STUDIES ON THE NATURE AND FUNCTION OF POLYGENIC LOCI IN *DROSOPHILA*

II. THE SUBTHRESHOLD WING VEIN PATTERN REVEALED IN SELECTION EXPERIMENTS

JAMES N. THOMPSON, Jr.

Department of Genetics, University of Cambridge, Milton Road, Cambridge CB4 1XH

Received 22.iii.74

SUMMARY

Vein patterns have been studied in lines of *Drosophila melanogaster* selected for increased and for reduced expression of the mutants plexus and net. Within each selection line, and in certain crosses involving these and other vein mutants and selection lines, the pattern of extra venation at all levels of mutant expression is highly specific. Measurements of the position and frequency of vein fragments allowed a series of profiles to be constructed which represent the relative tendency to form veins at various positions on the wing. The general phenotypic effects of many vein mutants as well as the overall action of selected polygenic modifiers of vein length are discussed in relation to the resulting model of subthreshold developmental potential. Correlations between this subthreshold pattern and the veins of related Diptera suggest that many of the subthreshold veins may be remnants of ancestral venation.

1. INTRODUCTION

THE study of pattern in developmental processes can be approached in a variety of ways. Waddington (1973, p. 501) has stressed that the "causal structure of a pattern . . . can be successfully investigated only when we can find ways of producing controlled changes in it". One way of producing such controlled changes is by artificial selection, for selection may make the experimental population approach or reach homozygosity for polygenic alleles that have the desired effects upon the character, and thus changes the phenotype in a predetermined direction by small steps.

It is the aim of this series of papers to investigate the development of quantitative characters and the developmental relevance of polygenic modifiers in natural populations. There are, of course, limitations to the application of quantitative genetics to problems of development. As Wallace (1968) has pointed out, an understanding of the precise action of genes cannot follow directly from an analysis of phenotypic frequencies. But the possible qualitative changes and some general patterns of phenotypic response can be shown by such an approach (Maynard Smith and Sondhi, 1960, 1961; Sondhi, 1961, 1962; Falconer, 1967; Carlson, 1970), and the technique of isolating and manipulating individual components of a polygenic system (Thoday, 1961) can take one further by showing the specific ways in which the development of the selected individuals has been altered to produce the observed phenotypic response (Spickett, 1963; Mohler and Swedberg, 1964).

Thompson (1974) has shown that polygenic backgrounds which had been selected to increase or decrease the expression of vein mutants have qualitatively similar effects upon other phenotypically related mutants.

This has been confirmed in chromosome substitution experiments, in which chromosomes from Long and Short L4 vein selection lines were found to produce qualitatively similar effects upon L4 vein length in non-homologous mutants (Thompson, 1973). It follows that at least a proportion of the polygenes which affect the expression of wing vein mutants act independently of the major mutant. They apparently produce their phenotypic effects indirectly, by affecting common steps in some developmental process leading to the formation of vein material.

In addition, Thompson (1974) demonstrated a direct relationship between the action of a selected background in vein-gap mutants, such as veinlet, and extra-vein mutants, such as plexus. Crosses between selected lines showed that the selected polygenic background which caused an increase in vein material in veinlet also increased the size and number of extra vein fragments in plexus. The converse was found in crosses between lines selected for a decrease in total vein material.

This paper explores the vein patterns which are revealed in selection lines of the mutants plexus, net, and in certain mutant combinations. The patterns are found to be similar in a wide variety of mutants and to show analogies with normal veins in related families of Diptera. These findings are discussed in terms of a subthreshold vein pattern model which provides a conceptual relation between the action of selected polygenic backgrounds and the phenotypes of the various classes of mutants which affect the veins in *Drosophila melanogaster*.

2. MATERIAL AND METHODS

Stocks of the mutant plexus (*px*, 2-100.5) and net (*net*, 2-0.0) were outcrossed to a newly captured wild type stock, Eversden-14, and reseeded to provide new mutant stocks with increased genetic variation. From each of these mutant stocks two lines were selected for increased expression of the mutant, *i.e.* for greater amounts of extra venation, and two were selected for decreased expression, *i.e.* for fewer vein fragments. All selection lines were maintained in duplicate cultures at $25 \pm 1^\circ$ C. in half-pint bottles on a standard oatmeal and molasses medium seeded with a suspension of live yeast.

Both of these mutants cause the formation of branches and fragments of vein material, particularly near the tips of the longitudinal veins and margin of the wing (Lindsley and Grell, 1967). The nomenclature of *Drosophila* wing cells and veins is complicated by the fact that a number of different terminologies are in use. Morphological terminology (fig. 4) is generally used in descriptions of wing development and by many systematists in assigning names to the veins which characterise the various families of Diptera. A non-morphological nomenclature (fig. 2, *a*) is generally used by geneticists and by some systematists, however. Since it is the terminology which is most familiar to geneticists in the description of *Drosophila* mutants, it will be used throughout, with the exception of one section in which comparisons are made among Dipteran families to which the non-morphological nomenclature cannot readily be applied.

All lines responded readily to selection. The positions in which vein fragments appear was studied in mounted wings and in photographs of samples from selected lines with differing degrees of mutant expression.

The actual responses to selection are not of primary importance to this discussion, and it is sufficient merely to describe the average phenotype of the extremes reached by the *px* and *net* selection lines at generation S-12, after they had reached a plateau.

Flies in the *px* Low lines often have no extra vein material, though small fragments in the marginal, second or third posterior cells, or a slight terminal bend in the L4 vein are observed in some individuals.

There is no overlap between the phenotypes in the *px* Low line and those in the *px* High line. The wings of flies from *px* High always have large branches from the L2 vein or long fragments in the marginal cell. A complete or nearly complete longitudinal vein is present in the third posterior cell, and there are always disturbances near the tip of the L3 vein. Although more extreme modifications often occur, those described here will serve to illustrate the differences in phenotype which distinguish the *px* Low from the *px* High lines.

The degree of difference between the *net* Low and the *net* High lines is similar to that distinguishing the *px* lines, except that the *net* Low lines have a slightly higher frequency of vein defects than the corresponding *px* Low lines. The *net* High flies look much like the *px* High flies in the amount and pattern of extra venation present. The wings of *net* High flies, however, often have blisters, *i.e.* areas in which the two wing surfaces have failed to join properly and one surface has torn or ruptured (Waddington, 1940). Blisters have never been observed in the *net* Low lines.

It should be emphasised that since the major mutants are homozygous in each selection line, the following discussions will be primarily concerned with relationships among the selected polygenic backgrounds.

3. SPECIFICITY IN THE PLEXUS VEIN PATTERN

The selection line responses showed that the amount of extra venation in homozygous plexus populations can be modified dramatically. But the most interesting fact which emerged from these lines is that at particular levels of mutant expression, fragments are present in rather specific positions on the wing. Regional specificity has been noted by others (*e.g.* Goldschmidt, 1945; Waddington, 1973). It appears, however, that as mutant expression is increased or decreased by selection, vein material is added to or removed from the wing in a reasonably predictable sequence.

The range in expression of the plexus phenotype is illustrated in plate I. The most extreme Low in these homozygous plexus flies is almost indistinguishable from wild type (not illustrated). The phenotypes which occur as mutant expression is increased can be summarised as follows.

(i) In flies with the smallest fragments, the extra vein material is present as (a) a small linear fragment in the marginal cell a little distal to the midpoint of the L2 vein, (b) two dot-like or small linear fragments on each side of the L5 vein in the second and third posterior cells, and (c) a slight bend in the distal section of the normally straight L4 vein (plate I, a and b).

(ii) In slightly more extreme individuals the marginal fragment is longer and often joins the L2 vein (plate I, c). Irregular crossvein-like structures often form between the distal region of the L2 vein and the anterior margin of the wing, which is the L1 or first longitudinal vein.

(iii) Next, small fragments appear in the submarginal cell near the distal

tip of, but seldom touching, the L3 vein (plate I, e). In addition, the linear fragment in the third posterior cell is lengthened proximally and forms a new longitudinal vein roughly parallel to the proximal part of the L5 vein.

(iv) With more extreme expression, the submarginal fragment forms a crossvein-like connection with the L3 vein. The marginal fragment may often be irregular in shape and may form additional connections with the L2 vein. The original second posterior cell fragment is variable, but often joins the L5 vein at the junction of the L5 and the medial crossvein (plate I, f). The medial crossvein, in turn, is gradually displaced to form a more acute angle with the distal part of the L4, while the terminal bend of the L4 vein becomes larger. Branches often extend from the L4 vein into the second posterior cell, often joining the distal tip of the L5 which bends into the second posterior cell near the margin of the wing (plate I, f and g).

(v) From this point, with one exception, flies with increasingly extreme expression are distinguished only by the lengthening of fragments in the marginal, submarginal and second posterior cells, and by increasing irregularity and thickening of the vein fragments. The exception is that in individuals in which the L4 terminal bend has become very large, a new linear fragment appears about halfway between the distal tips of the L3 and L4 veins (plate I, g-l). This fragment becomes larger until it joins the distal margin of the wing. In these selection lines, however, it does not increase further in size. In some flies a small branch also forms from the small anterior, or radial-medial, crossvein.

4. EXTRA-VEIN PATTERNS IN OTHER MUTANTS AND MUTANT COMBINATIONS

The positions of vein fragments in other mutant selection lines and in crosses among mutants show that the pattern, as described for plexus, is a fairly general feature of the *Drosophila* wing. For example, the mutant net typically has a greater amount of extra vein material than does the corresponding plexus base stock or selection line. The same general venation patterns are observed, however, in most areas of the wing. This can be illustrated by comparing a sample wing from the *net* High line (plate II, f) with wings of comparable degrees of extra venation in plexus homozygotes (plate I, k and l).

Indeed, even in non-plexus-like mutants, parts of this pattern can be observed. In sublines of the mutant short vein (*shv*), which usually has terminal gaps in the longitudinal veins, fragments sometimes appear when the mutant line is successfully selected for decreased expression, *i.e.* for longer veins. This selection for increased vein material produces fragments as well as lengthening the veins. These fragments are found near the tip of the L3 vein and in the marginal cell in precisely the same positions as the fragments described in the plexus line having a low degree of mutant expression (plate II, e).

The manipulation of polygenic variation by artificial selection can, thus, be used to demonstrate differences between anatomical regions of higher and lower vein-forming potential. But appropriate selection lines can also be used to investigate the developmental relationships between classes of mutants, such as the relationship between those mutants which cause vein gaps, *e.g.* veinlet and short vein, and those which cause extra vein fragments, *e.g.* plexus and net.

Drosophila vein mutants can be broadly grouped into a number of classes (table 1). The majority of these mutants have pleiotropic effects, such as upon bristles, wing shape, or facet arrangement in the compound eyes. With regard to vein effects, however, there appear to be three main phenotypic classes: (1) those that form vein fragments, (2) those that shorten one vein or otherwise have only local effects in the reduction of vein material, and (3) those that shorten all of the veins.

With the exception of the class with L4 and L5 gaps only (which I have included in group 2), those mutants reported to cause gaps in two or three of the longitudinal veins probably fall into the third class (Thompson, unpublished), for upon closer examination several of the recorded phenotypes have been found to be incomplete in that the mutants may affect more veins when they are crossed into different genetic backgrounds or selected for increased expression.

TABLE 1

Classification of mutants of Drosophila melanogaster arranged according to the veins they affect (compiled from Lindsley and Grell, 1967)

Add veins (plexus-like)	Shorten veins				Number in class	Effects
	L2	L3	L4	L5		
x	—	—	—	—	38	} local + general
—	x	—	—	—	7	
—	—	x	—	—	2	} local
—	—	—	x	—	10	
—	—	—	—	x	8	
—	—	—	x	x	7	
—	?	x	?	x	1	} general
—	x	?	x	x	3	
—	?	x	x	x	1	
—	x	x	x	x	5	

The class of mutants which shorten both the L4 and L5 veins has been included in group 2, because the L4 and L5 veins are in the developmentally distinct posterior half of the wing (Garcia-Bellido, 1972). These mutants may, therefore, be similar to L2 gap mutants or L4 gap mutants in having a local effect, rather than a general effect, upon the total venation system.

Waddington (1940) suggested from histological evidence that all processes of wing formation are related, so that those mutants causing fragments and those causing vein gaps are likely to be opposite ends of a spectrum of possible alterations of a single developmental process.

Waddington's view was supported by the results from crosses among vein mutant selection lines reported by Thompson (1974). Selection lines of vein gap mutants and extra vein mutants were crossed together in all combinations and the frequency of vein defects was scored in the double heterozygotes in the F_1 generation. In general, when both lines in the cross had originally been selected for more vein material, such as *ve* Long $\times$ *net* High, the F_1 included a high proportion of individuals having fragments of extra vein material in otherwise normal wings. However, when both contributing lines had been selected for shorter veins or for fewer vein

fragments, such as *ve Short* $\times$ *net Low*, the F_1 flies had few, if any, vein fragments. Crosses between two vein gap mutants, such as *ve Short* $\times$ *shv Short*, often produced flies having gaps in the longitudinal veins. Crosses between increased and decreased expression lines, such as *ve Long* $\times$ *net Low*, were intermediate, indicating that overall modifier effects were not simply due to dominance of one set of modifiers.

These results suggested that the polygenic modifiers which cause an increase in vein material, regardless of the mutant in which the modifiers were originally selected, are similar in their effect upon the general vein-forming process. The formation of gaps in the veins and the formation of fragments in otherwise normal wings are apparently opposite extremes in a single process. Further, when fragments occurred in the normally wild type double heterozygotes, they appeared in those positions which the study of the plexus pattern had shown to be the regions of highest vein-forming potential (plate II, d and h). It is, therefore, proposed that there is a subthreshold pattern of vein-forming potential, which may or may not be strictly equivalent to a prepattern, and that it is this pattern which is revealed in these selection experiments.

5. PROFILE OF THE SUBTHRESHOLD VEIN-FORMING POTENTIALS

(i) *General description of the profiles*

An analysis of the position of veins and vein fragments, the sequence in which they appear, and the way in which they become larger allows one to draw a three-dimensional model of the probability of vein formation, in which the wing surface is two-dimensional and the probability curves are the third dimension. This is similar to a topographic map, except that the height of each profile represents the concentration of some necessary substrate or any similar factor or set of factors below which no vein is formed. A similar procedure has been used in other studies (for example, Maynard Smith and Sondhi, 1961) and serves primarily as a means of aiding one to visualise relationships among veins and to discuss hypotheses of the determination of the observed patterns. In this discussion the curves are profiles of expected vein phenotype rather than probability curves in the mathematical sense.

If the wing diagram is transected and the curve representing expected vein appearance for a specific region is viewed in cross section, relationships among veins and regions of high and low vein-forming potential can be illustrated as in fig. 1. A diagram of a wing having extreme venation (fig. 1, *a*) has been vertically transected at three points (A-A', B-B' and C-C'). For each point at which the transect cuts a vein or vein fragment a curve is present in the corresponding transect diagram. The curves are quite narrow in most instances, since the width of the vein seldom changes. But where the vein becomes wider, as at the tip of the L4 cut by transect C-C', the curve is correspondingly wider.

If a vein is generally present at a certain point in the wild type or mutant fly, the curve representing that vein is high. If a vein fragment often appears at that position in slightly more extreme phenotypes, the curve is lower. Finally, if a fragment does not appear until the extra venation phenotype is very extreme, the curve is quite low. In the wild type fly with normal venation, it is presumed that all of the relevant deter-

minants for subthreshold veins are present. In these flies, however, the threshold of vein expression (horizontal dotted lines in fig. 1) is higher than any of the individual competence levels with the exception of those for normal longitudinal veins and crossveins (fig. 1, *c*, *d*).

Although this is simply a conceptual model in which observations can be summarised, there are a number of direct and indirect ways of testing its validity in interpreting vein development, especially in respect to the phenotypic effects of polygenes. This is one object of future studies. One

FIG. 1.—Theoretical profiles of the likelihood of vein formation: *a* and *b*, subthreshold pattern in plexus enhanced; *c* and *d*, wild type. Arrow indicates direction of increased amount of vein. See text for explanation.

immediate point can be made, however, by suggesting the ways in which various mutant classes (table 1) might be supposed to produce their effects upon vein formation.

It is clear from table 1 that each longitudinal vein is, at least to a degree, developmentally independent of the other veins. If a mutant decreases or eliminates a factor that is required for the formation of a particular vein, such as the L2, only that vein will be shortened. In terms of the model presented here, this can be visualised as a decrease in the relative height of the competence curve representing the L2 vein (fig. 2). The expression threshold, on the other hand, may be thought of as representing a regular distribution of some common precursor. Providing the expression threshold remains unchanged in this example, an L2 gap phenotype like that of

radius incompletus or one of the other six L2-specific mutants will be produced.

If, however, a mutant alters the expression threshold, the effect upon the veins will be a general one. If the expression threshold in this model is decreased, which is meant to imply nothing about the nature of the biochemical reactions involved, the likelihood of observing extra vein

FIG. 2.—Theoretical profile of the likelihood of vein formation in the mutant radius incompletus. See text for explanation.

fragments will be increased, and a plexus-like phenotype will result. If the expression threshold is raised above individual competence levels, gaps will begin to appear in all of the longitudinal veins, and phenotypes will be produced like those which characterise veinlet and short vein (Lindsley and Grell, 1967).

The profiles for the normal longitudinal veins have been simplified and illustrated as having uniform heights. These profiles are not in fact uniform, however, but their study is complicated by the fact that different mutants have different profiles, as might be predicted for region-specific mutants.

Plate I

Wing samples which illustrate the range of venation phenotypes exhibited in homozygous plexus High and Low selection lines.

Plate II

Wings of *Drosophila melanogaster*: a, wild type with nomenclature, (a) marginal cell, (b) sub-marginal cell, (c) first posterior cell, (d) second posterior cell, (e) third posterior cell, (f) anterior or radial-medial crossvein, (g) posterior or medial crossvein; L2, L3, L4, L5 are longitudinal vein designations; b, *net* Low; c, *px* High grown at 18° C.; d, double heterozygote from cross of *shv* Long × *ri* Long; e, *shv* Long; f, *net* High; g, *net* High grown at 18° C.; h, double heterozygote from cross of *px* High × *shv* Long.

Selected polygenic complexes or their component genes could affect vein length in two ways. They could alter the expression threshold and, thus, produce general effects upon venation in all areas of the wing. Or, they could alter a particular competence level and produce a local effect upon vein length. A set of polygenes which lowered the expression threshold would be seen to increase the amount of vein material in the wing, whether this was increasing vein length in veinlet or increasing the size and number of fragments in plexus. The phenotypic effects of the polygenic modifiers would then be independent of the major mutants in which their effects were measured. This is precisely what was observed in the crosses among selected lines reported by Thompson (1974).

With respect to local phenotypic effects, it is interesting to note that the subthreshold pattern can be dissected by growing selection lines at low temperatures. Although the general level of expression is little affected by temperature, at 18° C. the new longitudinal vein in the third posterior cell is almost completely removed (plate, II c). When small traces of this vein remain as in the *net* High lines at 18° C., however, the fragments are found near the posterior crossvein and at the base of the wing in areas of highest vein-forming potential (plate II, g).

(ii) *Quantifying vein profiles*

The relative likelihood that vein material will be formed in various wing regions can be quantified by a method similar to that used by Carlson (1970). Wing samples are mounted on microscope slides and their images are projected on to a sheet of graph paper using a camera lucida. Equally spaced lines are drawn on the sheet, which is then positioned to cover an entire segment of the wing. This compensates for variations in the size of the wings and veins. When a set of 21 parallel lines is properly aligned, it divides the vein region into 20 equal spaces. The presence or absence of vein material in each of these divisions can then be recorded and summed over a large sample to give curves such as those in fig. 3. The curves differ from those of Carlson in that a transformation is not used, since these curves will not be interpreted to represent concentration directly.

It can be seen from the figure that fragments appear in the marginal cell at the same time as they appear in the third posterior cell. In both regions the fragments increase in size by being lengthened proximally. The fragment at the tip of the first posterior cell does not appear until the marginal cell and third posterior cell fragments are quite large. The small fragment near the anterior crossvein appears later.

6. DISCUSSION

The specificity observed in the location and sequence in which vein fragments appeared during selection in several mutant lines and in mutant combinations is consistent with the hypothesis that the wings of *Drosophila melanogaster* have regions of high vein-forming potential. A simple model has been proposed in which each vein-forming region is represented by a profile. The higher the profile, the more likely it is that vein material will be produced at any particular degree of mutant expression. Indeed, the subthreshold pattern may be equivalent to the prepattern (Stern, 1968),

FIG. 3.—Quantified pattern profiles for the fragments which appear in the marginal cell (a), the first posterior cell (b), and the third posterior cell (c). The exact region of the wing which is represented in each profile is illustrated at the top.

but this could only be tested with mosaics, and it is not desirable to assume it at present.

Since the subthreshold vein pattern appears to be a reasonably general component of *Drosophila* wing development, one possibility is that it marks regions in which ancestral veins once occurred. If this is so, correlations might be expected between the positions of subthreshold veins and the positions of normal veins in related families of Diptera similar to the bristle

FIG. 4.—Wings of Diptera: *a*, family Trypetidae; *b*, *Drosophila melanogaster* showing morphological terminology for the longitudinal veins; *c*, family Empididae. (After Oldroyd, 1970.)

correlations reported by Maynard Smith and Sondhi (1961) in a *Drosophila subobscura* selection line. In fact, the number of correlations with even quite distantly related Diptera is striking. Two examples will serve to illustrate this point (fig. 4). The only venation difference, other than small crossveins, between *Drosophila* and the family Trypetidae is a longitudinal vein (Cu_{1+1A}) in the third posterior cell which appears to be analogous to that observed to extend from the medial crossvein to the base of the wing in the subthreshold vein system. Flies in the family Empididae

also have this longitudinal vein in the third posterior cell, and in addition have a branch from the fifth radial vein ($R_5 = L3$ vein) like that found in the subthreshold vein system (plates I, j; II, c).

It is not yet known how this conceptual model of vein-forming potentials is related to the actual development of the wing or to developmental compartmentalisation of the wing disc (Garcia-Bellido, *et al.*, 1973). Waddington (1940) described the histological development of several extra-vein and vein-gap mutants and found that the mutant effects were first expressed as the presence or absence of basal processes between the cell layers in wings of 19- to 28-hour-old pupae. Although he attributed certain distortions to mechanical causes, he found that the presence of vein material depended upon the forming and maintaining of spaces between the two cell layers of the pupal wing. In the final stage of vein development, extra chitin is deposited in all areas in which the two layers are not in contact. When basal processes form, the two cell layers join and form a central membrane. Where basal processes have not formed, spaces remain between the layers and extra chitin is later deposited there. Mutants with extra veins appear to have the basal processes inhibited in certain parts of the wing, while in short vein mutants, the spaces are erased when basal processes appear soon after the normal vein canals have been formed.

In studies of individual veins, House (1953) and Carlson (1970) have used the simple relationship between basal processes and vein canals to propose a model in which vein formation is an all or none reaction involving a theoretical substance which controls the outgrowth of cytoplasmic processes from the pupal wing cells.

Scharloo (1962) has proposed a similar model for the formation of the L4 vein in cubitus interruptus Dominant (*ci^D*). In his model there is a proximal-distal gradient of competence for cells to react to a second substance which he calls the vein-inducing substance. If the two components in Scharloo's model are extended to the vein system as a whole, his model and the model of developmental potentials discussed in section 5 are equivalent. The uniform concentration of "vein-inducing" substance in Scharloo's model is equivalent to the expression threshold in section 5, while the gradient of cell competence in the L4 vein region is equivalent to the individual vein competence profiles diagrammed in fig. 3. It is encouraging that, although these studies are based upon different types of experimental results and concern different vein regions and mutants, the resulting models have the same basic components and general simplicity.

Thus, the manipulation of polygenic variation by artificial selection has allowed us to describe the normally unexpressed developmental potentials for vein formation in many parts of the *Drosophila* wing. The phenotypes of certain major mutants and the phenotypic effects of selected polygenic backgrounds were related to a conceptual model of vein formation. Finally, the subthreshold veins have been correlated with normal veins in related Diptera, with the implicit conclusion that one way in which the *Drosophila* vein pattern might have evolved was by the selection of polygenes or mutant alleles which erased the ancestral veins, leaving only the subthreshold potentials.

Acknowledgments.—I would like to thank Professor J. M. Thoday, Dr Michael Ashburner, Dr Peter Lawrence and Mr David Skibinski for their helpful discussions and comments on

the manuscript. I also thank Mr Brian Curtis for preparing the photographs. During this work I was supported by a Marshall Scholarship.

7. REFERENCES

- CARLSON, J. H. 1970. Genetics of an L2 venation mutant in *Drosophila melanogaster*. II. Pattern effects. *Ohio J. Sci.*, 70, 365-371.
- FALCONER, D. S. 1960. *Introduction to Quantitative Genetics*. Oliver and Boyd, London.
- GARCIA-BELLIDO, A. 1972. Pattern formation in imaginal disks. In H. Ursprung and R. Nöthiger (eds.), *The Biology of Imaginal Disks*, Results and Problems in Cell Differentiation, Vol. 5, pp. 59-91. Springer-Verlag, Berlin.
- GARCIA-BELLIDO, A., RIPOLL, P., AND MORATA, G. 1973. Developmental compartmentalisation of the wing disk of *Drosophila*. *Nature New Biology*, 245, 251-253.
- GOLDSCHMIDT, R. 1945. A study of spontaneous mutation. *Univ. California Publ. Zool.*, 49, 291-550.
- HOUSE, V. L. 1953. The interaction of mutants affecting venation in *Drosophila melanogaster*. II. Additive and pattern effects of combinations of Hairless, engrailed, and cubitus interruptus. *Genetics*, 38, 309-327.
- LINDSLEY, D. L., AND GRELL, E. H. 1967. *Genetic Variations of Drosophila melanogaster*. Carnegie Institution of Washington Publ. No. 627.
- MAYNARD SMITH, J., AND SONDHI, K. C. 1960. The genetics of a pattern. *Genetics*, 45, 1039-1050.
- MAYNARD SMITH, J., AND SONDHI, K. C. 1961. The arrangement of bristles in *Drosophila*. *J. embryol. exp. Morph.*, 9, 661-672.
- MOHLER, J. D., AND SWEDBERG, G. S. 1964. Wing vein development in crossveinless-like strains of *Drosophila melanogaster*. *Genetics*, 50, 1403-1419.
- OLDROYD, H. 1970. Handbooks for the Identification of British Insects: Diptera. I. *Introduction and Key to Families*, Vol. 9, Part 1. Roy. Entomol. Soc., London.
- SCHARLOO, W. 1962. The influence of selection and temperature on a mutant character (*ci^D*) in *Drosophila melanogaster*. *Arch. Neerl. Zool.*, 14, 431-512.
- SONDHI, K. C. 1961. Developmental barriers in a selection experiment. *Nature, London*, 189, 249-250.
- SONDHI, K. C. 1962. The evolution of a pattern. *Evolution*, 16, 186-191.
- SPICKETT, S. G. 1963. Genetic and developmental studies of a quantitative character. *Nature, London*, 199, 870-873.
- STERN, C. 1968. *Genetic Mosaics and Other Essays*. Harvard Univ. Press, Cambridge, Mass.
- THODAY, J. M. 1961. Location of polygenes. *Nature, London*, 191, 368-370.
- THOMPSON, J. N., JR. 1973. General and specific effects of modifiers of mutant expression. *Genet. Res., Camb.*, 22, 211-215.
- THOMPSON, J. N., JR. 1974. Studies on the nature and function of polygenic loci in *Drosophila*. I. Comparison of genomes from selection lines. *Heredity*, 33, 373-387.
- WADDINGTON, C. H. 1940. The genetic control of wing development in *Drosophila*. *J. Genet.*, 41, 75-139.
- WADDINGTON, C. H. 1973. The morphogenesis of patterns in *Drosophila*. In Counce, S. J. and C. H. Waddington (eds.), *Developmental Systems: Insects*, Vol. 2, pp. 499-535. Academic Press, London.
- WALLACE, B. 1968. *Topics in Population Genetics*. W. W. Norton and Company, Inc., New York.