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In Pursuit of a Gene

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The pursuit of a wild animal begins with tracking the spoor. In the case of certain wild animals which have never been seen, such as the Abominable Snowman and the Gene, this is as far as anyone has ever gotten—in the Himalayas, tracks in the snow, probably grossly enlarged and deformed in shape by melting and evaporation; in the phenotype, traces of hereditary effects altered and confused by penetrance and pleiotropy, polygenic modifiers and major suppressors or enhancers, thresholds, temperature and other environmental factors, and, of course, sex. Yet here we perforce begin—with the somatic trail left behind as the gene slithers from one generation to the next, through the secret paths of the germ plasm.

In these days of the operational definition, we should quite properly not be speaking of "genes" at all, but of units of mutation, recombination, or function —to use Benzer's colorful terminology, of "mutons," "recons," and "cistrons," respectively (*1*). Nevertheless, it is my conviction that in ordinary scientific conversation the term *gene* will continue to be used for these several units, since they are after all not altogether independent. The trail we pick up and trace through its effects upon the phenotypes of our animals (or other organisms) is detectable because of alterations in the *functions* of hereditary units which originate through mutation and are localizable in the chromosomes through the analysis of recombination. Just as the term *enzyme* remains convenient and meaningful despite the fact that the supposedly unitary enzyme has broken up into composites of apoenzyme, coenzyme, and chelated metal ions, with enzyme function and specificity depending on the exposure and configuration of one or more "sites" which can vary independently of one another, so the concept of the gene will remain useful despite its compound complexities and its nebulosity at each end where it melts into the genetic continuum of the chromosome.

In following the trail we must of course be canny and not dash off like any unseasoned hound on the first fresh cross trail. There are many genes, and there are many trails not made by genes at all. Note therefore that my title is "In pursuit of *a* gene," not "In pursuit of *the* gene." Some 15 years of tracking one particular gene in that relatively well known countryside delimited as *Drosophila melanogaster* have afforded me a certain amount of insight into the habits of the elusive quarry I have been pursuing. I cannot claim even to have glimpsed it, as yet; but I can testify that the assiduous pursuit of such game has many surprises, and what I have learned about the habits of the quarry is possibly of more general significance than what I might have turned up had I followed every fascinating fresh scent into the underbrush. At any rate, certain major problems have been illuminated to some extent in my own mind, and it is to these that I wish to confine my remarks.

One problem is the question regarding the time during life when genes act, and the modus of obtaining a clear-cut answer to the question. A second problem is that of the nature of gene action, and particularly of the action of suppressor genes that produce (or restore) the normal phenotype by a different mechanism or channel from that utilized by the normal allele of the mutant which is suppressed. I am quite convinced that the further analysis of the action of suppressor genes will afford us more insight into the nature of gene action than the analysis of almost any other genetic type of interaction. Third, I shall come to an evolutionary consideration of paramount significance: What is the meaning of the widespread distribution in natural populations, within single species and within related species, of diverse genetic means of attaining the same end, the production of a particular "normal" phenotype?

The Time of Gene Action

Fifteen years ago I had occasion to x-ray the eggs of two different strains of *Drosophila melanogaster* with the moderate dose (for *Drosophila*) of 1000 roentgens (*2*). Nothing very remarkable was noted when the treated individuals of one strain emerged as adults, but all or nearly all of the individuals of the other strain emerged with grotesque growths in the center of each eye (Fig. 1). As fully grown larvae, the individuals of this second strain also contained numerous free melanotic tumors in the body cavity, or hemocoele (Fig. 2). After a preliminary flurry of excitement over the thought that perhaps directed mutation had been achieved, it turned out that when these treated individuals with erupt eyes and melanotic tumors were mated among themselves, their offspring lacked tumors and had perfectly normal eyes.

The induced effects were therefore not hereditary—or so it seemed until crosses were made between untreated individuals of the strain that responded to the x-rays and the strain that did not. It was then found that, by retaining the third pair of chromosomes but replacing the second pair of the responding strain by those from the other, one could extract a stock that, without any treatment at all, consisted of flies with full-blown eye growths. Conversely, by replacing the third chromosome pair of the responding strain with those derived from the other, while keeping the second chromosome pair, a stock was obtained in which every larva develops melanotic tumors. The original responding strain was thus shown to carry two mutants, one for each of the two types of abnormal growth described and, at the same time, two specific suppressor genes that usually inhibit the manifestation of the presence

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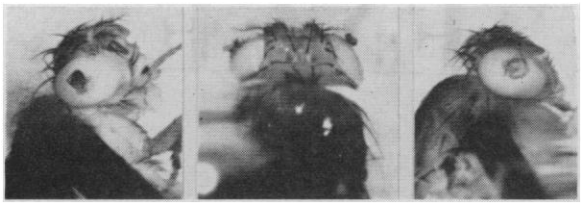

Fig. 1. Erupt-eyed *Drosophila melanogaster*. Expression extreme. Eye color, brown-scarlet, phenotype nearly white.

of the mutants but fail to do so when the eggs are x-rayed. The arrangement of the mutants and their suppressors is a reciprocal one (Fig. 3). The strain is hereafter referred to as the "double-suppressor" strain.

As a means of determining when the suppressor genes act during development, not only eggs but also larvae of various ages were treated with a dose of 1000 roentgens (*3*). This investigation has been completed only with respect to the suppressor of erupt, and further discussion of the time of action of these genes is limited to the erupt-suppressor-erupt system. Interpretation was greatly simplified by the fact that, no matter what the time of treatment with x-rays, no recovery occurred thereafter. Treatment was effective until the period when the eye of the fly actually begins to differentiate, during the third larval instar.

The effect of the x-rays, in other words, was of an all-or-none type during the embryonic period and the first larval instar, and it began to diminish only in the middle part of the second larval instar (Fig. 4). X-rays applied late in the third instar were without effect on the suppressor-erupt system. When an effort was made to push back the time of treatment to as early a moment as possible during cleavage, H. L. Plaine and I were eventually able to collect and irradiate a sufficient number of eggs within 16 minutes after their fertilization and deposition. Inasmuch as meiosis in *Drosophila* is blocked in Metaphase I, and completion of the meiotic division occurs only after fertilization, it follows that in these 8 ± 8-minute-old eggs the sperm and egg pronuclei had not yet united and cleavage had not yet begun when the x-ray treatment was administered. Nevertheless, full inhibition of the suppression of erupt by the x-rays was observed, as usual.

However, when unfertilized eggs or spermatozoa, or both, were irradiated, even with a dose of x-rays 4 times as high, no interference with the suppression of erupt could be observed at all. It is therefore evident that the x-rays effectively destroy some hypersensitive substance or system related to the suppression of the erupt phenotype, and that this sensitive reacting system comes into existence upon fertilization and is not replenished during the remainder of the life-cycle but remains unutilized until the third instar, when, at the time of the differentiation of the eye, it is gradually depleted.

Is the sensitive substance (or system) then to be identified as the primary product of the gene, or is it on the contrary an essential substrate or precursor for the gene's action? If the first is the case, then clearly the gene we are studying may exert its primary action enormously in advance of any visible differentiation affected by it and in advance of such critical periods (temperature-sensitive period, chemical-sensitive period) as have been regarded by many gene physiologists as indicating something about the time of gene action. But if the sensitive substance is substrate, rather than primary product, the action of the suppressor gene might actually be concurrent with differentiation of the eye. To distinguish between these alternatives is not easy.

In current biochemical genetic theory, the gene is conceived as determining the specificity of some single enzyme. Thus, in the classic case of the eye-color mutant vermilion in *Drosophila,* the conversion of tryptophan to kynurenine is blocked, presumably through a failure in the production of one of the enzymes required in this three-step process. As a consequence, no brown eye-color pigment is produced. But the formation of kynurenine, as is shown by the classic studies of Beadle, Ephrussi, Tatum, and others, does not take place in the eye at all but in the gonads and other organs, whence it diffuses into the eye discs. The time of gene action in that instance is not at all at the time of pigment differentiation but at some time prior to, or during, the formation of the enzyme concerned. A preliminary investigation in our laboratory by Frank Erk (*4*) has indicated the presence of kynurenine even in the *Drosophila* embryo. This early-formed kynurenine may never be available for the formation of eye pigment in the pupa. It may be destroyed long before the development of the eye makes utilization of the kynurenine possible.

Thus the enzyme may be present and active long before the conditions for the utilization of its product are fulfilled. Or, the enzyme may be present but its substrate inaccessible to it, as is apparently true in some portion of the intervening period, when kynurenine is not to be found. The question we have posed thus becomes the following: When do the genes determine the specificity of the enzymes they control? Is it even incredible to suppose that all the enzymes are made at, or shortly after, fertilization but, like the inducible enzymes of microorganisms, become abundant and enter into activity only when supplied with substrate?

To approach the question from another angle, we might consider the action of the genes in the case of "autonomous" characters in organisms possessing a mosaic type of development, such as *Drosophila.* As Stern (*5*) and others have shown, a somatic mutation or segregation of a gene affecting body or eye color or bristle growth may produce a very small area—even a single cell—characterized by the mutant phenotype. Is this not proof of the late action of the genes concerned? What it actually shows, it seems to me, is merely that a *change* in genetic constitution can bring about a *change* in phenotype even at so late a period in development. To wit: the enzyme concerned may be maintained in the cell only through the continual ministration of the controlling gene. As in the so-called "abortive transductions" in *Salmonella* and other bacteria (*6*) or in the maintenance of kappa in *Paramecium* (*7*), if the gene is removed or changed, the enzyme (or other gene product) disappears or is fundamentally

Fig. 2. Larvae of the tumorous nonsuppressor-tumor strain, with one to numerous melanotic tumors of various sizes in each.

altered. But the reaction governed by the enzyme may well have been occurring from the beginning, and the action of the gene have been continuous. Thus neither the diffusible nor the nondiffusible products of gene-controlled reactions really tell us when the gene is acting.

If, however—to return to the suppressor-erupt system—the x-ray-sensitive substance is not the primary product of the reaction controlled by the gene but is, instead, an essential substrate or precursor, it might be possible to answer the question by injecting body fluid or extracts from the nonreacting strains (*su-er*$^{+}$) into irradiated recipients of the reacting strain—namely, suppressor-erupt individuals in which the suppression of erupt had been blocked by destruction of the precursor but in which the remainder of the system was intact and able to use any fresh supply of the precursor. For if the sensitive substance is a precursor and not a product of the gene in question, it ought to be present in individuals of various genotypes, irrespective of their possession of suppressor-erupt and nonsuppressor alleles. These contemplated experiments are technically of great difficulty, because of the necessity for injecting material or transplanting tissues into very young and small *Drosophila* larvae. The third instar larvae customarily used as recipients are much too advanced in differentiation to serve in the present case. Still, it is hoped that indicative results can be obtained.

Nature of the Action of Suppressor Genes

Another approach would be to identify the exact chemical reaction controlled by one of these suppressor genes and then to determine whether the reaction is blocked by x-rays because of the destruction of the specific precursor or product. The analysis of biochemical, lethal mutations in *Neurospora* and in bacteria lends itself to this type of attack; but the small amount of differentiation existing in such organisms and the lethal nature of the mutations employed do not render it surprising that enzymes—such as tryptophan synthetase, to take an example—are active throughout most, if not all, of the life-cycle. Practically all that can be deduced is that when, on account of mutation, an essential gene is altered to an ineffective counterpart, the specific enzyme under the control of the gene disappears gradually, over the course of several cell generations, as though it at once had ceased to be renewed and was undergoing serial dilution to extinction.

A biochemical approach to the analysis of the action of the *Drosophila* suppressor genes was opened when it was discovered that an increase in the amount of tryptophan in the food upon which the larvae feed—up to 1 percent of the dry weight of the medium—would lead to an inhibition of the erupt-suppressor and tumor-suppressor genes nearly comparable in degree to that produced by x-raying the eggs or larvae with a dose of 1000 roentgens (*8–10*). Plaine further demonstrated that both the x-ray effect and the tryptophan effect of cysteine (*11*).

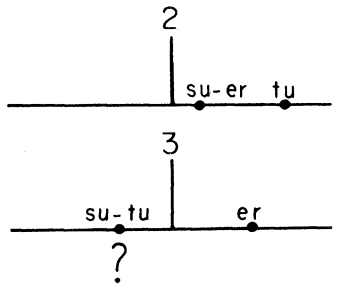

Fig. 3. Diagrams of chromosomes 2 and 3 of the double-suppressor strain. The approximate locations of the two mutants *tu* and *er* and their respective suppressors are indicated. *Su-tu* has not been located very exactly.

medium supplemented with 0.5 percent can be nullified by feeding larvae on a

The clue seems to be provided by the fact that the initial step in the degradation of tryptophan is its conversion to formylkynurenine by a coupled peroxidase-oxidase reaction. Cysteine, by reacting with the hydrogen peroxide or organic peroxides formed by ionizing radiation in tissues, would be expected to reduce the formation of formylkynurenine from tryptophan; or it would reduce the amount of peroxide normally present in tissue, so that, in the case of an excess supply of tryptophan, the latter substance could not be utilized in this particular reaction. It has thus become possible, by controlling the diet of larvae of the double-suppressor strain, to turn the action of the suppressors off and on again, at will. From this observation there has emerged a hypothesis that the suppressors themselves regulate the utilization of tryptophan in various competing pathways.

For animals, tryptophan is of course an essential amino acid. It cannot be synthesized through the coupling of indole and serine by tryptophan synthetase, as in plants and microorganisms. It is utilized in a number, perhaps a large number, of different ways (Fig. 5). It is incorporated into proteins; it is a source of the potent hormone serotonin (5-hydroxytryptamine); by way of kynurenine, it leads to the production of the brown eye-color pigment of *Drosophila* and other ommochrome pigments in other animals, and in plants to nicotinic acid and nicotinamide; and in plants, if not in animals, it is a source of the auxins which control various aspects of growth and tropic behavior. Tryptophan is thus the center of a nexus of biochemical reactions having profound consequences.

If the supply of tryptophan is normally limited in amount, the several channels of utilization must in a sense be competitive, and normal growth and differentiation will require a well-coordinated timing in the opening and closing of these channels, or the enlargement or restriction of flow along them. Thus meaning can be seen in the observations of Erk that kynurenine is present in the self-contained embryo but absent later; or that a free pool of tryptophan cannot be demonstrated to exist in the embryo, larva, or adult but is plentiful in the

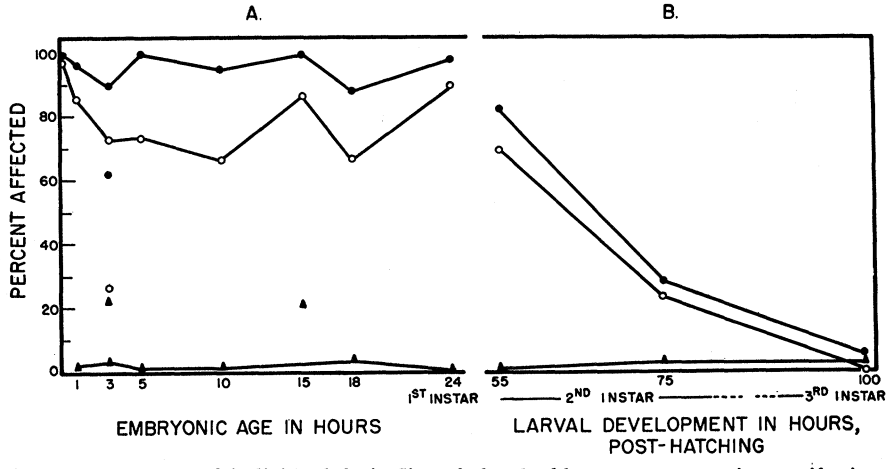

Fig. 4. Percentages of individual fruit flies of the double-suppressor strain manifesting erupt eyes when treated with 1000 roentgens of x-rays at various ages. ●, Percentage manifesting erupt to any degree, x-rayed series; ○, percentage manifesting extreme erupt, x-rayed series; ▲, percentage manifesting erupt to any degree, untreated control series (Glass and Plaine, *3*).

pupa; or, as has been mentioned, that kynurenine in the course of larval life is formed only in the gonads and Malpighian tubules.

Would it then be surprising if, as I have suggested elsewhere, the primary action of suppressor genes is exerted on quite different processes from those suppressed or enhanced? If the biochemical blocks produced by mutants in such a nexus of related processes stemming from a single point are not absolute, but, in the biochemical geneticist's phraseology, are "leaky," then the damming up of one channel may be sufficient to increase the flow past the leaky block. The evidence is clear that tryptophan itself does not accumulate to any great extent in the larvae. It is perforce used in one channel if not in another. The utilization in different ways consequently cannot be independent, and a mutant blocking one channel must influence the flow along others. A mutant blocking one channel might, in other words, act as a suppressor of a mutant blocking, or partially blocking, a different pathway. This is a more general explanation of the frequently observed nonspecificity of suppressor genes than to suppose that a suppressor gene must in some way remove an inhibitor from or repair a deformity in the very enzyme controlled by the mutant suppressed. In cases of suppressor specificity, the latter mechanisms might apply; in cases of nonspecific suppressors, a more general relationship must be sought.

A finding of paramount importance in this study was that the application of x-rays to embryos of the double-suppressor strain (*su-er; er*) produces erupt eyes, whereas the application to wild-type heterozygous for erupt (*su-er*$^+$; *er*$^+$/*er*) does not (*3, 12, 13*). In other words, the x-rays affect the action of the suppressor-erupt gene but do not affect the action of the normal allele of erupt —whence it may be concluded that the normal allele of erupt and the suppressor of erupt do not produce the normal eye phenotype by the same mechanism. The same end-result but distinct paths, one sensitive to x-rays, the other not!

We were therefore impelled to look for alternative or competing biochemical pathways. With these considerations in mind, E. Glassman and I began the search in the double-suppressor strain of *Drosophila*, and in the wild-type and other mutant strains as well, for the enzymes responsible for the conversion of tryptophan to kynurenine, 3-hydroxykynurenine, and the brown eye-color pigment (*14*). The vermilion mutant type is blocked in the formation of kynurenine from tryptophan. An enzyme system capable of producing kynurenine from formylkynurenine was readily found, its presence serving to confirm the belief that kynurenine is produced in *Drosophila* from tryptophan by way of formylkynurenine. However, the enzyme was omnipresent, in all stages of life and in all wild-type and mutant strains tested.

On the other hand, painstaking tests served only to confirm the surprising negative results of other workers: tryptophan peroxidase could not be found at all, even in pupae when there is a free pool of tryptophan, or in third instar larvae, when kynurenine is known to be formed in the gonads. Presumably, formation of the enzyme is extremely restricted, both in *site* and in *time*. Since the vermilion mutant form does contain kynurenine formamidase (the enzyme that converts formylkynurenine to kynurenine), the *v* block cannot be at that step but must involve the prior conversion of tryptophan to formylkynurenine. Green (*15*) has reported an accumulation of tryptophan in vermilion-eyed flies, but our tests show that this accumulation either is not sufficient to bring about an inhibition of the tumor and erupt suppressors, or more likely occurs only in the pupal and adult stages of life when the formation of melanotic tumors and differentiation of the eyes are past. Although it has also not been possible to show any effect of the tumor and erupt suppressors on the expression of vermilion, a distinction between the tumor and erupt suppressors has been found in the effect of feeding kynurenine to the larvae, for the feeding of kynurenine fails to inhibit the suppressor of erupt but does overwhelm the tumor suppressor (*8, 10, 16*).

In the still fruitless effort to isolate those enzyme systems responsible for converting kynurenine, by way of 3-hydroxykynurenine, to subsequent intermediates on the pathway to the brown eye-color pigment, Glassman discovered another phenomenon of great interest (*17*). This was an influence of tyrosinase and tyrosine on the disappearance of kynurenine or hydroxykynurenine in the formation of a dark-colored pigment *in vitro*. When dihydroxyphenylalanine was supplied, tyrosinase and tyrosine proved to be unnecessary. Experiments then demonstrated that a similar formation of pigment would occur spontaneously whenever any quinone was combined with an aromatic amine, such as kynurenine or hydroxykynurenine.

The production of these "aminoquinone" pigments suggests a simple explanation of a puzzling nonspecific suppressor gene action which has become virtually a classic case—namely, the suppression by a single *Drosophila* suppressor gene of both the vermilion eye-color and sable body-color mutants. The puzzling element has been the lack of any known connection between the biochemical formation of the ommochrome pigments, derived from tryptophan, and the melanins, derived from tyrosine.

Why should a restoration in the production of the brown eye-color pigment, which is an ommochrome, result simultaneously in a diminution in the production of melanin? If, according to the scheme depicted in Fig. 5, the brown eye-color pigment is an aminoquinone complex, formed from a quinone derived from tyrosine as well as from the aromatic amine hydroxykynurenine, and if the available supply of the quinone in the body is limited, then obviously a restoration in the blocked supply of kynurenine brought about by the suppressor of vermilion, either directly or indirectly, would once again draw upon the supply of quinone and consequently reduce the amount available for deposition in the hypodermis under the influence of the mutant sable.

This is clearly not the entire story. It fails to account for the tissue-specific aspects of pigment formation: Why is om-

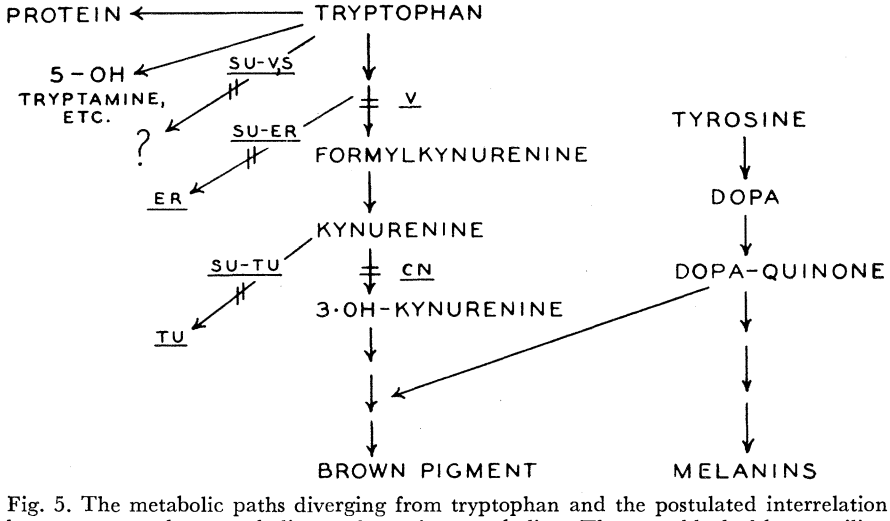

Fig. 5. The metabolic paths diverging from tryptophan and the postulated interrelation between tryptophan metabolism and tyrosine metabolism. The steps blocked by vermilion and cinnabar are marked by transverse bars, and the postulated steps blocked by various known suppressor genes that affect tryptophan metabolism are similarly indicated.

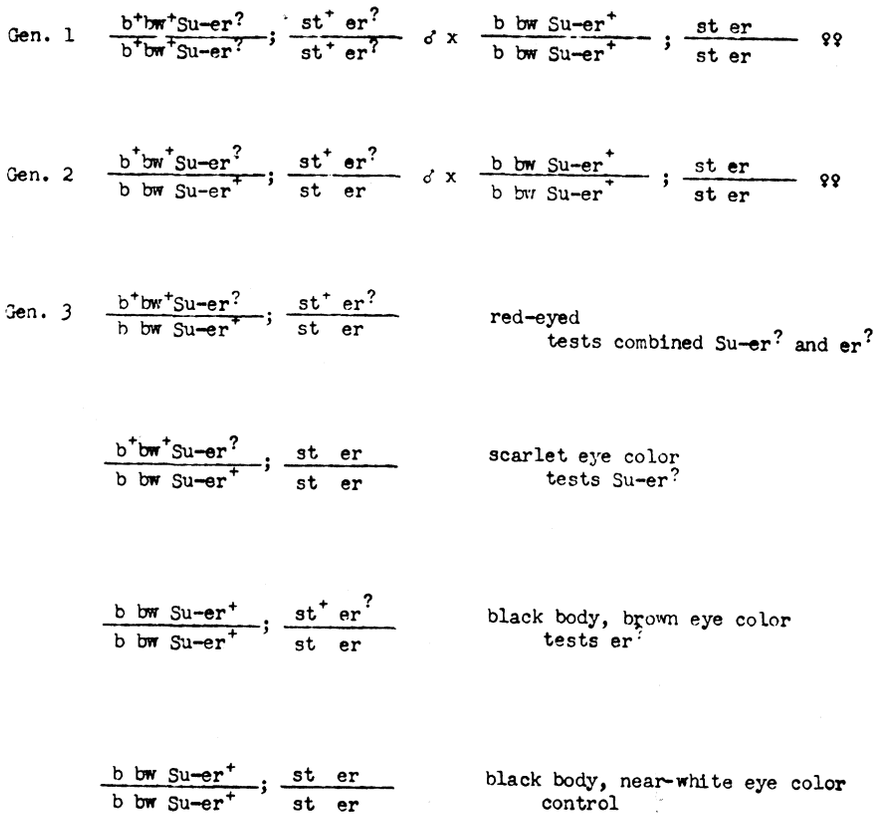

Fig. 6. Scheme of the crosses used to investigate the constitution of wild-type strains with respect to the alleles at the suppressor-erupt and erupt loci. *b,* Black (body color); *bw,* brown (eye color); *st,* scarlet (eye color); *er,* erupt eye; *Su-er,* suppressor of erupt eye. + Superscript: +, wild-type alleles of the aforementioned mutants; ?, alleles of unknown nature and potency which are being tested.

mochrome formed only in the eye, and melanin chiefly in the hypodermis? Or why does the suppressor of vermilion not reduce the melanin pigmentation of the wild-type or of other melanic mutants such as ebony and black? But such differences may lie at later stages of each pathway; certainly the tissue-specific effects must do so.

What is most encouraging is the way in which the discovery of the long-suspected connection between the formation of pigments from tyrosine and tryptophan permits an explanation of the possible mode of action of nonspecific suppressor genes that supports the hypothesis previously developed on the basis of the study of the erupt and tumor suppressors. The gene interactions postulated here resemble some of the theoretical schemes drawn up by Strauss and reinforce his conclusion that "the gene interaction obtained is not in any sense an interaction of the genes themselves, but is rather an interaction of the gene-controlled step reactions in non-genic parts of the organism" (*18*).

General Distribution of Suppressor Systems

The high frequency with which, in *Neurospora,* bacteria, and *Drosophila,* apparent reverse mutations have been found to be in actuality the result of mutations at a different locus that suppress the expression of the mutant—or, put otherwise, restore the normal or original phenotype—is well known. Surely every geneticist who works with such phenomena must be impressed with the remarkable capacity of developing organisms to achieve the same goal by various means.

These suppressor genes are not limited in occurrence to laboratory experiments. The discovery in my laboratory of the existence in a single strain of *Drosophila* of two independent suppressor systems, concealing the presence of two presumably detrimental mutants, made me, of course, curious to know how many other wild-type strains (insofar as erupt eyes and melanotic tumors are concerned) might be concealing these mutants by virtue of their possession of the suppressors. Assuming that the expression of these mutants is to some degree detrimental, would they not be entirely neutral when suppressed? Might they not even have effects which, not altogether suppressed, could in certain circumstances become advantageous? In other words, what would be the relative selective merits of the nonsuppressor coupled with the normal allele of the mutant, as against the suppressor coupled with the mutant?

The analysis has so far been carried out only with the suppressor-erupt system (*12, 19*). Because of the fortunate placement of the suppressor and the mutant in different chromosomes, already marked in the original strain by the mutant eye colors brown and scarlet, it was possible without great difficulty to develop a test strain for the erupt system. This test stock carried *bw* in chromosome 2 but no suppressor of erupt; in chromosome 3 it carried *er* and *st.* Flies of different wild-type laboratory stocks and samples of different natural populations could be crossed to the tester stock, and F_1 males could then be backcrossed to the tester strain. The resulting progeny (Fig. 6) would segregate into four eye-color types (wild-type dark red; scarlet; brown; and very pale brown scarlet), representing the inheritance from the wild-type strain, respectively, of both chromosomes 2 and 3, of chromosome 2 only, of chromosome 3 only, and of neither.

The fourth of these classes is the reconstituted tester strain and serves as a control to rule out any significant effect of either the sex or fourth chromosomes on the expression of the character. Fifteen wild-type strains collected from various parts of the world and kept in laboratories with more or less inbreeding for a period of years were tested in this way; and to them were added some freshly collected samples of wild populations from St. Louis, Mo. The results may be summarized very briefly.

No evidence was found for any suppressors of erupt in the X- or fourth chromosomes. Although the evidence is not conclusive that the suppression of erupt by the various second and third chromosomes tested was entirely located at the two loci of the suppressor of erupt in chromosome 2 and of erupt in chromosome 3, these loci must at least be responsible for the principal amount of effect. Great differences were found between strains and also within strains. No stock appeared to be homogeneous for erupt suppressors, except the long-inbred Florida-19 stock. Some strains possessed strong suppressors in chromosome 2 with potent "normal" alleles of erupt in chromosome 3. Some possessed potent "normal" alleles of erupt but weak suppressors. Others possessed strong suppressors in chromosome 2, but very weak "normal" alleles of erupt, even to the point of producing the erupt phenotype. From a number of stocks, such as Swedish-b and Urbana, it has proved possible to isolate erupt itself. Eggs of these wild-type strains, when x-rayed with 1000 roentgens, produce characteristic frequencies of erupt-eyed flies. In short, not only the laboratory-kept wild-type strains, but also the freshly collected ones, are almost universally seeded with the erupt mutant; but because of the sup-

pressors and wild-type alleles also present, the erupt phenotype was never observed, or at least reported, prior to my own analyses.

By comparing the relative strengths of the second chromosome suppressors of erupt (Fig. 7), and also those of the several normal alleles of erupt of differing potency (Fig. 8), it can be concluded that at least five significantly different strengths of suppressor and a like number of different normal alleles of erupt exist in a variety of naturally occurring combinations. Particularly noteworthy is the fact that in every case the combination of a suppressor in chromosome 2 and the normal allele in chromosome 3 (individuals of the wild-type eye color in our four-type segregation) produces a far greater suppression of erupt than would be expected from an additive effect of the two (Fig. 9). Consequently, when erupt does occur in a population and is heterozygous, as is usually the case, it is completely suppressed because of the multiplicative interaction of its own normal allele with the two genes at the suppressor locus.

Several studies by other geneticists have revealed a similar situation in *Drosophila*. Gardner *et al.* have reported the existence of a considerable variety of modifiers of two tumorous-head genes in eight tested wild-type strains (*20*). More similar to the erupt and suppressor-erupt phenomena is a situation described by Sturtevant (*21*) at the meeting of the American Institute of Biological Sciences in Storrs, Conn., last summer. Isoalleles of scute and of achaete, derived from different strains, were found to vary in dominance over a wide spectrum in potency. The normal alleles of the mutant also differed in potency. Sturtevant estimated that at least 4, and more likely 10 to 15, wild-type alleles of scute of different potency could be isolated from these strains. The quite distinct wild-type alleles of achaete were similarly of different strengths. These phenomena are strikingly like the existence of the numerous wild-type isoalleles of erupt and the occurrence of suppressor-erupt alleles likewise of different potency.

This sort of genetic situation must therefore be regarded as not rare, in fact, as probably a very common one. In my own experience, which includes the investigation of a number of loci not discussed here, it is commoner than the polygenic type of modifier system which has been so much emphasized in recent years. What, then, is its evolutionary significance?

To me it seems to fit clearly into the category of phenomena regarded by Schmalhausen as the product of "stabilizing selection" (*22*). To quote: "In the course of evolution due to a severe elimination of all deviations from the well-adapted standard form, a more or less complicated system of regulating (inclusive of buffering) mechanisms is created. This system tends to preserve normal development when the deviation from the standard of both internal and external factors is not too great" (*22*; quotation cited from Lerner, *23*, p. 103). And elsewhere, Schmalhausen has said, "Stabilizing selection produces a stable form by creating a regulating apparatus. This protects normal morphogenesis against possible disturbance by chance variations in the external environment and also against small variations in internal factors (i.e., mutations)" (*24*). And Schmalhausen cites Gershenson as having demonstrated that *Drosophila* populations may contain "dominant mutants whose appearance is suppressed in the genotype of the given population or whose appearance is attenuated considerably."

Another way of characterizing the effect of such a system is to relate it to "genetic homeostasis." If they are prevalent in natural populations, such systems must be extremely important in buffering the genotype against the effects of frequent, critical mutations. Particularly important would be the suppression of mutations that alter the "switch genes" which control the differentiation of serially homologous structures, and which when mutated produce that extraordinary category, the homeotic mutants. Thus Buzzati-Traverso has observed a progressive amelioration of the *Drosophila* mutant aristopedia subsequent to its origin by mutation (*25*). (It is possible, one might note, to regard erupt itself as a homeotic mutant, one in which a portion of the eye develops as an antennalike structure—but this may be a rather superficial view.) In any case, it seems to me that a new chapter might be added to Lerner's book on *Genetic Homeostasis* (*23*) in order to deal with this kind of system over and above the polygenic ones so well analyzed in it. Thus Lerner (*23*, p. 103) comments that Schmalhausen failed to visualize developmental homeostasis "as the specific property of heterozygotes." It seems, however, that it is also a property of epistatic, that is, suppressor-systems, and the distribution of these in Mendelian populations in differently balanced combinations satisfies Lerner's definition of genetic homeostasis as "the property of the population to equilibrate its genetic composition and to resist sudden changes" (*23*, p. 2).

If organisms can maintain the "normal" phenotype by a variety of genetic systems which restore the balance between competing biochemical pathways, then the evolution of isolated populations might well be expected to diverge in respect to possession of suppressor systems of different composition. The divergence might be virtually at random and might be promoted by random genetic drift, unless the particular components of the gene system in question have subsidiary phenotypic effects which would subject them to the action of natural selection. In the latter case, just as new genes have been postulated to arise through the divergence in function of duplicated genes,

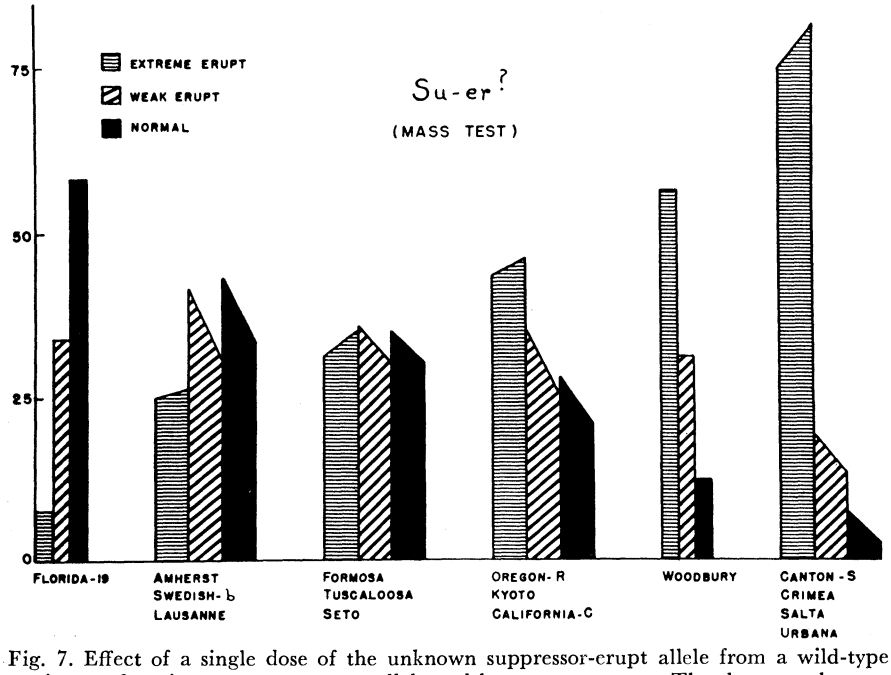

Fig. 7. Effect of a single dose of the unknown suppressor-erupt allele from a wild-type strain tested against a nonsuppressor allele and homozygous erupt. The slopes at the tops of the bars represent the difference between the highest and lowest values for the strains in each group.

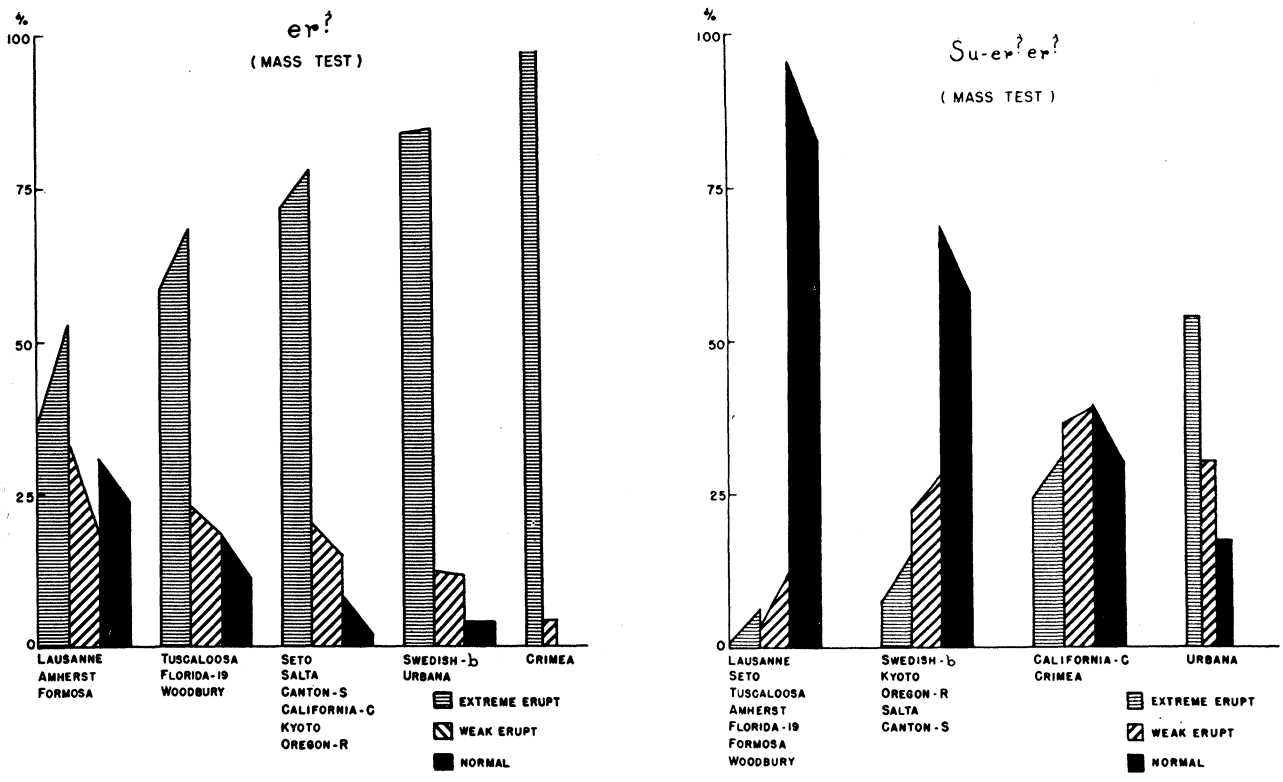

Fig. 8 (Left). Effect of a single dose of the unknown erupt allele from a wild-type strain tested against an erupt allele and homozygous nonsuppressor. Fig. 9 (Right). Interaction of single doses of both the unknown suppressor allele and the unknown erupt allele from a wild-type strain, tested against one nonsuppressor and one erupt. The slopes at the tops of the bars represent the difference between the highest and lowest values for the strains in each group.

so too new genes might arise through the divergence of balanced "suppressor-plus-mutant" systems. A mutant gene that is neomorphic in nature and that is inadequately suppressed in respect to its adverse effects by its own normal allele may be tolerable when it is suppressed by a gene at another locus and may then have an opportunity to become established on the basis of its subsidiary, advantageous effects, if any.

With this reasoning in mind, an attempt was made to determine whether *Drosophila simulans* and other *Drosophila* species carry an established erupt-suppressor-erupt system (*12, 19*). Hybrids between *D. simulans* (from several geographic regions) and *D. melanogaster* erupt proved to be mostly wild type, but 2 to 8 percent were strongly erupt, in respect to the eye. The results were very similar to those from the majority of the *D. melanogaster* wild-type strains. In other words, *D. simulans* does in fact carry an established suppressor-erupt gene. (An earlier report to the contrary (*12*) is attributable to the fact that many of the hybrids between *D. simulans* and *D. melanogaster* have very disarranged eye facets, which may be confused with erupt.) *Drosophila simulans,* as well as several species which cannot be hybridized with *D. melanogaster,* were further tested by exposing the embryos to 1000 roentgens of x-rays. This test, of course, would detect only the presence of the entire system consisting of the suppressor plus the erupt mutant. The results were somewhat inconclusive, but in general no clear sign of the presence of erupt in combination with its suppressor was found in these species. The suppressor is thus more widely distributed than erupt itself, and the balanced system as a whole is of recent evolutionary origin.

The trail leads on into more and more engaging areas of investigation. Let me then conclude by reasserting my belief that in the pursuit of the gene there is merit in sticking to a single trail until the quarry is treed. At least in some instances, most of the major unsolved questions of genetics and development will become involved, and some measure of enlightenment may follow (*26*).

References and Notes

1. S. Benzer, in *The Chemical Basis of Heredity,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1957), p. 70.
2. B. Glass, *Genetics* **29**, 436 (1944).
3. B. Glass and H. L. Plaine, *Proc. Natl. Acad. Sci. U.S.* **36**, 627 (1950).
4. F. Erk, unpublished results.
5. C. Stern, *Genetics* **21**, 625 (1936).
6. See P. Hartman, in *The Chemical Basis of Heredity,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1957), p. 408.
7. T. M. Sonneborn, *Harvey Lectures Ser.* **44**, 145 (1950).
8. B. Glass and H. L. Plaine, in *Amino Acid Metabolism,* W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1955), p. 940.
9. B. Glass and H. L. Plaine, *Proc. 9th Intern. Congr. Genet. Caryologia* **6**, suppl., 1163 (1954).
10. H. L. Plaine and B. Glass, *J. Genet.* **53**, 244 (1955).
11. H. L. Plaine, *Cancer Research* **15**, 151 (1955).
12. B. Glass, *Proc. 8th Intern. Congr. Genet. Hereditas* suppl. 582 (1949).
13. B. Glass and H. L. Plaine, unpublished.
14. E. Glassman, *Genetics* **41**, 566 (1956).
15. M. M. Green, *ibid.* **34**, 564 (1949).
16. B. Glass and H. L. Plaine, unpublished data.
17. E. Glassman, *Arch. Biochem. and Biophys.* 67, 74 (1957).
18. B. Strauss, *Am. Naturalist* **89**, 141 (1955).
19. B. Glass, unpublished data.
20. E. J. Gardner and G. H. Stott, *Genetics* **36**, 72 (1951); E. J. Gardner and F. J. Ratty, *Genetics* **37**, 49 (1952).
21. A. H. Sturtevant, personal communication.
22. I. I. Schmalhausen, *Zhur. Obshchei Biol.* **6**, 3 (1945).
23. I. M. Lerner, *Genetic Homeostasis* (Wiley, New York, 1954).
24. I. I. Schmalhausen, *Factors of Evolution,* I. Dordick, translator (Blakiston, Philadelphia, 1949), pp. 79–80.
25. A. Buzzati-Traverso, *Natura* **38**, 45 (1947).

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