THE GENETIC BACKGROUND OF CHEMICALLY INDUCED PHENOCOPIES IN DROSOPHILA

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FOUR FIGURES

INTRODUCTION

It is well known that Rapoport ('39) found that a number of chemicals, many of them known to be enzyme inhibitors, produce phenocopies of Drosophila mutants when given with the food. The most important feature was that one or a few mutant effects were phenocopied in 100% of the individuals when the poison was administered in sublethal dose and that these effects were specific for the different substances (acting upon one wild-type stock used). A number of authors have since repeated these experiments with, in the main, the same results and some, like Gloor, Bodenstein and Abdel-Malek and Schultz et al. have added data of the same type, though the details are not quite as simple as Rapoport claimed. During the past 10 years we also have repeatedly made such experiments but interrupted them for other work. Like others we found in a general way that Rapoport's experiments were reproducible. But quite a number of individual facts were recorded which hinted at complications which might seriously affect the interpretation of the experiments. The work was therefore taken up again, restricting it finally, for the time being, to a single substance, sodium tetraborate, and centering the attention upon the facts which complicated the basic effect.

The most important and elaborate work on the subject (limited to a single substance) was done by Sang and McDonald ('54) who made a complete quantitative study of the

action of sodium metaborate which was known to produce the phenocopy of eyeless (and also aristopedia and antennaless). We shall mention below their results which relate to the eyeless character. Here we point only to some findings which play a minor role in their work but are important for ours. 1. They worked with two Oregon strains, one of which gave good results, while the other, highly inbred one, had a much lower reactivity to the treatment. 2. Later work — mentioned only in passing and not specified further — with different strains of flies showed that some were more sensitive than others to the effects of the boron and that they differed considerably in the frequency with which the different developmental systems were modified by the salts. This, then, means an influence of the genetic background upon the phenocopic effect.

Already in Goldschmidt's old work with heat shocks ('35) he had found that the genetic constitution of the treated flies made quite a difference for the results. A table was compiled, showing the varied reactions when different wild-type or mutant stocks were used. In a later paper (Goldschmidt, '37) a case was also described in which phenocopic treatment brought out visibly a subthreshold mutant of vestigial (no or almost no penetrance), found to be present in the stock. Similar features will be in the foreground of the present work.

In the earlier experiments we used different borates but later we applied only sodium tetraborate ($Na_2B_4O_7.10H_2O$). Sang and McDonald had shown that the curve of action of the borate, proportional to the quantity, is more regular and the effect more powerful if the medium contains only dead yeast, as live yeasts take up the salt and by their divisions reduce the concentration taken up by the larvae feeding on yeast. This difference is important for the quantitative work done by these authors but not essential for most of our work, which was therefore done with our standard Drosophila food (corn meal, agar, molasses medium with Tegosept M added) mixed with the borate in solution. The concentration usually em-

ployed was 0.06 weight per cent/volume (2 cm^3 of a 3% borate solution were added to 100 cm^3 of food). This had to be varied from 0.04–0.10% for a good effect on less or more sensitive strains. In view of the size and number of experiments they were not performed with counted eggs. A sufficient measure of lethality produced = sensitivity (which is usually though not always proportional to the effect or reactivity as measured in terms of percentage of affected flies) was obtained by comparing the number in the controls to those of the experimental bottles (details below). One pair of flies (virgin females) which had been mated in vials for 24 hours were left for three days in the control bottle and then transferred to the experimental medium. All cultures were raised at 25°C. The count of the experiments was finished on the 17th day.

1. The qualitatively different phenocopies characterizing different stocks

It turned out that the old description of the borate effect as producing the phenocopies of eyeless, aristopedia and some wing defects (Rapoport, '39) is true only for some stocks. In others many other mutant types (including also other eye mutants) are phenocopied and, further, phenocopies which are rare in one line may be preponderant in another and vice versa. The manyfold results in regard to the characteristic reaction of different genetic stocks and lines have been obtained with the just described identical standard treatment i.e. three days in control bottle for one pair, then transfer to 0.06% borate food. To be counted as phenocopy the aberrant phenotype must be absent in the controls and present in all or a number of bottles of the same experiment and, in most cases, also in such repetitions as were made after some months. (The reason why sometimes not all bottles of the same experiment showed the effect are: too small numbers or unexplained general failure of borate action.) Abnormalities appearing only in a single bottle as one or two

individuals (including those which turned out to be mutants) were not counted (though recorded) as they might have been chance products not referable to the treatment. When many phenocopies were produced, individual flies might show only one of them, or more than one or even many. Thus once a fly was found which showed simultaneously the phenotypes of Bar, aristopedia, antennaless, double antenna, dachs, curved, lanceolate, Beaded. As it is impossible to publish the tabulations of all results, we give, in table 1, one example of a typical set of experiments with the very reactive stock Formosa, while for all other stocks only the general results will be tabulated in table 2.

We see in table 1 that the lethality was not considerable in these experiments, as there were more experimental than control flies (the average expectation without lethality is, according to tests made, experiment : control = 60 : 40%). Further two groups are discernible in table 1: the first three cultures reacted strongly with over 70% phenocopies. The last two reacted weakly with only about 33% phenocopies. Therefore the two groups were summarized separately for our purposes. We shall meet repeatedly with this variability and try to analyze it in one instance. Thus far no completely satisfying explanation of this phenomenon has been found. Ten different phenocopies (possibly 11) were found, the most frequent ones of which were those of Bar and curved, followed by lanceolate, dachs and Beaded. In the lower columns the percentages of each are given both among all and among affected flies. We see that all or almost all affected flies showed the eye effect. When a fly was Bar, lanceolate, curved each character was counted separately. The comparison of the total number n with the individual numbers permits to estimate the amount of simultaneous phenocopies in one individual. If e.g. one eye was Bar, the other normal — the effects are frequently onesided — the fly was registered as Bar, but if one side of the head showed aristopedia while the other side showed antennaless both characters were counted separately (this applies only to the

TABLE 1

Line Formosa treated with 0.06 borate

NO.	n	PHENOTYPES										
		+	Bar	Curved	Lanceolate	Dachs	Beaded	Dumpy	Antenna-less	Aristo-pedia	Scutenick	Control
3076	76	28	48	11	6	3	1		1		2	?
3077	110	35	74	33	7	1	4				3	89
3079	92	18	69	18	6	4	4	2		2		83 [1]
Sa.	278	81	191	62	19	8	9	2	1	2	5	172
3080	204	180	24	14								65
3081	220	103	117	29	9	3			2			84 [1]
Sa.	424	283	141	43	9	3			2			129
% of n	No. 3076–79			22.3	6.9	2.9	3.2	< 1	< 1	< 1	1.8	
% of phenoc.	No. 3076–79			31.4	9.6	4.1	4.6	1	< 1	< 1	2.5	
% of n	No. 3080–81			10	2.1	< 1			< 1	< 1		
% of phenoc.	No. 3080–81			30.5	6.4	2.1			1.3	1.3		

[1] Some double scutellars.

TABLE 2

Characteristic phenocopies of wild type lines. — a few scattered individuals, +[1] = a few per cent, +[2] up to 50% in the best experiment, +[3] the majority affected. All 0.06 Borate except when noted at right. Sensitivity: I more viable than controls, no phenocopies. II few phenocopies. III many, IV majority of flies, V most flies and many types. VI lethal.

NO.	STOCK	n	PHENOCOPY OF (OR PHENOTYPE)																							NOTES
			Eyeless	Kidney	Lobe	Bar	"Dispersed"	Aristopedia	Antennaless	"Frontless"	Double ant.	Arch	Curved	Beaded	Lanceolate	Puff	Dumpy	Intersex	Podoptera	Scutenick	Polychete	Polychetous	Dachs	"Double arista"	Divergent	Sensitivity	
1	Riverside	2365	—																—							II	ey at 0.09 +[2] pod at 0.09 +[1]
2	Orinda I	3607	—																+[1]							II	only 1 ey
3	Samarkand	1334		+[3]					—					—	—		—		+[2]	+[3]						III	pod see special table
4	Lausanne S	972	+[2]					+[2]				+[3]							—							V	
5	Florida	521			+[1]														—							II	
6	Florida 19	730			+[1]			—						—						—				+[1]		II	
7	Salta	1412																								I	
8	Corona	664			—						—			—					—							II	Lobe or eyeless?
9	Bikini	>4000			+[1]			+[1]	—		+[1]	+[1]							+[1]			+[1]				I	at 0.08 still very fertile and large, phenoc. above 0.08 only
10	Sweden	116	+[2]					+[1]											+[1]							II	
11	Orinda II	2546	+[2]																+[1]							II–IV	some low, some high reactivity
12	Canton S	1000's	+[3]						+[3]	+[3]	—								—							II–IV	from 0 to 100%, see special tables
13	Oregon Mohler	280	+[3]					+[1]																		V	
14	Oregon Dempster	1325	+[1]					+[1]												+[3]						VI	very weak, phenoc. from 0.04 to 0.05 once 0.07
15	Urbana S	177	+[2]					+[1]			+[1]														+[2]	III	"double antenna" a different type
16	Big Ridge	2833					+[1]	+[2]						—					+[1]							I–II	broods up to more than 500. aristop. very low
17	Quicksand	1046			+[2]			+[1]	+[1]					+[1]				+[1]	—	+[1]	—					IV	
18	Formosa	908				+[3]		—	—	—			+[2]	+[1]	+[1]		+[1]					—	+[1]			V	
19	Sevelen	370		+[2]				—				+[2]				+[2]			—							III	some arch in control
20	Idaho Falls	655			+[3]			+[2]			—															V	almost all low L = ro and angular, aristop. very low
21	Amherst	809	+[2]					+[2]				+[3]		+[1]												IV	aristop. highest grade of all

summarizing tables, while our records show the combinations). A more exact method would be counting half flies, but for the purposes of the present paper, the study of the genetic aspect of phenocopy, this was not deemed necessary.

In the general tabulation (table 2) a dash indicates a few individuals scattered over some broods, e.g. Scutenick in Formosa (see table 1); a + means a few per cent even in the most responsive individual experiments e.g. Beaded in Formosa. The sign ++ designates that in the successful experiments up to 50% of the flies show the type (e.g. curved in Formosa) while +++ means that the majority shows the effect (e.g. Bar in Formosa). With successful experiments we allude to such cases in which some individual broods are not reactive for unknown reasons and therefore not included in the overall appraisal of reactivity as presented in table 2. The Formosa experiments can serve as an example. When the effects are obtained only with other than the standard concentrations a footnote is added in explanation. Such cases cannot be used for the quantitative side of the effect, though giving information on its quality. Clearly Bar, curved, dachs, lanceolate and Beaded are characteristic for the Formosa stock, while Bar and dachs are as far as our experiments go, completely specific for this stock, i.e. not found otherwise.

We describe first all the phenocopies obtained regularly — testing still more stocks would certainly add to the list — and mention for each the typical incidence in our experiments. In the table summarizing the results (table 2) only the total number for each experiment is tabulated and the order of magnitude of the effect is described.

A. Phenocopies of the eye.

Most frequently borate affects the eyes with or without simultaneous effects upon the other derivatives of the cephalic complex. In different stocks the eye effect is not always the same i.e. a phenocopy of eyeless. Rather a number of known (one unknown) eye mutants are copied each one found always in one or more of the different stocks tested. Some of those

phenocopies can be identified clearly with known mutants, others may imitate more than one mutant type and again others do not match exactly any known type. But it must be kept in mind that some of the mutants used for comparison overlap considerably among themselves, and depend in the details of expression upon sets of modifiers; in addition multiple alleles of these mutants have different expression. A complete morphological comparison would hardly lead to reliable results. Thus, what is presented is an evaluation of the average type in each case to the best of our ability.

1. Eyeless. This is the most frequent phenocopy produced in many different lines. The phenotypic effect is identical with that of our stock of eyeless, ey^2. The lowest effect is either moderate roughness in one eye or a small inward depression in the contour of the eye. Next both eyes are affected but usually not symmetrically. The eye becomes more or less restricted either smaller without change of shape or with irregular indentations of great variability. In the higher grades segments may be split off or odd, spider-like shapes produced. In the still higher grades a small group of facets, frequently bulging out, remains at different points of the eye site and finally the eye is completely gone. Different stocks of the mutant ey^2 differ in penetrance and expressivity. But our ey^2 is 100% penetrant with a majority of flies in the higher and highest grades of expressivity as opposed to the stock used by Sang and McDonald. In the phenocopies expressivity parallels the penetrance (the latter being dependent upon concentration of borate and the susceptibility of the genetic lines; see below). A 100% effective treatment produces practically the same phenotype as our stock ey^2. The eyeless reaction was found in the following stocks: both lines of Oregon, Riverside, Canton, Orinda I and II, Urbana, Lausanne, Swedish, and Amherst. Their different reactivities will be studied below.

It seems that some kind of phenocopic eye-effect is typical for borate treatment. Thus far no stock was found without any action upon the eye. When the standard treatment did

not produce an effect, a higher borate concentration would do it. But in a number of stocks the eye effect was typically one different from the eyeless phenotype and simulated eye mutants other than eyeless. In such stocks the phenocopy in question appeared in every individual experiment, whether the stock was a highly susceptible one for the treatment or not, or whatever concentration of borate was used. We never found mixed results, as far as the normal overlapping of many eye mutants permits to state, only different grades (expressivity) of the same phenocopy characteristic of that stock. The following additional phenotypes were discovered.

2. Kidney. We call the type kidney, though it would be more correct to speak of one of the kidney-like mutants. The lowest grade of expression is roughness, much rougher than in ey^2 and characterized by a tendency of the disturbed rows of facets to converge toward an anterio-posterior line. At the anterior end of this line, or more ventral of this point, the first indentation appears which becomes in a higher grade a crater shaped groove inside of which a knob with or without hairs erupts, as in the mutant erupt. In a still higher grade the kidney shape of the eye may overlap with one of the many individual variants of eyeless. Thus far the kidney phenocopy characterized only our lines of Samarkand and Sevelen.

3. Lobe. Although very variable in different lines and alleles this mutant (L), overlapping with eyeless in higher grades, has a tendency to increase the eye surface which folds and creases in different ways up to the formation of horn-like excrescences. One allele is characterized by the frequent combination with double aristae or antennae (see Zimm, '51). The phenocopy of Lobe is more or less easily distinguished from that of eyeless (though single individuals may overlap) and where it was found, it was also combined with the double antenna in some individuals. The lobe phenocopy is typical for the stocks Florida-19, Quicksand, Corona, Idaho Falls, Bikini and also for our mutant stock of spineless (ss). There are stocks in which almost all treated flies have slightly rough eyes which might be called a phenocopy of

one of the rough alleles. Idaho Falls is a typical example. But here and in all other less extreme cases a few flies were typically Lobe and therefore the roughness was considered a low expression of Lobe. It should be emphasized again that many of the genuine eye mutants are characterized by their location in the chromosomes rather than by the phenotypes. These overlap or are frequently indistinguishable and could not be picked out of a mixture. Thus our designation of phenocopies does not mean more than the fact that they are characteristic and discernible from others. But in some instances they might be called phenocopies of one or another similar looking mutants. The one we chose for designation means only that the resemblance seems to us to be close. Of course we have not seen all eye mutants described, a drawback which applies to all such experiments.

4. Bar. The different grades of the mutant Bar eye in different alleles and compounds are well known to every geneticist and they do not overlap much with other eye mutants. Only one of our stocks, Formosa, gave the phenocopy of Bar and this constantly; but it must be said that the phenotype is not completely identical with that of Bar. It certainly overlaps with it and is completely different from that of all other eye types which were produced as phenocopies.

5. Dispersed. A very characteristic phenotype of the eye after borate treatment has not been described as yet as a mutant as far as we are aware. The reduction of the eye begins with a separation of dorsal and ventral halves in a straight line with a narrow rimlike bulge of the epidermis between. In higher grades the eye is broken up into two to four parts, some of which may be far displaced on the head. If only one splinter is left in the highest grades it may be found in odd locations on the head, different from comparable expressions of eyeless. Very typical is the following arrangement: the eye chitin devoid of facets is clearly delineated. The upper half of the eye has very rough facets. In the non faceted lower half a tiny group of facets is located at the

ventral posterior edge. In front of it a dense brush of hairs, bristles and vibrissae is located inside the eye area. This type is specific for the borate treated stock of Big Ridge.

B. Phenocopies of the antenna.

Phenocopies of antenna mutants may appear simultaneously with eye phenocopies, but either of the two parts of the original cephalic disk may be affected independently. Of course, in the case of high incidence of eye effects the chance for simultaneous occurrence is a greater one. Only in one case we observed that all antennal effects were accompanied by eye effects.

The most frequent antennal phenocopy is that of aristopedia.

1. Aristopedia as a mutant (ss^a) exists in a number of alleles. The most extreme one is characterized by 100% flies with a perfect tarsus instead of an arista; lower alleles may show all transitions from arista to tarsus. A still lower one usually called aristopedia Bridges ($ss^{a\text{-}B}$) shows only an inflated base of the arista of varying length from a tiny segment to about $\frac{1}{3}$ of the length of the shaft. The phenocopy of aristopedia has frequently the phenotype of $ss^{a\text{-}B}$ but when a stronger effect is produced most or all of the shaft is inflated and even the beginning of tarsal segmentation appears, while the tip of the arista still bears a tuft of hairlike branches. Among the hundreds of specimens only a single one was found with one perfect tarsus replacing the arista on one side. As table 2 shows many stocks react upon borate with the aristopedia phenocopy varying from the phenotype of $ss^{a\text{-}B}$ to that of lower grades of ss^a. As a rule the incidence follows that of the eye-effect, but not always. Thus the line Canton-S with extreme eye reaction and also extreme reaction of the antenna (see next paragraph) never produced a single aristopedia. Four stocks were characterized by the production of a very high percentage, some even surpassing the eye effects (Lausanne, Big Ridge, Idaho Falls and Amherst). Of these some showed only the lowest type; the most reactive line was

Amherst with almos all ss[a] phenocopies being bilateral and of the most extreme type encountered in this work, i.e. short of a true tarsus. Thus the incidence or absence, and the grade of the aristopedia phenocopy characterize each stock, as the table shows.

2. *Antennaless and frontless.* The mutant antennaless seems rather variable. In the available stock we observed all degrees of degeneration of one or both antennae up to their complete absence. The phenotype seems rather modifiable as we found in one set of single-pair control bottles exclusively the total absence. In the phenocopic experiments we found all these grades. But the total absence of the antenna was still more extreme than in the mutant stocks. We called it frontless and registered it separately because also the small tissue remnants of the front, still found in the mutant, were absent and the head in front of the eyes was completely missing and the head frequently ended anteriorly with a deep furrow between the eyes. This effect was found as a rule combined with the eyeless effect, but there were also many individual cases of antennaless and frontless with completely normal eyes. Table 2 shows that the phenotype appears in small numbers in different stocks. But as a mass phenocopy it is completely specific for the Canton-S stock, in which each successful experiment produces this phenocopy, sometimes in 100% of the flies.

3. *Double antenna* is known as a pleiotropic mutant character in a number of stocks, but we do not remember a mutant with this character only. In one of our Lobe mutants (that studied by Zimm l.c.), the double antenna was always present with variable penetrance. In the phenocopy experiments individual cases of considerable variation were found in different stocks, specially in the presence of the Lobe phenotype. Usually the effect is asymmetrical but among the many grades and variants also a perfectly symmetrical double antenna on each side was found. It is typical that in the presence of other phenocopies (e.g. aristopedia, double arista) one of the doubled antennae may be aristopedia, one normal

etc. Double antennae were also characteristic for a borate treated aristopedia — Bridges line, where sometimes the two antennae were arranged in tandem. A relatively large number of double antennae was found in the Bikini and Urbana experiments, one accompanying Lobe, the other eyeless phenocopies.

4. Double arista. We are not aware of such a mutant though it probably exists. An occasional individual in the phenocopic experiments may have been overlooked. Only once this type was characteristic for an entire experiment, namely with the Florida-19 stock.

C. Phenocopies of wing mutants.

Abnormal wings of more or less undefined type are rather frequent in the experiments. We did not register them in the table when they did not appear as typical results. Such are poorly expanded or irregularly blistered wings. But quite a number of wing types were produced as typical phenocopies.

1. Arch. The mutant arch has downward arched wings of an opaque texture but in some flies they arch upwards. This type of wing is produced as a typical phenocopy after treatment with arsenate (not to be studied in this paper). In the borate experiments occasionally a single fly with arched wing appears, which is not registered because it is not certain that it is due to the treatment. But there are lines in which the borate treatment results in arched opaque wings of different degree from almost normal to typical strongly arched and opaque wings in almost 100% of the survivors.

The most extreme case is that of Lausanne, where almost all flies were arch, from high expressivity of the trait through all transitions to more or less normal. In Amherst a large number of arch phenocopies were produced, many of them with upturned wings like the mutant Ski. The stock Sevelen showed a high percentage of arch phenotypes. But here the controls contained a small percentage of the same phenotype, which, then was enhanced by the treatment. We shall discuss this problem later.

2. Curved as a mutant has some similarity to arch but the arching of the wing surface is more extreme. In addition many, sometimes all, flies of the mutant curved have somewhat spread wings of a very characteristic look. As phenocopy this type appeared only in the line Formosa, for which it is thus far specific (see numbers in table 1).

3. Beaded is one of a number of mutant types in which the wings are scalloped, and, in some cases, beaded along the anterior margin. Some scalloped mutants like Beaded and Beadex overlap in phenotype, others like cut and Xasta have a very distinctive phenotype, again others like the vestigial alleles overlap in some respects with Beaded, facet and Notch but are distinctive in others. The phenocopies produced with borate are not easy to classify because of considerable variability. In some stocks only the type with a nick in the wing tip is produced, resembling the phenotype of the lower vestigial alleles, also of facet. In others scalloping of the posterior wing edge is always present which is characteristic for the lower grades of the mutant Beaded. Only twice (stocks Formosa and Quicksand) the beading of the anterior edge together with scalloping was typical, resembling the phenotype of medium and high grades of the mutants Beaded and Beadex. We are certain that an elaborate statistical study of these phenocopies would permit to assign the phenotypes to copies of at least three mutants. As this has not been done as yet we describe here all types of scalloped wings under the name of Beaded. Individual scalloped or nicked wing flies are apt to appear everywhere (see former analysis by Goldschmidt, '37). But there are a number of stocks in which a small percentage of "Beaded" phenocopies always occurs. Here belong Quicksand (with up to 16% of all phenocopies), Formosa and Amherst, all with real Beaded types in addition to lower grades of scalloped. It seems that in most of these cases there is also a tendency to produce other wing mutants. Good examples are Quicksand and Formosa, also Samarkand, though here the percentage incidence is small.

4. *Lanceolate* is a very characteristic mutant of the wing shape. Low grades show only an inward curving of the anterior wing edge, in higher grades the wing becomes lancet shaped. Again certain stocks produce this character as a phenocopy in small numbers, usually in the presence of other wing phenocopies. Examples are Samarkand and Formosa. In the latter stock one might call the phenocopy of lanceolate a characteristic reaction as in some brooods up to 10% of all affected flies have the lanceolate type.

5. *Dumpy* is the well known truncated mutant (or polygenic group of mutants). As phenocopy it appeared only in one wing, the other one being either normal or lanceolate. It has been obtained thus far only in small percentages in Samarkand and Formosa, both of which produce also the lanceolate type. (This interrelation is paralleled also by a mutant which we described [Goldschmidt, '45] as having typically one wing truncated, the other pointed and called bran^{dp} or poi : dp.)

6. *Divergent* is a mutant with spread and blistered wings. The phenocopy characterized the line Urbana where it was produced in large numbers.

7. *Puff* being a blister in the 5th posterior cell was only once produced as a phenocopy in large numbers (and in each brood), namely in the stock Sevelen. There are a number of mutants of such a type known which could be used as comparison, the best being puff (puf), which is completely identical with our phenocopy.

8. *Podoptera* a group of multifactorial mutants is a special case to be studied below in detail. The lowest grade of expression is one wing held at a right angle, the highest is replacement of the wing by a leg-like structure. As a phenocopy usually only the lower grades appear but in a few cases the higher ones were found. One type of expression both in mutant and phenocopy is a kind of hemithorax. Small numbers of podoptera are obtained in many stocks (details below). Only one stock has been studied thus far (actually two different lines of Samarkand), in which large numbers of podoptera are produced by borate treatment. Clearly this is an

enhancement of an already present genetic condition as will be discussed in detail. Table 2 shows that the majority of the treated stocks show the podoptera effect, either absent or present in very low numbers in the controls.

D. Phenocopies of the thorax

The podoptera phenotype already involved a change of both, wings and thorax. A mutant and phenocopic effect affecting especially the dorsum of the thorax is Scutenick.

1. Scutenick. This is a mutant which in its lower grades affects the posterior edge of the scutellum, the scutellar bristles, the ocelli and according to description also the eyes. We obtained the type as constant though not frequent phenocopies in the stocks Florida 19 and Quicksand. It is most characteristic for the line Oregon Dempster, in which in some cases more than half of the flies showed the character. In such cases the higher grades appear as well, which seem to be unknown for the mutant Scn. As this phenocopy let to experiments with remarkable results, a special section will be devoted to it below. Another stock with rather high incidence of the phenocopy is Samarkand. It could be added that the spineless mutant stock also reacted to borate treatment with production of this phenocopy.

2. Polychaete. This phenocopy does not copy exactly the mutant polychaetoid but is a combination of polychaetoid, humped, grooved, and cloven characters as far as we can see. We do not know of a single mutant resembling it. It has thus far been specific for the stock Quicksand although it occurs only in small numbers. In good specimens the dorsocentrals are multiplied to form a bundle of bristles and also the surrounding hairs are irregular in arrangement and longer than usual. Simultaneously the anterio-lateral part of the thorax buckles up and the depression between the buckles may become so deep that the thorax is cleft but without any absence of chitin (as in hemithorax types). In addition frequently in the anterio-lateral corner in front of the wings a group of hairs and bristles appear, looking like a duplication

of a part of the thorax. The type deserves a detailed morphological study.

3. Polychaetous. The mutant of this name shows duplications of bristles, especially of the dorsocentrals and scutellars. The phenocopy has most frequently duplicated, even triplicated dorsocentrals, usually on one side. One of the scutellars may be also duplicated. A rare but characteristic phenotype shows three, or even four, tiers of scutellar bristles on one or both sides. Single individuals with bristle duplication are frequently found but were not recorded. A conspicuous reaction of this type was only found in the stock Bikini (requiring a concentration of borate up to 0.1%), a smaller number also in the stock Formosa.

4. The phenotype of hemithorax has not been entered in table 2, though it was found very frequently in the experiments. The reason is that the genetic type described by this name occurs in very different mutants, some of which can be distinguished. Thus the hemithorax condition belonging to the mutant podoptera as one of its variations is characterized by the complete absence of one or both halves of the thorax, including the wings. When this type was encountered in the simultaneous presence of typical podoptera, it was recorded as podoptera. But there are also hemithorax mutants known based upon one recessive locus. One could assume the presence of phenocopies of this mutant, when quite a number of such hemithorax flies are produced in the absence of typical podoptera. In view of the frequent occurrence of podoptera such a situation is hardly expected to be met with. A preponderance of hemithorax in the presence of some podoptera does not suffice to separate the two phenocopies. A kind of hemithorax is also part of the Scutenick syndrome (see below), in this case the wings remain in spite of the total or partial absence of dorsal thorax differentiation. This type was rather frequent in the experiments with Quicksand; it was recorded as Scutenick.

E. Phenocopies of leg abnormalities

Abnormal, crippled, undergrown or missing legs are frequently found in many of the experiments. In the presence of podoptera flies in the culture they are considered a part of the phenotype i.e. expression of podoptera. Otherwise such phenotypes, appearing irregularly as single specimens were not recorded. One stock was met with (Riverside) which contained an incompletely penetrant mutant with crippled legs, which could be isolated. Thus the only real phenocopy of a leg mutant encountered was the phenocopy of dachs legs.

1. Dachs. This was specific for the stock Formosa where it was produced with standard treatment in conspicuous numbers, varying through all grades, just beginning to extreme, of this well known mutant type.

F. Phenocopies of mutants affecting the sex organs

The only typical phenocopy of this kind was male (XY) intersex. Single intersexes (also males with rotated genital armature) were repeatedly found but not counted as phenocopies. Only in the stock Quicksand did this type appear as a true phenocopy. It occurred in 5 out of 9 broods and in one of them almost 20% of all individuals were intersexes. The phenotype varied from abnormal or absent armature to the presence of a genital cone and reduction of the sex comb.

These data show clearly:

1. The susceptibility of different stocks to the standard treatment is typical for each one. The susceptibility becomes visible as sensitivity i.e. degree of lethal action and reactivity i.e. amount of phenocopic effect. The quantitative problem of sensitivity and reactivity will be taken up in a subsequent chapter. Table 2 shows in the last column a general appraisal of susceptibility as explained there.

2. The quality of the reaction i.e. the types of phenocopy produced in each tested stock is characteristic and different from stock to stock.

3. Some phenocopies are produced in a considerable number of stocks tested e.g. eyeless, aristopedia, podoptera;

others are still frequent like Scutenick and Beaded, others are rather rare, and some have, thus far, been found only in a single stock e.g. dachs and curved.

4. Every stock is characterized by the types of phenocopies obtained in all or most individual experiments. Most of them have also one preponderant type and a type which is rare in one stock may be frequent in another. Thus each stock has its typical pattern of quantitative and qualitative response.

5. It is worth mentioning that the most frequent phenocopy, that of eyeless is typical for old laboratory stocks, while the rarer eye-effects are found in less widely distributed stocks (also in mutant stocks not tabulated here). This might be due to chance or not.

2. Genetic variation of sensitivity and reactivity

We mentioned the exact quantitative work of Sang and McDonald with the eyeless phenocopy, also including their short note without details on their finding that different lines reacted differently to the treatment. This was what we also had found independently and which made us embark upon an analysis of the genetic side of the whole problem. As the details in regard to dependence upon concentration or time of action of the borate in a reactive Oregon stock were analyzed completely by the Edinburgh authors, also the relations of degrees of lethality to penetrance and expressivity, we did not repeat the exact quantitative work on these topics; such results as appeared incidentally in our special work, were completely in accord with theirs. We concentrated instead on the genetic differences for the production of the effect, using always the simple feeding technique in a medium with 0.06% borate (as explained above) as the standard for comparison of different stocks, where possible. Our quantitative results thus relate to this basic and identical procedure, and are only supplemented by the use of different concentrations of borate, where indicated e.g. by extreme sensitivity.

In table 2 a column is found with a rough estimate of the quantity of susceptibility to borate treatment as explained in the legend. It indicates how differently the individual stocks react to the borate. Special experiments attempting the analysis of the genetic background of this variation were made with many of the stocks which produce the typical eyeless phenocopy. We summarize first the genetic types encountered:

1. Stocks and lines which gave always high incidence of the eyeless phenotype, the grade of expression usually being proportional to the percentage penetrance i.e. lower grades of eye reduction with lower percentages in incidence and extreme eyeless types with high incidence up to 100%; as well as lethality, proportional to effect, in the majority of cases as Sang and McDonald had stated in detail. But, exceptionally, broods combining extreme effect with little lethality are found; sometimes a low grade of eyelessness was combined with high incidence. Thus sensitivity and reactivity are usually correlated, but not always.

2. Lines which in some individual experiments (one pair broods) produced only a small percentage of individuals with lowest effect, if any, while in other broods a high eyeless effect appeared.

3. Lines which at 0.06% borate showed no effect at all. At higher concentrations, however, eyeless was produced, but, even with near lethal doses, the percentage of eyeless flies remained low.

4. Lines which never exhibited a typical eyeless effect, even with highest, almost lethal doses, while they yielded regularly a different eye effect.

5. Lines which showed only small effects upon the eye while other phenotypes were frequent.

Table 3 shows examples. The first column contains the total number of flies treated for the purposes of the present problem, not including experiments made while studying other problems with the same procedure. The second column states the number of eyeless phenotypes among these. The percentage of the eyeless phenocopies is found to the right.

TABLE 3

Phenocopies in wild-type stocks of different reactivity

NO.	LINE	BORATE %	n	PHENOTYPES							%		S.I.
				Eyeless	Aristopedia	Antennaless	Kidney	Dumpy	Beaded	Lanceolate	Scutenick	Eyeless	Aristopedia	
1	Riverside	.06	1846	2								.1		0
2	Riverside	.07	845	26								3.1		2
3	Riverside	.08	441	30								6.8		12
4	Riverside	.09	233	34	3	2						14.6	1.3	26
5	Ore R. Mohler	.06	280	257	5							93.8	1.8	0
6	Ore Dempster old	.06	723	26	12						236	3.6	1.5	0
7	Ore Dempster old	.07	31	8	4						7	26	1.3	?
8	Ore Dempster new	.04	348	2							6	.6		0
9	Ore Dempster new	.04	200	49	14						54	24.5	7	0
10	Ore Dempster new	.05	23	5							11	21.7		?
11	Samarkand (inbred)	.07	101			3	8	1	1	1	26			37
12	Samarkand (inbred)	.07	290								3			2
13	Samarkand (inbred)	.075	134			2	6	1	3	1	9			17
14	Samarkand	.06	29			1	18			1	8			37
15	Samarkand	.06	780				3				5			0
16	Samarkand	.07	750								2			0
17	Orinda I	.06	2056	1								.05		0
18	Orinda II a	.06	436	106								24.3		0
19	Orinda II b	.06	801	9								1.02		0
20	Florida a	.06	42	20								50		18
21	Florida b	.06	479	1								.2		9
22	Canton S a	.06	115	90		22						85.7		0
23	Canton S b	.06	1486	10								.7		0
24	Canton S c	.08	378	185		28						50		28

In this table also the numbers of other phenocopies have been entered which were only evaluated in table 2. For one of them, aristopedia, also the percentage incidence is added for later use.

Another column contains a sensitivity index (S.I.). It indicates the amount of lethality in the experimental bottles as estimated from the number of survivors in relation to that of the controls. This index permits a comparison of the amount of phenocopic effect (reactivity) with the amount of sensitivity to treatment. Such knowledge is sometimes needed, e.g. in order to show that in a certain case absence of phenocopic action is not due to experimental error. But it should be stated at this point that unfortunately the usual correlation between sensitivity as measured by the amount of lethality and number and grade of the phenocopies i.e. reactivity does not obtain in every case. As a rule the presence of dead larvae and pupae is a good indicator for the amount of phenocopic effect. But in some experiments there is, for unknown reasons, no lethality, but high incidence of phenocopies; in others a high number of phenocopies is combined with generally low expressivity. Such exceptions will be pointed out. They might indicate unknown experimental errors, or, unknown genetic specificities.

The index S.I. is of course useful only when the usual rule of positive correlation between sensitivity and reactivity obtains and it measures the deviation in viability from normal in the treated bottles i.e. the relative sensitivity. Special tests showed that under the standard procedure of our experiments the three day controls contain on the average 40% of all flies and the bottles to which the parents are transferred 60% of all flies, if transfer is made to bottles without borate. (There might be some differences between stocks in the rate of egg-laying etc. Certainly a more exact index could be obtained; but ours suffices for our purposes.) Therefore absence of borate action could be assumed if, in an experiment, the experimental flies are 3/2 as many as the control flies (or more)

and lethality after treatment can be measured by the difference from this expectation. One way of measurement is to subtract from 60% expectation the actual percentage of experimental flies among all flies i.e. $60 - \frac{100\, n^e}{n^e + n^{co}}$ % (where n^e is the number of experimental flies and n^{co} that of the controls). If the value is 0 or less no lethal effect was found and a positive value measures the intensity of destructive effect, + 60 being total lethality.

We see at once that an Oregon stock (Oregon R Mohler) no. 5 is highly susceptible to phenocopic action (94%) though this is one of the cases where lethality is absent (high reactivity without sensitivity). It might be noted that Sang and McDonald and Gersh also worked with Oregon stock.

The line called Oregon-R-C Dempster reacts differently. Through long inbreeding this stock has become very weak and even the controls are very small. We shall see below that this stock has a specific phenocopic response apart from eyeless (namely Scutenick). When our first experiments were made many years ago this stock could stand the standard treatment of 0.06 borate and gave hardly any eyeless effect (no. 6) which was increased at the almost lethal concentration of 0.07% to 26%. When the work was resumed with the still more inbred stock all experiments with 0.06% resulted only in dead larvae and pupae, while a concentration of 0.04 acted as before 0.07 had done. The now almost lethal concentration of 0.05 acted as 0.07 did before (no. 7, 10), (no. 8 is one of the experiments which for unknown reasons falls out of line). At the other end of the series stands the Riverside stock. It is still practically normal at 0.06% borate (no. 1) and remains so almost up to 0.08% which kills most other stocks (no. 2, 3). Even at 0.09% (no. 4) which is an unusually high concentration the eyeless effect reaches only 14.6% with high mortality (S.I. = 26). The constancy of this result is best illustrated by a new check made many months later with flies from the stock kept in mass culture without selection or

inbreeding. Among 764 flies only 7 were eyeless of the lowest hardly discernible grade.

Orinda I (no. 17) is just as refractive. (Here a repeat test was made after mass breeding for a long time. Among 1745 flies only 2 showed a trace of eyeless effect.)

Completely different is the behavior of our Samarkand stocks (no. 11–16, table 3). We tested two stocks: one had been kept by ordinary laboratory stock mass breeding after an earlier history of inbreeding, the other one was inbred by one pair brother-sister matings for 250 generations. The sensitivity to treatment was variable as the survival index shows. But even with the highest concentration of borate tolerated no eyeless types appeared. Other phenocopies were produced instead (see table and below) and among them an eye-effect which resembles the kidney-like mutants, namely a crater shaped nick at the anterior-ventral edge of the eye circumference, inside which a tuft of hairs may be found on a more or less high excrescence. In two of the 6 groups no eye effect was found, in the others it varied, being 0.4, 4.5, 8.0 and in one case of high sensitivity (only 29 survivors) 62%.

A number of other stocks behaved more irregularly. Thus Orinda II was very insensitive and unreactive in some experiments (table 3, no. 19, vs. 18). In other cases with equally absent lethality (column S.I.) a considerable eyeless effect was found but never a very extreme one (no. 18). No. 18 and 19 separate clearly bimodal groups within all experiments. A new test was made also with this stock at a later time which may have been more efficient because of intervening unintentional selection (see below description for Canton) and yielded 28% eyeless flies. A comparable behavior was found for the Florida line (no. 20, 21) with a majority of experiments yielding hardly any eyeless flies but with one brood of 50% eyeless. In view of these results obtained from unselected stocks it is very improbable that the small unavoidable variants of procedure i.e. purely environmental factors are responsible for the variation. It is more probable that we are

dealing here with stocks in which genetic factors for more or less sensitivity are segregating in some irregular way.

We have tried to learn more about this point by a closer study of one stock with a strangely varying type of sensitivity, Canton-S, bred for a long time as a mass culture. With the standard treatment the majority of the early experiments yielded hardly any eyeless flies (table 3, no. 23); but a few individual broods gave near to 100% eyeless phenotypes without any conspicuous lethality (no. 22). Treatment with the highest tolerated concentration (0.08) gave only 50% eyeless in spite of high lethality (S.I. = 28). The predominance of the two extremes: no or almost no effect, or, almost 100% effect comes out best if we plot the variation of all experiments made with the Canton stock (whatever the individual procedure to be detailed at once). It turns out that out of 64 individual one pair experiments 20 yielded no eyeless phenotypes or only a few, 20 more contained 80–100% eyeless types and only 24 fall in between with peaks around 25 and 50%, which latter peaks may or may not have a meaning. These preliminary facts suggest the presence in the Canton-S stock of a simple genetic condition for an all or none response and an environmental factor and (or) segregating modifiers, interfering with the alternative. We shall return to this test, applying it to another set of experiments.

It was tried to find the genetic basis of reactivity by a set of selection experiments, all environmental conditions being as uniform as possible. These were started with 9 pairs from the stock: 5 of them produced only normal flies without any lethality (up to 348 flies in one brood not counting the controls!); 3 bottles contained a small percentage of eyeless phenotypes (0.2, 10, 15% respectively). One bottle had high lethality and 100% eyeless phenotypes. From this sensitive and reactive culture 4 pairs were selected for the same standard treatment. They produced 100%, 100%, 100% and 87% eyeless phenotypes among 129 offspring (high lethality). This looked very encouraging and in favor of a simple genetic background. But the offspring of the sister broods which had

been normal or contained only a few eyeless types did not agree with this simple assumption. From one of the completely normal broods, actually the one with 348 normal flies 7 pairs were subjected to the standard treatment. All of them showed a rather high eyeless effect fluctuating around 50%, namely 33, 40, 53, 54, 56, 64, 68% with corresponding degrees of lethality. From one of the parental broods which had reacted most strongly (15% eyeless) offspring was raised both from normal and eyeless phenotypes. From the normal parents 26.5 and 30% eyeless were obtained respectively. Eight pairs of low eyeless phenotypes produced after standard treatment 1.4%, 17.1%, 22.2%, 26.5%, 30%, 86%, 100%, 100% eyeless offspring i.e. 3 out of 8 gave the two extreme reactions and 5 about the same results as the offspring of normals. It seems obvious that these are again not chance results; but it is impossible to draw simple conclusions upon genetic or environmental factors involved. There seemed to be a certain amount of dominance of low reactivity. But also additional factors inherent in the procedure of experimentation could be involved. Thus we tried first to isolate such features before continuing with the study of the genetic basis.

A number of occasional observations suggested two phenomena which might obscure the selection results. The first is the possibility that the age of the mother influences the sensitivity of the eggs. The second is the observation that frequently later counts of the same bottle contained a higher proportion of, or exclusively normal flies, even when the first counts showed a very high percentage (up to 100%) of eyeless phenotypes. This might be based upon the age of the mother or upon the progressive loss of concentration of the salt eaten by the larvae, because the yeast takes up the salt but continues to divide and thus dilutes it (as Sang and McDonald had found). Thus an experiment was made for testing both of these sources of error in different lines.

In the standard experiment the parents are 4 days old when put on the borate medium (3 days laying as controls plus one day in the vial). In the present check the parents

were put directly into the experimental bottles (after the usual 24 hours in the mating vial). Afterwards these parents were transferred six times at 2 days intervals to fresh borate bottles, so that one may assume that the larvae were always exposed to the same borate concentration. The first counts were always made on the 10th day and no flies hatched as a rule after 2–3 days, meaning that the developmental time was rather constant in all cases. To introduce the genetic angle 5 different stocks were used, reactive lines isolated from the stocks Canton and Oregon Mohler, the resistant stocks Riverside and Orinda I and the variable stock Orinda II. The results are shown in table 4. We see first (last column) that the reactivity to treatment is the same as known from former experiments; i.e. Orinda I and Riverside show practically no effect of the treatment; Oregon Mohler and Canton-S a high reaction. Next we see a considerable tendency in the highly reactive stocks to produce no or only a few flies in the first bottles though dead larvae and pupae were abundant. This means, that the eggs from young mothers are much more sensitive to the treatment, complete lethality being the highest degree of sensitivity. But there is considerable variation. Two of the 5 Canton broods have a large number of flies in the first counts though the overall eye-effect is rather high. On the other end of the sensitivity scale two of the Riverside bottles have no flies in the first counts, and most of them rather few in the second counts (no flies meaning always dead pupae present). Thus there is some relation between lethality and age of mothers, though not a very clear one, and one which hardly has a great influence on over-all results.

One observation was made in these and other experiments; we meet sometimes with lines in which in the absence of action or with a low action of the borate an immense viability of the offspring is observed. The normal expectation for untreated or resistant flies is a progeny of about 200–300 flies per bottle in our setup. Numbers of up to over 700 (in Orinda I) are clearly an enhancement of viability. We shall meet with this phenomenon again and draw only attention to the

TABLE 4

An experiment on the influence of the age of the mother and of unlimited supply of borate upon the eyeless effect of stocks of different reactivity. Parents transferred to new borate food every second day

NO.	LINE	TRANSFERS 1		2		3		4		5		6		Total		
		n	% ey	n	% ey	n	% ey	n	% ey	n	% ey	n	% ey	n	% ey	
2711	Canton S			1	100	63	94	33	91			33	30	130	87	
2712	Canton S	2	100	38	34	35	71							75	53	
2714	Canton S					21	100	1	100	4	100	9	89	35	97	
2736	Canton S	59	60	59	93	9	100	64	97	16	75	2	50	209	83	
2737	Canton S	101	56	92	95	12	92	95	85	2	0			302	78	
2715	Oregon Mohler							17	100	32	20	25	60	74	51	
2716	Oregon Mohler			1	0	18	71	2	100					21	70	
2717	Oregon Mohler			1	100	22	100							23	100	
2718	Oregon Mohler			29	60	175	36	1	100	7	14			212	40	
2746	Orinda II	29	0	67	9	29	31	19	37	72	3	40	3	256	15	
2747	Orinda II	7	0	25	8	26	15	31	0	29	24	25	0	143	14	
2749	Orinda II	50	72	110	63	56	30	8	100	18	50	84	13	326	44	
2719	Riverside	2	50	1	0	123	1	37	5	31	3	200	1	394	.5	ey actually kidney
2720	Riverside	1	0	37	0	52	0			20	0	86	1	196	.5	ey actually kidney
2721	Riverside			3	0	48	0							51	0	ey actually kidney
2722	Riverside			11	0	94	0	17	0	1	0			123	0	ey actually kidney
2744	Orinda I	32	0	95	0	61	0	55	0	27	0			270	0	
2745	Orinda I	105	0	175	0	174	0	154	0	71	0	61	0	740	0	
2743	Orinda I	95	1	182	0	171	1	192	1	31	0			671	.4	

well known fact, that different chemicals e.g. growth substances may act as strong inhibitors in a high concentration but as enhancers in a low concentration.

It might be possible that not only the eggs of young flies are more sensitive but that the sensitivity of the eggs decreases gradually with the age of the mother. In this case a decrease of eyeless phenotypes should be observed in table 4 with increase of the number of transfers. There is a little indication of this in a few of the Canton bottles and also in Orinda II. But it is not conspicuous enough as to mean much. Therefore we have good reason to assume that the increase of normals in the later broods with standard treatment, even when the first broods were 100% eyeless, is due to the yeast phenomenon described by Sang and McDonald.

This last point, the increase of normals in later counts of reactive stocks under standard treatment, is more significant for the outcome of selection experiments. In order to realize this we must introduce now the expressivity of the effect, namely the grades from a little nick or bend in the circumference of one eye to complete eyelessness. It turned out that as a rule this expressivity is correlated to the percentage of the effect i.e. reactivity. Usually a low expression is combined with low reactivity, and high phenocopic effect (in terms of percentage of phenocopies produced) is combined with high expressivity. The latter is variable in case of relatively low lethality, and is more uniform when lethality is high. When a large number of experimental bottles of Canton-S were checked in daily counts from the 12th to the 17th day and the grades of expressivity were noted a series of different types could be recognized which are represented in table 5 by six examples selected from hundreds of similar ones and illustrated in the diagram figure 1.

In the diagram the variations of phenotype from normal to extremely eyeless are marked on the abscissa and the limit between normal and the first trace of eyeless is marked by a vertical line. It must be added that in all cases in which the grade first trace of eyeless is recorded, the grading is reliable.

TABLE 5

Examples of different reactions to borate in Canton-S selected for high reaction. Parents taken from a brood with 100% eyeless phenocopies and put into borate food without previous controls. Counts 12th – 17th day

NO.	1ST COUNT		2ND		3RD		4TH		5TH		6TH		n	IN DIAGRAM 1	% ey
	+	ey	+	ey	+	ey	+	ey	+	ey	+	ey			
														curve	
3042	—	5	—	15	—	12	—	11	—	11	—	33	87	I	100
3041	4(?)	5	—	26	4(?)	42	21	36	37	10	64	6	255	II	49
3045	3	38	2	47	26	32	36	13	39	1	51	2	290	III	46
3033	2	80	2	70	49	11	35	—	24	—	—	—	273	IV	59
3035	78	12	45	2	41	—	15	—	31	—	—	—	224	V	35
3044	24	19	44	5	31	1	33	—	28	—	40	—	225	VI	11

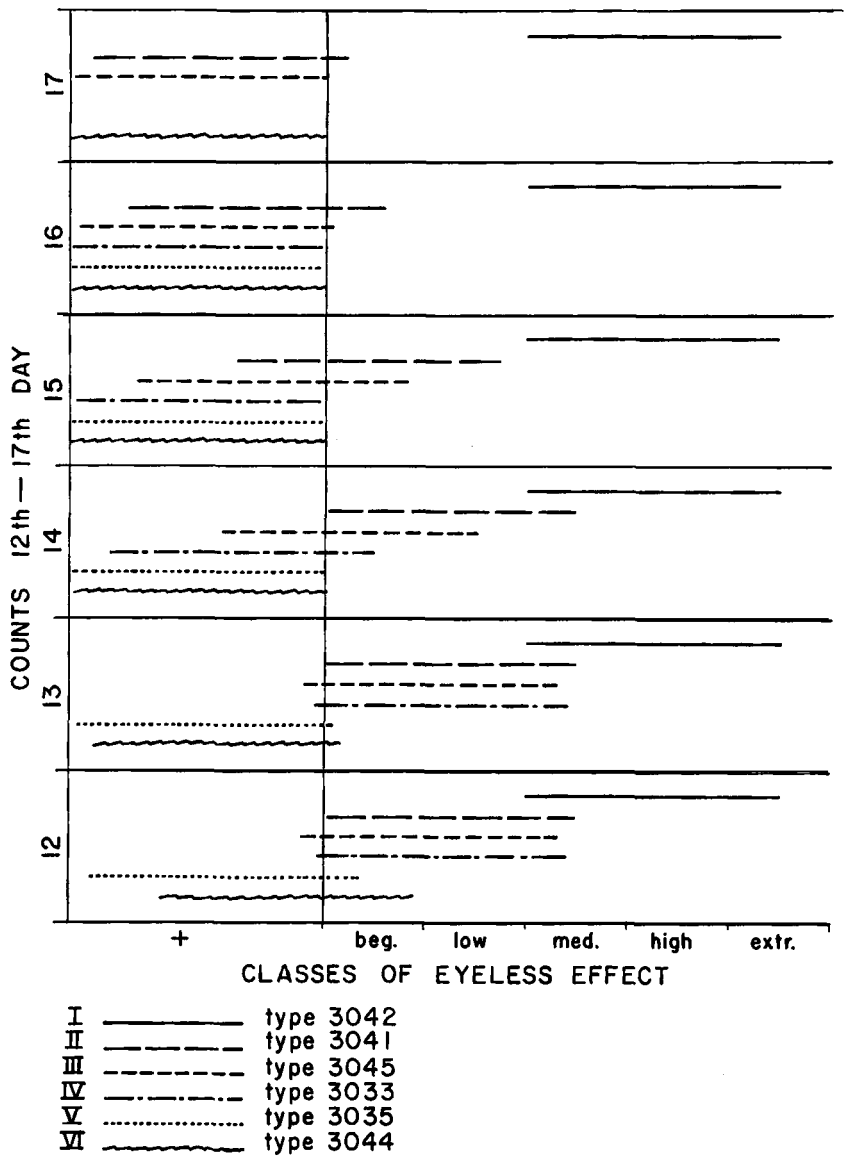

Fig. 1 Graphical representation of the 6 types of eyeless effect as recorded in table 5.

But when a few normal individuals are registered among many eyeless ones as in the 2nd to 5th example represented in table 5 the possibility cannot be excluded that the individuals were not really normal. A slight depression of the edge of the eye merges so imperceptibly into a normal contour that a decision is sometimes difficult to make and lies, therefore, within the limits of the personal error. On the ordinate of the diagram are marked the six successive counts. Each of the six types of broods selected for table 5 is represented by a line of equal length which measures 100% of the individuals. The location on the abscissae indicates the variation of the phenotype as observed. Thus some of the curves are located with 100% within the eyeless phenotype or the normal phenotype, while others indicate different percentages of normal and eyeless types as seen by the stretching across the dividing vertical line. The diagram and the actual data in table 5 can be easily cross-checked and similar data could be extracted from the hundreds of cultures.

We see at once that in four of the six selected types the first count contains 100% or almost 100% of eyeless phenotypes. But only in one, no. I, the extreme phenotype is represented, the one without shift into normal in all counts. In type II the first three counts contain only eyeless phenotypes but of a low to medium grade and even a few doubtful ones. In the 4th count a minority of normals appear; this increases to a majority in the fifth count and in the sixth only a few eyeless phenotypes are left. In the following types III and IV a few normals appear already in the first two counts and their numbers increase more or less in the third count. The increase continues through 4th and 5th count in type III, 100% normals appearing only in the sixth count; in type IV this condition is already reached in the 4th count. The fifth type has only a few eyeless phenotypes in the first count as well as in the second, afterwards all flies are normal. The sixth type is similar with more eyeless in the first count. In all cases the expression of eyeless is correlated with its overall percentage.

In interpreting these results it should be stated first that all these broods are derived from parents belonging to an experiment in which a selection for 100% reactivity (type I) seemed to have succeeded. Actually 16 pairs of this origin (sisters and brothers from the selected brood) were tested. Of these 6 gave again offspring of type I, 2 of type II, 4 of type III, 1 type IV, 1 type V, 2 type VI. An interpretation must take into account (1) that in most cases the first two counts showed only (or almost only) eyeless phenocopies; (2) that only such broods which from the beginning contained the extreme grades of effect remained throughout the counts 100% affected; (3) that all those which lost the effect in time started already with lower grades of effect; (4) that the loss of the effect in time, more or less slowly in the different types, is correlated with the grade of expression. These facts require a set of collaborating conditions. We assume that the selection of the parents for maximum reactivity to treatment was successful and that the line is genetically homozygous for, possibly, a one locus difference, separating reactivity from non-reactivity. The next factor at work is the one causing continuous decrease of the effect with the age of the bottle. We assume on the basis of Sang and McDonald's results that this is generally due to the progressive dilution of the borate by action of the yeast, as mentioned before. But this does not suffice, as there is no reason to expect the different types described on this basis alone. Neither the different initial effect nor the differences in fading out in time nor the correlation of reactivity with expressivity can be understood in their regularity as caused by direct environmental action alone. The facts seem to require the presence of segregating modifiers for all primary reactivity. Type VI should appear (apart from the yeast action) if minus modifiers are accumulated, type I as a result of accumulation of plus modifiers. Consequently it should be possible to select for these types and selection for extreme eyeless effect should therefore be also a selection for plus modifiers and in selected material the types II to VI should finally disappear, if caused exclu-

sively by genetic modification. But it is possible, even probable (see the yeast effect) that in a homozygous highly sensitive line environmental conditions, i.e. effects of the general experimental procedure like the yeast action or other unknown environmental variants could produce some normal flies and, therefore, the types II and III might still be found after successful selection. For example if lethality is high and therefore growth of yeast enhanced we should expect such a result. All this shows that in a variable line like Canton-S overall results obtained by pooling all counts give wrong information by obscuring the fact that primary reactivity or absence of it is controlled by a simple, possibly unifactorial, difference. In the present work we are interested only in demonstrating that the phenocopic effect requires definite genetic conditions. This is true also for variable lines as the foregoing data suggest whatever the details of more complicated additional genetic and environmental actions may be. Now we can consider the details of selection for reactivity.

Selections for high phenotypic effect — with a few counter-selections — were made starting with the 16 bottles of offspring from 100% sensitive parents, from which the types of table 5 were derived. The highest grade eyeless phenotypes and also + phenotypes, were selected for breeding the next generation, which was exposed to borate treatment like the parents. The only difference from the former series was that counts for ascertaining the type of reaction were made in two day intervals only i.e. 3 counts.

The diagram figure 2 shows the results. The parental generation was, as said before, one bottle of type I effect obtained among a series of trials with unselected stock. From this bottle 16 pairs were treated in the usual way. Only 6 showed full effect of selection i.e. type I, the others exhibited types II to VI as indicated. For the next generation selections were made from all types. From type I to III only eyeless phenotypes were selected, from types IV and V both normal and eyeless phenotypes and from type VI only normals. In the selections from type IV and V it did not make much

difference whether the parents were normal or eyeless. Actually from type IV eyeless were obtained, 2 I, 1 III, 1 VI and from normals 1 I, 2 V, 1 VII (VII meaning only a few eyeless flies); from type V eyeless derived 2 VII, 2 VIII (= all normals) and from type V normals 3 I, 1 VI, 1 VII. The normal parents of type VI also produced types I to VII in the offspring. Thus the phenotype of the selected parents is unimportant, what counts is the ancestry. This F_2 shows that type I is obtained from all parental types. But from

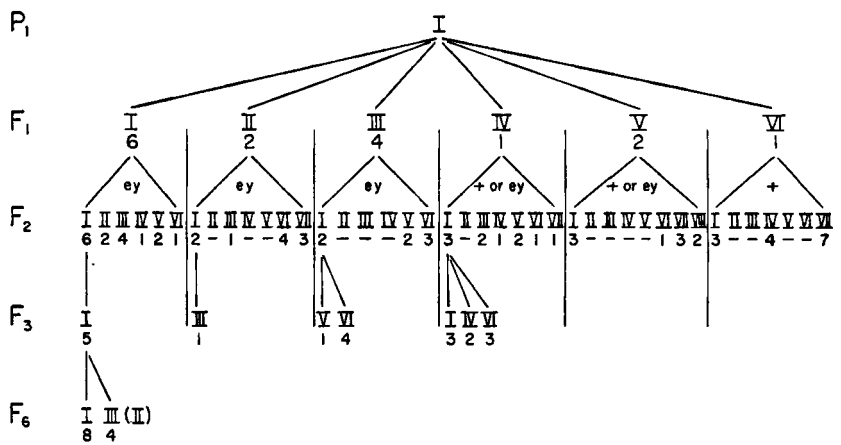

Fig. 2 Selection for reactivity to ey-phenocopy in Canton-S. Roman numerals = types as in table 5 and figure 1; arabic numerals = number of broods. I in P derived from unselected stock.

parental type I the majority of broods is found in the 3 lowest types, while the offspring from the higher types is predominantly of the higher types. This suggests, as we concluded before, that the entire line contains a rather simple, perhaps monofactorial condition for high reactivity to treatment and in addition segregating modifiers which lower the reactivity. (Unknown environmental factors — one might think of food selection by larvae — would probably work in the same direction). In the next generation only selection for high reactivity i.e. type I was made in the first 4 of the F_2 groups. In the first group, the one which had complete ancestry of type I,

only type I resulted; in the other selections types I–VI appeared. From one of the successful selections of F_3 a mass culture was made and kept as a standard stock, supposed to be homozygous for the main factor or factors. From this stock 12 pairs were selected after 2–3 generations of mass breeding. Of these, 8 which were treated simultaneously gave all type I, 4 more were treated subsequently and behaved like type II–III. In these latter ones possibly some modifiers still segregated. But it is also possible, even probable, that by chance more yeast was present which diminished the borate concentration. The difference between the two batches made at different times suggests such an explanation or another one entailing unknown differences in experimental procedure. Altogether the data bear out the interpretation, though there are certainly some misgivings; as we were not interested in selection experiments per se but only in proving the hereditary status of reactivity to phenocopic action, the experiments were not continued.

Another way of appraising the data on selection may be used. In the types I–VI which we described, to which type VII, meaning only a few eyeless flies, should be added, the percentage of eyeless flies in the sum total of each type follows roughly the order of sequence of the types I to VII i.e. with gradual decrease (see table 5 and the diagram 1). Thus we may take the eyeless percentage as an approximate measure of the effect, with small deviations due to the environmental effects (e.g. yeast). Thus it is expected that in the selection experiments in toto the positive selection effect should appear as a crowding of the high eyeless percentages in the counts of all broods, a relatively even distribution of intermediate effects due to the segregation of the modifiers and some crowding towards the low effects due to the group of counter selections made for low effect modifiers. In figure 3 a histogram for the percentages of eyeless phenotypes in all these selections is presented which fairly agrees with expectation. (A very similar distribution of reactivities in a preliminary

analysis (see p. 151) was reported above and used for identical conclusions.)

3. Phenocopy or premutation

Before continuing with the description of the experiments a short theoretical discussion is required in order to make the following facts more meaningful. The facts presented and still to be presented in this paper raise the question

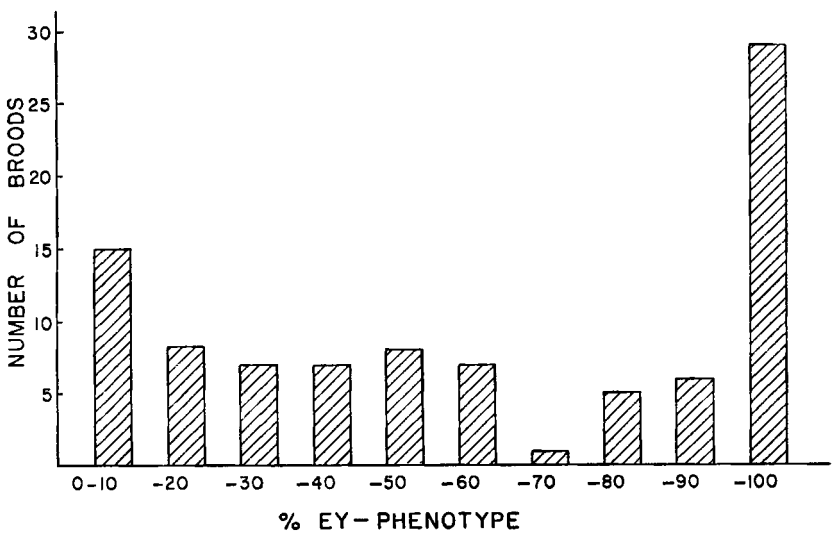

Fig. 3 Histogram of eyeless phenocopies in per cents, in 4 generations of selection from broods assumed to be homozygous for the main factor.

whether the morphological changes produced by borate treatment are phenocopies in the original sense of the term i.e. modifications of development by interference with specific processes of growth. The identity of the phenotypes with known mutants has in this case to be explained in terms of development. The additional feature that the genetic constitution of the material influences the phenocopic effect quantitatively and qualitatively could be explained by the well known fact that the phenotype of any hereditary trait is also dependent upon what has been called the internal environment,

meaning the sum total of all other genetic actions. Seen from the side of the genic action of a definite locus, all the other genic actions present simultaneously may be called the internal environment or the specific modifier system which interferes, as the case may be, with the genic action under scrutiny. It may be assumed safely that the different wild-type stocks contain different modifier systems. Just as these would affect differently a given genic action, they would also modify differently the parallel phenocopic action of the poison. Our result, the dependence of quality and quantity of phenocopy effect upon the genetic line or stock used, is thus to be regarded as the expected consequence of the ever present small genetic differences between different wild-type (or other) stocks, which provide in each case a somewhat different developmental system for the reaction to the treatment.

This first possibility has been expressed recently by Landauer thus (when discussing the fact that in his chickens the tendency to sporadic [non hereditary] appearance of the phenotype of a mutant and phenocopy, facilitates the production of that phenocopy): "It is well recognized that the development of every part and organ, the maintenance of all vital functions is controlled, if often in a roundabout fashion, by multiple genes, thereby providing dynamically equilibrated safeguards. The occurrence of several independent mutations with similar phenotypic effects is presumably evidence for the conclusion that the corresponding normal sequence of developmental events is in precarious equilibrium, and this may well be true for more than one link of the chain of developmental events. In the same sense our evidence leads us to conclude that sporadic defects as well as experimental phenocopies are the results of events through which ordinarily hidden weaknesses of developmental equilibria become manifest and that these weaknesses have a definite, if complex, genetic basis."

But a number of facts to be studied below suggest another alternative which would require a change in the definition of

phenocopy. This is the possibility, that the different effects of treatment upon different genetic lines are due primarily i.e. apart from the differences in the modifier system, to the presence of sub-threshold alleles (we prefer this term to isoalleles) of the mutants which are phenocopied; and that their action is raised above the level of visible effect by the treatment. Phenocopy, in this case, would not be a modification of development in the complete absence of the copied mutant (forgetting about the always present action of the internal environment which affects the reacting system); but phenocopy would rather mean a bringing to light (phenotypic visibility) of the action of an otherwise sub-threshold mutant already present in the experimental material. It is known that all unselected populations contain any number of hidden heterozygous mutants. We know also that "isoalleles" i.e. subthreshold mutants are found when looked for (e.g. at the vestigial and cubitus interruptus loci). Thus the idea that subthreshold mutants are widespread in populations is not objectionable. Actually Lerner ('54) has suggested this and assigned a major role in selection to this phenomenon. Therefore we must keep this alternative in mind when studying the genetic aspect of qualitative and quantitative suceptibility to phenocopic treatment.

There is a third possibility. As many phenotypes are known which are produced more or less identically by very different mutant loci e.g. wing scalloping or eye defects, it is imaginable that all these loci or some of them are present as subthreshold mutants in the wild-type stocks and, as such, act as genetic modifiers for the frequency and quality of a phenocopic effect. In this case the two alternatives just discussed would become more or less one and the same thing. Landauer (l.c.) had perhaps a similar idea in mind, though he expressed it in developmental rather than in genetical terms, when he wrote: "It is presumably no coincidence that the kinds of phenocopy effect, which can be most readily obtained, and often by a multiplicity of means, are variants which also occur as a consequence of more than one gene substitution."

As examples he mentions rumplessness and micromelia in fowl. For Drosophila we could point to our former examples of eye reduction and wing scalloping. But a very frequent phenocopy after borate treatment is aristopedia, though this mutant effect is known only for one locus; a fact which cautions against generalizations derived from the study of eye defects. But even in this case one might point to the existence of the very variable antennipedia mutants (an entire leg for an antenna) which have not yet been studied sufficiently and which might be considered, in the same way as our examples of different mutants for scalloping and eye defects, as parts of a group of antennal transformations which include also aristopedia.

4. Data derived from experiments with heterozygotes

The most conspicuous and reliable phenocopic effect after borate treatment is the phenocopy of eyeless. As the phenotype of the phenocopy is completely identical with that of our eyeless^2 stock, this phenocopic effect should be very useful for testing the different interpretations discussed in the last section.

A. The heterozygotes with ey^2

Sang and McDonald had already described that heterozygotes for the mutant eyeless (ey^2) if treated with borate show a high production of the eyeless phenotype. This may be described as a change of dominance. But as the normal homozygotes +/+ already produce the eyeless effect after borate treatment, dominance of ey^2 hardly enters the problem. What has to be explained is rather the action of the plus allele of eyeless under the influence of the borate and in the presence of a single eyeless allele, an action simulating that of an eyeless allele with the result of a compound like effect. If the phenocopic effect of the normal strain were actually based upon a subthreshold eyeless allele (which is the one alternative which we are testing) the

heterozygotes ey/+ would be a genuine compound with an intermediate genetic tendency for eyelessness i.e. ey^2/iso-ey. If this were the case it would be expected that the hybrids with a highly borate-reactive normal strain would produce the greatest effect after borate treatment and those with the non-reactive strains the lowest. Even the untreated F_1 (the controls) might show some amount of dominance when the highly reactive strains are used in the crosses with ey^2, assuming that this amounts to making a compound with a high subthreshold allele. On the other hand, if no eyeless subthreshold alleles were involved but only some general reactivity to boron, not connected with the ey-locus itself, it is improbable that the reactivity of the heterozygote would follow the order of reactivity of the stocks introduced into the heterozygote. Only in case of complete dominance of all modifiers acting as determiners such a parallelism could occur also without isoalleles. Such dominance is not borne out by the experiments with Canton-S reported above.

It should first be stated that our ey^2 stock has 100% penetrance and a very high expressivity (as opposed to Sang and McDonald's strain). If we divide the expression into the classes: just beginning (only rough eyes or a small dent or wrinkle in one eye), low (small effect on both eyes), medium (eyes about half size with varying types of indentations) high (only small rudiments) and extreme (no eyes), this stock varies from medium to extreme grade with a majority tending to extreme expression. The borate treatment of ey^2/ey^2 does not affect the eyes, while other effects as upon the antennae, are produced (see table 6 no. 1). The most conspicuous effect of the borate is that in many individuals, especially those of the highest grades, the group of bristles and vibrissae between eye and proboscis unites into a brushlike structure, sometimes with an elevated base. Seen from above this looks like a mustache, as it is called in the table, which shows also the absence of this phenotype in any but the treated ey^2/ey^2 stock.

TABLE 6

Crosses of wild-type stocks × ey², controls and standard borate treatment in order of reactivity of wild-type stocks. Only eyeless and aristopedia phenocopies tabulated

NO.	CROSS	EXPERIMENT			CONTROL		NOTES
		n	% ey	% ss[a]	n	% domin.	
1	ey² stock	576	100	1.5			Many mustache
2	Orinda I × ey²	1552	12	.06	1671	0.0	Reciprocal crosses included
3	Riverside × ey²	1467	21	2	993	0.2	Reciprocal crosses included
4	Samarkand × ey²	965	36	.3	1343	0.2	Reciprocal crosses included; eyeless or kidney?
5	Canton low × ey²	1275	17	0	975	6.5	
6	Orinda II × ey²	982	47	0	1250	1.3	
7	Canton high × ey²	614	73	0	554	1.3	
8	4 k iso Oregon [1] × ey²	149	97	0	806	4.0	Many ± headless
9	Oregon Mohler × ey²	827	23	2.3	352	0	Many ± headless

[1] = 1, 2, 3 Canton 4 Oregon.

The column Control in table 6 contains the information on dominance of eyelessness in the untreated F_1, calculated as the percentage of flies showing some eyeless effect. It is usually of the lowest grade with a small dent in one eye but is occasionally also of the class called above low up to almost medium. The results can be visualized in the graph figure 4. On the abscissa the crosses are arranged in the order of the reactivity of the wild type stocks to borate treatment. As

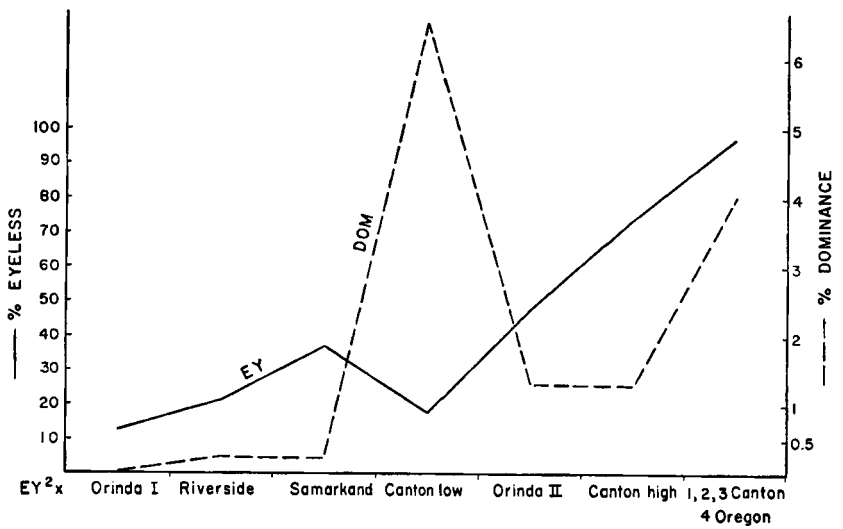

Fig. 4 Phenocopies of eyeless in $ey^2/+$ in ascending order of reactivity of the wild-type lines crossed with ey^2 and treated with borate. Broken curve gives amount of dominance in per cents of all flies (ordinate on the right side).

tabulated above, Orinda I and Riverside are unreactive. We have entered here also the crosses with Samarkand, though the phenocopic eye effect is of the kidney type. Under the assumption of subthreshold mutants of ey in compounds with ey^2, this heterozygote would be really ey/+ and should show no dominance if ey is completely recessive, a point which cannot be decided, obviously. The next hybrid is the one with Canton-S of low effect. But this is not a selected low line. Therefore, all individual crosses were separated into groups with low and high reactivity, assuming that the two

distinct groups are due to the presence in the unselected Canton stock of more and of less reactive types. Then follows the intermediate stock Orinda II and the separated Canton line of high reactivity and finally one special stock, not mentioned before, made up by a combination of chromosomes 1, 2, 3 from Canton with the fourth from Oregon. This line had always shown extreme reactivity.

It is obvious that the percentage of eyeless phenocopies in the heterozygote is fairly proportional to the known reactivity of the parental wild type line.

In the same graph the amount of dominance in the controls is shown in the broken curve, according to the scale marked on the right ordinate. The curve shows a good correlation to the curve for phenocopies for all crosses with nonreactive lines (Orinda I, Riverside, Samarkand) and a good correlation also for the heterozygotes with sensitive lines (Orinda II, Canton high and 1, 2, 3 Canton, 4, Oregon). The last combination, with about 20 times more dominance than the insensitive ones show, is the one which in the phenocopic experiments produced the most remarkable reaction, a phenotype going beyond that of the eyeless stock; most flies belonged to the most exreme eyeless type and a considerable number had in addition the entire head reduced to a rudiment. In between the low and high dominance groups is located the cross with the Canton line of low reactivity, which is completely out of line with over 6% dominance in the controls. We have no explanation to offer except to point out the complicated modifier systems discussed above for the Canton stock. Thus we may say that table 6 and graph 4 show altogether a good correlation between reactivity of the wild type stocks, percentage of eyeless phenocopies in the heterozygote with ey^2 and dominance in the untreated heterozygote. This would be the expected result if the grade of reactivity as well as the eyeless phenocopy as such were based upon the presence of different subthreshold mutants at the ey-locus. But it cannot be denied that this conclusion is not yet proven beyond doubt, and we shall try to find further ways of attack.

In view of these results it was very disconcerting to find that the highly reactive line Oregon-Mohler, crossed to eyeless^2 gave no dominance effect in F_1 (see no. 9 in table 6). (Actually the set of experiments not recorded in table 9 had 2.2% dominance in the controls; this was not recorded because the two controls with one ey fly each belonged to experiments with complete lethality.) This disconcerting result was, however, accompanied by other features, which set this Oregon line apart and which will have to be analyzed further. One such feature is the unusual sensitivity in some bottles: in one set 3 of 6, in another 5 of 6 bottles were without flies (though plenty of dead pupae were present). In spite of complete lethality the fertility was high. Another fact is that in reciprocal crosses with eyeless one gave 94% ey-flies, the other only 17%. We lumped these crosses because in all other examples of reciprocal crosses differences went in one or the other direction, without any rule. A third fact was that one of the crosses showed a considerable heterosis with over 400 control flies. A repetition of the cross (without treatment) produced an extreme heterosis: Already the first two counts contained 200 flies per bottle with enough pupae left for 400 more. The flies were so vivacious that it was most difficult to shake them out of the bottle and they were very resistant to etherization (we found other cases of such heterosis, e.g. in Orinda I $\times$ Orinda II). In view of these special features it is possible that the falling out of line of the ey^2 $\times$ Oregon-Mohler cross has special reasons, which we shall try to find.

B. The eyeless-deficiency tests

A recessive mutant opposite a deficiency shows the haploid effect, i.e. the phenotype of the recessive, sometimes with the exaggeration phenomenon, and the mutant ey falls in line, as we convinced ourselves. We received from Professor C. Stern a stock (M-4[ci]-24) with an eyeless deficiency which acts like a Minute if heterozygous and is thus easily classified. When put opposite ey^2 or ey^R the exaggeration effect is

observed (with ey^R a few survivors eyes were exaggerated, with ey^2 the ey/Df flies did not hatch, but could be checked by dissection of pupae). We cannot predict with certainty how a subthreshold mutant opposite a deficiency would act. The possibility exists that the exaggeration phenomenon would lift the haploid action above the threshold for the eyeless phenotype. For our purpose, the testing for presence of different subthreshold mutants in different lines, only a positive result would be significant and in order to be convincing it would in addition require that the nonreactive lines and the reactive ones would behave in the compounds in an

TABLE 7

Eyeless effects in the hybrid M (4) ci-24/+ =Df (4) ey/wild type, slight effect means small irregularities which are difficult to describe. The percentages are calculated for both a + b and a + b + c columns

NO.	ONE CHROMOSOME 4 FROM	MINUTES	WITH EYE EFFECT LIKE			% ey + k	% all 3
			(a) ey	(b) k	(c) slight		
1	Orinda I	437	6	2	..	1.8	..
2	Riverside	809	4	..	..	.5	..
3	Samarkand	110	..	1	1	.9	1.8
4	Formosa	96	..	3	10	3.0	13.5
5	Canton high	478	16	25	16	8.5	11.9
6	Oregon Mohler	377	1	5	2	1.6	2.1

orderly way i.e. parallel to the known reactivity. It must be realized that in the present case, controls cannot be made; the control should be Df/+, but when the + chromosome may contain a subthreshold ey-allele the only control possible is an indirect one, the comparison of different wild-type chromosomes in the compounds. The same applies to the possibility that the deficiency itself has an eyeless position effect.

The results of the tests are found in table 7. The flies with the eyeless deficiency are easily recognized as Minutes and sufficient numbers were obtained in this set of experiments. The visible effect upon the eyes was classified as "eyeless like" (which was always a low type of eyeless), "kidney like," meaning an indenture in the front of the eye, and "slight,"

meaning slight irregularities in shape and surface texture which might or might not represent a grade of eyelessness. The percentage of eyeless types was therefore stated without as well as with the "slight" type included.

The results would be simple and convincing if only the tests for the nonreactive lines (Orinda I and Riverside) were compared with the highly reactive and selected Canton line. They would clearly indicate the presence of subthreshold eyeless alleles of a lower and a higher grade. But the rest of the results does not fall in line. There is again the cross with the highly sensitive Oregon Mohler line, which shows only a small effect. This same line fell out of line before, in the crosses with ey^2 (table 6). In the former case we pointed to the specific features observed which might be responsible for the unexpected result also in the present experiment. We included in the table crosses with the Formosa and Samarkand lines, both of which do not produce the eyeless phenocopy but rather that of Bar (Formosa) and kidney (Samarkand). If they were, therefore, devoid of an eyeless some effect was found in both heterozygotes, one of them isoallele no deficiency effect should be expected. Actually with an unreactive line (Samarkand), the other with a reactive one (Formosa). There is no reason why these lines should not have an eyeless allele in addition to whatever genetic condition causes the Bar and kidney type of phenocopy and there remains still the unsolved problem of a position effect of the deficiency. The statements show how difficult it is to come to a definite conclusion when no genuine controls are feasible by the very nature of the experiments. Thus the set of experiments would speak in favor of the subthreshold mutants if only the crosses 1, 2 and 5 in table 7 existed. But the results of the other combinations are still open to explanation.

C. Spineless, aristopedia and their heterozygotes

Most remarkable facts were found when the frequent incidence of the phenocopy of aristopedia suggested a study of

the mutants at the ss-locus and their hybrids. The phenocopy of aristopedia appears in many lines after standard treatment with borate in more or less small numbers. It is more frequently found together with the eyeless phenocopy but there is no strict correlation as aristopedia appears also with normal eyes. Table 2 (p. 132) shows aristopedia in 13 out of 21 treated lines, among them 4 with rather high incidence. In table 4 (p. 154) numbers and percentages are found for the much used lines and a comparison with the reactivity for the eyeless effect can be made. In the insensitive and non reactive line Riverside a few aristopedia appear only at the highest borate concentration of 0.09%. In the other insensitive and nonreactive lines, Samarkand and Orinda I, they were

TABLE 8

Stocks with high incidence of aristopedia phenocopies after standard treatment

NO.	LINE	n	% EYE EFFECT	% ss^a	
1	Lausanne S.	742	16.8	4.6	
2	Big Ridge	1502	2.6	16.8	
3	Idaho Falls	655	100	5.5	
4	Amherst	809	24.6	16.0	All high grade

missing, but also absent in reactive and nonreactive broods of Orinda II and Canton. But in both Oregon stocks and lines a small percentage of aristopedia phenotypes is produced. The percentage of the effect is of the same order of magnitude as in the stocks marked — and + in table 2. The above table 8 shows the effect in stocks for which a high incidence of aristopedia phenocopies is characteristic. In the most sensitive stocks, especially in Amherst also the grade of expression is highest. This means that in the other stocks as a rule only one of the antennae is affected and the phenotype is that of the mutant aristopedia of Bridges ($ss^{a\text{-}B}$) with a variation from $ss^{a\text{-}B}$ to a grade between this allele and fully expressed ss^a. But in the Amherst phenocopies almost all individuals show bilateral expression and a grade almost resembling ss^a (a complete tarsus). More data on the incidence

of the phenocopy are found in table 6 (p. 168) with results similar to those for the stocks recorded in table 3.

As already pointed out above, the mutant aristopedia differs from eyeless and other eye abnormalities in one important aspect: the mutants affecting eye shape and structure are very frequent e.g. Bar, eyeless, Deformed, Lobe, small eye, but their effects are, we may say, variations on the same theme, and show more or less overlapping. But aristopedia, the replacement of the arista by a tarsus, is known only from one locus and has a most specific effect. Other mutants of the antenna as thread, aristaless, antennaless belong morphologically and developmentally to a different category and thus may not be combined with ss^a into a group like the eye mutants. We mentioned above the mutants "antennipedia" (a whole leg instead of an antenna, including many transitions). Thus far no information on their genetics is available. Therefore, the phenocopy of aristopedia is of special interest for our present problem and this suggested a borate experiment with the mutants at the ss locus and their heterozygotes with wild-type stocks producing the phenocopy.

We used the following mutant stocks: spineless (ss), characterized by the almost complete reduction of bristles, has completely normal antennae; the stock ss^a = aristopedia which we used showed a perfect tarsus in place of the arista with complete penetrance and little variation; the allele $ss^{a\text{-}B}$ (aristopedia of Bridges), which was available in two lines (probably of identical origin). One, from Pasadena, showed a typical effect in almost all individuals, an inflation of about a quarter of the length of the shaft of the arista, with not much dislocation of the branch filaments. The other line, taken from Professor Stern's stocks, had an apparently normal arista and seemed to have lost the mutant phenotype, except for the bristles on the body which were sometimes normal, sometimes shortened to different degrees. But there is one troublesome fact which made it difficult to distinguish between a normal arista and the first beginnings of the aristopedia phenotype. The basis of the arista is usually

pigmented and of a structure like the rest of the shaft. But frequently the basis is inflated and transparent and glistens in a light beam like a pearl. This pearl is more or less large and it is hardly possible to distinguish it from a beginning aristopedia effect. Only when a considerable section of the shaft is inflated and transparent (as in the Pasadena stock) we are certain that we have an aristopedia phenotype. Now the $ss^{a\text{-}B}$ stock of Stern has only the pearl, but it turned out that a number of the stocks used in the present work, like spineless, Samarkand, different Oregon lines, Amherst had all a variable pearl, mostly in the females, but also in males. Thus the boundary between normal arista and a beginning aristopedia effect is hardly discernible. Therefore we considered all pearls as a normal phenotype when evaluating the results of the present set of experiments. We did not study the bristle effects of these mutants because they turned out to be very irregular and not in conformity with former descriptions (quotations in Bridges and Brehme). Only one note on a conspicuous bristle effect is found in table 10.

These lines were subjected to standard borate treatment. The stock aristopedia ss^a was rather unreactive to 0.06 borate. A concentration of 0.08% was usually lethal but one brood survived and showed remarkable results (all controls typical ss^a). Among 174 individuals 40 had different kinds of abnormal wings but all eyes were normal. One hundred forty-six flies had ss^a antennae without any change, but 28 had affected antennae, namely: two with one antenna ss^a the other side antennaless; three with one antenna ss^a the other normal; 18 with both antennae like an intermediate between $ss^{a\text{-}B}$ and ss^a; 5 with one antenna ss^a the other aristaless. Clearly a certain amount of shifting away from the aristopedia effect towards more or less normality had occurred.

The line aristopedia Bridges from Pasadena reacted in a more complicated way to standard borate (the controls were typically as described above, with little variation). The results are found in table 9. On the right the other phenocopies are

tabulated in order to show the considerable reactivity to treatment. The effect on the arista went mostly in the direction of making it more normal or completely normal. But it must be added that here the difficulty of assaying the transitions between a typical $ss^{a\text{-}B}$ antennal shaft and pearl detracts from the value of the counts; though a shift towards normal is undoubtedly found. But in two individuals a more extreme type of aristopedia was present! Further, in many individuals, difficult to classify because of the transition, the antennal joint to which the arista is attached laterally was changed in shape in such a way that the arista attachment became continuous with the tip of the joint. This is a typical feature of high grades of aristopedia, produced here independently

TABLE 9

$SS^{a\text{-}B}$ Pasadena treated with borate 0.06

BOTTLES	n	ARISTAE		+	MORE EXTREME	DUPL. ANTENNA	ANTENNALESS	EYES LOBE	PODOPTERA	INTERSEX	WING ABN.
		Like control	More normal								
11	391	105	123	117	2	34	10	274	18	8	8

of the change of the arista itself. Thus the phenocopic effect changes the phenotype of this allele both towards more extreme and more normal. In addition there were many antenna duplications and antennaless flies. The latter are interpreted as independent phenocopies, the duplications as a pleiotropic effect always found when the phenocopy of Lobe was produced as is the case here.

The $ss^{a\text{-}B}$ line of Stern turned out to be rather unreactive. No effect was produced with 0.06 borate. Only with 0.09 and 0.095% phenocopies appeared, among them a few aristopedia of medium grade, inferior to that produced after treatment of many wild-type stocks. This is clearly not significant and therefore no details are given.

The allele ss never affects the arista. After treatment with borate a high percentage of aristopedia phenotypes with a rather high expression (but no real tarsus) is produced.

TABLE 10

F_1 ss and ss^a × different wild-types, treated with borate and controls

NO.	CROSS	REACTIVITY OF + PARENTS	CONC.	n	% ss^a	% EYE EFFECT	OTHER PHENOCOPIES (AND NOTES)
1	ss × Ore Mohler	high	.06	225	12.4	40.0	
2	ss × Ore Mohler	high	.07	268	25.0	50.0	Beaded, Scutenick, podoptera, antennaless.
3	ss × Samark.	low	.06	200	2.0	5.5	
4	ss × Samark.	low	.08	38	25.5	37.7	Scutenick, intersex, Beaded.
5	ss^a × Ore Mohler	high	.08	141	17.8	35.0	Scutenick, podoptera, antennaless, double antenna.
6	ss^a × Samark.	low	.08	723	1.0	7.4	Beaded, frontless, lanceolate, antennaless, Scutenick, many polychaete.
7	Control Ore Mohler	high	.06	280	1.8	93.8	
8	Control Samark.	low	.06–.075	2084	0	0	
9	Control ss × ss^a	low	.06	324	0	0.3	(Bristles almost ss, but longer in untreated control.)
10	Control ss	low	.06	692	6.3	4.6	
11	Control ss	low	.07	488	8.4	3.7	
12	Cntrol Amherst	± high	.06	809	15.9	24.1	
13	ss × Amherst	± high	.06	757	16.2	high	ss^a up to 35% in individual broods without one large insensitive brood among 6, ss^a = 23% average.
14	ss^a × Amherst	± high	.06	1059	0.2	high	ss^a low grade.

even segmentation of the shaft is found. The data are presented in table 10 no. 10, 11. The eye effect of the Lobe type is small but the arista effect occurs in over 6% of the individuals. This is to be compared with 1.8% in the highly reactive Oregon Mohler line (table 10 no. 7) and none at all in Samarkand (table 10 no. 8). In addition the quantitatively strong effect in ss is also combined with much higher expressivity than that in the stocks mentioned as controls. Thus the first idea is that the borate treatment has brought out in ss the hidden potency for an arista effect of ss, which normally is only present in the different ss^a alleles (the thus far not-studied possibility that a series of "pseudoalleles" at the ss locus exists of which one, ss, has a strong bristle effect but no arista effect, the others vice-versa, will not be discussed, because this, if found one day, would not affect the interpretation of the borate effects). But it must at once be realized that wild-type lines exist which also produce a strong aristopedia reaction after borate treatment, actually in one case a stronger one both in penetrance and expressivity than recorded for ss. The extreme thus far encountered is the stock Amherst (table 10, no. 12) with almost 16% very high grade aristopedia phenocopies. Therefore, the result with spineless could also mean that ss is highly sensitive to the production of aristopedia phenocopies, like Amherst, without any relation to the genetic potentiality for aristopedia effects suggested by the ss^a alleles of ss. If this were so, it would amount to an almost malevolent coincidence, which, however, cannot be ruled out. This is, of course, only worthy of discussion if the aristopedia produced in the Amherst stock are genuine phenocopies. If the effect is based upon subthreshold mutants, as we are trying to prove, the alternative interpretations of the effect in spineless stock become one and the same. As we want to use the ss-effect as a demonstration of subthreshold mutant effects we must discuss it as if the Amherst effect were a genuine phenocopy.

In order to reach a decision the F_1 of ss (and ss^a) with different wild type lines, chosen for reactivity and nonreac-

tivity in regard to both the eye effect and the antenna effect, were treated with borate. The results are tabulated in table 10. As a measure of susceptibility to phenocopic treatment the percentage incidence of an eye effect (eyeless, Lobe, or kidney) is stated, and, where conspicuous, also the other frequent phenocopies are mentioned; the column reactivity of + parent relates to the eye effect.

Though the results seem to be orderly their interpretation is not easy. If the strong aristopedia reaction in the ss stock amounts to an enhancement of a subthreshold potentiality it ought to be absent or lower in the heterozygote, provided the parental wild-type stock does not contain itself a subthreshold allele of ss^a. If the latter were the case, the hybrid would be a compound of two ss^a alleles and an intermediate effect would be expected. In table 10 nos. 1–8 we find the crosses with the reactive Ore-Mohler stock and the nonreactive Samarkand stock, and the controls. The Ore-Mohler stock (no. 7) had a fair aristopedia effect (1.8%), Samarkand (no. 8) none, even at the highest borate concentration tolerated. F_1 (no. 1) ss $\times$ Ore has a medium eye effect, about intermediate between that of the two parental lines. But the aristopedia effect is high above that of both parental lines, and is doubled again if the borate is increased to 0.07% (no. 2). In the Samarkand crosses the aristopedia percentage may be called intermediate (no. 3). In a single small brood (no. 4) which survived at 0.08% borate treatment, both the eye and antenna effects were much increased. The greatest interest attaches to the ss $\times$ Amherst crosses, as Amherst has the highest aristopedia effect of all wild-type lines (no. 12). The hybrid has an insignificantly higher effect of 16.2%. But actually one of 6 broods was highly insensitive and unreactive to treatment as shown by the large number of surviving flies and the low phenocopic effect for both eyes and antenna (as it happens over again in all experiments for unknown reasons i.e. as a variant of experimental procedure). If this aberrant brood is left out the incidence of the aristopedia phenotype is 23%. A clearcut decision between the two

possibilities, i.e. bringing to light the subthreshold arista effect in spineless vs. chance presence of an Amherst-like reactivity for the aristopedia phenocopy, is hardly possible, as stated already, because the expectations cannot be stated unequivocally, whether we assume that the wild-type stocks contain a subthreshold ss^a-allele of different strength or not. But it may be pointed out that the considerable increase of the effect in all hybrids with a reactive stock (Oregon and Amherst) is more in favor of ss being genetically capable of an aristopedia effect which is somehow blocked in development; in compound with a subthreshold allele in a wild-type partner chromosome both potentialities would be brought out additively.

The F_1 between wild-type stocks and the mutant aristopedia, treated with borate might be helpful. In the controls, ss $\times$ ss^a treated with borate, an enhancement of the spineless bristle effect beyond that of the untreated compound is visible, but no aristopedia phenotype occurs. One should think that this compound would react strongly to the treatment, at least as strongly as ss/+. There must be a special reason for this failure. The almost complete absence of an eye-effect might lead to an explanation. Actually the mutant ss^a treated with 0.06 borate does not show any eye effect. This points to a dominant low reactivity of ss^a as the source of the strange result. Actually the compounds of ss^a with the wild-type stocks show no reaction both for eyes and antennae when treated with 0.06 borate. But at the highest tolerated concentration of 0.08% both eye and arista effect appear in approximately the same quantity (nos. 5, 6) as that produced in the ss heterozygotes at 0.06%, a fact which may be quoted in favor of the idea that ss has the genetic potency for the aristopedia effect rather than a general high reactivity. If, as we are trying to find out, the high reactivity of the stock Amherst is based upon a subthreshold allele, both alternatives discussed before become the same thing, as already pointed out. This is a logical situation, which

applies unfortunately to much of our general argument and makes it so difficult to produce genuine proofs for our interpretation. There is finally the $ss^a \times$ Amherst cross (no. 14). Again the aristopedia effect is practically nil, but the eye effect is high! No interpretation is possible at present, though one might play with the idea that a proof of pseudo-allelism of ss and ss^a with different norms of reaction for both, would provide an explanation.

5. *Experimental enhancement and reduction of expressivity*

We saw already in the experiments with the ss^a-alleles that the treatment with borate sometimes enhances and sometimes reduces the expression of the mutant character, even in a single experiment. The enhancement was one of the facts which pointed to the possibility that phenocopies might be based on increasing the action of subthreshold alleles. There are also more unequivocal cases of such action than those just mentioned to which we turn now.

A. The Scutenick experiments

Years ago a series of experiments on chemical phenocopies had been started, among them also work with borates. In one set made with an Oregon stock brought from Professor E. R. Dempster's laboratory after our old Oregon-R stock was lost a new phenotype appeared apart from the typical eyeless effect: Many of the treated flies showed abnormalities of the scutellum clearly forming an ascending series of the same effect, beginning with one or both scutellars missing, shortening of the scutellum, posterior indenture, followed by destruction of parts of the scutellum starting laterally and posteriorly and through many variants up to complete destruction of the scutellum. As the lower grades corresponded to the classical description of the mutant Scutenick we speak of a Scutenick syndrome. That this was correct was shown later when also another typical Scutenick character was observed, namely the displacement or suppression of individual ocelli and occasional

presence of a fourth ocellus far laterally near the eyes. We speak of a syndrome because the effects go far beyond those described for the mutant Scutenick (which is said to be very variable) but with such transitions that clearly a single syndrome is involved. The highest grades of it beyond those just described affect the whole thorax. It becomes indented in front, furrowed in the mid-line, the two halves separate and chitinize only in part, or not at all on one side, while the bristles become very irregular and misshapen. A kind of hemithorax condition follows, which is different from genuine hemithorax, especially because wing base and wings are not affected (except by poor expansion). In the original experiment these extreme grades were very frequent. In the repetition to be reported they were missing, but they appeared again in still other experiments.

Besides Oregon-Dempster the only wild-type stock which gave the phenocopy of Scutenick regularly, though in small numbers is Quicksand. But a relatively large number of Scutenick individuals, and mostly of the higher grades, were produced in the borate treated F_1 ss $\times$ Oregon Mohler, ss^a $\times$ Oregon Mohler and ss $\times$ Samarkand. Though it seems that the ss-partner must be responsible, the phenocopy was not found in the treated stocks of ss and alleles.

The old experiments had been performed with an "Oregon stock" which at that time was considered to be identical everywhere and therefore was not named Oregon-Dempster as it should have been. The controls were normal, as far as the experiment went. Identical results were obtained with sodium tetraborate and perborate. When the work, interrupted at that stage, was taken up again years later, experiments with "the" Oregon stock, i.e. that available among our stocks, Oregon-R. Mohler, failed to reproduce the results. At that time it was realized (as also found by Sang and McDonald) that the eyeless effect of the borate treatment differed much in different stocks. Thus the suspicion arose that the old experiments had been performed with a different

Oregon stock which genetically was fit to produce the Scutenick phenocopy. We were able to locate the stock used in our former experiments. The research assistant, Rui D'Cruz, had brought an Oregon stock from the genetics department which should have been called Oregon Dempster. When the experiments were repeated now with this line — a stock which meanwhile had been still more inbred and had become rather

TABLE 11

Phenocopies in Oregon-Dempster

NO.	EXPERIMENT	CONCENTR.	n	% EYE	% SCN	PHENOCOPIES OTHER	NOTES
1	Old	.06	723	3.6	33	Aristopedia	Scutenick percentage minimum because lower grades missed in part.
2	Old	.07	31	26	23	Aristopedia	Scutenick percentage minimum because lower grades missed in part.
3	New	.04	200	24.5	27	Aristopedia (3.5%) podoptera, tetraptera	
4	New	.05	23	21.7	50		Extreme type.
5	New	.04	348	.6	1.7		

weak and very sensitive to borate — the Scutenick syndrome was again produced! While the old experiments had succeeded with the standard treatment of 0.06% borate the stock as found now after more inbreeding was completely infertile at that concentration, meaning that the food in the bottle was covered with dead, first instar larvae. Thus lower concentrations had to be used which still permitted only relatively few survivors. Also the controls were very poorly viable.

The data are found in table 11. While the old data included among 7 broods only two without the Scutenick types, the new work with the since longer inbred Oregon Dempster line showed a large number of individual bottles which at a workable concentration gave only or almost only normal flies (no. 5). It seems that the borderline between viable non-sensitivity and sublethality with phenocopic effect had become very narrow, so that only experiments which hit by chance below the borderline were successful and only one (no. 4), with a usually lethal concentration of .05, gave as extreme a Scutenick-like effect as the old experiments nos. 1, 2. The column eyeless per cent shows that a high Scutenick effect may coincide with a relatively high eyeless effect (no. 2) or with a low one (no. 1) or none at all. The decisive point is that it is proven that Oregon Dempster reacts to borate typically with the Scutenick syndrome, which is otherwise rare, as mentioned above.

After these facts were established it became necessary to compare the phenotypes of the "phenocopy" with that of the mutant Scn (fourth chromosome, homozygous lethal), kept balanced over eyeless-dominant, ey^D (a fourth chromosome duplication). It turned out that our Scutenick stock was phenotypically completely normal (and we found since that also the Pasadena stock has no more expressivity of Scn) in rather large numbers checked, though it showed ey^D. It is well known that mutant stocks, if not selected, return frequently to normal phenotype or retain only some of the original mutant characters (see e.g. the case of blistered and balloon in Goldschmidt, '45). It is a fair assumption that selection of modifier systems pulling toward normalcy is involved. This Scutenick stock Scn/ey^D was now subjected to the standard borate treatment. It must be added that in our stock the dominant eyeless has a rather poor expression insofar as in many flies only one eye is affected and the high grades of indented or highly reduced eyes typical for ey^2 are missing (other ey^D stocks are different). After borate treatment a number of completely eyeless flies appeared and

most of these were visible Scutenick! Borate thus brings out a completely nonpenetrant mutant known to be present in the stock. The remarkable fact is illustrated in table 12 which shows also the great quantitative variation of the effect. We mentioned that the flies showing the Scutenick phenotype had also reduced eyes, far beyond the phenotype of ey^D in this stock and in the not Scutenick sister flies. In the old description of the mutant Scn eye reduction is mentioned as one of its effects, which then is added here to the rather weak eye effect of the balancer.

TABLE 12

Scn/ey^D treated with borate; 6 more bottles normal with different concentrations of borate

NO.	CONC.	n	% SCN	
1	.06	29	41.4	
2	.06	80	1.3	
3	.07	55	21.8	A few Scn only ocelli effect
4	.07	50	10	A few Scn only ocelli effect
5	.07	224	3.1	A few Scn only ocelli effect
6	.08	131	3.8	A few Scn only ocelli effect

The conclusion is, of course, that it is a fair assumption that in the Oregon Dempster case the specific "phenocopy" Scutenick is actually the presence of the subthreshold Scutenick allele or a Scn allele plus a counteracting modifier system which is brought out phenotypically by enhancement of the mutant action by borate or by counteracting the suppressor system. One could compare this action to the enhancement of the penetrance of a poorly penetrant dominant like Bd (Beaded) with only a few per cent penetrance in Bd/+ which can be increased to 100% if certain inversions or one of the Minutes are simultaneously present. It would not be unexpected therefore, if, in the case of recessive mutants, chemical treatment affected the penetrance of a homozygous mutant of variable penetrance. Another example which comes to mind is Glass' erupt suppressor which prevents the visibility of the erupt phenotype, which however comes out when the

suppressor mutates. Actually Plaine and Glass ('55) showed that the penetrance of erupt eyes in the Suppressor-erupt strains is increased from 9 to 35% by L-tryptophane treatment, to 50% if oxygen treatment precedes the L-tryptophane. [As Hinton, Noyes and Ellis ('51) had found that L-tryptophane produces phenocopies of tumors and eye effects the facts parallel ours.] But we must add again that the conclusion upon subthreshold mutants brougth out by phenocopic treatment is possible but not proven, since the restoration of the expression of Scn by counteracting a system of minus modifiers with borate treatment, could be a different process from bringing out the Scn-phenocopy in the Oregon Dempster stock. But the accumulation of such cases may be claimed to be in favor of the genetic interpretation via subthreshold mutants; and it may be added that this Scutenick case sheds also light upon the spineless-aristopedia effect in so far as it adds to the argument in favor of the idea of bringing out a subthreshold potency of ss.

B. The podoptera phenotype

We were first led to the problem of subthreshold mutants in the wild-type stocks by observations on the podoptera phenotypes (pod.). These had been shown (Goldschmidt, Hannah and Piternick, '51) to be almost ubiquitous though usually exhibiting such a low penetrance as 1–3% or less. The pod effect [also the related tetraltera (tet) effect] is not based upon a single locus but on one major locus in the 2d chromosome and minor ones in all chromosomes. The specific penetrance in the different pod and tet lines, which had been found could not be changed by selection in some, changed only slightly in other lines, while in tetraltera selection up to 100% penetrance was possible. (Details in l.c. and Goldschmidt, '53.)

In the present experiment the many wild-type stocks studied threw in the controls a few podoptera flies and in the majority of phenocopic experiments pod flies appeared as can be

seen in table 2 (p. 132). The percentages were usually small. Only in the Samarkand stocks the borate treatment increased the podoptera type immensely. In table 13 the experiments with some representative stocks are tabulated in more detail. The percentage incidence of pod is given for controls and experiments, both with standard and higher borate concentrations. The column mult. contains the multiplicator of increase in the experiments. This is a negative value when the controls contain more pod flies. If the control contained no pod we calculated nevertheless a multiplicator assuming a value of 0.06% pod otherwise found as a minimum in the experiments, in order to have a finite multiplicator. S.I. is the survival index as measure of sensitivity, explained in p. 148. For comparison with other known reactivities the reactivity for phenocopic effect upon the eye is recorded in the last column. This table shows: in all 5 lines the controls contain a small percentage of podoptera, as expected from former work. This percentage varies in the individual groups of experiments from nothing to less than 1%. In every group except one (no. 13) this percentage incidence is increased many times by borate. The greatest effect was found invariably in the two Samarkand lines. As the last column shows, this is not at all correlated to the reactivity for the eye effect. There is also no correlation to the lethal effect of the treatment (measured in column S.I.) as seen easily in the Riverside group. Thus Samarkand has a genetic constitution which allows a very high enhancement of the pod effect. This may mean that the pod factors, or some of them, react favorably (in regard to penetrance) to the borate treatment. But there is an alternative interpretation, namely that the borate produces the pod effect purely as a phenocopy and that the different lines used have a different reactivity for this effect, i.e. Samarkand a high one, Riverside a very low one (under standard conditions). In this case the small genetic pod effect in the controls would remain unaffected, but would be added as an indistinguishable part to the phenocopies in the treated flies. The data reported thus far (table 13) are

TABLE 13

Podoptera in some wild-type lines

NO.	LINE	CONC.	CONTROL		EXPERIMENT				REACTIVITY OF EYE EFFECT
			N	% Pod.	N	% Pod.	Mult.	S.I.	
1	Samarkand	.06	1403	.07	2106	7.2	103	0	Very low
2	Same, later rep.	.06	962	.10	1530	1.3	13	0	Very low
3	Same, one sensitive brood	.06	298	0	138	40.6	677	45	Very low
4	Samarkand inbred	.06	380	.78	125	32.00	41	35	Very low
5	Samarkand inbred	.07	648	.30	376	9.04	30	24	Higher
6	Samarkand inbred	.075	178	0	134	36.00	600	17	Higher
7	Orinda I	.06	2918	.06	3607	.21	3.5	9	Lowest
8	Riverside	.06	989	.15	1864	.47	3	0	Very low
9	Riverside	.07	615	0	845	2.38	46	0	Very low
10	Riverside	.08	565	0	428	1.40	9	17	Very low
11	Riverside	.09	455	.44	176	9.02	20	32	Very low
12	Orinda II	.06	831	.36	1307	1.68	5	0	Medium, mixed
13	Canton-S	.06	1260	.40	2843	.28	..	0	High, mixed

clearly open to both interpretations, but the independence of the effect from lethality and from the eyeless effect i.e. sensitivity and the most conspicuous reactivity, are not in favor of the non genetic explanation.

As the lumped numbers from all individual broods in the table might not give a complete picture, correlation between lethality and enhancement of pod effects was studied for individual broods. On the lowest and highest level of the two

TABLE 14

Podoptera lines treated with 0.06% borate

NO.	LINE	CONTROL		EXPERIMENT				EYE EFFECT
		N	% pod.	N	% pod.	Mult.	S.I.	
1	Pod G	885	2.9	1802	3.44	1.2	0	None
2	Sel. for pod F_1	145	7.58	308	5.84	..	0	None
3	Sel. for pod F_2	451	2.0	337	5.28	2.6	17	None
4	Sel. for pod F_3	334	2.69	473	5.70	2.1	0	None
5	Sel. for pod F_4	832	1.92	513	7.02	3.2	22	None
6	Sel. for pod F_5	1456	2.74	915	3.50	1.3	22	None
7	All selections	3118	2.69	2546	4.43	1.7	14	None
8	One F_3	112	2.7	15	74.7	28.0	48	None
9	One F_5	108	3.7	5	100	27.0	56	None
10	Pod M 124	854	16.31	476	22.5	1.4	29	None
11	Pod (2) K	111	1.8	267	5.25	2.9	0	None
12	Tet y cv	295	68.13	428	50	..	0	None
13	Tet Bd	2416	36.9	2286	35.9	..	10	None
14	Tet 100	616	72.1	839	74.8	1.04	0	None

effects correlation was good, but absent in between which is more in favor of the genetic interpretation, but one requiring a polygenic setting.

The following experiments are probably more decisive. First we are interested to know how borate treatment acts upon homozygous podoptera lines with different penetrance. We studied the same lines which we had used in our podoptera monograph ('51). Table 14 contains the results, the columns being the same as in table 13. The pod-G stock is homozygous for pod with a penetrance around 3% which cannot be increased any more by selection. This formerly established

fact was again confirmed in the present experiments in which phenotypically pod flies from borate bottles were selected for breeding over 5 generations (column controls nos. 1–7). With exception of one F_1 in which the controls had unusual high penetrance (multiplicator minus, no. 2) the experimental broods showed a high percentage of pod, though much less than in the Samarkand experiments. In three of these groups the viability was not impaired and in three others there was quite a reduction of viability, but the two groups did not differ in regard to the borate effect. The phenocopic eyeless effect was always absent just as in the Samarkand experiments. Thus the borate action was approximately of the same order of magnitude as in the totality of the non-Samarkand experiments of table 13 and it is safe to conclude that in these pod-G experiments the action of known homozygous pod factors was enhanced to about double their effect.

The high effect in the Samarkand line, which has a much lower spontaneous incidence of podoptera than the lowest podoptera stock, is therefore a special feature added to the general enhancing effect of the borate. As said before, this effect may be due to the presence of pod factors with a different norm of reaction; or of specific modifiers of high reactivity; or of a high purely phenocopic reactivity independent of the presence of pod and its modifiers. As the Samarkand stock is non-reactive for the eyeless phenocopy as are also the podoptera lines, the last alternative would require different and independent reactivities for the pod and eyeless effects. Actually a very large body of material shows that the pod effect after borate treatment in the wild-type lines does not require a simultaneous eyeless effect. But when the eyeless effect is present the number of pod flies which are simultaneously eyeless is many times higher than expected on a chance basis; frequently all pod flies are eyeless. Thus the third possibility is very improbable.

There are a few facts recorded in table 14 which make it very probable that the chance presence of specific modifiers accounts for the high pod effect in the Samarkand experi-

ments. In table 14 two individual experiments (meaning one brood from one pair of parents) are reported (nos. 8, 9) one in the third generation selection and one in the fifth: In both the lethality of the experimental flies was very high (though no eyeless phenotype appeared!), and the survivors were 75 resp. 100% pod. As the known pod factors were homozygous and a mutation was excluded by the unchanged controls it must be assumed that a chance selection of plus modifiers was accomplished, acting only in the presence of the borate. (All old experiments with pod-G showing that a selection beyond the normal penetrance is impossible might be added to the controls.)

In table 14 additional experiments with other pod stocks are reported (nos. 10, 11). One shows little, one more enhancement but neither case was followed up. It seemed interesting to test also some of the tetraltera stocks with high tet incidence nos. 12–14. It turned out that here the borate treatment decreased somewhat the penetrance of tet in the overall results. The reason is obvious. In these tet stocks the higher grades of tet are present and it is known that the vitality of tet flies decreases with the expressivity of the character. Thus tet flies are more liable to be killed by the borate than their normal siblings.

The conclusions in regard to the causes of the typical results in the Samarkand experiments find a strong support in selection experiments with the highly reactive (in regard to pod) inbred Samarkand line. Treated Samarkand flies showing the pod effect were selected over 6 generations with continued borate treatment. From F_3 on appeared individual broods which showed high lethality of the treated flies together with unusually high percentages of pod. Table 15 contains only these broods out of a large number of experiments, which showed the extreme effects. Thus this entire group of experiments points strongly to the interpretation that the pod enhancement effect is actually enhancement of the action of the pod factors and of genetic modifiers sensitive to borate

action while the alternative of a pure phenocopic effect added to an already present genic effect becomes very improbable.

C. Data on the mutants aristaless and antennaless

As the phenotypes of aristaless and antennaless, with all transitions between the two were frequently produced as phenocopies (see table 2) it was of interest to study the reaction of the mutants of these types to borate treatment, of course with the problem of enhancement of subthreshold mutants ultimately in mind. The different lines of aristaless (al) used differed somewhat in phenotype. One, which was considered good (al b c sp) had aristae reduced to $\frac{1}{3}$–$\frac{1}{2}$ of

TABLE 15

Selection of pod phenotypes from treated broods of Samarkand inbred, only broods with high lethality (see S.I. column) are tabulated

NO.	GEN.	CONTROL		EXPERIMENT		
		N	% pod.	N	% pod.	S.I.
1	F_3	89	..	57	36.9	20
2	F_4	144	.7	61	50.0	30
3	F_4	156	.7	52	44.2	35
4	F_5	36	..	9	33.3	40
5	F_6	37	..	4	50	50

their normal length with hardly any variation. In one experiment with this line, producing a considerable eye effect with 0.06 borate, out of 243 flies the antennae of 160 were unchanged, but in 73 one or both aristae were still further reduced or completely absent. Thus the borate had enhanced the mutant effect. In one of the other al experiments also some beginnings of the antennaless types were observed, but this might be a direct phenocopic action independent of the al-mutant present, an explanation which does not apply to the al-effect, which, thus far, never was produced to such an extent in normal lines.

The experiments with the antennaless mutant had a surprising result. In all controls made with one pair bottles the mutant was 100% penetrant and expressive. After borate

treatment many antennae were present on one or both sides i.e. the mutant effect was counteracted. But it turned out that in the mass culture bottles the same was found. Borate had thus the same effect as crowding. Here is a fact which might be studied further, if comparable results should turn up in future work.

DISCUSSION

If it would turn out that the interpretation of the facts in terms of enhancement of subthreshold mutants could be proven beyond doubt, this could undoubtedly touch at the roots of many important genetic problems: phenocopy, mutation, genetic composition of populations, genic action, polygenes, pleiotropy, structure of the genic material. In view of such far reaching consequences not only great caution is advised in interpreting the results obtained thus far, but in addition much further work along the same lines is needed, covering all aspects of former work on phenocopies, chemical and otherwise. Therefore at this point of our work a discussion cannot do much more than state the problems and hint at the possible direction of future solutions. While reporting the facts we weighed them already for possible explanations, which had been presented before introducing the facts (p. 163). Former work of other investigators was also mentioned in the text. Most of this deals with the specific phenocopic effect of different chemicals upon a standard wild-type line (e.g. Rapoport, Bodenstein and Abdel-Malik, Gloor, Hinton and coll., Plaine and Glass). To these can be added now a paper by Schultz and coll. undertaken for the study of completely different problems, which required treating Drosophila with a series of 28 chemical compounds used for cancer chemotherapy. Oregon-R was the stock used and for each of the compounds the degree of lethal effect and the typical morphological effects were studied. Each compound produced a characteristic pattern of different phenocopies (described as "morphoses"), which agrees with Rapoport's original

findings for other chemicals. The extensive material presented was not further analyzed for the problem of phenocopy. We mentioned already that in the work of Sang and McDonald the first steps toward an analysis of the genetic side of the problem is found.

Much nearer to the present work comes Landauer's and collaborator's work with chickens, where Insulin and a few other substances, among them boric acid, injected into the young embryo, produce the phenocopies of rumplessness, micromelia and beak abnormalities. The effect varies qualitatively and quantitatively with different breeds and a parallelism of these effects is found with the tendency to natural occurrence of the characters as rare non hereditary variations in the different breeds. A review and bibliography is found in Landauer ('57). We quoted above already Landauer's interpretation (p. 164) and opposed it with our own (as also expressed in an invited discussion to Landauer's symposium paper). Therefore we summarize only shortly the situation as it appears at present.

It is proven that the genetic constitution of the material of phenocopy experiments is of greatest importance for the results, and that the qualitative and quantitative character of the effect has a genetic basis; further that different wild-type stocks and lines may react differently, both quantitatively and qualitatively; further that low penetrant mutants can be enhanced by phenocopic treatment; further that mutants which have lost their phenotypic expression may have it restored by the phenocopic treatment; further that mutants lacking a phenotypic effect found in another allele (ss and ss^a) may show this effect after the same phenocopic treatment. Already at the present level of the work the problem appears: are all these facts based upon the same underlying cause or not? Is the effect of the treatment simply the production of a phenocopy by changing the development in the same way as a mutant does, while the genetic elements in the effect are nothing but modifying factors changing the details of

the basically single tracked phenocopic action, just as the general genetic background affects all genic action to a larger or smaller degree? In other words, are we dealing with genuine phenocopies as expressed upon different genetic backgrounds? This would mean that the different enhancing actions of the borate treatment upon penetrance, expressivity or even the mere appearance of phenotypic effect copying that of mutants do not carry information useful for understanding the modification of development by the phenocopic action identically with that of mutant action. If this were true nothing would have to be changed in the general notion of specific chemical phenocopies (thus far relating only to those produced by boron), except the realization that genetic modification of the effect plays a more considerable role than expected.

The other possibility which appears after the experimental results are considered in their totality is that the qualitative and quantitative genetically controlled differences in reactivity to the boron treatment are directly based upon the presence of specific subthreshold mutants (isoalleles) of just those loci, which appeared to be phenocopied. This would mean that all over the chromosomes of Drosophila subthreshold mutants (without visible effect), of many loci and of many subthreshold degrees at each locus could be present in different homo- or heterozygous compounds. The apparent phenocopy would then be an enhancement of the action of these isoalleles, quantitatively (penetrance and expressivity), as controlled by the concentration of the salt and by the potency of the isoallele below the threshold level for visible action; while the quality of the effect would be controlled alone by the chance presence of one or another isoallele and its reactivity to one or another chemical. In this case the proven enhancing of known low penetrant or subthreshold mutants would be a part of the entire story and thus a proof for the correctness of the interpretation.

Finally we may point out some problems which might be attacked when further work is done. In the foreground will

always be the question of developmental interrelations which make possible the similarity of phenocopic and mutant actions. One will think of such facts as the different effects of chemicals in different concentration upon growth phenomena (see Thimann, '56); the details of differential growth in the development of organs (see Vogt, '46, '47); the possibility of the existence of channels or notches in development into which alone developmental processes can snap, as it were, controlled perhaps by chemical equilibria and by different substrate affinities. A solution of the causation of such features of developmental determination, both by mutant or environmental action, may clarify many problems of developmental genetics. We think for example of mutators and mass mutation, which might mean a general or widespread effect upon many thresholds simultaneously exercised by a special mutant locus which results in shifting a number of thresholds in a parallel way. Another general problem, discussed already by Lerner ('54) is the meaning of subthreshold mutants for population genetics. Again another one is the relation to pleiotropy and to "polygenes," a relation which would be suggested if isoalleles are responsible for phenocopy. From the point of view of genetics (apart from phenocopy), these problems have been discussed by Haldane ('30), Lerner ('54), Grüneberg ('54). Altogether we think that the further study of phenocopy can become a major tool of research in many directions.

APPENDIX

List of wild-type stocks

Amherst-34, from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

Big Ridge, Tenn., from Lexington: University of Kentucky, October 1955, kept by mass mating.

Bikini Atoll 1947, from Lexington: University of Kentucky, October 1955, kept by mass mating.

Canton-S, from C. Stern, Department of Zoology, University of California, Spring 1954, kept by mass mating.

Corona, from Division of Genetics, University of California. Trapped at Corona, Riverside County, California, kept by mass mating.

Florida 26–24, from Aloha Alava, Department of Zoology, University of California. A line from the Florida stock of Columbia University, was inbred by single pair matings in 1945–1946 now kept by mass mating.

Florida-19 (inbred), from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

Formosa, from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

IF-37, Idaho Falls, Idaho, from D. F. Poulson, Yale University, October 1955, kept by mass mating.

Lausanne-Special, from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

Oregon-R Mohler, from D. Mohler, July 1955. Stock had then been inbred by single pair matings for 165 generations. Kept by single pair mating.

Oregon-R-C Dempster, from Division of Genetics, University of California, 1955. Stock had then been inbred by single pair matings for 151 generations. Kept by single pair mating.

Orinda I, from D. Mohler, 1955. Trapped at Orinda, California, November 1954, kept by mass mating.

Orinda II, from D. Mohler, 1955. Trapped at Orinda, California, November 1954, kept by mass mating.

Quicksand, Ky. 1954, from Lexington: University of Kentucky, October 1955, kept by mass mating.

Riverside, from D. Mohler, 1955. Trapped at Riverside, California, November 1954, kept by mass mating.

Salta, from Division of Genetics, University of California, July 1953. Trapped at Salta, Argentina, February 1950. Kept by mass mating.

Samarkand-inbred, from B. Hochman, November 1954. Stock had then been inbred by single pair matings for 250 generations, kept by single pair mating.

Samarkand, from C. Stern, Department of Zoology, Berkeley, California. A line taken from the inbred Samarkand stock in 1953. Kept by mass mating.

Sevelen, from D. F. Poulson, Yale University, October 1955, kept by mass mating.

Swedish-b-6 (Swedish-b cleaned of inversions), from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

Urbana-Special, from Cold Spring Harbor: Carnegie Institution, June 1955, kept by mass mating.

The authors wish to thank Mr. Benjamin Hochman for many helpful suggestions and for making available the stocks from Cold Spring Harbor, Yale University, Oak Ridge, the University of Kentucky, Corona and Salta and several special 4th chromosome stocks. We also thank Dr. Aloha H. Alava, Dr. Curt Stern, Dr. Everett R. Dempster and Dr. James D. Mohler for the stocks they contributed.

SUMMARY

1. The phenocopies produced in Drosophila by action of sodium tetraborate were studied as to the genetic basis of the effect.

2. A large number of different wild-type stocks behave differently, but typically, both in regard to sensitivity and reactivity to the treatment i.e. quantitative response, and to the quality of the effect i.e. the characteristic phenocopies produced.

3. Each stock and line reacts to the treatment with one or more phenocopies. Their type — over 20 were studied — relative frequency and combination characterizes each stock and line. Some phenotypes like eyeless, podoptera, aristopedia are frequently found, others like dachs or Bar characterize a single stock; also the total spectrum and the relative frequency of the different phenocopies are characteristic of each stock and line.

4. The quantitative response i.e. sensitivity and reactivity (to the identical treatment) is genetically controlled and different in different stocks, some being very reactive, others highly refractive. The degree of lethality i.e. sensitivity is frequently proportional to the amount of phenocopic effect. But this parallelism is not obligatory; probably both are determined independently.

5. In some lines with varying reactivity it is possible to select for high response. It seems that the genetic basis is a simple, perhaps even monofactorial, main difference plus a multifactorial modifier system.

6. Two major explanations offered themselves: the presence of different modifier systems which control the developmental system so that it permits or does not permit quantitative and qualitative aberrations; or the presence or absence of subthreshold mutants at many loci, the action of which can be shifted above the threshold of visibility.

7. Tests for a decision were made in different ways. One is the action of borate upon heterozygotes of the different wild-type lines with phenocopied mutants like eyeless or aristopedia. If subthreshold mutants are present the heterozygote should behave like a compound and the order of reactivity of the wild-type parental stocks should be paralleled by the order of effects in the hybrids. A number of data are according to expectation; others do not agree.

8. For the phenocopy of eyeless direct tests could be made by putting the different wild type loci opposite an ey-deficiency. Again some experiments were in favor of the theory of subthreshold mutants, others did not fit.

9. Comparable tests were made with spineless and aristopedia mutants. Homozygous spineless treated with borate produces many phenocopies of aristopedia. The compounds with wild types follow to some extent the expectations for the presence of subthreshold alleles. But some wild-type stocks like Amherst have alone a very great phenocopic aristopedia reaction. A comparison of all ss, $ss^{a\text{-}B}$ and ss^{a} crosses with wild-type stocks, treated the same way, again favors a decision for the subthreshold mutants, but does not prove it.

10. Scutenick is a phenocopy typical for some wild stocks. A stock of Scn/ey^{D} showed no visible Scutenick effect. After treatment with borate the phenotype reappeared. Thus we could compare this Scn to a subthreshold mutant and use the facts in favor of the interpretation of the other experiments under discussion.

11. The best material for demonstration that penetrance of a low penetrant mutant (down to less than 1%) can be raised by borate treatment is the podoptera phenotype. Its incidence could be doubled in many wild-type stocks, where

the almost subthreshold mutant is present and also in podoptera lines homozygous for low penetrance. One wild-type stock, Samarkand, contained a modifier system which permitted an immense increase of podoptera by standard treatment. These facts may be considered to show that a bringing out of subthreshold mutants is possible.

12. A few irregular results with other mutants which are phenocopied are added.

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