations with ST and CH chromosomes fed on Fleischmann's yeast. Their data yield a chi-square of 29.38, which, for 24 degrees of freedom, has a probability of about 0.2. An unpublished experiment of Dobzhansky and Pavlovsky, in which two populations with Arrowhead and Chiricahua chromosomes were observed simultaneously, gave a chi-square of 3.15; for 6 degrees of freedom this has a probability of about 0.8. Combining all the above replicate experiments, we obtain a chi-square of 60.11; for 54 degrees of freedom this has a probability of about 0.27. If anything, this indicates a surprisingly good reproducibility for experimental results in which very many variables are involved. And yet the adaptive values of the karyotypes are so sensitive to

environmental factors that apparently trivial differences sometimes produce large effects. In the unpublished experiments of Louis Levine, the populations are kept in cages of a type different from ours, which have certain technical advantages but which cause the frequencies of some chromosomes to undergo fluctuations which are much wider, on the average, than those observed in our cages. As shown above, the populations fed on *Zygosaccharomyces* behave very differently from those fed on *Kloeckera* at 21°, but no difference is noticeable at 25°.

Summary and Conclusions.—The data reported in this article demonstrate an exquisite sensitivity of the adaptive values of certain karyotypes in *Drosophila pseudoobscura* to environmental changes. Third chromosomes with the ST and CH gene arrangements derived from the populations of Piñon Flats, California, have been used. At the temperature 25° C., the ST/CH heterozygotes are definitely superior to the homozygotes in the environment of our population cages and on food containing either of the three yeast species tried. Lowering the temperature by only 4° to 21°, causes no great change in populations fed on *Kloeckera* (and apparently not in those fed on *Saccharomyces cerevisiae*[1]), but with food containing *Zygosaccharomyces* the heterosis disappears. The heterozygotes ST/CH are now equal in fitness to the ST/ST homozygotes, and both karyotypes are superior to the CH/CH homozygotes. A further lowering of the temperature, to 16°, changes the situation completely. The heterosis disappears or becomes so weak that no perceptible changes in the compositions of the populations occurs within two to three generations—a period of time amply sufficient to produce changes if the temperature were only some 5° higher. Furthermore, the relative fitness of a given set of karyotypes may change appreciably when some of them are eliminated from the population or when new karyotypes are introduced in the population.[5]

The biological functions of these exceedingly delicate mutual adjustments between the karyotypes are obscure at present. We know that many natural populations of *Drosophila pseudoobscura* undergo seasonal changes in relative frequencies of some of the karyotypes. The karyotypes evidently have different ecological optima, and the population responds to changes in its habitat by altering its genetic structure in conformity with the conditions prevailing in a given place and at a given time. This genotypic plasticity is however, combined with a genetically controlled homeostasis (Dobzhansky and Wallace[10] and unpublished data), which enables the carriers of a single genotype to become adjusted to a variety of environments by means of adaptive phenotypic modifications. Data are accumulating that show an intimate relationship between heterosis and homeostasis, at least in *Drosophila* populations. Further work in this field may bridge the gap between population genetics and developmental genetics—two branches of the same science between which there has been little contact.

The authors take pleasure in expressing their gratitude to Mrs. O. Pavlovsky, who has prepared all the slides of the larval salivary glands on the study of which this paper is based. Mr. Louis Levine has diagnosed the gene arrangements in the samples which were taken during the summers of 1952 and 1953. Professor H. Phaff, of the University of California, and Professor A. B. Da Cunha, of the University of São Paulo, have furnished the strains of the *Zygosaccharomyces* and *Kloeckera* yeasts and have instructed the junior author in the techniques of yeast culture.

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*POSTZYGOTIC ELIMINATION OF GENETIC FACTORS IN ESCHERICHIA COLI**

BY THOMAS C. NELSON† AND JOSHUA LEDERBERG

DEPARTMENT OF GENETICS, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

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Segmental Elimination.—Genetic recombination analysis has been related to a typical haplobiontic life-cycle in *Escherichia coli*, strain K-12: the vegetative proliferation of the haploid phase alternates with occasional syngamy to form a transient diplophase. This, in turn, segregates almost immediately to restore the vegetative haplophase,[1, 2] barring rare diploid exceptions.[3] The exceptional diploids have been disqualified as representative of the primary zygote on two counts: some were homozygous for certain factors, usually heterozygous, and all were hemizygous for the factors *Mal* and *S*. The first peculiarity can be explained if the diploids as recovered are not simply unreduced zygotes but nondisjunctions of segregant genomes after meiosis. A similar process, accelerated by ultraviolet light, also leads to automictic homozygotes from established heterozygotes.[4, 5] The second aberration is probably a feature of all crosses in *E. coli*, strain K-12, and is reflected in anomalies of segregation and mapping.[3–6]

Hemizygosity for the Mal_1 locus was initially inferred from the finding that diploids from crosses of $Mal_1^- \times Mal_1^+$ were invariably pure for this marker, though segregating for many others. The types carrying the Mal^- allele were tested further by a reversion analysis[3, 5] which showed that purity for Mal^- represented a hemizygous, not a homozygous, state at this locus. That is, the diploids are imperfect, and a segment (or chromosome) including the Mal_1 locus is represented only once, though most of the other genetic factors are of course represented twice. The problem is thus narrowed to the contingencies by which the full genetic content of both parents fails to be represented in each diploid. Two hypotheses had been considered: (1) preliminary exclusion of the segment from a gamete or (2) its subsequent elimination from a complete zygote previously formed from intact gametes. The former interpretation, although superficially simpler, was doubted from the first because the diploids were invariably hemizygous for *Mal* but might carry the allele from either one of the two parents. This indicated that, in any