

THE GENETIC BACKGROUND OF CHEMICALLY INDUCED PHENOCOPIES IN *DROSOPHILA* II

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In the first part of our work ('57) it was reported that the phenocopies produced in *Drosophila melanogaster* after treatment of developing eggs and larvae with sodium tetraborate were more or less specific for wild type lines of different provenience; that the reactivity to such treatment is genetically controlled; that dominant loci which had lost their phenotypic effect in a stock had it restored by borate treatment; and that borate treatment brought out in a member of a series of alleles an effect characteristic of another member of the series. In spite of a considerable series of positive results we did not succeed, however, to prove unequivocally that the specificities of the phenocopic effect were due to the presence, in the different stocks, of subthreshold alleles (iso-alleles) of the mutants which were phenocopied. Searching for such proofs a set of further experiments were performed, some of which extend the ones already reported, while others attack the problem from a new angle.

The specific reactions in compounds of different lines

Wild type lines which had shown characteristic reactions to borate treatment by producing one (or more than one) phenocopy in larger numbers and more extreme grades than did other lines; or which produced specific phenocopies completely absent in other lines, were crossed with other wild type lines of known reactivity and the F_1 eggs and larvae subjected to the standard treatment with 0.06% borate as detailed in

the former paper. We call these hybrids "compounds" under the hypothesis that they contain different subthreshold alleles of mutants.

Before describing the results, it should be stated that we have refrained from calculating percentages of the individual phenocopies. The reason is that the variation in the individual bottles of the same crosses is hardly a random variation, but is influenced by the chance presence of modifiers, sometimes in an all or none way. Averages would, therefore, not have much meaning beyond what the inspection of the tables shows.

The Formosa crosses

The line Formosa is unique (1) by producing simultaneously a great variety of phenocopies so that once even a single fly showed 8 different phenocopies; (2) by producing as a phenocopy an effect, resembling (but not completely identical with) heterozygous Bar; (3) by a large number of phenocopies of "curved" (c); (4) by the otherwise not found phenocopy of "dachs;" (5) by relatively frequent occurrence of "lanceolate" (11). Table 1 summarizes the results of treatments of F_1 between Formosa and some other wild type lines, as well as with some stocks of dominant and recessive eye mutants. In the column \pm ey are found all eye effects, including those of the crossed in dominants which will be analyzed below. \pm F eyes means more or less of the typical Formosa eye-phenocopy resembling Bar and counted already with the not specified eye effects.

No. 1. $F \times Canton-S$. The hybrid shows in the majority of flies more or less of the typical Formosa eye phenocopy. The other typical F (= Formosa) reactions are absent. Instead a significant number of antennaless and podoptera phenocopies appear, both of which characterize the Canton line, antennaless more so than podoptera. Thus the typical reactions of both parents appear, in part, in the compound.

No. 2. $F \times Samarkand$. The latter line is characterized by low general reactivity, kidney-like eye-effect, if any, and high incidence of podoptera, further, occasional phenocopies of

TABLE 1

Crosses of formosa (F) with different other lines after standard borate treatment

CROSS	+	± ey (incl. dom.)	± P-type eyes	ll	Bd	c	Antl.	Scn	ss ^a	pod	double ant.
1. F x Canton	30		140				4		1	5	
2. F x Samarkand	69	129		12	10		7	1	1	11	3
3. F x Idaho Falls	many	many		5			1				
4. F x Ore Mohler	11	69	198	4	2		10		5	3	
5. F x ey ²	412			1	6	1	4	1	22		
6. F x L		600		2			10	2			1
7. F x B		1000			2		15	1			
8. F x Dfd/Cx	432	72		9	2	3	6				

lanceolate and Beaded. The reaction of the hybrid is very characteristic. From F comes the high reactivity of the eyes, but no typical Bar-like eye-effect was seen. The high incidence of ll and Bd phenocopies comes from the F parent, but the curved effect typical for Formosa is missing. The few other phenocopies are not characteristic of either stock, but the high incidence of podoptera is typical for the Samarkand parent. Thus again the reactions of both parents are found in an incomplete way in the compound.

No. 3. F × Idaho Falls. The latter is characterized by an abnormal eye shape (polygon) in the untreated stock, low reactivity of the eyes, and absence of special phenocopies. Among many (i.e. about 200) flies one half were normal, one half showed eye effects which ranged up to an extreme eye reduction, but neither of the parental types appeared. The ll effect from the F mother was visible. Thus the compound did not react in the simple way observed in the foregoing cases i.e., not exhibiting dominance of the parental types.

No. 4. F × Ore Mohler. This Oregon line is highly sensitive to treatment reacting with a special type of eyes (see below) and comparatively many ss^a phenotypes. In this F₁ the eye effect resembles mostly that of the Oregon parent; in some flies, however, a more elongated eye is found, suggesting a Formosa effect. The Bd and ll effects come from Formosa. The many antennaless phenocopies are probably due to the influence of both parents. Thus the former results, i.e. part dominance of the reactivities of both parents, are found again.

The series contains three more such crosses between F and Riverside, Amherst, and Big Ridge, not entered into the table. They are heterotic with large numbers of normal flies. We stated already in the former paper that some crosses between certain lines show heterosis which simultaneously removes the reactivity to the standard treatment (see below discussion of Ore Mohler x ey² crosses).

No. 5. F × ey². The ey² mutant reacts upon standard treatment with extreme eyelessness up to the condition described as headless, a special type described in the former paper as

“mustache” and the appearance of many antennaless flies. It is remarkable that in this heterozygote with F the reactivity of the eyes is much lower than in either parental line. The types of eye effect were very variable and showed mostly the phenotype of ey^2 , but in many individuals also the F type or combinations of both and in addition the eye type described in the first paper as dispersed were unusually frequent. Thus we may say that both parental reactions come through in the hybrid, but also combination effects and effects absent in both parents. From the F parent came the tendency to a great variety of phenocopies. In addition to those recorded in the table, 4 ocelliless, 1 plexus, 1 polychete phenocopies were found. Bd, ll, and a high percentage of ss^a were contributed by the F parent. The results thus fall in line with those obtained in wild type compounds.

Nos. 6-9. F \times dominant eye mutants. All show more or less of the F reactivity in the appearance of the phenocopies of ll, Bd, c, antennaless, which are absent in the other parents.

Altogether these groups in table 1 show that in treated crosses with Formosa the reactivity of Formosa is more or less dominant. Least of this is visible in the eye-effects, which follow mostly the type of the other parent, though frequently showing an inkling of the Formosa type. But there is also a cross in which a type of eye present in neither parent is produced.

A dominant reactivity of the Formosa parent is clearly seen in the multiplicity of phenocopic types, in the relative frequency of such types which are typical for Formosa as ll, Bd, c as well as those like antennaless and ss^a , which are rather frequent in Formosa. But when the not Formosa parent has a typical effect, like podoptera in Samarkand or frontless in Canton, this may also be dominant in the heterozygote. In the case of the Samarkand cross, we have the situation that the ll and Bd phenocopies are typical for both parents and F, has a high incidence of both. Thus it appears that in spite of much variation the typical reactivity of both parents is dominant in the compound in a more or less regular way.

The Amherst crosses

This line is conspicuous for the high incidence and high grade of phenocopies of aristopedia, *ss*^a. The reactivity of the eyes is not very high, but frequently a phenocopy of the wing mutant arch (also *ski*) is found. The Beaded type is also found in small numbers. Table 2 contains the results of standard treatment of Amherst (A) heterozygotes.

TABLE 2
Crosses of Amherst (A) after standard borate-treatment

CROSS	PHENOTYPES					
	+	eye effects	<i>ss</i> ^a	pod	antl.	Bd
1. A x Big Ridge	400	1		1		
2. A x Canton	13	108	14	3		
3. Canton x A	194	350	53	4	6	
4. A x Samarkand	100			14		
5. Samarkand x A	273	104	22	2		1 1 intersex
6. A x Idaho Falls		44				
7. Idaho Falls x A		456	37			
8. A x Ore Mohler	597	59	13			
9. Ore Mohler x A	203	165	28		1	1 double ant: 1 dachs
10. A x Deformed	137	110	17	2		

Nos. 1-9 are reciprocal crosses with different stocks. It is visible at once that in the majority of cases the high incidence of *ss*^a phenocopies (also their high grade) is dominant. The exceptions are the crosses with Big Ridge, which are heterotic (as repeatedly found for Big Ridge crosses), with almost complete absence of phenocopies; further, A x Samarkand with considerable dominance of the pod effect, typical for Samarkand, and A x Idaho Falls with high lethality. The reactivity of the eyes follows largely the parent with the higher sensitivity but with considerable irregularity.

The arch effect of Amherst never appeared in the hybrids. In one of the Samarkand crosses the podoptera effect had the high incidence typical for this line, but not so in the reciprocal

cross. The group of antennaless flies in one Canton cross agrees with Canton's tendency to produce this phenocopy, but is again missing in the reciprocal cross.

No. 10 is a cross with the dominant eye mutant *Dfd/Cx*. Again the *ss*^a incidence is dominant.

Altogether, in spite of some unexplained irregularities, the same phenomenon is observed as in the Formosa crosses, i.e. more or less dominance of the reactivity of both parents.

The Samarkand crosses

Samarkand (highly inbred) is characterized by a low sensitivity to borate treatment, and the infrequent eye-effect being of a kidney type; further by a strong or even very strong podoptera effect. We saw already in table 1, No. 2 and table

TABLE 3

F₁ Samarkand (S) × different lines after standard treatment

CROSSES	PHENOTYPES								
	+	ey	ss ^a	pod	Scn	antl.	double ant.	Bd	ll
1. Ore Mohler x S	100	68	6	23	1	1	1		
2. S x Ore Mohler	3	26	1	9		5			
3. S x Canton	32	33		7	1	16		1	2
4. Canton x S	175	129		40		27			
5. Idaho Falls x S	3	36		8		1			
6. S x Idaho Falls	388	12	2	13	1	12	1		
7. S x Big Ridge	203			28	1	5			
8. Big Ridge x S	157			59		12			

2, No. 4, the dominance of the pod effect. Samarkand has also a slight tendency to produce Bd, ll, and dp-phenocopies. Table 3 contains further data on Samarkand crosses with standard treatment. The eye effect is more or less high when the other parent was reactive (Ore, Canton). It is low—so low that the first beginnings can hardly be safely recorded—where it is low in the other parental line. Again the podoptera effect is dominant in all heterozygotes. There is a generally high antennaless effect, and again it is dominant when Canton is one of

the parents. The appearance of Bd and ll might be dominance or chance. Altogether the results fall in line with the preceding ones.

Crosses of lines with less specific characteristics

1. Crosses with Oregon Mohler

This line has, as a rule, a very high reactivity for eye effects, but shows some variations in spite of almost 200 generations of inbreeding. The most conspicuous one is that frequently the early hatching flies are normal and all later ones show the eye effect. (Usually the opposite phenomenon occurs due to the behavior of the yeast as described in the first paper.) In addition Ore Mohler has a fair tendency for the production of the ss^a phenocopy. A new check of the line, with standard treatment made at the time the present experiments were performed gave:

179 + (all but 11 found among the first hatchers)

324 \pm eyeless types (see discussion of phenotypes below)

29 ss^a

In table 4, F_1 crosses with this line are recorded except those already contained in tables 1-3.

No. 1. OM \times Big Ridge. The latter line has a tendency to extreme fertility. After treatment many eyes are dispersed. (See description in the former paper. This type has since been described as a mutant by Becker, 1956.) In the treated hybrid almost all eyes vary unclassifiably from rough eyes to different grades of dispersed and others described as Lobe-like. Thus the eye effect of Big Ridge is dominant. But the unusually high percentage of the ss^a phenocopy comes from the OM parent.

No. 2. The reciprocal cross shows the high fertility and F_1 luxuriance frequently observed in Big Ridge crosses; one of the 4 summarized in the table contains 315 normal flies; only one in four has fewer flies and a slight eyeless effect. The difference between reciprocal crosses is obviously a chance

result as the luxuriance was also observed when Big Ridge was the father (e.g. table 2).

Nos. 3, 4. Reciprocal crosses with Idaho Falls. The latter line has already, in untreated flies, a tendency to irregularly shaped eyes marked as polygonal. After treatment they become rough, varying up to a Lobe-like eye. Further, the *ss*^a phenocopy is rather frequent, also antennaless and double antenna (which latter always goes with Lobe-like eyes). In the experiment No. 3 all flies have polygonal eyes or transitions to a Lobe type, which can hardly be classified. This is essentially a dominance of the Idaho Falls type with no recognizable OM effect. But the reciprocal cross is very different,

TABLE 4

Phenocopies in Oregon Mohler (OM) crosses (1-6) and a few additional combinations

CROSS	PHENOTYPES							
	+	± disp.	eye effects	<i>ss</i> ^a	antl.	double ant.	pod	others
1. OM x Big Ridge	9	118		31	3	3		
2. Big Ridge x OM	932		12					
3. OM x Idaho Falls			474 ¹		1	1		
4. Idaho Falls x OM			418 ¹	23		27		
5. OM x Canton			185	3	54	1	15	1 Sen
6. Canton x OM	519		430	21	10		13	
7. Idaho Falls x Big Ridge			332 ¹	5	7	10	5	
8. Big Ridge x Idaho Falls			1000+ ¹			all extr. L		
9. Canton x Big Ridge	231		313		17		6	3 ll, 1 Bd
10. Idaho Falls x Canton	22		516	2	13	1	2	

¹ See text.

though identical in all 4 individual experiments which are summarized in the table. Again all transitions from polygon to Lobe are present in all flies. But in addition a large number of flies show an extreme Lobe type which is very characteristic: At the site of the eyes no eye tissue is present, so that only the axis of the head is left. But the posterior edge of the head is drawn out into a short or sometimes, rather long horizontal excrescence at the tip of which a tiny piece of pigmented eye is located, always on one side only. It is impossible to say whether this is an extreme exaggeration of the Lobe reaction in Idaho Falls or a combination product of the OM and Idaho Falls reaction. In addition this group contains a rather high number of ss^a phenocopies which are typical for both parental races and also double antennae, which are known to occur with the types of Lobe, both in the L mutant and the L phenocopy.

No. 5. OM x Canton. The typical Canton effect is the phenocopy of antennaless varying up to what was described in the former paper as frontless and headless. The effect is here rather extreme, which might indicate that the Canton type of reaction is not only dominant but is increased by the high sensitivity to the eye effect (which leads up to headless types) contributed by both parents. In addition many flies registered as ey have a type to be described later for extreme Deformed types. Thus the Canton reaction is present and enhanced and also the OM reaction, together with what looks like a combined effect. The relatively high incidence of podoptera comes from the Canton parent.

No. 6. The reciprocal cross is different in so far as it shows hybrid heterosis (one individual experiment contained 320 flies). Correspondingly a great many normals are present. The eyeless types do not differ from those produced in the parent lines. The other types are the same as in the reciprocal cross, though present in a smaller percentage, corresponding to the lowering of the phenocopic sensitivity by heterosis, namely ss^a from the OM parent and podoptera and antennaless

from Canton. The OM crosses already tabulated in table 1, No. 3 do not show any special features.

2. Other combinations

In table 4 some more combinations have been added, involving Big Ridge, Idaho Falls, Canton, and Samarkand. No. 7 and 8 are very remarkable. Big Ridge has typically a very high fertility and not much eye reaction to borate treatment; while Idaho Falls has a little higher eye reactivity with a tendency to produce Lobe-like types, also ss^a, antennaless, double antenna and podoptera phenocopies. In addition Idaho Falls has the polygonal eye type even in the untreated stock. Both reciprocal crosses contain practically no normal flies. All transitions are found from polygonal, rough, medium eyeless, eyeless, extreme Lobe types, with only a posterior speck of the eye left on a socket. The last type has usually double antennae. Thus a high sensitivity, much higher than that of either parent, is present, and, in No. 7, dominance of the Idaho Falls phenocopies. But while in No. 7 each experiment contains about 120 flies there are over 250 in each of No. 8, i.e. the high fertility is here dominant. But while high fertility due to heterosis is usually combined with more or less absence of phenocopies, here the eye-effect reaches 100%, indicating that not heterosis but dominance of the high fertility of the Big Ridge parent is being observed.

Nos. 9, 10 finally give the results of the treatments of Canton heterozygotes with Big Ridge and Idaho Falls. No. 9 shows the eye effect quantitatively intermediate between that of the parental lines, also fertility is intermediate but the expressivity rather low. The majority of affected eyes are only very rough, and a few more or less of the eyeless type. Thus the low reactivity of Big Ridge is more or less dominant. But the presence of many antennaless flies (up to the extreme called headless) is a typical Canton reaction, also the rather low percentage of podoptera belongs to the Canton type, while the lanceolate and Beaded phenocopies characterize neither

parent. No. 10 shows again the Canton dominance in regard to the antennaless effect. The almost 100% eye reactivity is due mostly to the polygon and rough effect characterizing Idaho Falls with very little of the Canton type eyeless effect visible.

Summary of Part I

The most conspicuous fact, appearing with great regularity in the borate treated hybrids between a number of wild type lines of known ability to produce different phenocopies, is that in a great many cases the hybrid shows dominance of the phenocopic reaction characterizing either parent. This is most conspicuous when one of the parental lines produces phenocopies which were not found otherwise, but which appear also in the hybrid, like the Bar-like eyes of Formosa. (But it happens also that such a type, like dachs in Formosa, does not show up in the hybrid.) Such dominance of reactivity is also conspicuous if a line like Formosa is characterized by reacting to treatment with a large variety of phenocopies and this peculiarity reappears also in the hybrids. The dominance of typical effects appears also in many cases when a parental line is characterized by an unusual frequency of some phenocopy which is rarer in other lines. Thus the different grades of antennaless, frontless, headless, are very typical for the Canton line and this reaction is dominant in most hybrids with Canton. The same is true for the high percentage and grade of aristopedia in the Amherst line, the high incidence of podoptera in the Samarkand line, lanceolate and Beaded phenocopies in Formosa, the Lobe and double antenna effect in Idaho Falls and the fertility in Big Ridge, all of which become visible in the different hybrids, treated identically. But there are also exceptions from this rule, which indicates that a definite genetic background is required if the dominance effect is to take place. It is obvious that both the regularities and the irregularities of the results must have a bearing upon the understanding of the genetic basis for the specificity of the phenocopic responses in different lines.

These facts relating to compounds of wild type lines will have to be compared now with those effects obtained when one of the parents is a mutant of the type which is phenocopied in the different wild type lines, the other parent one of the wild type lines.

Hybrids with eye-mutants

A. ey^2

In the former paper the hybrids of some wild-type lines with the eyeless mutant (ey^2) were studied in detail. It was found that the heterozygote $ey^2/+$ (i.e. the control for the borate experiments) showed a little dominance of ey^2 , the degree of which was fairly proportional to the sensitivity and reactivity of the different wild-type lines to produce eyeless phenocopies. This is in agreement with the assumption that the wild-type lines contain an isoallele of eyeless, which we are trying to prove. The same correlation was also found for the phenocopic reactions of the $ey^2/+$ heterozygotes. The homozygous mutant ey^2 reacted to the same treatment by an increase in the degree of eyelessness and the production of still more extreme types with head reduction (headless). But there was one wild type line, Oregon Mohler, inbred for many generations (192 when the present experiments were started), which did not fall in line. Though this OM line showed a strong eye effect in the phenocopic experiments, actually perhaps the strongest, there was no dominance in the controls $OM \times ey^2$ and the actions of borate upon the hybrid were irregular. Also the hybrid of OM with a line containing a deficiency for ey did not take its place in the series, which otherwise agreed fairly with the hypothesis. Thus the experiments were repeated in order to search for the reasons of the inconsistency.

It turned out first that the Oregon Mohler line, which should be homozygous after 192 generations of brother-sister breeding, had some special features, which were not reported in the former paper. The most unusual feature (already mentioned above) is that the treated flies hatching during the first days were normal while all the later hatchers showed the eye effects.

This is the opposite from typical behavior. For the latter explanations were discussed in the former paper. In the recent test among 503 flies altogether, 64.5% eye effects were found. But the last 2 counts (16th and 17th day) contained 94.6% abnormal eyes. Furthermore, a classification of the eye effects showed what had not been recorded formerly, that they were rather different from those in such lines as Canton-S and other Oregon lines, with which they had been lumped as resembling eyeless more or less. Actually, the eye effect resembling the phenotype of the mutant ey^2 was rather rare. Instead, a great variety of aberrant types appeared. There is a general tendency of restricting the eye differentiation to the upper part, while in the ventral half undifferentiated eye epidermis remains into which frequently long tongues of differentiated tissue protrude. Dorsally, the row of vibrissae forms a brush-like structure. Frequently the anterior part of the eye or even the head is missing and small eye rudiments are pushed way back and are situated on warts or peduncles. These may also appear as palps without eye tissue and finally the type described as headless is found. This indicates to us now that in Oregon Mohler we have no reason to assume the presence of a subthreshold eyeless allele. Obviously some other genetic constellation in regard to eye determination had been selected by inbreeding and this might be the reason why in the former work this line did not fit into the series of expected results, expected if an isoallele of ey were present.

The new experiments on $OM \times ey^2$ crosses bear this out. There was again no dominance of ey^2 in the controls. In addition the eye effect in the treated F_1 was completely different from that in other crosses. All the types just described for the line occurred in addition to many others. Many could be described as dispersed (see description in former paper), others resembled the erupt-mutant. Thus clearly the eyeless-type was absent and the Ore Mohler type of phenocopy was brought out in the hybrid. As was the case in other crosses with ey^2 the sensitivity was very high, around 90% of eye effects. In addition a relatively high incidence of ss^a phenocopies was noticed,

which characterizes also Ore Mohler. The other phenocopies are not typical for either parent. Table 5 shows another feature already reported in the former paper for Ore Mohler, namely the completely irregular production of heterosis. Three out of the four groups of crosses gave normal effects, with individual experiments yielding less than 100 surviving flies. But in one series most of the individual crosses showed heterosis with up to 423 flies; correspondingly the eye effect was quantitatively much lower. It is difficult to explain why heterosis appears or is absent without any definite rule.

TABLE 5

F₁ ey² × Ore Mohler and reciprocal with standard treatment

	PHENOTYPES						
	n	+	eye effect	ss ^a	pod	Bd	others
ey x OM	225	28	197	12	14	2	2 antl. 2 cleft
another set	250	38	212	37			
OM x ey	2194	1382	812	45	1	1	1 Sen
another set	77	8	69	15	1		

Though these and other observations of heterosis and its irregular ways do not belong to the topic of this paper, we like to draw attention to them. Here might be found an interesting material for such quantitative studies of heterosis as Robertson and Reeve are pursuing. We quote only the correlation between heterosis and "resistance to environmental variability" observed by Robertson and clearly present in our material. The use of the borate method for a quantitative study of heterosis might lead to interesting results. (See also the findings of heterosis in other OM and Big Ridge crosses.)

A few other experiments concerned ey² crosses with Canton-S and Idaho Falls. The results are similar to those of crosses of different wild-type stocks: the specific characteristics of both parents come out in the compounds.

Dominant eye mutants

1. *Bar*. Homozygous Bar itself was not affected by standard borate-treatment. Heterozygotes of Bar with different wild type lines (i.e. the controls) are typical heterozygous Bar, only B/Canton had somewhat smaller eyes than usual. (Of course modifiers for the Bar effect have been known to exist.) But after standard treatment the heterozygotes showed characteristic effects. The crosses were always $+ \times B$; thus the males could show only the standard eye effect of the wild-type stock, both for X-chromosome and autosomes; while the females could reveal an effect upon heterozygous Bar and, possibly, also an additional effect based upon the rest of the genome. Two of the heterozygotes, namely with Canton and Idaho Falls, did not show any effect of the treatment. As both of these wild-types react typically and, as we saw, retain their typical effects in heterozygotes with other wild-types, the lack of any reaction both in females and males is probably a heterosis effect, i.e. due to lessened general sensitivity to treatment. All other heterozygotes react typically. We must treat separately the eye effects in the presence of B/+ and the other phenocopic effects characterizing the different wild-types. The latter effects, like podoptera in the presence of Samarkand, are produced as in wild-type heterozygotes. But there is one important exception. We saw in Amherst and its compounds with other wild-type stocks a typical high penetrance and expressivity of the ss^a phenocopy. But in the heterozygote Amherst \times Bar, which showed otherwise considerable phenocopic reaction—almost all males had eye phenocopies of the type described for Amherst—not a single aristopedia fly appeared, in either sex. The Bar genome must have introduced something dominant inhibiting the ss^a reaction and this condition must be autosomal.

We come now to the eye effect in the heterozygotes. In the crosses with Big Ridge, Samarkand, and Amherst always a variable effect was found, namely an increase of the Bar action up to the phenotype of double Bar. The variability may be

due to the presence of different modifier systems. Thus the result for females Big Ridge x Bar was: one experiment mostly like controls, but a few flies with eyes like homozygous Bar; one experiment with about 30% like B/B; one experiment with most females like B/B and many like double Bar. Thus the effect of the treatment is to increase the B/+ effect to the phenotype of homozygous double Bar in the extreme case. In some flies, one eye was like B/B, the other like B/+. Only once did the effect not remain completely within the quantitative series of Bar effects. This happened in one female with one eye like B/B and the other completely eyeless. But one could say that eyelessness might belong also to the B series, if still more B-sections could be piled into an X-chromosome. (Rapoport 1940, had succeeded to do this and only a few facets were left.)

In the crosses with Samarkand two experiments showed no effect, in two others all flies looked like B/B. In the Amherst crosses one was unaffected, in one most females had the phenotype B/B and some that of BB/BB; in a third about half each of the flies were of these two phenotypes (a separation BB/B from BB/BB phenotypes was not tried).

As in former examples the highly inbred Ore Mohler stock gave aberrant results in the hybrid females (while the males not having the Bar duplication reacted as described before for Ore Mohler). The females were very variable with all phenotypes B/+ to B/B and BB/BB down to complete absence of eyes. But in addition there were types without facets in the Bar region of the eye but small patches of facets posteriorly, reminding of some of the dispersed eyes described before. Clearly here not only the Bar effect was increased up to eyelessness but in addition the aberrant eye types characterizing the Ore Mohler stock became somewhat dominant in the heterozygote after removal of the Bar effect. This is, of course, very interesting for an attempt at embryological interpretation, which would have to take into account the separate determination of different regions of the eye surface. Such an interpretation would require much more detailed

knowledge of the fields of determination in the eye anlage, than available at present. This is also true for the finding of one single fly with the dorsal half of the eye like B/B and the ventral half in the shape of a broad clover leaf, i.e. a combination of Bar and dispersed.

Thus we see that the heterozygous Bar action upon the eye is increased in the direction of so-called Bar mutants (B, BB, BBB) by the standard treatment. While the other typical reactions of the different wild-type lines are found, the Bar effect remains usually dominant. Only in the B/OM combination both the B effect and the dispersed-like OM effect can be dominant simultaneously, i.e. combine and produce combination effects. Remarkable is the absence of ss^a -phenocopies in the Amherst/B compound. As the non-Bar males are included it is not the B locus which prevents the otherwise typical effect but some autosomal condition of the B-stock. In table 2 containing the Amherst crosses also three cases of absence of the ss^a effect are found. One shows heterosis with low phenocopic action, but the two others have good eye reactions and should also exhibit ss^a . The explanation might be the same as for the Bar series.

2. *Deformed*. Dfd (3-47.5) is known for its variable effect upon the eyes. If typical the eye has a kind of V shape if seen from above. Another frequent effect is an eye of which only the posterior part has developed as a pigmented knob, so that the rest of the head looks rudimentary, but with normal antennae attached in front (different from headless where also antennae and anterior head tissue are missing). The vibrissae tend to form tufts, which is sometimes the only effect visible with otherwise normal eyes.

In our stock Dfd/Cx bred pairwise at 25°C the eyes are practically normal; only a few individuals have smaller eyes and a considerable number have more or less tufted vibrissae. After treatment of Dfd/Cx with borate about 1/2 of the flies are unchanged, the other half shows different degrees of the typical eye effect up to the extreme one with the tiny posterior eye-knobs. (A few antennaless and aristopedia phenocopies

are also present.) In the heterozygotes with the different wild-type stocks already the controls *Dfd/+* are very variable. (The balancer *Cx* has no phenotypic effect. As in some controls 1/2 of the flies are visibly *Dfd* it is obvious that *Cx/+* is also normal.) Table 6 shows this variation of the controls *Dfd/+*.

TABLE 6

Controls + × Dfd/Cx

+ parent	eyes
Ore Mohler	All +
Idaho Falls	1/2 <i>Dfd</i> , incl. only tufts
Canton	1/2 <i>Dfd</i> , lower grades
Samarkand	the same
Big Ridge	only 3% eye effects, all grades up to extreme
Amherst	8% eye effects, all low

TABLE 7

Different wild types × Dfd/Cx with standard treatment

WILD TYPE PARENT FROM	PHENOTYPES										
	+	low	<i>Dfd</i>	Extr. <i>Dfd</i>	ssa	front- less	ll	Bd	c	double ant.	pod
Amherst	137	65	8	37	17						2
Big Ridge	218	210	39		19	34	4	2	3	2	3
Samarkand	1	10			2						1
Idaho Falls	175		30		2						2
Ore Mohler	172	205	17		15	13				1	
Canton	1/2		1/4	1/4		many					

It shows that in some controls *Dfd* is completely recessive, in some a slightly dominant and in others completely so. No correlation exists between these variants and the known sensitivity to phenocopic treatment or the types of eye phenocopies in the respective wild lines. Obviously chance modifiers are responsible, as is also indicated by the behavior of the cross with the highly inbred Ore Mohler.

The results of the borate treatment of the heterozygotes are presented in table 7. It shows again in some cases that the typical effect of the wild-type parent is present in the heter-

ozygote, e.g. the ss^a effect in Ore Mohler and Amherst, the frontless effect in Canton, the polygon effect in Idaho Falls (which makes classification of the + and low grade effects difficult). Surprising is the result for the Big Ridge cross. This line has low sensitivity and no specific reaction; but in the heterozygote an unusual array of phenocopies appears, though the effect upon penetrance of Dfd is rather small. Another feature is that the heterozygotes with Canton and Amherst produce so many high grade Dfd types, while those with Oregon Mohler, which has a high sensitivity for eye defects, do not contain the extreme Dfd phenotype.

If we try to categorize these results of the borate treatment we may say:

1. A low eye effect could be due to weak penetrance of Dfd, but it could also be a phenocopic eye effect of the eyeless type produced in the absence of a Dfd effect. But as it seems that the Cx/+ siblings remain normal, we may safely assume that the observed low grade effects are to be attributed to penetrance of Dfd. Thus, generally speaking, Dfd action is enhanced. But just as in the controls an irregular dominance of Dfd occurs, the enhancement in the experiments is also irregular, pointing to the interaction with complicated modifier systems.

2. As a rule the presence of Dfd does not interfere with the dominance of the specific phenocopic reactions of the wild-type lines.

3. In view of the variability of the Dfd type it is very difficult to decide whether the eye types produced in the experiments are exclusively increases of Dfd penetrance or whether the specific eye reactions of the wild-type parent became also visible. In the Bar crosses this was partly the case. Here no eye types were seen which were outside the range of variation of Dfd.

4. It is not understood why the Big Ridge/Dfd combination shows such a variety of phenocopies while the wild-type parent is rather insensitive.

5. It was noticed that all ss^a flies in the Amherst and Ore Mohler crosses were also high grade Dfd, which indicates that the Cx/+ combination gave no ss^a reaction, i.e. the eye effect predisposes also for the antennal effect. Such a general correlation was already noticed in the data of the first paper.

3. *Lobe* (L, 2-72.0). Lobe and its alleles have a very variable phenotype which overlaps eyeless, erupt, Deformed. In the stock used here (the same as analyzed by Zimm 1951), eyes increased in size or decreased, folded, even tumourous looking ones are frequent and so are double antennae. Homozygous Lobe subjected to the standard borate treatment reacted by increased Lobe effects leading to eyelessness, the condition described before as headless and increase of the extreme types with tiny posterior eye rudiments on stalks and double (or triple) antennae.

Lobe is a homozygous viable dominant. But the controls, heterozygotes of L with the different wild stocks, are extremely variable. The extreme is the heterozygote with Ore Mohler. As also observed in other crosses with this highly inbred line, F_1 is heterotic and simultaneously almost 100% normal i.e. hardly any dominance of Lobe visible. At the other extreme are the heterozygotes with Samarkand, Canton, Big Ridge, which vary in individual bottles from a high dominance of L down to the presence of about 50% normals and a corresponding low expressivity of the Lobe character.

The results of the experiments with the heterozygotes are tabulated in table 8. We see first a repetition of the former observation that the specific reactions of the wild-type lines may come out also in their heterozygotes. Thus the Amherst cross has the highest incidence of ss^a phenocopies and the Canton cross the same for antennaless and frontless. The latter cross is especially remarkable because almost half of all flies show the extreme reaction of the front region of the head, frontless and headless. We saw that pure Lobe also reacts to treatment with increase of the Lobe effect and appearance of the headless type. Thus in the heterozygote a typical Canton reaction from one parent and the Lobe reaction

from the other add up to an extreme reaction of the same developmental type.

In all crosses except the one with Ore Mohler the eye-effects are 100% dominant (or almost so), (though very variable in the controls). The expressivity parallels rather closely the sensitivity of the wild-type parents in regard to eye effects. Thus the Samarkand crosses contain mostly the lower grades of eye effects; the Idaho Falls heterozygotes the medium grades; the Canton crosses the high grades and the Amherst crosses the extreme grades. Big Ridge dominance is complete in spite of high fertility, which is already known to us as a

TABLE 8

Experiments with Lobe heterozygotes

WILD TYPE PARENT FROM	PHENOTYPES						NOTES
	n	+	± L	± headless	ss ^a	pod	
Idaho Falls	249	20	229	7		11	
Samarkand	124		124	5	1	7	
Canton	149		149	67	3	2	
Big Ridge	1076		1076			1	
Ore Mohler	1216	1216			5	1	
Amherst	258		258		13		95 like L 163 like Dfd extreme

genetic character of the stock, which is dominant in the crosses; but the expressivity varies from almost normal to extreme. In the Ore Mohler combination, in different bottles, normal flies are present in varying, usually high, numbers, and the affected eyes vary over the entire range. Here heterosis is clearly involved.

DISCUSSION

In the first part of this work it was shown that the production of phenocopies by chemical treatment is not as specific in regard to the chemical used as had been assumed from Rapoport's work, but, rather unexpectedly, that it is much

more specific for different genetic lines undergoing the same treatment. It was shown for a number of wild-type stocks that they reacted differently to the same treatment, both quantitatively and qualitatively. When it was found, in addition, that mutant phenotypes which had disappeared through accumulation of modifiers toward the wild type reappeared again after phenocopic treatment; further that a phenotype characterizing one allele but absent in another could become visible in the latter after treatment; the idea occurred that the specificity of the phenocopic effects might be due to the presence of sub-threshold alleles (isoalleles) in the different lines. But it turned out to be very difficult to prove such an interpretation beyond doubt. A number of facts agreed with it, others did not. The present paper continues the search for proofs or disproofs of the basic idea.

One of the difficulties encountered in the former paper was that one stock, Oregon Mohler, derived from a standard Oregon stock by intensive inbreeding, fell out of line in a number of experiments testing for a sub-threshold allele of eyeless. In the heterozygotes with both ey^2 and an eyeless-deficiency, this line, supposed to give a high eyeless effect after borate-treatment, did not fall into the proper place when correlated to other lines of less extreme sensitivity. It was shown now that the specific eye reaction of this line is of a very different type from that of the mutant eyeless and that, therefore, it could not be expected that the correlation sought for existed.

The most conspicuous results of the present work demonstrate that many of the typical and specific reactions of the different wild-type lines occur also in heterozygous condition, e.g. the line Amherst produces always under standard treatment a large percentage of relatively high grade aristopedia phenocopies and the same is true for almost all heterozygotes with Amherst, whether with different wild type lines or mutant stocks. The Canton line is characterized by antennaless (and the extreme type frontless) phenocopies in large numbers and so are the crosses with Canton. Samarkand and its heterozygotes react similarly in regard to the podoptera phenocopy.

The Formosa line is characterized by manifold otherwise rare phenocopies and the heterozygotes show the same. Further, in the same heterozygote the types of phenocopies favored by both parents may be produced. This astonishing dominance of the types of phenocopic reactivity is very difficult to explain. There is no doubt that these facts demonstrate that there is a considerable hereditary element in the entire phenomenon. But again the dilemma arises, whether this dominant effect can be explained by the general genetic background, i.e. the chance modifier systems; or whether the presence of individual subthreshold mutants is required. We feel that the considerable regularity of the phenomenon points more to the explanation by individual subthreshold mutants. In this case all the F_1 combinations would be genuine heterozygotes for a series of such subliminal mutants brought in from both parents. Now we know (also from some of the new work e.g. the data on Bar) that the standard treatment can enhance in many cases the dominance, or, expressed more correctly, the heterozygotes expressivity of a number of mutants. From such facts one could extrapolate upon our problem and conclude that the dominance of the typical effects in the heterozygotes with the different lines should also be the result of enhancing the expressivity of a real heterozygote, real because of the presence of a subthreshold mutant. Thus we consider the probability that this explanation is correct to be enhanced by the present work, though a final proof would require another technique which is being tried.

In the present work a further example was found for the disappearance of visible dominance of a well known dominant mutant and its bringing to light again by the standard treatment; this group comprises now Scutenick and Deformed. Nearly related are the cases of enhancement of effect of the heterozygotes (Bar) and both hetero- and homozygotes of dominants (Lobe). Most probably the induction of the aristro-pedia-effect in the allele spineless (reported before) belongs to the same group of phenomena. A real explanation which would have to be given in terms of embryological factors or,

even, biochemical ones, is not yet visible, though it might be restated from former discussions, that it seems much more probable that the basic common action is one upon the chemical kinetics of development, rather than upon the quality of underlying chemical reactions.

SUMMARY

1. This continuation of the phenocopy experiments with tetraborate treatment tries to find proofs for the assumption that the specific effects upon different wild-type *Drosophila* lines could be due to the presence of subthreshold mutants (isoalleles).

2. A number of lines of known and characteristic reactivity were crossed among themselves and the "compound" subjected to standard borate treatment. In spite of a certain amount of variation, it turned out that many, though not all specific phenocopic reactions of both parents appeared in the treated hybrids.

3. This was very characteristic for the Bar-like eye effect of the Formosa line, the aristopedia effect of the Amherst line, the antennaless effect of the Canton line, and the podoptera effect of the Samarkand line.

4. But some specific effects, like curved in the Formosa line, did not show this dominance.

5. Another set of experiments was made with heterozygotes of the same lines with recessive and dominant eye mutants. In addition to the dominance of the typical reactions of the individual lines, a number of facts were found:

(a) The discrepancies noted in the former work in regard to the Oregon Mohler x ey^2 hybrids could be resolved when it turned out that this wild-type stock reacts to the borate treatment with a phenocopy different from that of eyeless, so that no compound action may be expected.

(b) The eye effect in B/+ was always increased up to the phenotype of double-Bar, with characteristic variations in the different crosses. Only in the heter-

ozygotes with Ore Mohler a combination of increased Bar effect and the OM type of phenocopy occurred. One remarkable fact was that in the Amherst-Bar heterozygotes the typical ss^a effect of Amherst was absent.

- (c) Deformed is a dominant mutant with variable expression, which, in our stock, was very low. But borate treatment brought out the Dfd-phenotype up to its highest expression in the balanced stock Dfd/Cx. In heterozygotes Dfd/+ the controls varied considerably, i.e. the dominance of Dfd was variable from complete absence in Dfd/OM to about 100% dominance with low expressivity in Dfd/Canton, which points to the action of chance modifiers. Also the borate treated heterozygotes show a variable effect without visible rule. In some the extreme type of Dfd is frequent, in others absent.
- (d) Homozygous Lobe treated with borate shows more extreme phenotypes than the controls. L/+ heterozygotes show different grades of dominance from almost complete absence in L/OM to 100% in L/Amherst. Most of the treated heterozygotes show a 100% eye effect, the expressivity of which is fairly proportional to the reactivity of the + parent. In the Canton heterozygote the antennal effect (frontless, etc.) is increased beyond the one typical for Canton alone.

6. The discussion points out that the facts are in favor of the presence of subthreshold alleles as prerequisite for specific phenocopic effects, without affording a final proof.

7. A number of interesting facts on heterosis were observed but not analyzed.

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POSTSCRIPT

After this paper had gone to press Bentley Glass published a most interesting survey of his recent, mostly unpublished work on the erupt-suppressor system. (*Science* 126, 1957: 683-689). While we had pointed in our first communication to some of the former work of Glass and Plaine, which was relevant for our present discussion, the new data raise important questions and might expose our data to a reconsideration under an unexpected point of view. It is known that Glass had been able to show that the eye mutant erupt is associated with a suppressor locus (in another chromosome) which prevents its phenotypic appearance. If this locus is removed by replacement of its chromosome, the mutant becomes visible. The same happens when larvae up to the middle of the second instar are irradiated with 1000 r. The latter experiment considered alone looks like production of a phenocopy by radiation (X-rays are known to be a phenocopic agent). But the genetic analysis made it clear that a suppressed mutant had become penetrant by stopping the action of a suppressor locus by irradiation. This original analysis led thus to the establishment of what might be called a "mock-phenocopic effect." Further work, reported now by Glass makes it imperative to inquire whether the suppressor action does not enter directly into the present analysis of the genetic basis of the phenocopic effect.

We pointed only briefly to Glass' discussion of the possible biochemical basis of the suppressor action, specific and unspecific, important as it is. More relevant for the present problems (i.e. at the present stage of the phenocopy work) is the following: In an inquiry into the selective value of the suppressor effect many wild-type stocks were checked for the presence of the suppressor locus. Only one strain, long inbred, was homogeneous for the suppressor. Some strains had strong suppressors in the second chromosome with potent normal alleles of erupt in the third, some had potent normal alleles of erupt and weak suppressors. Others had weak normal alleles, so that erupt became visible. Altogether erupt mutants were present almost everywhere but did not become visible because of suppressors and plus alleles. The combinations made with different wild-type lines show further that at least 5 alleles of different strength can be located both for the erupt and the suppressor locus. Glass mentions also facts which show that

the occurrence of alleles of different strength is a widespread phenomenon and discusses the evolutionary meaning of suppression or incomplete suppression of frequent dominant mutants in populations. The presence of such suppressor systems would be important in buffering the genotype against the effects of critical mutations thus stabilizing homeostasis (in Lerner's sense) without the need for multiple modifiers.

It is obvious that these facts suggest the possibility, that what we tended to consider as different subthreshold mutants, brought to light by the phenocopic treatment, might be a combination of subthreshold isoalleles for different loci with a set of different more or less specific suppressor loci, again of different potency, the action of which is more or less canceled out by the same treatment. It will have to be seen whether further work will show that the two lines of work, the suppressor and the phenocopy work, will come together as aspects of a single phenomenon. Only one point made by Glass should still be mentioned in support of such possibilities. He points out, that from the point of view of evolutionary significance the suppression of mutants of the type represented by the homoeotic mutants would be of special importance. Should it be significant, then, that the aristopedia and podoptera phenocopies are so preponderant in our work? Finally one suggestion presents itself. The biochemical deliberations of Glass point to the possibility that unspecific suppressors (e.g. known for vermilion and sable) affecting completely different pathways may act via one common precursor e.g. tryptophan. If this is true, rare suppressors might exist which affect a large group of loci with a common precursor. A removal of such a suppressor (e.g. by mutation) would produce the rare, thus far unexplained but certainly observed, phenomenon of mass mutation, which then, is paralleled by mass phenocopy as described for the Formosa line. Thus the work of Glass might supply leads into a number of unexpected directions.