Expression of hybrid vigour in Drosophila subobscura

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GENE STRUCTURE AND ACTION IN RELATION TO HETEROYSIS

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The working hypotheses proposed for the interpretation of heterosis in terms of gene action can be reduced to two essential types. One is that of interaction between non-allelic genes; the other that of interaction between alleles. Examples of both types of action are, of course, known in physiological genetics. But while many examples of gene interactions have been analyzed in biochemical terms, only two of ‘one-gene heterosis’ have been so analyzed: sickling in man (Pauling, Itano, Singer & Wells 1949), and Pab in Neurospora (Zalokar 1948).

Physiological genetics does not provide any crucial argument to choose between a model of heterosis based on gene interactions and one based on allele interactions. Furthermore, advances in our knowledge of gene structure and action in recent years have led to the realization that there may be no absolute distinction between alleles of one gene and alleles of different genes. If this is so, the distinction between the two types of models for heterosis is one which has no longer a precise meaning, though it may still be useful at certain levels of approximation.
In earlier genetics the tacit assumption has been that the three ways in which the gene could be defined—unit of crossing-over, unit of mutation and unit of physiological action—were co-extensive. Even the discovery by Sturtevant (1925) of the position effect did not lead to the rejection of this tacit assumption; the position effect could be considered the consequence of localized interactions between distinct genes (Muller 1938). We owe it to Goldschmidt (review 1951) to have stressed for long that, on the contrary, the position effect made it necessary to re-appraise our views on gene structure and action. But we owe to Muller (Raffel & Muller 1940) the clearest statement of the previous conceptual limitations.

In the part dealing with the divisibility of the genetic material, the paper by Raffel & Muller analyzed the problems of gene structure and action in a way which has been ignored repeatedly but not surpassed by later workers. Raffel & Muller used X-ray-induced rearrangements in the *scute* region of the X-chromosome of *Drosophila* and came to the conclusion that chromosome breakage could differentiate at least four sections within this region, mutants at each acting as allelic of the others. The previous work of Dubinin (1929) had also suggested a highly compound and integrated structure for the *scute* region.

In the work of all three of these schools (Goldschmidt, Dubinin and Muller), chromosome regions behaving as a unit in hereditary transmission were shown to be divisible by chromosome breakage or separate mutation. Since 1945 there has been progress in three new directions. First, further examples of divisibility by mutation, e.g. D. Lewis's (1949) work on mutation of the incompatibility gene in *Oenothera* and Stadler's (1951) work on *R* in maize. Secondly, divisibility by crossing-over, as exemplified by the work in our laboratory in *Aspergillus* and *Drosophila* (summaries, Pontecorvo 1952a, 1954); and thirdly, divisibility by 'position effect' as exemplified by the work of E. B. Lewis (summary, 1954) on 'position pseudoallelism' in *Drosophila*. An additional point is that the properties first found in the *scute* region of *Drosophila* seem to be the rule rather than the exception. These properties have already been verified partially or completely in at least seven regions in *Drosophila*, five in *Aspergillus*, two in *Neurospora*, two in maize, and probably overlooked or interpreted differently in many other cases. Indeed, they have been found in all cases in which they have been looked for with techniques capable of detecting them.

Let me summarize the present position with one example out of the five investigated in our laboratory in *Aspergillus*. This example is based on work of Pritchard (1955). It consists of the analysis of a group of seven independently arisen mutants, all of them differing from wild type in one gene, all of them requiring adenine for growth, and all of them very closely linked to a visible marker (*y*) with which they give less than 1 per 1000 recombination. All these mutants are recessive in the diploid as well as in the heterokaryon. Five of them are phenotypically indistinguishable from one another. Two are distinguishable from the others by being able to grow to a certain extent even in the absence of adenine. On criteria both of localization relative to other markers and of physiological allelism, we would call the seven mutants allelic to one another. We refer to them as the alleles of the
Gene structure and action in relation to heterosis

Using the Drosophila terminology we would go even further and say that five of them are the result of recurrence of the same mutation, while the last two are the result of mutation to different alleles. Pritchard tested, two by two, four of these alleles (ad-16, ad-11, ad-8, ad-10), and by means of the closely linked marker y on one side, and of another marker, bi, 5 cM (= 5 centimorgans) away on the other side, he found that adenine-independent cross-overs arise with frequencies varying from 3 in $10^5$ between ad-10 and ad-8, to 1-4 in $10^3$ between ad-16 and ad-8. The four alleles can be localized within a region less than 1 cM in length, in the linear order in which I have enumerated them above. Three more mutants are located between y and ad-8, but they have not yet been tested in all possible combinations with the others and among themselves.

That crossing-over and not some other process accounts for the origin of the adenine-independent recombinants was shown by an ingenious technique devised by Roper (Roper & Pritchard 1955). This technique makes use of a property of alleles, viz. the position effect discovered by Sturtevant (1925) for the somewhat special case of Bar in Drosophila, and then shown by E. B. Lewis (1945) to be of more general occurrence in Drosophila even in cases in which unequal crossing-over is not involved. In the case of Star, Stubble, bithorax (E. B. Lewis 1945; 1950), vermilion and lozenge (Green & Green 1949; Green 1954), white (MacKendrick & Pontecorvo 1952, E. B. Lewis 1952), all in Drosophila, as well as in the three fully analyzed cases in Aspergillus (Roper 1950, 1953; Pritchard 1955), a double heterozygote in trans $(+m_2/m_1-)$ is mutant or more mutant, while the heterozygote in cis $(++/m_2m_3)$ is non-mutant or less mutant. I propose that this phenomenon should be called the 'Lewis effect', instead of 'position pseudoallelism', as E. B. Lewis himself suggested because he was implying a special rather than a ubiquitous feature of alleles.

The technique by Roper mentioned above consists in making a diploid Aspergillus heterozygous in trans for any two alleles of independent origin. In the specific example which I am giving here this heterozygote requires adenine. If a mitotic crossing-over occurs between the two alleles the two complementary cross-overs will carry, respectively, in one chromosome both mutant alleles and in the other chromosome both non-mutant. In other words, mitotic crossing-over in a heterozygote in trans will give origin to nuclei heterozygous in cis; the former determine adenine-requirement, the latter adenine-independence. It is therefore only a matter of plating large numbers of cells of the heterozygote in trans on a medium lacking adenine, and the cross-overs, no matter how rare, will be selected. Once so obtained, the heterozygotes in cis can be analyzed and their genotype ascertained. In this way Roper & Pritchard (1955) have been able to make sure that it is really crossing-over between alleles which gives origin to new alleles from a heterozygote in either cis or trans.

In the case of alleles of the ad-8 series, Pritchard (1955) identified four distinct 'mutational sites' (Pontecorvo 1952a), separable by crossing-over, for the first four mutants analyzed. The three remaining represent certainly a mutational site or sites different from two of these four, but we do not yet know whether some or all are different from the other two and/or from each other. We have, thus, at least
four and at most seven mutational sites for seven independently arisen mutants. All this within a region less than 1 cM in total length.

Earlier examples of the same kind of analysis were the bi and paba regions studied by Roper (1950, 1953). In the case of the bi region three phenotypically identical mutants of independent origin represented three sites of mutation distinguishable by crossing-over. In the case of the paba region two independent mutants represented two sites of mutation. Taking into account two further not yet fully analyzed examples in Aspergillus—the ad-9 and the pro regions—and those in other organisms, it looks as if any one such region, less than 1 cM in map length, contained usually several, perhaps very many, mutational sites separable by crossing-over. That the mutational sites are more than just a few is suggested by the data mentioned earlier; in fact we have as yet no case, among the fully analyzed material, of two allelic mutants not separable by crossing-over. Mutation at each of the sites gives origin to alleles not necessarily distinguishable in phenotype from those at other sites. The mutant alleles within the whole region behave, physiologically, all as alleles of one gene and show the Lewis effect in combination with one another.

I wish now to consider another type of relation: contiguous chromosome regions which, though having similarity of phenotypic effect, behave as complementary in the heterozygote. Dunn (1954) calls situations of this kind ‘complex loci’. There are over sixteen examples in Drosophila (Komai 1950), at least one in the mouse (the t series, Dunn 1954) and many in various other organisms (Pontecorvo 1952a; Stephens 1951). The situation is, in these cases, the same as in the case of alleles except that there is no Lewis effect and the double heterozygote, either in cis or in trans, is non-mutant or less mutant than the homozygote for either or both mutant alleles. In individual cases, crossing-over may or may not have been observed within such ‘complex loci’.

In older terminology, ‘complex loci’ would have been classified as pairs or clusters of closely linked different genes with similar phenotypic effects; they can be subdivided by mutation, and by different, though related, activity as shown by the complementary effect in the double heterozygote. In some cases they have also been subdivided by crossing-over. Of course, separability by crossing-over is purely an operational criterion dependent on the resolving power of the experiments which one can and is prepared to do. For instance, in the case of the t series in the mouse, Dunn (1954) has failed to obtain any recombination between pairs of mutants in a total of 20000 gametes tested. On a sample as small as this, in many of the cases of allelism in Drosophila and Aspergillus crossing-over would not have been detected.

Clearly, the distinction between alleles within one of the regions mentioned before, and alleles at ‘complex loci’, in Dunn’s definition, is one which rests exclusively on mode of action. In one case we have the Lewis effect, in the other case we have complementarity.

For a working model of the organization of the chromosome over minute regions it may be useful to assume that the difference between the two types of relation is a consequence of spatial organization, in first approximation of distance apart. In a very crude way, it could be supposed that mutational sites very close to one another
Gene structure and action in relation to heterosis

175

other give origin to alleles showing the Lewis effect, and mutational sites farther apart give origin to alleles showing complementarity. The assumptions behind this model are the ones which I proposed some years ago (Pontecorvo 1950, 1952a, b). If we consider stepwise reactions occurring on the surface of a chromosome in an assembly-line fashion—i.e. ordered in space sequence correspondingly to the time sequence—a rate of millimicromolar order of the reactions, instability or non-diffusibility of the intermediates could all account singly or in combination for the Lewis effect. However, the Lewis effect could no longer operate when the distance apart between two mutational sites is greater than the average distance between two homologous chromosomes.

An alternative model, not very different, is one of systems of reactions occurring within an enclosed microvessel along the chromosome. In a very naive way we can think of a pipe with a number of holes: at one hole an abundant substrate gets in and goes through successive reactions as it moves along the pipe and gets out at the next hole. The mutational sites within the pipe in a stretch between two successive holes would give origin to alleles showing the Lewis effect, but mutational sites in two different stretches of the pipe would be complementary. Clearly, one could substitute for this ultra-mechanistic picture one based on physical chemistry in which the enclosed vessel is only a fiction standing for the greater probability of a molecule of substrate going from one adsorption to the successive one rather than diffusing away.

As far as I am aware, regions, say 1 cM in length, in which some alleles show the Lewis effect and others show complementarity, have not yet been described; a possible exception is that of amz andlz in Drosophila. Up to a short time ago we did not have any such example in Aspergillus. We had a number of regions where the mutants behaved as allelic with the Lewis effect, and at least one (ad-1, ad-3, Pontecorvo 1952 a) where the mutants behaved as complementary. We seem to have now two regions which show both kinds of relation and intermediate relations, respectively. One is a region (pro) mutation at which determines requirement for proline. In it Mr Forbes has found some mutants to show allelism and others complementarity. Another is a region (ad-9) mutation at which determines requirement for adenine. In it Dr Calef has found two mutants to behave as incompletely complementary; there is no detectable complementary effect in the heterokaryon in trans, and there is only a partial one in the heterozygote in trans. The analysis of both these regions is still too incomplete to say more than that it looks promising.

There is one important point to consider now. How far we can go to identify qualitatively distinct action between alleles. The clues come from two types of facts. One is the specificity of suppressors, the other is immunological diversity. That suppressors may be specific for some but not others of a series of alleles showing the Lewis effect was first demonstrated by Green (1954) in the case of the vermilion region in Drosophila. It was then confirmed by Pritchard (1955) for the ad-8 region in Aspergillus. In both these cases the suppressor is not closely linked to the region—vermilion or ad-8—which it ‘suppresses’. But MacKendrick (1953) has found in the white region of Drosophila a situation which is more readily interpreted as
a partial suppressor within that region. A double mutant—i.e. mutant at two sites within the region—has a less extreme mutant phenotype than a mutant at one only of these sites.

While it is easy to think of models which could harmonize the assembly-line model mentioned before with effects of suppressors within the region which they ‘suppress’, it is less easy to do so for suppressors located elsewhere. In any case, the detailed study, both genetical and physiological, of suppressors and back mutants is in such a preliminary stage that not much can be said at present.

Genic effects on immunological specificity are probably the most suitable material for the analysis of the ultimate organization of minute chromosome regions. Unfortunately, in this field so far the immunology and the genetics are out of step. On the one hand there are investigations like those of Fox (1954) in Drosophila which are not sufficiently refined on the immunological side, while they are on the genetical side; on the other hand, there is the excellent work on blood antigens in birds and mammals, which is very advanced immunologically but not so genetically (e.g. Race & Sanger 1954).

Let us assume, for instance, that the ABO region in man has an ultimate architecture of the same kind as that of, say, lozenge in Drosophila or ad-8 in Aspergillus, and that the configuration of the ABO antigens reflects the configuration of this chromosome region. Changes in one or more mutational sites along this region will result in changed antigenic specificity, or perhaps in no antigen at all; the antigens, and their absence, will behave as alleles in heredity. Mutational changes could be distinguished qualitatively by the immunological reactions of the resulting antigens. If the antigenic configuration were also active enzymatically, and our methods of distinguishing one allele from another were a growth response or a test of enzyme activity, we could distinguish the various alleles only quantitatively: those with full activity we would call ‘wild type’ and the others hypomorphic mutants.

This situation, which occurs in all the examples mentioned before in this paper, does not imply that alleles differ only quantitatively, but that, except in the case of antigenic effects, our methods of distinguishing them detect only quantitative differences.

We see now why the genetics of antigens—in organisms in which the genetical part of the story is manageable—would be of crucial help in probing the mechanism of the Lewis effect. If antigens A and B were the consequence of different configurations in the corresponding chromosome region, a heterozygote in trans should produce two different molecular species just as a heterozygote for sickling produces two kinds of haemoglobin. A heterozygote in cis should also produce two molecular species, but their immunological properties would not necessarily be predictable from those of the two antigens in the trans heterozygote. They might be AB and no antigen, or two antigens either or both different from A and B. If antigens do really reflect a configuration of the corresponding chromosome region, there seems to be no reason to suppose that the cis arrangement should determine an antigen which is merely the sum of the two determined by the trans arrangement; a new configuration may well arise, though not necessarily always, from the juxtaposition of two different halves. However, the hybrid antigen found by Irwin (1947) in
Gene structure and action in relation to heterosis

birds heterozygous in trans for certain antigen-controlling genes, and the supposed Lewis effect found by Race, Sanger, Levine, McGee, Van Loghem & Van den Hart (1954) in the Rh region in man could be taken as examples against the preceding hypothesis. But in neither case are the genetics and the immunology sufficiently complete.

In conclusion, the first steps for an understanding of heterosis, and what is more important, rational use of it in animal and plant breeding and marriage counselling, should be towards a better understanding of gene structure and action. Only then would the approach at the higher level of population genetics become illuminating.

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