EXPERIMENTS ON HYBRID SUPERIORITY IN DROSOPHILA MELANOGASTER.
I. EGG LAYING CAPACITY AND LARVAL SURVIVAL

GERT BONNIER

Institute of Genetics, University of Stockholm, Stockholm, Sweden

Received March 9, 1960

IN the present paper experiments are described on the capacity of egg production and of the sensitivity to temperature stresses in *Drosophila melanogaster*. A temperature of 25°C may usually be taken as an optimal temperature for this fly; with an increase of a few degrees, larvae and pupae meet markedly increased difficulties to survive. In order to get enough material, oviposition and hatching of the eggs were allowed to take place at 25°C in all cases, The sensitivity to temperature stresses is measured by the proportion of larvae which survive to eclosion, and this is made for two temperature levels *viz.* 25°C and 30°C.

MATERIAL AND METHODS

All flies emanated from three wild type stocks. One of the stocks was American (Oregon), and two were Swedish (Karsnäs and Skaftö); they will be symbolized as stocks E, K, T, respectively. From each of these a number of strains, homozygous for the three major chromosomes, were produced, and one strain from each of the stocks was kept for the experiments; they will be symbolized as strains e, k, t, respectively. The method used in the process of homozygotization is shown in Figure 1. As may be seen, the homozygous strains got their chromosomes 1, 2, 3, and Y from the corresponding stock. The generation on the bottom line in Figure 1, i.e., the first generation in which homozygous wild type flies were mated, will be taken as generation 0. The generations following it will, consequently, be regarded as generations 1, 2, 3...

The homozygous flies of strains e, k, t were, in generation 0, few in number, and it was necessary to propagate them for some generations. It is known (MULLER 1954) that spontaneous mutations occur in early embryonic life, during the period of meiosis, and in ageing spermatozoa; during ageing of the flies themselves, however, practically no mutations occur. Because the experiments should be made on a rather large scale all crosses and tests could not be made simultaneously. They were, therefore, repeated in ten runs starting on ten consecutive weeks. In order to minimize the occurrence of spontaneous mutations the following procedure was in principle adopted. Some of the flies from generation 1 were propagated for four generations. In this, and all other propagations, females were allowed to oviposit during a maximum time of four days after their first matings. Other flies of generation 1 were aged for two weeks. In this, and all subsequent

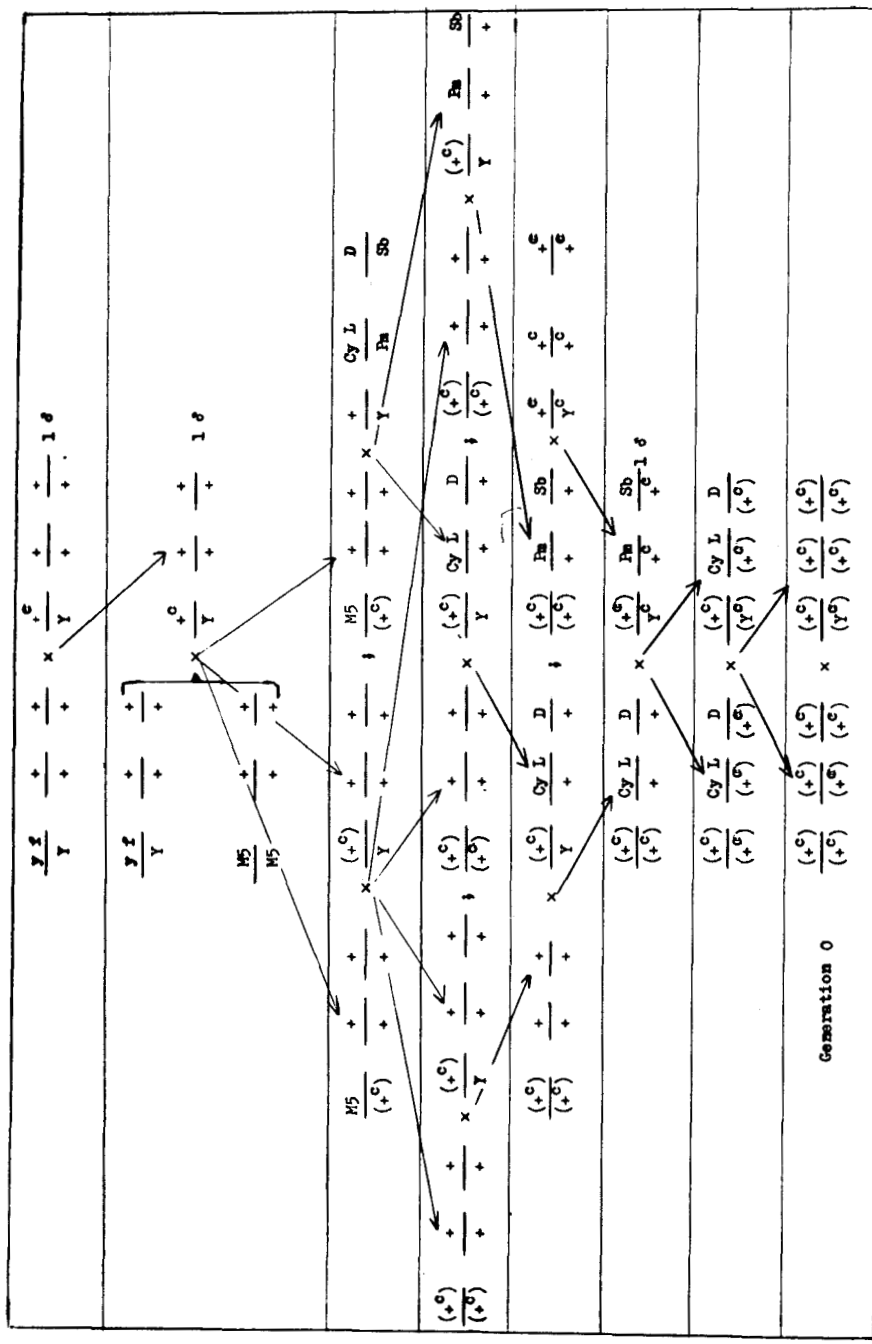

FIGURE 1.—Method used for producing homozygous strains which are isogenous for chromosomes 1, 2, 3, and for Y. The female parent is in all crosses to the left and the male parent to the right. Those wild type chromosomes made homozygous are shown within parenthesis. The letter c indicates that the chromosome emanates from the specific wild type stock used. Females with X chromosome *y f*/Y are so-called double attached with an extra Y chromosome. M-5, *Cy L*, *Pm*, *D*, and *Sb* are crosssing over reducing dominant markers. The method avoids the use of females which are heterozygous, simultaneously, for M-5 and for the second and third chromosome markers. The use, in the second chromosome, of both *Cy* and *L* gives a good guarantee that crossovers would be detected. *D* covers fairly well the whole of chromosome three.

ageings, the females were aged as virgins, and the males were kept with other females during their ageing. After ageing, females and males were mated. One part of the progeny was propagated for three generations and one part aged for two weeks. Following this system, a large number of wild type flies was produced on five occasions between each of which two weeks had elapsed; all of these flies belonged to generation 5. Each of the lot of flies so produced was used for starting two consecutive runs of the experiment. The ten runs could, hence, more correctly be said to be made up of five double runs.

The stock flies E, K, T were for each double run taken afresh from the laboratories' stock bottles. In order to get enough material to start the runs using E, K, T simultaneously with the runs using e, k, t, the E, K, T were propagated for some generations in advance.

Experiments were performed with flies e, k, t and their six possible hybrids (including reciprocal crosses); and also with flies E, K, T and their six possible hybrids. No crosses were made between E, K, T and e, k, t. Table 1 shows the different items of one run, exemplified for flies e and for crosses between e females and k males. The runs with the other strains and crosses were performed in an identical manner.

TABLE 1

Description of one run of the experiment, using as example e flies and e females mated to k males. All other genotypes were treated in exactly the same way. The left part of the table contains matings made for a study of the egg laying capacity of pure females and of the survival of pure and hybrid larvae. The right part contains matings made for a study of the egg laying capacity of hybrid females

Item	Length of time between consecutive items	Content of items	
1		Beginning of collection of e females and e males of generation 5 and keeping them together in vials.	Beginning of collection of virgin e females and k males of generation 5 and keeping females and males in separate vials.
	7 days		
2		Transferring the flies to cages with food in Petri dishes.	Mating e females to k males in cages with food in Petri dishes.
	3 days		
3		Inserting Petri dishes with black food into the cages.	Inserting Petri dishes with black food into the cages.
	16 hours		
4		Removing the Petri dishes from the cages.	Removing the Petri dishes from the cages.
	28 hours		
5		Collection from the Petri dishes of freshly hatched larvae of generation 6 and transferring them to vials: 75 larvae per vial.	Collection from the Petri dishes of freshly hatched larvae of generation 6 and transferring them to vials: 75 larvae per vial.
	8 days		
6		Collecting e females and e males and keeping them together in new	Collecting hybrid females and males from the cross e female by

TABLE 1—Continued

Item	Length of time between consecutive items	Content of items	
		vials. Collecting virgin e females and k males and keeping females and males in separate vials.	k male and keeping them together in new vials.
	2 days		
7		Transferring the non virgin e females and e males to new vials, each vial with one female and two males. Transferring the virgin e females and k males to new vials, each vial with one female and two males. 30 vials were made up and numbered from 1 to 30.	Transferring the flies to new vials, each vial with one female and two males. 15 vials were made up and numbered from 1 to 15.
	4 days		
8		The flies of the 25 first vials in which the female still was alive were transferred to 25 empty vials into which a spoon with black food was inserted.	The flies of the 13 first vials in which the female still was alive were transferred to 13 empty vials into which a spoon with black food was inserted.
	16 hours		
9		Flies removed from the vials. The number of eggs counted on each spoon separately. This was made on the spoons of the 20 first vials in which the female still was alive.	Flies removed from the vials. The number of eggs counted on each spoon separately. This was made on the spoons of the ten first vials in which the female still was alive. (End of the experiments on the egg laying capacity of hybrid females.)
	28 hours		
10		Freshly hatched larvae of generation 7 collected from the spoons and transferred to vials, 25 larvae per vial. This was not made separately from each spoon but collectively from the 20 spoons containing larvae of the same genotype. Half the number of vials so produced was incubated in 30°C and the other half in 25°C.	
	7 days		
11		*30° series* First count of adults. All vials containing the same genotype were counted collectively.	*25° series*
	1 day		
12			First count of adults. All vials containing the same genotype were counted collectively.
	3 days		
13		Second (= final) count of adults.	
	1 day		
14			Second (= final) count of adults.

As may be seen, items 1 to 4 include flies belonging to generation 5, and items 5 to 9 include larvae and flies of generation 6. The capacity of egg production in the case of homozygous flies was, therefore, tested on females of generation 6. The reason for not testing this capacity one generation earlier was to secure, as far as possible, similar conditions for all compared genotypes, including conditions during larval growth. Table 1 shows also that it was the larvae of generation 7 which were tested with regard to their capacity of reaching the adult stage at 25°C and at 30°C.

The amount of food in the vials (Table 1) was always about 12 ml per vial (though not measured in individual cases).

RESULTS

Treatment of the results consists in most cases of a comparison of nonhybrids with hybrids. Flies from the E and K stocks (i.e. "pure" flies) are, for instance, each compared with their two reciprocal hybrids. This gives four comparisons. In the same way one gets four comparisons from E and T, and, likewise, four from K and T. Hence there are 12 comparisons to be made with the stock flies. But as the studies comprise only nine groups of stock flies, all 12 comparisons are not independent. This must, of course, be borne in mind when judging the results of the statistical analyses.

Egg laying capacity: Item 6 of Table 1, in the left column, shows that the e females which were intended for crossing with k males were, for several days, kept as virgins. This was not the case with the hybrids shown in the right column, which from their eclosion were kept together with males. Therefore, these hybrids may, in a more satisfactory way, be compared with the pure e flies which, according to the left column, also were kept with males from the beginning of their adult life. Comparisons of egg production capacities are, hence, confined to these kinds of groups. Item 9 of Table 1 indicates that 20 of the pure females and ten of the hybrids were tested for each run. However, in spite of the margins used (see items 7 and 8) there were some instances in which less than 20 females were alive to be tested. Moreover, flies of the E stock, in which the females were very poor in egg laying capacity, were not available for the first double run (runs 1 and 2).

The results of the egg counts are shown in Table 2 in which also coefficients of variation are given. Stock E females are, by far, the poorest layers. Hybrids produce more eggs than pure females (Table 2). In the case of homozygotes there is only one comparison in which the homozygote did produce more eggs than one of the hybrids (Table 4), though quite insignificantly so (k *versus* hybrids from e females crossed to k males). Of the remaining 11 comparisons with homozygotes, three are without statistical significance, whereas eight are significant. All hybrids between stock flies produce more than the pure ones, which is in good agreement with observations made by GOWEN (1952). Among the 12 comparisons there is only one in which the probability level exceeds the five percent point (T *versus* hybrids from E females crossed to T males). None of the three com-

TABLE 2

Average number of eggs produced by single females during 16-hour periods of oviposition

Female parent	Male parent	Number of females tested	Number of eggs	Coefficient of variation	
				Within runs	Between runs
e	e	191	18.5 ± 3.9	0.92	0.90
e	k	100	46.4 ± 6.6	0.45	0.51
e	t	100	58.4 ± 5.8	0.32	0.30
k	k	200	47.0 ± 4.8	0.46	0.27
k	e	100	64.6 ± 5.4	0.26	0.17
k	t	100	67.5 ± 5.6	0.26	0.16
t	t	197	46.9 ± 4.7	0.44	0.31
t	e	100	64.3 ± 5.9	0.29	0.35
t	k	100	57.6 ± 6.1	0.34	0.38
E	E	160	9.9 ± 3.9	1.77	0.52
E	K	80	56.5 ± 7.0	0.39	0.37
E	T	80	64.8 ± 6.1	0.30	0.14
K	K	200	37.7 ± 5.1	0.60	0.40
K	E	100	60.4 ± 7.6	0.40	0.32
K	T	100	69.7 ± 6.9	0.31	0.25
T	T	184	48.3 ± 6.7	0.53	0.34
T	E	100	66.8 ± 5.8	0.28	0.28
T	K	100	70.2 ± 6.2	0.19	0.22

Using the symbols s^2_w = mean square within runs; s^2_b = mean square between runs; n = number of females tested per run; $\bar{x}$ = general average, the following formulae are used:

$$\text{standard error of mean} = \sqrt{\frac{s^2_w}{n}}$$

$$\text{coefficient of variations within runs} = \frac{\sqrt{s^2_w}}{\bar{x}}$$

$$\text{coefficient of variations between runs} = \frac{\sqrt{\frac{s^2_b}{n}}}{\bar{x}}$$

In the few cases where there was unequal numbers of females per run, n is substituted by the harmonic mean of the different n:s.

parisons between stock flies and their corresponding homozygotes give statistically significant differences.

The coefficients of variation within runs (Table 2) are larger for pure females than for hybrids. This may to some extent also be true for coefficients of variation between runs, though not as conspicuously as for those within runs.

Larval survival in 25°C and 30°C: Experiments testing the possibility of the larvae to survive to the adult stage at 25°C and 30°C are described in items 10–14 of Table 1. The number of eggs varied very much between the different genotypes as well as between runs, and as the larvae were collected in groups of 25 (Table 1, item 10) they varied from run to run. The results arrived at after the last run are given in more detail in Table 3.

The primary purpose of the present investigation was to study the temperature sensitivity, and, as a measure of this sensitivity, the proportion was chosen of the number of freshly hatched larvae which, under the given circumstances, were able to reach adult stage (Table 1, items 10–14) From Table 1 (items 11–14)

TABLE 3

Larval survival in 25°C and in 30°C. Total material

Female parent	Male parent	Number of larvae collected for rearing in 25°C	30°C	Proportion of larvae reaching adult stage in 25°C (p_{25})	30°C (p_{30})	p_{30}/p_{25}
e	e	1225	1225	0.878	0.816	0.929
e	k	1400	1400	0.952	0.908	0.954
e	t	1400	1400	0.966	0.929	0.962
k	k	3750	3750	0.970	0.746	0.769
k	e	1475	1500	0.942	0.950	1.008
k	t	2675	2675	0.945	0.938	0.993
t	t	3225	3225	0.945	0.872	0.923
t	e	1850	1875	0.956	0.922	0.964
t	k	2675	2675	0.977	0.939	0.961
E	E	525	500	0.930	0.784	0.843
E	K	775	800	0.956	0.945	0.988
E	T	425	425	0.962	0.936	0.973
K	K	3075	3075	0.943	0.878	0.931
K	E	950	975	0.947	0.962	1.016
K	T	2675	2675	0.973	0.935	0.961
T	T	3325	3350	0.933	0.913	0.979
T	E	1200	1225	0.962	0.935	0.972
T	K	3175	3175	0.960	0.959	0.999

it is seen that all adults of each separate genotype were counted collectively. This was done because otherwise the routine work would have taken too much time. But, as a consequence, variation within genotypes could not be observed. Hence, the only part of the variance which in the statistical analysis could be estimated was that due to random (binomial) causes. It should be observed, however, that the experiments for each run were performed simultaneously for all genotypes (though with the exception that there were no E flies available at runs 1 and 2). This will tend to cancel environmental differences between the genotypes. Hence, if, in a comparison, there is no real difference between two genotypes the variance of the difference between the genotypes would be equal to the sum of the random variances. An ordinary t test, based on the difference between the genotypes and on the sum of their randomly caused variances, would, as a rule, give a reliable statistical test. It is now known that a proportion, p, is distributed sufficiently close to normality if np (n being the number on which p is based) exceeds a certain value, e. g. 15, and that the same holds good for $n\ (1 - p)$. Let now n_{25} and n_{30} be the total number of larvae collected from a certain genotype for rearing in 25°C and 30°C, respectively, and let f_{25} and f_{30} be the number of larvae reaching the adult stage, and denote the proportions of these larvae, f_{25}/n_{25} and f_{30}/n_{30}, by p_{25} and p_{30}. As these proportions in most cases are close to the limit 1, it is necessary for the analyses that $n_{25}\ (1 - p_{25})$ and $n_{30}\ (1 - p_{30})$ each exceeds, say, 15. But $n_{25}\ (1 - p_{25}) = n_{25} - f_{25}$ and $n_{30}\ (1 - p_{30}) = n_{30} - f_{30}$. We must, therefore, have $n_{25} - f_{25}$ and $n_{30} - f_{30}$ larger than 15. This is the case for all

TABLE 4

Summary of significance tests

Type of test	Homozygotes versus their hybrids: Number of cases in which homozygotes < hybrids	homozygotes > hybrids	significance	Stock flies versus their hybrids: Number of cases in which stock flies < hybrids	stock flies > hybrids	significance	Homozygotes versus stock flies: Number of cases in which homozygotes < stock flies	homozygotes > stock flies	significance
Egg production	8	..	+[1]	11	..	+[5]	..	2	—
	3	..	—	1	..	—	1	..	—
	..	1	—	..	..	..	..	..	..
Larval survival in 25°C (p_{25})	6	..	+[2]	9	..	+[6]	1	..	+[9]
	2	..	—	3	..	—	..	1	+[10]
	..	3	+[3]	..	..	..	..	1	—
	..	1	—	..	..	..	..	..	..
p_{30}/p_{25}	10	..	+[4]	9	..	+[7]	2	..	+[11]
	2	..	—	..	2	—	..	1	+[12]
	..	..	..	..	1	+[8]	..	..	..
	24	..	+	29	..	+	3	..	+
Total	7	..	—	4	..	—	1	..	—
	..	3	+	..	1	+	..	2	+
	..	2	—	..	2	—	..	3	—

	P between 0.05 and 0.01	P between 0.01 and 0.001	P less than 0.001		P between 0.05 and 0.01	P between 0.01 and 0.001	P less than 0.001		P between 0.05 and 0.01	P between 0.01 and 0.001	P less than 0.001
1)	3	1	4	5)	5	.	6	9)	.	.	1
2)	.	.	6	6)	3	2	4	10)	.	.	1
3)	.	1	2	7)	1	1	7	11)	.	.	2
4)	1	1	8	8)	1	.	.	12)	.	1	.

When the significance level, P, is equal to or less than 0.05 this is indicated by the sign +; otherwise by the sign —. The distribution of the observed magnitudes of P is, in cases of significance, shown at the very bottom of the table.

genotypes when using the whole material (Table 3), i.e., the material available after ending the tenth run. The analyses are, hence, based on the total material.

When analysing proportions, the computations are often much simplified by substituting, for the proportions themselves, their natural logarithms. Moreover, by using logarithms, a possible metric bias due to proportionality between variances and averages would probably be minimized. It is now found that

$$\text{variance of log nat } p_{25} = \frac{n_{25} - f_{25}}{n_{25} \cdot f_{25}}$$

and

$$\text{variance of log nat } p_{30}/p_{25} = \frac{n_{30} - f_{30}}{n_{30} \cdot f_{30}} + \frac{n_{25} - f_{25}}{n_{25} \cdot f_{25}}$$

Table 4 contains the significances which are based on t tests for differences of log nat p_{25} and for differences of log nat p_{30}/p_{25}.

Looking now at p_{25}, the larval survival in 25°C, for the homozygotes (Table 3) it is found that larvae from e have a significantly poorer survival than three of its hybrids (in two of which the differences are significant); while larvae from k have a significantly better survival than three of its hybrids. Turning to the stock flies and their hybrids it is seen that larvae from the pure stocks in all cases are inferior to their corresponding hybrids; in nine of the 12 comparisons the differences are significant. Comparing, finally, stock flies with homozygotes, one finds that larvae from E are significantly superior to those from e; that larvae from K are significantly inferior to those from k; and that larvae from T are inferior to those from t, but only on the verge of significance.

One of the principal aims of the present investigation was to study the relative sensitivity to increased temperature, and this has, as mentioned above, been measured by the ratio of the survivals in 30°C and in 25°C, p_{30}/p_{25}. The results for the total material are shown in Table 3. All of the 12 comparisons between larvae from homozygotes and larvae from corresponding hybrids show superiority for the hybrids; this superiority is significant in ten of the cases. It is noteworthy that larvae from k, which in several cases were superior to their hybrids when developing in 25°C, show the most conspicuous relative decreases when comparing the survivals in 30°C and 25°C. In the case of larvae from stock flies, those from E and K are significantly inferior to their corresponding hybrids, while those from T are significantly inferior to only one of its hybrids. The comparison of larvae from stock flies with those from the corresponding homozygotes show that larvae from e are significantly superior to those from E.

Condensed survey of the results of the present experiments: Three types of tests are studied: (1) egg production, (2) the proportion p_{25} of larvae reaching the adult stage in 25°C, and (3) the relative decrease in larval survival measured by the ratio, p_{30}/p_{25}, of larval survival in 30°C to that in 25°C. Each of the three homozygotes was compared with the four hybrids into which they entered as parents, making 36 tests. Likewise, there were 36 tests for individuals from the stocks. Finally, the homozygotes were compared with the corresponding stock flies, making nine more tests. Table 4 summarizes the results. Though not all tests are independent (see first paragraph of the present section), it is clearly seen that in the great majority of tests, the homozygotes are significantly inferior to the corresponding hybrids. The same is true with regard to the comparisons between stock flies and their hybrids. The comparisons between homozygotes and stock flies give no clear picture.

To these results should be added that, with regard to egg laying capacity, pure flies (including homozygotes and stock animals) varied more within runs than did hybrids.

DISCUSSION

The characters studied in the present investigation, *viz.* egg laying capacity and larval survival, are certaintly of importance from a selectional and evolutionary point of view. The flies used in the experiments all emanated from stocks which for a very long time were kept at room temperature in ordinary stock

bottles. Mass propagation of the stocks was used, i.e., with intervals of between 15 and 20 days the content of the bottles were shaken over into fresh bottles. The flies produce usually heavy numbers of larvae, and larval competition within the bottles was probably as strong as under natural conditions. The number of flies shaken into fresh bottles was never counted but may be estimated to be around 200 of each sex. The relative smallness of this number may induce some degree of inbreeding (remember the low egg production of flies from the E stock), but otherwise the structure of the stock bottle populations may be considered to be rather similar to that of natural populations.

Concerning the fitness of wild Mendelian populations, the discussion mainly concerns two hypotheses, often called the "classical" and the "balance" hypotheses (see DOBZHANSKY 1959). In a study of the effects of low dose irradiation on *Drosophila melanogaster*, WALLACE (1958) gives a diagrammatic representation of the genetic content of an average individual according to the two hypotheses. The chief difference, as visualized in the diagram, is as follows. According to the classical hypothesis there is a large proportion of dominant homozygous loci, few heterozygous loci, and a limited number of alleles per locus; while, according to the balance hypothesis, there are few homozygous loci, many heterozygous loci, and a large number of alleles per locus. It is known (SPASSKY, SPASSKY, LEVENE and DOBZHANSKY 1958) that a large amount of genetic variability may be released through recombination, though this amount may differ between different chromosomes. It seems, therefore, inevitable to conclude that, at least within the species studied, the individuals have large numbers of heterozygous loci. This does not, however, in itself invalidate the essentials of the classical hypothesis. If one supposes that there is a high frequency of spontaneous mutations—probably much higher than hitherto assumed—and that each individual mutant gene has only a very low detrimental effect in heterozygous condition, one would, within the population's chromosomes, find a large number of long-lived mutant genes. But such a situation would not prevent the importance of one of the most characteristic attributes of the classical hypothesis, expressed by MULLER (1956) in the sentence ". . . the exceptional cases of the heterozygote being superior are probably represented for the most part . . . by adaptations that have not yet stood the test of geological time". According to this sentence, one has to postulate that homozygotes for genes which have stood the test of geological time, have received a homeostatic plasticity in magnitude equal to or surpassing that of heterozygotes in their capacity to withstand the stresses of changed environmental conditions.

The occurrence of hybrid superiority was known in pre-Mendelian time, and was considered in genetic analysis independently by SHULL and by EAST as early as 1908. In spite of this there is still no unanimity about its causes. (Hybrid superiority is known both from selfing plants and from outbreeding species. The discussion here will be restricted to the latter category.) The classical hypothesis is bound to the assumption that the average viability of individuals which are heterozygous for a specific locus never, or only in exceptional cases, exceeds the average viability of both of the two homozygotes. This is, thus, understood to

mean that hybrid superiority practically always depends on the interaction of nonallelic genes. The balance hypothesis is, in contradistinction to this, bound to the assumption of an effect *per se* of heterozygosity, that is to say that the average viability of individuals which are heterozygous for a specific locus may be superior to the average viability of both of the two homozygotes. This assumption has several names; here the word *overdominance*, proposed by HULL (1945) will be used. It should be stressed that adherents of the overdominance assumption certainly neither deny the presence of many instances where hybrid superiority is due to the covering effect of nonallelic dominant genes, nor do they pretend that all single gene heterozygotes show overdominance. From the point of view of evolution and of population dynamics one may, therefore, formulate the two opinions by saying that those who deny the truth of the balance hypothesis think that overdominance is at the very most of insignificant importance; whereas adherents of the balance hypothesis think that overdominance—though not the sole agent in provoking hybrid superiority—is common enough to play a profound and widespread rôle.

If there is hybrid superiority *within* a population, it is plausible to imagine that selection acts in favour of heterozygotes and so keeps their frequency on a high level. But when hybrids *between* unrelated populations, natural or artificial, show an increased viability in comparison with the parental populations—as has been proved to be common in several species of Drosophila—then it can hardly be a question of selection. In a series of studies VETUKHIV (1953, 1954, 1956, 1957) showed this to be the case for larval survival in *Drosophila pseudoobscura*, *willistoni*, and *paulistorum*, and for egg laying capacity and for longevity in *pseudoobscura*. Since the intercrossed populations were isolated from each other, he concluded that selection could not have been active, but that his results were in agreement with the assumption of an heterozygosity *per se* effect. Likewise, BRNCIC (1954) who worked with *pseudoobscura* found that the survival rate—i.e., the proportion of wild type flies after certain crosses involving marker chromosomes—was higher for population hybrids than for the parental populations.

NICOLETTI and SOLIMA (1959) found that, when starting each of five populations from 30 females and 30 males, and then comparing the number of flies after 60 days, the superior population was one which originated from a cross in which females and males descended from different populations. The three populations E, K, T, of the present investigation have been mass cultured at room temperature in our laboratory for several generations, ranging from about 200 (population K) to 600 or more generations (population E). "Room temperature" is usually between 18°C and 20°C; in summer time it may rise to about 25°C. A room temperature of 30°C, if it ever has occurred, is of very short duration and can certainly be excluded as a selectional stimulus. In spite of this, such a physiologically complicated character as relative resistance in larval survival was in most cases more marked for stock hybrids than for the parental stocks.

The superiority of hybrids between homozygotes over their parental forms is probably due to similar causes as that of hybrids between different populations. But the high level of viability of the former has not the same weight as the latter

when discussing the *per se* effect of heterozygosity. MAYNARD SMITH, CLARKE and HOLLINGSWORTH (1955) working with *Drosophila subobscura* found, among other things, that hybrids between two inbred lines descending from different populations had a more superior longevity than the parental inbred lines. BONNIER, JONSSON and RAMEL (1959) showed that the time span from egg hatching to eclosion was, in *Drosophila melanogaster*, shorter for larvae which were heterozygous for the two long autosomes than for those who were homozygous for these chromosmes.

In reviewing the subject MATHER (1955) expresses doubts against the assumption of an effect of heterozygosity itself. He says: "Even . . . in outbreeding species, like Drosophila, where heterozygotes characterstically show heterosis, its level is not always proportional to the degree of heterozygosity." This is true if one emphasizes the word *always* in this sentence. But when STRAUS crossed flies of *Drosophila melanogaster* from one population with flies of an inbred line of another population and then ordered the results according to the 27 possible combinations of hybridity and nonhybridity in the three pairs of major chromosomes, he got the following results for daily egg yield (arranged after an article by GOWEN 1952): homozygosity in all three pairs (eight combinations) 38.2 eggs; homozygosity in two and heterozygosity in one pair (12 combinations) 51.5 eggs; homozygosity in one and heterozygosity in two pairs (six combinations) 62.6 eggs; and heterozygosity in all three pairs (one combination) 76.9 eggs. In the study mentioned above, NICOLETTI and SOLIMA (1959) found a correlation between degree of heterozygosity and rate of growth of the different populations; there was, however, one exception: the least heterozygous population showed, at least to begin with, the fastest growth in number of individuals. This population was started from flies which had been inbred for 400 generations. (It is known that inbreeding not always is a reliable method for producing homozygosity; it would be interesting to know what would happen if real homozygous strains, by aid of an inversion marker method, were derived from this inbred population.)

MATHER (1955) points to the fact that: "the observation of overdominance has often been claimed and seldom, if ever, proved in relation to hybrid vigour, for it is not easy to distinguish . . . from interaction between nonallelic genes". In a recent paper, MUKAI and BURDICK (1959) reported on the viability of homozygous populations of *Drosophila melanogaster* into which they had introduced a single second chromosome lethal. The experiment is very interesting from the point of view of persistency of a lethal gene, as they find a high equilibrium frequency which is independent of genetic background and starting frequency. It is also possible that their case is an example of overdominance, even if one hesitates to say that they have proved this to be true. It seems uncertain that they, by using a very contracted technique for the process of homozygotization, really have developed homozygosis in chromosomes one and two. (Judging from a paper by BURDICK and MUKAI (1958) the authors seem to be aware of this.) Their method for determining the frequency of the lethal seems, from the description of their technique, to be open to question. Moreover, the second chromosome with

the lethal originated from the same population as that from which the homozygous population was produced. But the second chromosome, containing the lethal, must nevertheless, have differed from the second chromosome of the homozygous population with respect to several genes, apart from the lethal itself. These genes, though without necessarily directly interacting with the lethal, may interact *inter se* and may so contribute to the high viability of heterozygotes for the lethal. The fact that the frequencies of the lethal, observed during a number of generations, fit well with an expected curve, calculated from the assumption of a single gene pair effect, does not exclude the possibility of constructing a more or less similar curve on the basis of multigene effects.

It will perhaps remain impossible to give, by genetic analysis, a definite answer to the question of whether or not overdominance can occur in an outbreeding species. One may, however, approximate "one gene" by a short chromosome segment. This was done by BONNIER, JONSSON and RAMEL (1959) by making females of *Drosophila melanogaster* wild type isogenous for the whole length of the X chromosome except for a segment including the *w* locus and being of a maximum length of 4.7 map units. The time from egg hatching to eclosion was compared for female larvae which in the X were either +/+ or heterozygous for + and one *w* allele. It was found that the homozygotes +/+ were superior, but it was emphasized that a different result might be found if a similar experiment were to be performed with a short autosomal segment.

It ought to be stressed once more that certainly not all heterozygous loci give overdominance effects. Several examples could be given. Suffice here to mention the studies on asymmetries of sternopleural bristle numbers in *Drosophila melanogaster* by THODAY (1955). He had shown that asymmetry was inversely correlated with viability. X chromosomes were derived from populations that had been adapted to two different environments, and it was found that whereas intrapopulational hybrids had a low degree of asymmetry in their own as well as in the foreign environment, the interpopulational hybrids had a high degree of asymmetry in both environments.

HAGBERG (1953) who has made a very thorough investigation on hybrid superiority (though mostly on selfing plants) emphasizes two different ways by which overdominance may be produced: "(1) the two alleles function as complements . . . together they result in a better effect in the heterozygote than each of them in a homozygous state are able to produce. (2) One of the alleles may be without effect, may even be a deficiency—the other allele has an optimal effect when in single dose and the homozygous state is an overdose". (With regard to the second possibility he refers also to FISHER 1918). But MUKAI and BURDICK (1959) point out that if one gene controls only one chemical reaction or produces only one enzyme, the first of HAGBERG's assumptions could not be valid. This remark seems, however, not to be essential in such cases where one of the genes is neomorphic to its allele. HALDANE (1955) refers to some cases in which it could be possible to explain overdominance on biochemical grounds. In one of these (the case of lozenge studied by CHOVNICK and FOX), it is known that the two alleles are pseudoalleles. But as one can hardly be sure that any pair of alleles,

will not with refined techniques turn out to be pseudoalleles, one may ask if this, in respect to the problem of overdominance, is of critical importance. One of the methods which PONTECORVO (1958) describes for selecting mitotic crossovers in *Aspergillus nidulans* is based on the fact that germinating conidia having certain combinations of requirements survive longer than those of strains having only one of these. He mentions, for example, that on a medium lacking adenine and biotin, double adenineless-biotinless mutants survive much longer than biotinless ones. Even if this may seem to be a peculiar event, it is a fact, and because of this, one has reason to agree with HAGBERG (1953) who said: "Complementary genes give together an effect which each of them alone cannot produce. It seems reasonable to assume that two alleles may be complementary in a similar manner, and there is nothing astounding or 'unnatural' in the phenomenon of superdominance. Rather, it would be 'unnatural' to assume that it could never occur".

CONCLUSIONS

Many cases of hybrid superiority are certainly due to interactions of nonallelic genes and many heterozygotes are certainly nonoverdominant. It is possible that every case of hybrid superiority could be explained without accepting overdominance. But such explanations would in many important instances, e.g. the superiority of hybrids between nonrelated wild type populations, probably be of quite a formal kind, involving complicated accessory assumptions and constructions of interactions between nonallelic genes. The single assumption of overdominance seems to suffice for making many accessory assumptions unnecessary. As it is known that the combination of inferior genes may make a superior product (see above about *Aspergillus nidulans*), one seems to be entitled to conclude that, as long as there is no explicit biochemical proof to the contrary, the assumption of overdominance—sometimes caused by stimulation of two inferior genes, so as to make their sum superior; sometimes caused by one of the homozygotes being an overdose—is the simplest and most probable tool that nature has evolved within many important fields of the dynamics of natural populations.

SUMMARY

1. Three unrelated wild type stock populations of *Drosophila melanogaster* were used in the present study. From each of these stock populations one homozygous population was derived. Viability experiments were made with flies taken directly from the stocks ("pure" flies) and with the six possible F_1 hybrids between them. The same types of experiments were made with pure homozygous flies and with the six possible F_1 hybrids between them.

2. The viability studies included three characters: (1) egg laying capacity, (2) capacity of larvae to survive to the adult stage in 25°C; this character was measured by p_{25} = the proportion of freshly hatched larvae which reached eclosion, (3) larvae were also reared in 30°C, and their capacity to survive was measured by the corresponding proportion p_{30}. The third character studied was, however, not p_{30} itself but the relative decrease in survival rate when comparing

survival in 30°C (strong stress) to survival in 25°C (no stress). This character was measured by the ratio p_{30}/p_{25}.

3. The hybrids were the superior ones in the great majority of cases and for all three characters.

4. After a discussion of the results and after reviewing similar results from the literature, emphasis is laid on the difficulties to explain the superiority of hybrids between unrelated populations as caused by selection. It is concluded that the single assumption of overdominance will make many accessory assumptions unnecessary. As long as there is no biochemical proof to the contrary, the assumption of overdominance seems to be the simplest and the most probable tool that nature has evolved within many important fields of natural population dynamics.

ACKNOWLEDGMENTS

The author is indebted to the Swedish National Science Council for the financial support of the present experiments. To PROFESSOR LÜNING and DR. RAMEL I wish to express my sincere gratitude for many fruitful discussions. The skillful and painstaking assistance by MISS U. B. JONSSON and MISS K. BENGTSSON has been a most valuable help in my studies, and I am greatly indebted to them. I wish also to thank MISS Z. STAMENOVA and MISS B. SCHUWERT for their able assistance.

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