The assumption that the new mass distribution is primarily located on the electric field in the whole space satisfies the obligation for relativistic transformations just as for the electromagnetic field itself. The simplified model with additional mass localized on the particle must be considered only as a simplifying approximation.

7. *Generalizations; Quantum Problems.*—The preceding method can be easily generalized to many other problems of "potential energy." The first step is to introduce a convenient type of field, propagating around each source. The next problem is to obtain the formula for the energy density, corresponding to equation (7), and then the calculation proceeds as in section 4. In such discussions, one should always beware of so-called "potentials," that are usually defined up to an arbitrary constant (or function), and directly lead to "gauge" troubles.

Quantum problems were discussed by W. Lamb, H. Bethe, J. Schwinger, and others, and their papers can best be found in Schwinger's book entitled *Quantum Electrodynamics* (New York: Dover, 1958). The method leads to corrections on the rest mass of particles, called "mass renormalization," and yields excellent numerical results. Quantum effects include electrostatic potential energy and all sorts of spin effects.

The present discussion proves that *mass-renormalization* is not only needed in quantum theories, but that it must already be introduced in classical Relativity, where it was completely overlooked by the founders of Relativity. Sommerfeld and Dirac were not aware of the difficulty, and their formulas must be very carefully revised. A first draft of the present paper was published in French.[1]

* Contract Nonr 266(56).

[1] Brillouin, L., "La masse de l'énergie potentielle," *Compt. Rend.*, **259**, 2361 (1964); Brillouin, L., "L'énigme $E = Mc^2$: Energie potentielle et renormalisation de la masse," *J. Phys. Radium*, **25**, 883 (1964).

---

*BICHROMOSOMAL SYNTHETIC SEMILETHALS IN DROSOPHILA PSEUDOOBSCURA**

BY THEODOSIUS DOBZHANSKY, BORIS SPASSKY, AND WYATT ANDERSON

THE ROCKEFELLER INSTITUTE

Communicated January 12, 1965

The mutation process is the ultimate source of the genetic raw materials from which evolutionary changes are compounded by natural selection. Without mutation, evolution would eventually be arrested. Populations of sexually reproducing, diploid, and polyploid organisms carry, however, enormous stores of potential genetic variability. This variability is gradually released by recombination. The release of the variability can be demonstrated experimentally. Populations of Drosophila carry many recessive lethal, semilethal, and subvital genetic variants, mostly concealed in heterozygous condition in "normally" viable individuals. Some of these lethals and semilethals arise by mutational changes in single genes, and perhaps by deletions of small blocks of genes. Other lethals and

semilethals are "synthetic." Synthetic lethal and semilethal chromosomes originate through crossing over between quasinormal chromosomes, which themselves give a viability close to "normal," i.e., to the average in a random-breeding population. Synthetic lethals and semilethals have been observed in several species of Drosophila. They occur more frequently in some species and populations than in others,[1-7] and some investigators failed to find any. This made it necessary to rule out the possibility that the apparently synthetic lethals arise through some peculiarly frequent mutations of single loci, somehow associated with crossing over. Dobzhansky and Spassky[8] have attempted to "desynthesize" twelve apparently synthetic lethals in the second chromosomes of *Drosophila pseudoobscura* by crossing over. This was accomplished in 10 of the 12 cases; the remaining two were either point mutations or synthetic lethals, the components of which were tightly linked.

A possibility not yet adequately explored is that synthetic lethals may arise not only through recombination of linked genes in the same chromosome, but also of genes in different chromosomes. An opportunity to observe such bichromosomal synthetics arose in the experiments which we have been conducting for about the last two years for a somewhat different purpose. These experiments are made as follows. Males, or sons of wild females of *Drosophila pseudoobscura* collected in their natural habitats, are crossed singly to females with mutant gene markers in their second and third chromosomes. One of the second chromosomes contains the genes upturned, bithorax, Bare (*Ba*), glass (*gl*), and an inversion which suppresses most of the crossing over; the other second chromosome has the gene Delta; one of the third chromosomes carries orange (*or*), Lobe (*L*), and an inversion (Santa Cruz); the other third chromosome has only orange. In the F_1 generation a single male, showing Delta and no other markers, is taken from each culture, and crossed back to females like his mother. In the following generation, females and males showing *Ba* and *L* are selected and mated.

The progeny of these matings should, theoretically, contain *Ba L*, *Ba*, *L*, and wild-type flies in a ratio 4:2:2:1 (*Ba* and *L* chromosomes are lethal in double dose). This theoretical expectation assumes, of course, that the four surviving classes are uniform in viability. This is far from always the case—the *Ba* class carries in double dose a third chromosome of the wild-collected ancestor, the *L* class is homozygous for its second chromosome, and the wild-type class is homozygous for both the second and the third chromosomes. Lethal, semilethal, or subvital gene complexes carried in the chromosomes of the wild-collected ancestors will cause some or all of the *Ba*, or *L*, or wild-type flies in the test generation to die. The observed segregation ratios will then depart from the ideal 4:2:2:1. Most interesting is the viability of the wild-type class in relation to the viabilities of the *Ba* and *L* classes. If the loss of the viability caused by homozygosis for a second chromosome is independent from that caused by the homozygosis for a third chromosome, then the viability of the double homozygote is predictable. Let the viability of the heterozygous class, *Ba L*, be unity, and of the single homozygotes, *Ba* and *L*, 1-*s* and 1-*t*, respectively. The viability of the double homozygote, the wild-type class, should be $(1-s)(1-t)$. Epistatic (synergistic) interactions between the second and third chromosomes may, however, reduce (or increase) the viability of the double homozygotes to $(1-s)\ (1-t)\ (1-i)$, where i is an interaction factor.

By the method described above, we have studied some 120 pairs of second and

third chromosomes from several natural populations of *Drosophila pseudoobscura*. The experiments continue, and we hope to report their results in due course. Here we report the data for only three pairs of chromosomes which give particularly striking interaction effects (Table 1). The strains S-19, T-157, and T-168 are derived from the population samples collected in Sonora, Mexico, and in the vicinity of Tucson, Arizona, and kindly sent to us by Professor W. B. Heed. The second and third chromosomes S-19 were tested in 13 replicate cultures, and T-157 and T-168 in 6 replicates each. The appearance of a few *Ba L or*, *L or*, and *Ba gl* flies is due to occasional crossovers in the inversion heterozygotes; in the calculations these crossovers are added to the classes with the respective dominant but no recessive marker genes.

TABLE 1

TEST CULTURES SHOWING SEMILETHALITY OF BICHROMOSOMAL HOMOZYGOTES

Chromosomes	Replicate	*Ba L*	*Ba*	*L*	Wild	*Ba L or*	*L or*	*Ba gl*	Total
S-19	A	22	8	7	1	1	—	—	39
	B	79	34	27	3	—	—	1	144
	C	68	32	25	5	3	—	—	133
	D	75	40	31	3	—	1	—	150
	E	50	22	27	3	—	—	—	102
	F	73	33	21	1	1	2	—	131
	G	36	19	13	2	—	—	—	70
	H	20	8	8	3	2	—	—	41
	I	63	35	19	4	—	—	—	121
	J	41	18	5	—	3	—	—	67
	K	39	12	11	1	1	—	1	65
	L	3	1	—	—	—	—	—	4
	M	38	15	9	1	1	—	—	64
	Total	607	277	203	27	12	3	2	1131
T-157	A	130	13	26	1	—	—	—	170
	B	81	11	23	2	—	—	—	117
	C	127	26	46	1	—	—	—	200
	D	89	11	5	1	—	—	—	106
	E	85	15	10	1	—	—	2	113
	F	60	6	5	—	—	—	—	71
	Total	572	82	115	6	—	—	2	777
T-168	A	19	2	5	1	—	—	—	27
	B	130	18	10	1	—	—	—	159
	C	48	7	3	1	—	—	—	59
	D	61	15	16	1	—	—	—	93
	E	55	16	9	—	—	—	—	80
	F	24	3	4	—	—	—	—	31
	Total	337	61	47	4	—	—	—	449

Most interesting in the data in Table 1 is the rarity of the wild-type class, which constitutes much less than one ninth of the total, and much less than one half of the *Ba* or *L* classes. Simultaneous homozygosis for the second and the third chromosomes reduces the viability of the double homozygotes much below what could be expected if the effects of these chromosomes were independent. To estimate the magnitude of the interaction factor quantitatively, one further circumstance must be taken into account. The gene markers, *Ba* and *L*, may have effects on the viability of their carriers. To measure these effects, a control experiment is made. *Ba L* females and males carrying second and third chromosomes of different wild progenitors are intercrossed. The progeny consists of *Ba L*, *Ba*, *L*, and wild-type classes; this last class carries, however, two chromosomes of each pair of independent origins. The viability of this wild-type class is normal by definition. The devia-

tions, if any, from the expected 4:2:2:1 ratios of the *Ba L*:*Ba*:*L*:wild-type classes measure in this control experiment the viability effects of the gene markers. The upper row of figures in Table 2 gives the frequencies of the four classes among the more than 58,000 flies counted in the control experiment. It is evident that the markers *Ba* and *L* are, under our experimental conditions, only slightly deleterious to their carriers.

TABLE 2

FREQUENCIES (%) OF THE FOUR CLASSES OF PROGENY IN THE CONTROL AND EXPERIMENTAL CULTURES

Chromosomes	*Ba L*	*Ba*	*L*	Wild	Flies counted
Control	44.07	21.94	21.93	12.07	58,249
S-19	54.73	24.67	18.21	2.39	1,131
T-157	73.62	10.81	14.80	0.77	777
T-168	75.06	13.59	10.47	0.89	449

Let *A*, *B*, *C*, *D* stand for the frequencies of the *Ba L*, *Ba*, *L*, and wild-type classes, respectively, in the control experiment, and *a*, *b*, *c*, and *d* for the frequencies of the corresponding classes in the experiments involving the chromosomal homozygotes. The selection coefficients, *s* and *t*, and the interaction factor, *i*, can be estimated as follows:

$$s = 1 - Ab/aB \qquad t = 1 - Ac/aC \qquad i = 1 - aBCd/AbcD.$$

From the data in Table 2, the following coefficients are calculated:

Chromosomes	*s*	*t*	*i*
S-19	0.0949	0.3313	0.7366
T-157	0.7051	0.5960	0.6796
T-168	0.6364	0.7197	0.5754

The relative viabilities of the homozygotes for the second (II), for the third (III) chromosomes, and of the double homozygotes (II + III), are:

Chromosomes	III	II	II + III
S-19	0.9051	0.6687	0.1594
T-157	0.2949	0.4040	0.0382
T-168	0.3636	0.2803	0.0433

The third chromosome of S-19 is mildly subvital, and the second chromosome is definitely subvital when homozygous; the double homozygote is well in the semilethal range. It is a clear example of a synthetic semilethal. With T-157 and T-168, both the second and the third chromosomes are semilethal in double dose; the corresponding double homozygotes are handicapped so severely that they seldom survive. The handicaps are greater than would be expected if the viability losses produced by homozygosis for the second and the third chromosomes were independent.

Epistatic interactions may, theoretically, have effects opposite in sign to those described above. The viability loss in the double homozygote may be less, instead of greater, than what is expected on the assumption of independence. A possible, though inconclusive, case of this sort in our materials is the chromosome pair T-183. In four replicate cultures there appeared 529 *Ba L*, 2 *Ba or L*, 130 *L*, 6 wild-type, and no *Ba* individuals. The absence of *Ba* seems to indicate that the third chromo-

some is here lethal in double dose, and yet 6 double homozygotes have survived. The possibility that the third chromosome is only semilethal when homozygous, and that no *Ba* individuals appeared by chance, cannot be ruled out completely. Be that as it may, evidence is rapidly accumulating[9-11] that genetic variants which reduce the viability of their carriers tend to act synergistically. Simultaneous presence in a genotype of several such variants results in a viability lower than would be expected if epistatic interactions played no part. Although the hereditary materials are discontinuous, the development, especially in higher organisms, has the continuity of an integrated process.

Summary.—A low viability, well in the semilethal range, is found in individuals which carry in duplicate (are homozygous for) a certain second and a certain third chromosomes. In the homozygotes for this second chromosome without the third, and for the third without the second, relatively slight reductions of the viability are observed. Simultaneous homozygosis for both chromosomes thus creates a "synthetic" semilethal. Two further examples are recorded, where a second and a third chromosomes, each of which is semilethal in double dose, give a very low viability in the double homozygotes. Epistatic (synergistic) interactions of the components of genetic loads in Drosophila populations appear to be widespread and important.

* The work reported here has been carried out under contract AT-(30-1)-3096, U.S. Atomic Energy Commission.

[1] Dobzhansky, Th., *Genetics*, **31**, 269–290 (1946).

[2] Wallace, B., J. C. King, C. V. Madden, B. Kaufmann, and E. C. McGunnigle, *Genetics*, **38**, 272–307 (1953).

[3] Spassky, B., N. Spassky, H. Levene, and Th. Dobzhansky, *Genetics*, **43**, 844–867 (1958).

[4] Spiess, E., *Genetics*, **44**, 43–58 (1959).

[5] Spiess, E., and A. C. Allen, *Genetics*, **46**, 1531–1553 (1961).

[6] Dobzhansky, Th., H. Levene, B. Spassky, and N. Spassky, *Genetics*, **44**, 75–92 (1959).

[7] Gibson, J. B., and J. M. Thoday, *Heredity*, **17**, 1–26 (1962).

[8] Dobzhansky, Th., and B. Spassky, *Zool. Jahrb. Syst. Okol. Geogr. Tiere*, **88**, 57–66 (1960).

[9] Dobzhansky, Th., B. Spassky, and T. Tidwell, *Genetics*, **48**, 361–373 (1963).

[10] Torroja, E., *Genetics*, **50**, 1289–1298 (1964).

[11] Malogolowkin-Cohen, Ch., H. Levene, N. P. Dobzhansky, and A. S. Simmons, *Genetics*, **50**, 1299–1311 (1964).

---

*CELL-TRANSFORMING ABILITY OF A TEMPERATURE-SENSITIVE MUTANT OF POLYOMA VIRUS**

BY MICHAEL FRIED

DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

Communicated by Renato Dulbecco, January 18, 1965

Conditional lethal mutants have been shown to be extremely useful in the study of the physiology of viral reproduction and maturation.[1, 2] Since such mutants are distributed over most of the genetic map,[1, 2] they could prove to be valuable in the study of tumor viruses. They may be used to "tag" almost all of the viral genes, a property of obvious physiological and genetical implication; they can also