VOL. 38, NO. 4 December, 1963

THE QUARTERLY REVIEW of BIOLOGY

THE BIOLOGICAL FOUNDATIONS OF ANIMAL PATTERNS

BY K. C. SONDHI

Gerontology Branch, National Heart Institute, National Institutes of Health, Bethesda, and Baltimore City Hospitals, Baltimore, Maryland

[Present address: Department of Zoology and Physiology, Rutgers University, Newark, New Jersey, and Department of Laboratories, Newark Beth-Israel Hospital, Newark, New Jersey.]

ABSTRACT

Pattern formation in morphogenesis has been a subject of considerable investigation by many biologists working in diverse fields. The present article takes into account various lines of inquiry on the genesis of regular patterns in terms of concepts of genetics, embryology, biophysics, biochemistry, and mathematics and attempts to determine their contribution to the emergence of a general concept. The main emphasis is laid on primary patterns from which the final patterns are derived. The development of a pattern can be brought about by two processes, one concerned with the formation of a 'prepattern' which determines the positions at which structures are formed and the other determining the amount of 'precursor' or differences in the competence of cells to respond to an underlying prepattern. On the basis of this morphogenetic model, some of the experimental results, involving the genesis of novel patterns, and variation in the numbers and positions of structures are interpreted. The mechanisms responsible for the genesis of prepatterns are discussed. The prepattern is considered as the distribution of an inducing substance with regions of high and low concentration; the regions of high concentration occur at sites where structures later form. The process whereby such a distribution could arise is described. Various other mechanisms by which regular patterns involving larger number of structures can be formed are discussed.

INTRODUCTION

Reality is the embodiment of structure;
Structure is the embodiment of properties;
Properties are the embodiment of harmony;
Harmony is the embodiment of congruity.

Ts' T'ien-ch'in
(in the "Kuan Tsi," fourth century B.C.)

ONE of the properties of living things is the ability to reproduce. Reproduction is the mechanism by which like begets like. How is the likeness of form or shape between parents and offspring maintained? The chromosomal theory of heredity, in its essential features, maintains that heritable differences between individuals are largely due to differences in the genes on their chromosomes. Development, in a sense, is the process of unfolding the genetic information contained in the fertilized egg. The property of copying molecules of their own kind during cell division resides in the DNA (deoxyribonucleic acid) molecules on the chromosomes by which the likeness of form or shape between parents and offspring is produced.

The structure of the DNA molecule provides a plausible basis for gene specificity, for gene replication, and for gene mutation. The variable characteristic of DNA structure is the sequence in which the four kinds of bases follow each other along the molecule, providing for a large number of different sequences which determine the genetic information. It is possible to embody the information required for different amino acid sequences of proteins in the sequence of the four bases of the DNA molecule, whereby biosynthesis during development can proceed. However, the synthesizing of required substances is only one of the essential features of development, since we deal here with the process of differentiation in time and space, where differently organized units forming different organs maintain definite relative positions. Thus, we are confronted with two questions: first, what causes apparently genetically identical cells to become different in appearance and function, and, second, how do different kinds of cells become arranged in orderly patterns in space?

An interesting clue to the origin of differences in genetically identical cells comes from the work of Sonneborn and Beale (1949). In *Paramecium aurelia,* three groups of genes are known to determine the antigenic character of this organism. The expression of any one of the three specific antigens is controlled by the state of the cytoplasm, which can be altered by external influences. Slight changes of temperature can alter the cytoplasmic state to favor the expression of any one of the three antigenic characters. These environmentally induced changes are transmitted to the daughter cells when the individual cell divides. The bearing of these findings on multicellular organisms is obvious, where cells carrying at least initially identical genetic information may become different as a consequence of environmental differences. There is reason to believe that in the fertilized egg itself there are regional differences, so that cytoplasm in various regions has unlike properties. It is likely that, on cell division, nuclei find themselves in different kinds of cytoplasm which may lead to differentiation. Another suggestion is that differentiated protoplasmic units may be propagated in the nucleus and may be remodelled in the cytoplasm (Weiss, 1950), a concept which assumes two entirely different processes which occur at different places. Once such differences have arisen in the embryo, the contact of one group of cells with another may induce further complexity. The intricate mechanisms at work during development, however, cannot be simplified to such an extent, but at least we can picture development as a process wherein structural complexity increases in a stepwise manner.

Causal relations during development, or epigenetic processes, can be studied by embryological or genetic methods or by both. In general the embryological approach is to study how structures develop and then to interfere with the normal process by transferring parts of the embryo from one position to another or to treat the embryo with chemical and physical agents. The genetic approach is to concentrate on gene differences between closely allied individuals and to examine how genes control these differences during development.

We are mainly concerned here with the second of the two questions raised earlier. The purpose of this article is to examine how patterns, or structures showing an orderly arrangement, are formed. What follows is not a complete review of facts or ideas but a presentation of various attacks on the pattern problem so far as they contribute to the emergence of a general concept. Insects are chosen as illustrative material for their merit in regard to the availability of genetic information and of patterns of varying complexity. Insects provide a variety of structural patterns, some regular, some semiregular, and some highly irregular. The development of these structures is easy to follow, and numerous genes are known to affect them in one way or another. The mechanisms by which some of the insect patterns are produced are considered in the following sections.

ENDOMITOTIC DIVISIONS AND THE WING SCALE PATTERN

Work on the lepidopteran wing pattern has uncovered mechanisms of considerable significance in pattern formation. Since most of the original work is written in German, perhaps it has not invited the attention that it deserves from English-speaking biologists. Therefore, it

may be of some value to consider the main features of this work here.

The pattern of scales in the wings of Lepidoptera has been studied in great detail (Kühn and Henke, 1932; Henke and Mertz, 1941; Henke, 1946; Henke and Pohley, 1952; Pohley, 1953; reviews: Goldschmidt, 1938, 1955; Henke, 1947; Kühn, 1955). In the wing of the flour moth, *Ephestia kühniella,* three main types of scales (Fig. 1a, b) can be recognized: cover scales, middle scales, and ground scales. The pattern of scales on the forewing and hindwing differs. The scales on the forewing stand free from each other and are arranged in regular rows, while those on the hindwing cover each other, the way shingles do, and form irregular and branched rows. In those parts of the wing where all three types of scales are present, the scales are often arranged in groups of four. The cover scales show variation of structure and of pigment. In the forewing, eight color types of scales (Fig. 1c) can be distinguished. There exists a close correlation between the nature of the pigment present and the shape of the scale. The wing-scale pattern of the Lepidoptera is of particular interest to a student of developmental biology, for, in the determination of the scale pattern, one studies not only differences in pigmentation but also in the structure of scales.

In the development of the scale pattern, two types of differentiation must be considered. One is the development of the scale itself from the stem cell, the lineage of which can be traced back to the scale epithelial stem cell; the other is the amount of polyploidy, by which the size of the scale types is determined. The main features of Henke's (1947) description of scale differentiation are represented in Fig. 2.

In *Ephestia,* the formation of scales can be recognized with the appearance of large cells, the scale-forming stem cells, in the epithelium of the young pupa. The primary scale stem cell, which may be designated the scale stem cell I, undergoes two divisions (Köhler, 1932; Süffert, 1937; Strossberg, 1937, 1938). The first division of the scale stem cell I gives rise to a scale stem cell II and a degenerating cell. The surviving stem cell II divides again into two, one of which gives rise to the scale proper while the other

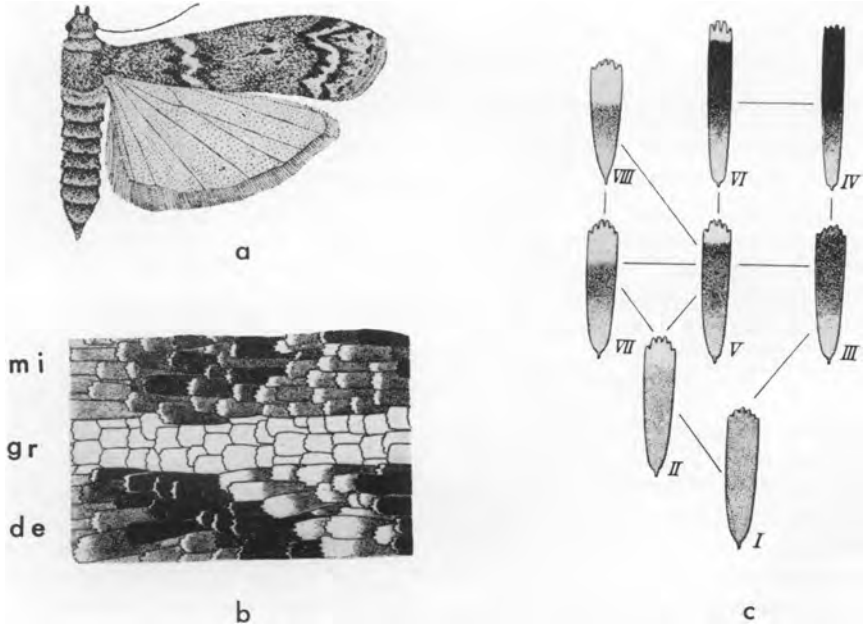


FIG. 1. WING SCALES OF *Ephestia kühniella*

a, part of the body with right forewing and hindwing; b, a piece from the upper side of the forewing; de, cover scales; mi, middle scales (cover scales removed); gr, ground scales (middle and cover scales removed); c, 8 types of scales with their characteristic form and pigmentation. (After Kühn, 1955.)

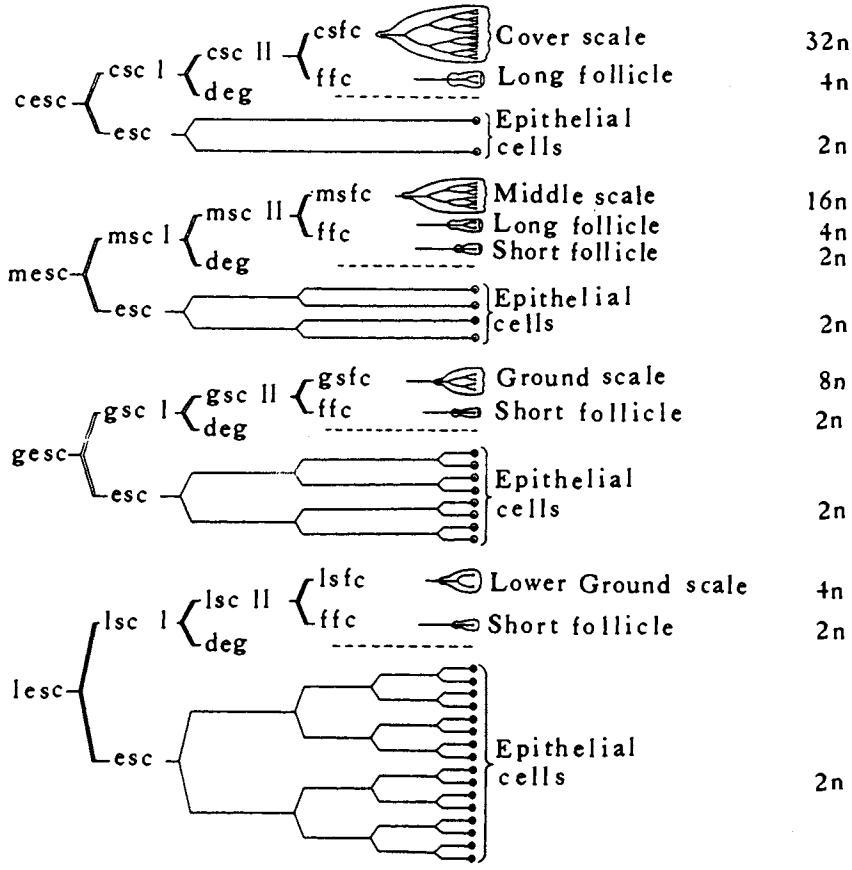


FIG. 2. SCHEMATIC REPRESENTATION OF DIFFERENTIATION OF WING SCALES IN *Ephestia kühniella*

Legend is arranged below to correspond to vertical columns. (After Goldschmidt, 1955; Kühn, 1955.)

cesc, cover scale epithelial stem cell
mesc, middle scale epithelial stem cell
gesc, ground scale epithelial stem cell
lesc, lower ground scale epithelial stem cell
csc I, cover scale stem cell I
esc, epithelial stem cell
msc I, middle scale stem cell I
gsc I, ground scale stem cell I
lsc I, lower ground scale stem cell I
csc II, cover scale stem cell II
deg, degenerating cell
msc II, middle scale stem cell II
gsc II, ground scale stem cell II
lsc II, lower ground scale stem cell II
csfc, cover scale-forming cell
ffc, follicle-forming cell
msfc, middle scale-forming cell
gsfc, ground scale-forming cell
lsfc, lower ground scale-forming cell

forms the follicle or socket of the scale. In general, the development of the butterfly scale is very similar to that of the *Drosophila* bristle (see p. 305), the degenerating cell in the former corresponding to the nerve cell in the latter.

An important clue to the mechanisms involved in the formation of scales of different sizes has come to light by measurement of the nuclear size of the scale-forming cells. There exists a correlation between the nuclear size and the scale size. Geitler's (1938, 1939-40, 1954) elaborate studies showed that nuclear growth can be the consequence of repeated division of chromosomes without a subsequent nuclear division, a process known as endomitosis. Since in endomitosis the number of chromosomes doubles in the absence of nuclear division, a corresponding increase in nuclear size is produced. By counting the number of chromocenters in the nucleus, the number of genomes can be ascertained. During development of the wing of *Ephestia,* countings are in good agree-

ment with the assumption that, starting with the normal epithelial cell with 2n chromosomes, nuclei with 4n, 8n, 16n, and 32n can be formed to give rise to lower ground scales, ground scales, middle scales, and cover scales, respectively. In Fig. 2, the large and small follicle cells are shown to be tetraploid and diploid, respectively, while 4- to 32-ploid nuclei are represented for different scale types.

A more careful examination of Fig. 2 reveals that, with increase in polyploidy of the scale-forming cells, the number of successive divisions of the epithelial stem cells decreases. The sum of the number of endomitotic divisions and the number of epithelial cell divisions remains constant. Thus, in the lower ground scales with 4n chromosomes, which have undergone only one endomitotic division, the epithelial stem cell divides four times; in the ground scales with 8n chromosomes, it divides three times; in the middle scales with 16n chromosomes, it divides two times; and, finally, in the cover scales with 32n chromosomes, the epithelial stem cell divides only once. These results suggest that in the formation of the scale pattern a compensatory principle is effective. This is a discovery of far-reaching significance, but comparable studies on other insects are badly needed to elaborate Henke's "law of compensation."

What conclusion could one draw from these findings? The analysis of the scale pattern, at its face value, suggests that the determination of a pattern might be the consequence of intranuclear differentiation of the genetic material, achieved through endomitosis. Closer examination, however, shows that this is not the case. Since the scale cell and the follicle cell arise at the last differentiating division, it is reasonable to assume that the determination of their difference in type has already been realized before endomitosis begins — that is, at the moment when epithelial and scale stem cells first separate. Now, it may well be argued that at the time of the first differential division a change in the chromosomal material might have occurred, and this may cause later cell generations to undergo endomitosis. Although the role of endomitosis in growth and form in insects is well established (Lipp, 1953, 1955, 1957; Geitler, 1954), there is not enough evidence to suggest that intranuclear differentiation is the cause of divergent determination.

SPEEDS OF DEVELOPMENT AND DETERMINATION STREAM

Another interesting feature of lepidopteran wing scales is their characteristic pigmentation. The color differences in scales are caused by pigments which appear during different times of development (Goldschmidt, 1923; Henke, 1933). Goldschmidt (1920) first pointed out that, in the development of the scale color pattern, long before the pigment is formed and the scales actually mature, detectable differences in the rate of differentiation of different parts of the scale pattern play an important role in the realization of the final pattern. The parts of the wing eventually forming dark-pigmented scales show slower differentiation in comparison with parts giving rise to light-colored scales. However, such differences in the differentiation of scales can only be detected during a short period. If the pupal wing is dried at an appropriate stage, the undifferentiated scales, which at this stage of development are soft bags filled with blood, collapse, but the differentiated scales remain erect. Since the main features of the scale pattern appear in the form of a relief of protruding and of flattened scales, this stage in wing development was termed the "relief stage" by Goldschmidt. The importance of Goldschmidt's findings lies in that they demonstrated for the first time that a primary pattern may be laid down before a pattern appears visibly.

Once it could be shown that the scales within different parts of a pattern are at different stages of differentiation, the concept of differential velocity of differentiation was extended farther to explain the differences in the scale pigmentation. Goldschmidt assumed that chromogens are present in the hemolymph only at certain specific periods and are incapable of entering entirely chitinized scales. This hypothesis was confirmed when Braun (1939) showed that in the relief stage, and even a considerable time before its appearance, the scales which are not entirely chitinized and which later form the dark scales are alone capable of yielding pigment when treated with tyrosine. Since pig-

ment is deposited in chitin in colloidal solution, a hardened scale is incapable of receiving more pigment; therefore, the speed of hardening, different in different areas, may lead to the pattern of pigmentation.

A similar situation was found to exist at a period preceding the relief stage when the scale-forming stem cells undergo two differentiating divisions. During this period two 'waves of mitosis' pass over the wing from the base toward the margin, but the wing regions which eventually form the dark scales show much more frequent mitosis than those which ultimately give rise to the light-colored scales (Köhler, 1932; Braun, 1936). This phenomenon is therefore called 'mitosis pattern' and corresponds to the stem cell divisions I and II (see p. 291). The frequency of these cell divisions is under genetic control (Braun, 1936). In this case the occurrence of more frequent mitosis indicates slower differentiation in comparison with those cells that had ceased dividing.

Further analysis of certain pigment patterns in the Lepidoptera (Goldschmidt, 1920, 1923, 1938) revealed that the amount and the specific arrangement of the pigment were under genetic control. In some cases it was found that the process of pigmentation started from one point and spread from there across the wing, following a definite course. This observation led Goldschmidt to suggest that visible color patterns may be due to the progress and nature of flow of an underlying determination substance. Adopting a concept introduced by Spemann, he called it a "determination stream" and suggested that genes controlling the structure and the color of a wing may act by controlling a determination stream of definite quantity, speed of progress, pattern of flow, and of action upon different processes in morphogenesis.

The concepts of differential velocity of differentiation and of the determination stream must be considered as first attempts to fill the gap between genetics and embryology, for they enabled one to picture development in terms of gene-controlled processes. Unfortunately, these concepts, when extended to explain the development of the lepidopteran wing pattern, proved to be unsuccessful. This was partly owing to Goldschmidt's assumption that during development the whole wing acts as a unit. The situation was clarified when Kühn and von Engelhardt (1933) not only demonstrated the existence of a determination stream in the developing wing of *Ephestia* but also showed the presence of a definite arrangement of areas or fields forming specific parts in the wing pattern.

PATTERN FIELDS IN THE WING

Schwanwitsch (1924) and Süffert (1925, 1927) first pointed out that the wing pattern of butterflies and moths must be divided into several independent systems consisting of pattern fields and that these should be regarded as belonging to the same system if they vary from one species to another concomitantly and independently of other pattern fields. Confirmation of this point of view came from the findings of Henke (1928) when he showed that individual fluctuations in the pattern fields belonging to the same system within a single species of *Larentia sordidata* do undergo concomitant variation. Finally, Schwanwitsch's (1925, 1928, 1929a, 1929b, 1931) elaborate studies firmly established that the complicated pattern of the lepidopteran wing is the result of different arrangements of invariably present constituent parts or fields. These morphological observations have served as a starting point for a detailed experimental analysis of wing patterns.

One of the thoroughly examined systems is the symmetrical system (Figs. 1a, 3k) present in the wing of *Ephestia kühniella*. In the symmetrical system, a central field is enclosed by proximal and distal bordering bands. Within the central field on the transverse vein of the wing, two black spots, with a white spot in between, are situated. At the margin of the wing there are five (or six) dark marginal spots lying between the veins. The unity of the central field is proved by the fact that it acts as a whole under modifying genetic or environmental influences.

Kühn and von Engelhardt (1933), by using the technique of microthermocautery, applied slight burns to the pupal wing of *Ephestia*. Two types of reactions, depending on the time of operation, could be sharply distinguished. In the first period, during the first day after pupation, the effects of burning on the wing

were entirely local. The bordering bands were pushed about the wing in a specific manner (Figs. 3b, c, d). Identical results were obtained on the black mutant race of *Ephestia* (Fig. 3e). Now, if the marks of cautery at different places in a number of wings are compared, the resulting pattern is such as would be caused if a determination stream (Fig. 3a) proceeded from the anterior and posterior margins of the wing toward the center and if the distal and proximal terminations of this stream determined the positions of the bordering bands limiting the symmetrical system.

In the second period, between 48 and 60 hours after pupation, when the determination stream is actually spreading, a cautery mark applied to the wing brings the spreading movements to a standstill (Fig. 3f–j). By stopping the determination stream at various stages of its progress, a series of adult wings can be obtained that reveal the entire course of the determination stream. From the course of the determination stream reconstructed in this manner, it becomes clear why the earlier operations produce their effect near the wing margin and later ones toward the center, and why still later ones, as would be expected, may only affect the contour of the bands which become transverse after the two streams have united. Similar results were obtained by Kühn and von Engelhardt (1936) on the currant moth, *Abraxas grossulariata*. The patterns formed in this way are called "spreading fields" (Henke, 1948). These findings suggest that, in the realization of a pattern, chemical diffusion and field arrangement are likely to be important factors. However, Schwartz's (1953) experiments on the wing pattern of *Plodia* indicate that the determina-

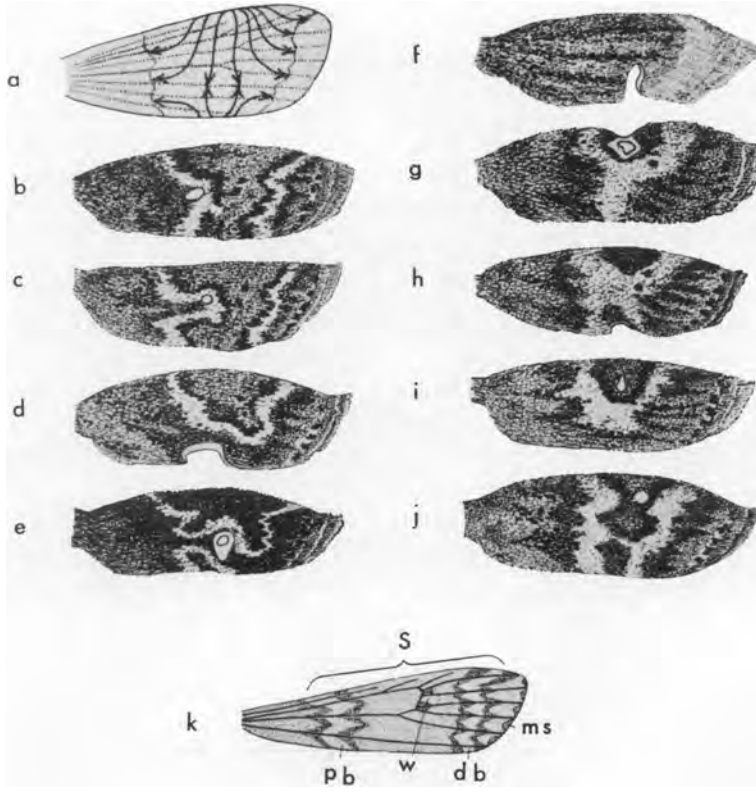
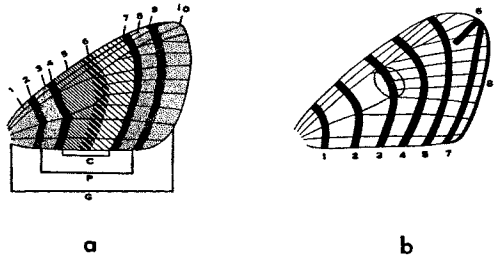


FIG. 3. DETERMINATION OF THE SYMMETRICAL SYSTEM ON THE UPPER SIDE OF THE FOREWING IN *Ephestia kühniella*

a-j, the effect of cauterizing the pupal wing during different periods of development; k, forewing of *Ephestia kühniella;* db, distal band; pb, proximal band; ms, marginal spots; S, symmetrical system; w, white spot located in between the two black spots. (After Kühn and von Engelhardt, 1933, and Kühn, 1955.)



a b

FIG. 4. GENERALIZED SCHEMES OF FIELDS AND PATTERNS IN THE SATURNID FOREWING

a, generalized scheme of the three main systems in the forewing. C, central field; G, general field; P, peripheral field; 1, basal field; 2, root field margin; 3, root field; 4, proximal band; 5, shadow field; 6, shadow; 7, distal band; 8, exterior field; 9, exterior field margin; 10, margin field. b, generalized scheme of pattern locations in the forewing. 1–8, the location of border patterns. (After Henke, 1948.)

tion stream is probably not due to chemical diffusion.

A comparative examination of wing patterns showed the existence of pattern fields occupying similar positions in the related groups of Lepidoptera. These findings enabled Henke (reviews: 1933, 1948) to draw a general scheme of the wing pattern of the Saturnidae. Fig. 4a, b shows schematically three fairly well-examined systems, which in Henke's terminology are referred to as "fields" and are known as the general field (Grundfeld), the peripheral field (Umfeld), and the central field (Zentralfeld).

The pattern of the lepidopteran wing is not determined as a whole by a uniform process but by a number of more or less partially independent processes. In a series of experiments on *Lymantria dispar*, Henke (1943) demonstrated that different fields are determined at different times during wing development. If cauterizations are made in the wing after a given element is determined, the wing shows only local defects. In cases where the pattern was not yet fully determined, its development was found to undergo modification, but, if cauterizations are made at different periods of development, some elements of the pattern may act as though they were fully determined at the time, while others are still labile and can be modified. Fig. 5 shows the effect of cauterizing the pupal wing at different periods. In the graph, age at operation is given as abscissa; the ordinate shows the percentage of cases in which the peripheral field or the central field behave in a mosaic manner, indicating that they have already been determined. The three wings drawn on the graph illustrate the results of cauterizing (a) at a period when only the marginal spots are determined, (b) at a stage in which the peripheral field is determined, the central field being still undetermined; and (c) a case in which the whole pattern is determined. Thus, the fields behave as independent units.

RHYTHMIC PATTERNS

Süffert (1927, 1929) described a certain kind of rhythmically repeated elements in the form of lines or dots in a lepidopteran wing and designated them "pearling patterns." Rhythmic patterns on the wing may be formed by dif-

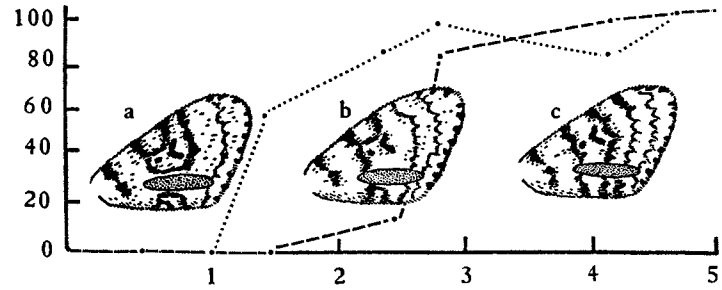


FIG. 5. THE EFFECT OF CAUTERIZATION ON THE DETERMINATION OF FIELDS IN THE PUPAL WING OF *Lymantria dispar*

Abscissa, age at operation. Ordinate, percentage of cases. Dotted line, symmetrical systems. Dashed line, middle shadow field. See text for description of the three cauterized wings illustrated. (After Henke, 1943).

ferent processes. Henke (1936, 1948) distinguished two types of rhythms: diffusion rhythms and simultaneous rhythms.

The diffusion rhythms represent several uniform lines or bands which form concentric figures. In some groups of Lepidoptera, symmetrical lines are seen on the proximal and distal sides of the wing, while in others a number of parallel lines are found. Although the appearance of one or more dark lines (Fig. 6a) in the white bands may give the impression that they are part of the symmetrical system, studies of Henke and Kruse (1941) have shown that these markings do not actually belong to the system in which they arise.

Henke (1936) suggested that diffusion rhythms in the central field may arise by a process of chemical arrangement, or Liesegang-phenomenon — that is, the phenomenon occurring if the precipitates are concentrically arranged in a colloidal medium. Mobilization occurs when the critical condition is being approached, with the material diffusing from the zones which become clear spaces to those which become rings. Becker (1937) has argued that the atypical pattern formed upon the tergites of aging wasp queens may be the consequence of Liesegang ring formation evoked mechanically by the irritation of the hypodermis. Henke (1948) has also attempted to explain the formation of a concentric pattern in the outer seed coat of the horse chestnut by means of Liesegang ring formation. In the incompletely colored pieces of the chestnut, one can see that the brown color and the formation of dark lines start from the radicle and the base of the cotyledon and proceed in a centrifugal direction. The dark lines always appear behind the extending brown color (Fig. 6b). This process corresponds with the formation of a Liesegang pattern as a consequence of chemical diffusion in a colloidal medium, starting from a center. Since the patterned area runs back into itself, concentric rings of decreasing size are formed on the margin. These may be centered at a single point (Fig. 6c), or they sometimes take the form of two circles (Fig. 6d). These suggested explanations are very tempting, but it must be pointed out that so far there is no genetic or experimental evidence to show that a Liesegang phenomenon is actually at work, although certain biological patterns apparently do indicate that such a possibility does exist.

There are other rhythmic patterns, such as simultaneous rhythms, which are characterized by the absence of any center of diffusion and therefore do not involve the mechanisms by which diffusion rhythms arise. In a simultaneous rhythm, as the name indicates, pattern-forming processes begin simultaneously in all parts of the wing. The development of wing scales in *Ephestia* provides a good example of a simultaneous rhythm, when two waves of mitosis pass over the wing from the base toward the margin, and the cellular processes proceed simultaneously in all parts of the

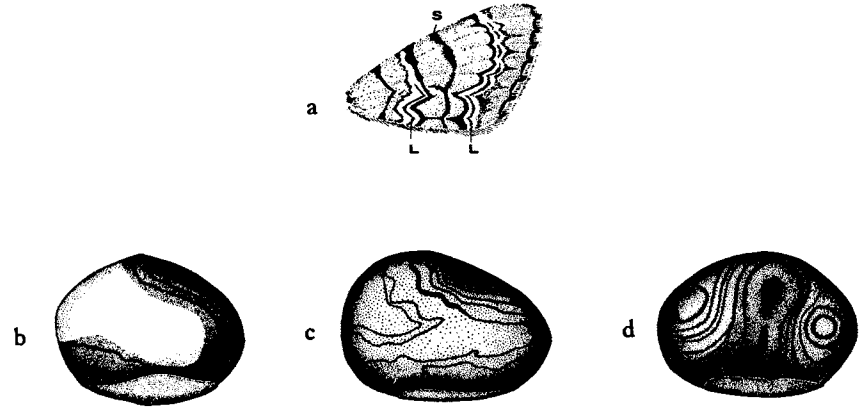


FIG. 6. PATTERNS IN THE FOREWING OF A MOTH AND IN A HORSE CHESTNUT

a, the pattern of lines in the peripheral field (L) and central field (S) in the wing of *Eupithecia venoscata;* b-d, the formation of a concentric pattern in the horse chestnut. (After Henke, 1948.)

wing. Furthermore, color spots on the wings of Lepidoptera show a great transition from irregularity to regularity. Henke was of the opinion that such patterns arise by a simultaneous rhythm. He invoked some sort of competition for a common substrate required by the cells forming the colored spots and argued that this interaction might transform originally irregular spots into a pattern of uniform structures. The main difficulty is that, although a competitive mechanism of the kind suggested by Henke might well account for patterns which vary from individual to individual, it would lack the precision required to produce regular patterns.

A HYPOTHETICAL SCHEME OF PATTERN FORMATION

Henke (1948) presented a hypothetical scheme (Fig. 7a–x) whereby various types of wing patterns in the Lepidoptera can be derived. In the formation of a simultaneous rhythm, a majority of elements are arranged in such a way that they divide the space uniformly. The size and form of these elements, as also their number and positions, depend on the conditions of the system. Henke suggested that in extreme cases these conditions could be of such a nature as would give rise to determination centers. This suggestion assumes that various patterns are a consequence of specific arrangement of pattern fields or determination centers which arise by simultaneous rhythms.

As an initial step, within an originally uniform area, accidentally arising inhomogeneity might produce irregular spots (Fig. 7a). Next, as a result of cellular competition for a common substrate, the irregular spots could be transformed into regular patterns of spots or bands (b-e). In extreme cases a single element might give rise to a band (f). If the concentration of the determining substance decreased toward the margin of the individual elements of the pattern, a gradient of color would appear in the final product. On this basis, concentric ocelli with different colors could arise (g).

If one assumed that a marginal pattern was formed through localization of a determining substance on the margin of the wing (h-k), its extension in the manner of a Liesegang process might produce a centric pattern (m). The appearance of different kinds of determination centers in the same region could give rise to concentric, heterogeneous bands (j-l). If a field were devoid of a color margin (n) and if a second marginal field is formed inside it, a marginal pattern, as shown in Fig. 7o, p, may be formed. If at the same time the smaller field gave rise to a color margin, a pattern with color zones (p) would appear. More complicated border formations (s) might arise along the lines of (p) and (q). It is also possible that a

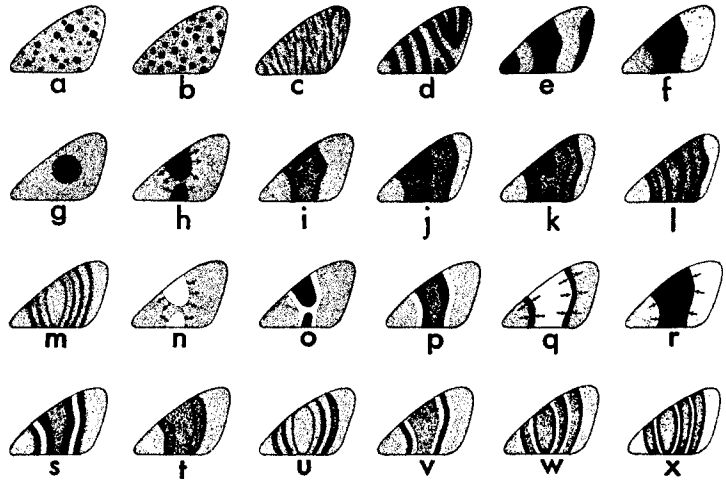


FIG. 7. HYPOTHETICAL SCHEME OF PRIMARY PATTERN FORMATION IN THE WINGS OF BUTTERFLIES

See text for explanation. (After Henke, 1948.)

field appearing earlier in development might modify the surrounding pattern. For instance, the amount of color-forming substance in a field may be reduced because of an adjoining zone that arose earlier in development, and weak coloration on the borders might result (r, v). In certain cases particular segments in a rhythmic zone might produce a concentration of color on their margins (t, u); these could be formed only in one direction (w) or uniformly in both directions (x).

The above scheme presented by Henke is of considerable interest. These studies began as a morphological analysis of pattern variability in related groups of Lepidoptera and subsequently led to numerous attempts to explain the origin of various patterns on the wing in terms of pattern systems, field centers, determination stream, diffusion rhythms, simultaneous rhythms, etc. Before drawing a general picture of pattern formation, the role of genes in the determination of the wing pattern, or of its constituent parts, must be examined.

GENES IN THE DETERMINATION OF WING PATTERN

Genetic and phenogenetic studies of the lepidopteran wing show that the pattern systems in the wing behave as independent units under modifying genetic or environmental influences (reviews: Ford, 1937; Goldschmidt, 1938; Caspari, 1941; Henke, 1947, 1948; Kühn, 1955).

The abnormalities which are induced by environmental stimuli such as temperature, X-rays, ultraviolet rays, and chemicals and which parallel in a striking manner the phenotypes of mutant genes were termed "phenocopies" by Goldschmidt (1935a). Studies of phenocopy effects provide information regarding the epigenetic system — that is, the entire system of causal relationships which is involved in the development of an organ or a whole animal. For each type of phenocopy there is a "sensitive period," or, occasionally, several sensitive periods, during which it can be relatively easily induced. Kühn (1926) showed that every morphological system in the wing of *Argynnis paphia* has its own distinct sensitive period. Later, the sensitive periods of different systems were thoroughly examined by Feldotto (1933), Köhler and Feldotto (1936, 1937), Kühn and Henke (1936), Stubbe 1938), and Wulkopf (1936). These studies established that elements belonging to the same morphological system have identical sensitive periods.

Different systems in the wing reveal developmental independence under the influence of genetic or environmental factors. For instance, there are certain genes which darken the central field without affecting the surrounding fields (Whiting, 1919; Kühn, 1937, 1939). Similar field-specific changes may be induced by chemical treatment (Strohl and Köhler, 1934). A number of genes have been described in *Ephestia* which act on the elements of one system only. There are genes which influence the symmetrical bands (Kühn and Henke, 1929, 1932; Hügel, 1933; Clausen, 1937; Schwartz, 1938); some genes affect the marginal spots (Kühn and Henke, 1929); others affect the central shadow (Clausen, 1937); and still others affect the central spots (Kühn and Henke, 1932).

Operations on the pupal wing, as also numerous mutants affecting the number of wing veins, show a direct dependence of the marginal spots upon the veins in *Philosomia cynthia* (Henke, 1933). Köhler (1940) suggested that the position of the marginal spots may be dependent upon the lacuna system in the developing wing of *Ephestia*. Pattern elements which show morphological dependence upon the structure of the wing, the veins, and the margin are termed 'dependent patterns.'

Numerous mutants are known to affect the shape and size of single scales (Fink, 1938) and the distribution of pigment (Kühn and Henke, 1932) in the single scale. Whiting (1919) and Kühn and Henke (1929) have described genes which replace light-colored scales by dark-colored ones without affecting the region of symmetrical bands and the central spots in the wing. In another mutant (Kühn, 1939), such replacement is restricted to the central field.

Kühn and Henke (1936) described two genes that influence the size of the central field and the positions of the symmetrical bands. The dominant gene *Sy* (lethal when homozygous) causes the bordering bands to approach each other, diminishing in this way the size of the central field. Another factor, *Syb* (intermediate

in the heterozygote), enlarges the central field by causing the bordering bands to diverge. Similar effects can be produced by applying a heat shock during the sensitive period for size of the central field in normal individuals. The effects of these genes and of temperature treatments during the sensitive period of corresponding regions in the wing are additive. An interesting feature of the *Syb* gene (Kühn and Henke, 1936) is that it shows the phenomenon of maternal inheritance. Thus, in reciprocal crosses between *Syb Syb* and *Syb syb* moths, the offspring originating from a cross of *Syb Syb* mother by *Syb syb* father has a broadened central field as compared with animals descended from *Syb syb* mothers. These results well illustrate the role of genes in the development of wing pattern.

The analysis of color patterns in the Lepidoptera is illuminating in several respects, for it reveals to a certain extent the nature of pattern-forming processes and their genetic control. These studies enable us to consider pattern formation in terms of processes which are stepwise in nature. In the development of a wing, the first pattern to be laid down is the pattern of the wing shape and of the wing veins, about which comparatively little is known. The second pattern is the pattern of future fields. The third pattern-forming process controls the limits of these fields, such as the bordering bands. Thus, a three-dimensional wing pattern is produced by a stepwise elaboration of an underlying pattern through processes which are four-dimensional in nature.

When we consider the final pattern as a derived expression of a primary pattern, numerous facts need to be explained. For instance, although there is enough evidence to show that various fields in the developing wing are under genetic control, there is no information as to how these fields with different constitutions arise. Least is known about the decisive point in the formation of those primary patterns which have been described as field centers and which arise as simultaneous rhythms or about diffusion rhythms which have been considered in terms of a Liesegang phenomenon. A great advance would have been made if the mechanisms underlying simultaneous rhythms or diffusion rhythms had been properly understood. Henke's suggestion that some kind of competition may produce a simultaneous rhythm is not very convincing. The mechanisms by which chemical rhythms with greater precision can arise will be described later. We turn now to the *Drosophila* wing pattern, which has given some information about the genetic mechanisms involved in pattern formation.

THE EPIGENETICS OF THE DROSOPHILA WING PATTERN

The role of genes in developmental processes can perhaps best be studied in *Drosophila,* owing largely to the availability of mutants of known phenotypic effects and also to the ease with which the development of certain organs can be followed. Among other organ systems, the wing pattern of *Drosophila* has been an object of intensive genetic analysis. The wing is a thin plate with a roughly oval outline. On the wing surface, five longitudinal veins and two cross-veins form a characteristic pattern.

Goldschmidt (1935b, 1937) and Waddington (1939, 1940, 1942b, 1942d) have described the development of the wing in the normal and in a number of mutant forms in *Drosophila.* A very generalized scheme of the stages in development of the normal wing is given in Fig. 8a–h. In the mature larva the prospective wing can be recognized as a thickened and folded area of the mesothoracic imaginal buds or discs. Shortly afterward, the folds elongate and the bud takes the form of a thin blade. Within the expanded blade the prepupal veins can be seen, but they are obliterated in the next stage when the wing is inflated and the two surfaces are forced apart. During pupation the wing contracts again and the two surfaces come together and leave spaces between them that thus form the pattern of the definitive wing veins. Finally, the fluid in the wing is forced out and the wing, not as yet completely mature, assumes the adult form.

During development the contracting wing is in a state of delicate balance, and during this period even minor genetic or environmental disturbances can produce large phenotypic effects, some of which are shown in Figs. 8i–p. Such periods in wing development have been termed 'epigenetic crises.' A number of genes

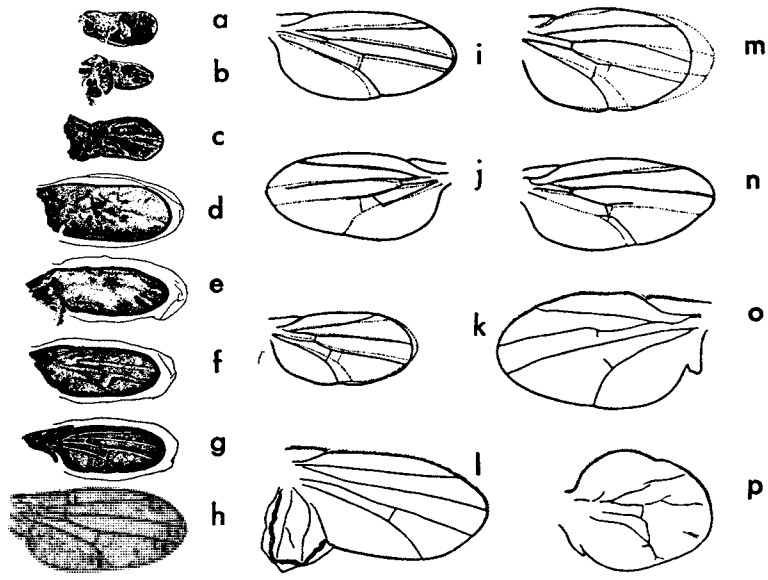


FIG. 8. STAGES IN THE DEVELOPMENT OF THE NORMAL WING OF *Drosophila* (a-h) AND THE EFFECTS OF CERTAIN MUTANT GENES ON THE WING-VEIN PATTERN (i-p)

i, *shifted-2,* the longitudinal veins pushed together; j, *veinlet,* showing elimination of a considerable part of the distal regions of the wing veins and a shift in position of the posterior cross-vein; k, *dachs,* causing a short square wing in which the veins diverge at a greater angle than normally; l, *blot,* producing a small mirror-image twin wing; m, *broad,* the wing blade relatively broader than normal, producing divergence of the wing veins; n, *cubitus interruptus,* the distal part of the fourth vein is eliminated and the fifth vein shifts forwards; o, *dachsous,* causing the wing to become large so that the cross-veins diverge at a greater angle; p, *dachsous-fourjointed-plexus,* causing abnormal wing-veins. L_1—L_5, longitudinal veins; the anterior and posterior cross veins are located between L_3 and L_4 and between L_4 and L_5, respectively. (After Waddington, 1956.)

tend to increase the wing's contraction in length or width or to affect the contraction in general. During the later stages of contraction, when the veins are in the form of cavities, some genes cause obliteration of veins; others produce extra veins.

The study of a large number of genes affecting wing development has provided a general picture of epigenetic systems, or the whole system of causal relations, involved in the development of the wing. This developmental sequence has been largely confirmed by producing mechanical defects in the wing at definite periods (Lees, 1941). Similarly, phenogenetic studies (Goldschmidt, 1935a; Henke, 1947; review, Waddington, 1950) have shown that a series of abnormalities, produced by treating the wing at definite periods with sublethal temperatures, parallel the phenotypes produced by mutant genes. In *Drosophila,* each epidermal cell on the surface gives rise to a single hair (Dobzhansky, 1929), and by counting these it is possible to discover alterations in cell number and arrangement following various modifications of temperature. A comparison of cell densities in mutants with those in the phenocopies gives a clear picture in terms of cell division, growth, and interaction of various factors which influence the epigenetic landscape. Although such studies yield some information about the period at which genes exert their influence during development, they do not reveal the nature of the primary gene activity or its mode of action.

An important fact emerging from the study of mutant phenocopies (Henke, 1947), is that the sensitive period of a mutant phenocopy usually coincides with the period at which the mutant diverges from the normal developmental course. This fact enables us to consider development in terms of systems which are unstable during sensitive periods and which, under genetic or environmental influences, may take up different developmental paths that are open to them. On the other hand, in normal indi-

viduals the developmental systems are well buffered against disturbing influences, so that in spite of minor variations a uniform end result is achieved. This is known as "epigenetic canalization" (Waddington, 1942a).

The sensitivity of an animal strain to environmental stimuli is under genetic control. Now, if selection is practiced for the capacity of a strain to respond to an environmental stimulus in a particular manner, genotypes can be produced which would develop into the selected phenotype in the absence of the particular, original environmental stimuli (Waddington, 1953b, 1956a, 1957a, 1960; reviews: 1957b, 1961). Thus, the *crossveinless* and *bithorax* abnormalities produced by environmental means, after selection had been practiced for some generations for responsiveness to a certain environmental stimulus, could be genetically assimilated. Bateman (1959a,b) and Scharloo (1961) have confirmed Waddington's findings. From this general picture of epigenetic processes in wing development, we turn to the specific pattern of the wing veins.

Intensive developmental and genetic analysis of the pattern of the wing veins in *Drosophila* has led to some important conclusions (reviews: Waddington, 1948, 1956b). Close examination of the pattern of five longitudinal veins (L_1–L_5) and two cross-veins (anterior and posterior) in the *Drosophila* wing (Fig. 8h) shows that it arises in a series of steps. Different veins appear during different periods of development. A number of mutants affect the wing vein pattern in different ways, some of which are shown in Fig. 8.

The genetic studies on the pattern of five longitudinal veins reveal that they do not always behave as a unit. A number of genes have effects on particular veins or particular parts of certain veins. House (1953a, 1953b, 1954, 1955) has shown that some genes with effects on particular parts of the wing vein may exert subthreshold effects on neighboring regions; in combination with other genes such genes may produce visible alterations in neighboring regions. These genes have pronounced effects on particular sections of the venation rather than on the whole wing system. However, there also exists a certain reaction of the whole wing-vein pattern to a local defect produced by a gene. In one mutant (Fig. 8n) that causes the absence of the L_4 vein, the tip of L_5 is found to move forward to fill in the space left by the absence of L_4. The posterior cross-vein in the wing pattern also exhibits a great tendency to adjust its position in relation to changes in the general surroundings (Fig. 8j, k, n). These facts suggest that the final wing must be considered as representing a state of equilibrium resulting from the interaction of numerous factors in a very complex manner.

There are reasons to believe that the seemingly simple pattern of wing veins may be the result of cooperation of a number of gene-controlled processes. This is well demonstrated in Timoféeff-Ressovsky's (1931) selection experiments on a mutant (*venae transversae incompletae*) that causes a break in the posterior cross-vein in the wing of *Drosophila funebris*. Timoféeff-Ressovsky was successful in isolating different stocks in which the break occurred anteriorly or posteriorly and a third stock in which the breaks in the posterior cross-vein occurred at both ends with equal frequency. In other words, modifier genes could alter the expression of the main gene. Thus, a number of genes with individual effects interact in the control of various developmental processes (see Scharloo, 1962). In some cases these processes may be disturbed to the extent that many parts of the vein may be missing or extra veins may be formed (Timoféeff-Ressovsky, 1934). Recent experiments (Waddington, 1955) on the posterior cross-vein in *Drosophila melanogaster* have given some information on the buffering of epigenetic systems in the cross-vein pattern.

The pattern of wing veins in *Drosophila* is highly instructive, for it reveals that, even in the determination of a small part of a vein, a number of processes may be at work (for the growth pattern in lepidopteran wing veins, see Weiss, 1925). Further, the ability of veins to adjust their position in relation to modifications arising in the neighboring regions indicates that the final pattern may be an outcome of a gradually developing equilibrium between a number of forces which may be complex. Although the number of such forces is bound to be large, only a few of them are likely to exert major effects. For instance, in the contraction and subsequent expulsion of the fluid during

wing development (for the role of hemolymph pressure during unfolding of the wing, see Stern, 1927), tension in the wing epithelia and the hydrostatic pressure of the body fluid must be some of the major factors with general effects on the process. That need not be surprising, for numerous examples in insect development can be given (Köhler, 1932; Henke, 1953b; Waddington, 1943, 1962) to show that such physical factors play an important part in pattern formation.

Certain facts, however, need further explanation if the genesis of such patterns is to be properly understood. The obvious question is to what extent the physical factors influencing a pattern can be controlled by genes. It may not be too optimistic to consider changes in the physical properties of developing systems in terms of changes in the properties of macromolecules, in turn controlled by genes. But we still have to provide an explanation for those factors which dictate that there are going to be only five longitudinal veins in the wing of *Drosophila* and not six, and that each one of them is going to occupy an assigned positon in the wing as a whole. Perhaps the answers to such questions could be sought better in simpler patterns, to which we shall turn.

CELL MIGRATION AND CELL AGGREGATION IN COLOR PATTERNS

The study of insect color patterns has revealed mechanisms of general importance in pattern formation. In some insects, in which the cuticle of the thorax or abdomen is transparent, the color may be due to the presence of pigment granules in the epidermis or in the peripheral fat body. When the cuticle is transparent, it is possible to photograph the pigment cells during development and to study the mechanisms involved in the formation of color patterns.

The changes in color pattern at metamorphosis brought about by the migration of chromatocytes have been examined in some detail in the Thaumaleidae (Hinton, 1958) and Simuliidae (Hinton, 1959). There is a relationship between the presence of skeletal muscles and the color pattern. The chromatocytes cannot be present between a muscle and the area of the cuticle in which it is inserted, and, therefore, changes in the disposition of the skeletal muscles necessarily impose changes in the pattern of the chromatocytes. The chromatocytes present in the mature larva persist in the adult, without any appreciable change in number, and thus are responsible for the color pattern of the pupa as well as for that of the young adult. In both families the establishment of color pattern involves a considerable migratory movement of chromatocytes, which form new aggregation patterns. The migrations are supposed to be due to a change in the hormonal balance, but no direct evidence is available to support this assumption. This type of formation of color pattern is exceptional and in some ways resembles the kind of color changes that are known to occur in some urodeles. For instance, in early stages of *Triturus torosus,* the melanophores, after their original dispersal from the neural crest, form new aggregation patterns (Twitty, 1945). The mechanisms by which such aggregation patterns in insects are formed await further analysis.

Another interesting case is that of changes in the color pattern brought about by migration and alterations of shape of pigment cells, a unique method developed by insects. In *Chaoborus (Corethra)* species, in their dark-adapted state, the chromatocytes cover the dorsal and dorsolateral sides of air sacs and prevent the larva from becoming conspicuous on a dark background because of light reflected by the transparent sacs. If the larva is transferred to a light background, the chromatocytes disperse and migrate to the ventral surface of the air sacs, leaving only four large black solid spots. Such a change from the dark-adapted state can be produced in 10 to 30 minutes and is reversible. The movements of chromatocytes are under endocrine control and can also be induced hormonally (Dupont-Raabe, 1957). However, in this case it is not known why a particular group of cells reacts differently to changing physiological conditions.

CELL SPECIFICITY AND CELL ORIENTATION IN CUTICULAR PATTERNS

Although during development of color patterns a considerable cell migration takes place, the work on *Rhodnius prolixus* well illustrates that cells retain the property of forming spe-

cific elements in a pattern. There are five nymphal stages in *Rhodnius,* during which the cuticular pattern of the abdomen is thrown into stellate folds and is pigmented with melanin. On the surface of the abdomen are bristles that arise from parts of smooth cuticle known as plaques. Wigglesworth (1940) noted changes in the cuticular pattern after burning the part between two lateral pigment spots in the third instar larva. An interesting outcome of these experiments was that the two spots united to form a continuous spot in the fourth and fifth nymphal stages. If one pigment spot is burned, it is replaced by unpigmented cuticle in the next instar. Further experiments confirmed previous observation that the cells which form a given element in the pattern retain the property of forming this element in spite of repeated division and migration, and these properties become manifest only under the influence of the appropriate hormones during metamorphosis. There are reasons to believe that the property of cells to form a given element in a pattern is likely to be under genetic control. Sobel's (1953, 1954) experiments on the *Abnormal Abdomen* mutant in *Drosophila melanogaster* have shown that the location of spots on the abdomen is influenced by a large number of genes.

Experiments on the bristle-bearing plaques showed that, when the cuticle was distended by artificial means, the cells forming the plaques were stretched also and thus formed comparatively larger plaques. On the other hand, in an unstretched cuticle smaller bristles were formed. The number of new plaques arising at a given molt was influenced by the nymphal stage from which the molting hormones were provided, a fact which shows that the number of plaques is not predetermined. But the number of new plaques appearing in each successive molt is determined by the number of cells intervening between existing plaques at the commencement of molting. Wigglesworth (1940) suggested that the bristle-bearing plaques exert an inhibitory influence on the surrounding plaques. It is supposed that, during the activity of the molting hormones, the plaque bristle-forming centers become active and draw some chemical substance or substances from the surrounding cells. The cells from which the chemical substances are drained off do not form these structures, but cells not lying in the immediate vicinity of existing plaques are capable of utilizing surrounding substances and therefore form bristles and plaques. In short, there is a sort of competitive mechanism that insures an approximately even spacing between irregular structures. It is not very different from the mechanism that produces streaks of cloud with clear zones in between; in the latter case no two streaks of cloud can survive too close while competing for condensed vapor. More precise mechanisms that determine the positions of bristles will be described later.

In *Rhodnius* the orientation of abdominal bristles is fairly regular. The constancy of bristle orientation is demonstrated by the fact that the bristles regenerating over a burned area show more or less normal orientation. This fact led Wigglesworth (1959) to conclude that there must be within the epidermal cell some kind of "cytoskeleton" which defines its anteroposterior axis. Piepho (1955) and Piepho and Marcus (1957) studied the orientation of scales in displaced implants in Lepidoptera and came to the conclusion that there exists some kind of "orienting force" in the general epithelium, while Picken (1949) showed that the orientation of the scales is due to the oriented protein polysaccharide of which the scale is composed.

The factors responsible for the orientation of the cuticle of abdominal tergites in *Rhodnius* have been investigated by Locke (1959, 1960). The larval cuticle in the abdominal region of *Rhodnius* has a uniform pattern of transverse ridges or ripples. The effects on the adult cuticle were studied by altering the orientation of pieces of larval integument in the developing insects. Grafting experiments within a segment showed that the pattern is only disturbed when grafts are interchanged in axis, a fact which suggests that the cells responsible for the pattern are similar from side to side but differ from anterior to posterior. There appeared to be an axial gradient within each segment, for the anterior segments show a greater ability to restore continuity of the distorted pattern than do posterior segments. Pieces of integument from different axial levels within a segment, when juxtaposed, affect the pattern according to the level from which they come. This in-

structive example shows that apparently similar elements within a pattern may possess a certain specificity of their own. The underlying process by which such individuality comes into being is not understood.

GENES IN PATTERN DIFFERENTIATION

We have discussed some of the important mechanisms in pattern formation. A better understanding of the problem can, perhaps, be achieved in simpler developmental systems which can be analyzed by applying precise genetic methods. In this respect the bristles of *Drosophila* provide a very suitable material. The bristles are of two types, microchaetae and macrochaetae. The microchaetae are smaller, numerous, and irregularly arranged. The macrochaetae are larger, fewer, and constant in number and arrangement. The bristles show patterns of varying complexity, some regular, some semiregular, and some highly irregular.

The development of the bristle organ has been studied in some detail. In insects, in general, the bristle organ is derived by two successive mitotic divisions of a single epithelial cell. These four cells form, respectively, the bristle proper, its socket, a sensory nerve cell, and its sheath (Lees and Waddington, 1942; Schwenk, 1947; Henke, 1951, 1953; Wigglesworth, 1953). In *Drosophila* the trichogen and tormogen cells arising out of the division of a single hypodermal cell give rise to a bristle and its socket, respectively. The bristles are innervated (Stern, 1938) by peripheral nerve cells which are bipolar and are situated below the hypodermis. The shape of a bristle depends on the growth of chitin chains of the cell wall in an orientation parallel to the long axis of the cell (Lees and Picken, 1945). Various mutants produce their phenotypic effects by interfering with developmental processes during different stages of bristle development (Lees and Waddington, 1942).

In *Drosophila*, the availability of numerous genes which influence bristle pattern in different ways makes it possible to study pattern formation in terms of gene-controlled processes. The dorsal surface of the head and thorax of *Drosophila* is covered by macrochaetae. These large bristles, in normal individuals, occupy 40 specific sites and form a constant pattern. Numerous genes are known to affect the differentiation of bristles at specific sites. Earlier investigations on pattern genes which cause supernumerary bristles (MacDowell, 1915; Neel, 1940, 1941, 1942) or on genes which remove bristles (Plunkett, 1926; Goldschmidt, 1931; Sturtevant and Schultz, 1931; Muller, 1932; Child, 1935; Ives, 1939) attempted to elucidate the role of genes in localized differentiation, but no satisfactory explanation could be provided.

A number of alleles at the *scute* (*sc*) locus eliminate bristles. Each allele acts at specific sites in the pattern of macrochaetae (Dubinin, 1929; Serebrovsky, 1931). Individuals that carry, for instance, alleles sc^1, sc^2, or sc^4 usually differentiate 26, 30, or 12 bristles, respectively (Fig. 9). Sturtevant (1932), realizing the importance of genetic mosaics in developmental analysis, first showed that *sc* tissue was autonomous in development. Stern (1954a, b) developed a hypothesis according to which the cells which form different numbers of bristles do so by responding differently to an unvarying prepattern. Thus, in individuals carrying sc^+ (wild-type) and sc^1 genotypes, a patch of sc^+ tissue in sc^1 surroundings responds to an unvarying prepattern by differentiating a normal ocellar bristle. On the other hand, a small patch of sc^1 cells in sc^+ surroundings fails to form a typical ocellar bristle (Stern and Swanson, 1957).

Experiments in which alleles of the achaete (*ac*) locus were employed revealed certain interesting facts. Individuals carrying the allele ac^+ differentiate all 40 bristles on head and thorax, but substitution of ac^+ by *ac* results in the elimination of certain bristles, including the two posterior dorsocentrals. In these individuals, although *ac* tissue usually did not form a posterior dorsocentral bristle, on a mosaic thorax sometimes a bristle of subnormal size did appear. However, such bristles were always found in the close neighborhood of ac^+ tissue (Fig. 10). These findings give the impression that diffusion of some substance from the ac^+ tissue into unresponsive *ac* surroundings might have caused the latter to respond to an underlying prepattern, but, in certain cases in which the ac^+ tissue did not cover the normal site of dorsocentral bristles but existed in its vicinity, the bristles differentiated at the abnormal site.

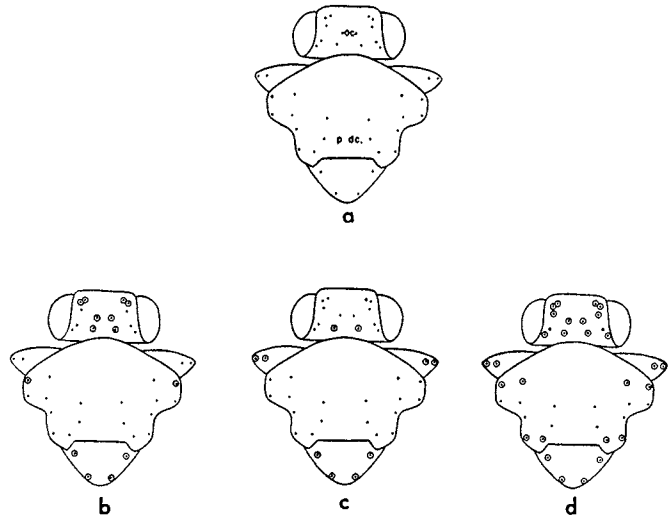


FIG. 9. THE POSITIONS OF THE LARGE BRISTLES ON THE HEAD, PROTHORAX, AND MESOTHORAX OF *Drosophila melanogaster*

a, dorsal view of head, prothorax, and mesothorax of the wild type, showing the positions of 40 bristles. oc, ocellar; p.dc., posterior dorsocentrals; b—d, the positions of bristles in sc^1, sc^2, and sc^4 respectively. Sites surrounded with a circle do not differentiate bristles. (After Stern, 1956a.)

These results suggest that the prepattern is distributed over a gradient field with a peak at a typical bristle site but is capable of evoking a response at lower levels (Stern, 1956a, 1956b).

The sex-comb of *Drosophila* provides an opportunity to study differences in the responsiveness of the two sexes. A male *Drosophila* is characterized by the presence of a row of thick bristles, known as a sex-comb, on the first tarsal segment of the foreleg. Females do not possess a sex-comb. In experiments involving genetic mosaics (Stern and Hannah, 1950), the differentiation of sex-comb teeth was found to occur whenever cells of male genotype were present in the regions of the foreleg, even though the majority of the tissue was of female constitution. These findings suggest that an identical prepattern exists both in male and female forelegs; only a certain region is capable of evoking sex-comb differentiation, and only in male cells but not in female cells. This interpretation is further attested and refined by cell lineage studies on gynandric male sex-combs (Tokunaga, 1962). The site of prepattern singularity for the primary sex-comb is recognized as a strip of tissue extending transversely in the distal part of the developing basitarsus. During this period, the differential responsiveness of this tissue to an identical male and female prepattern is determined. If it is to form a sex-comb, then the male tissue undergoes a shift in its position and occupies the site of the primary sex-comb. In female tissue, no change of position is involved. However, the reason that male tissue normally undergoes a shift of position to occupy the region of the prospective sex-comb, whereas the female tissue is com-

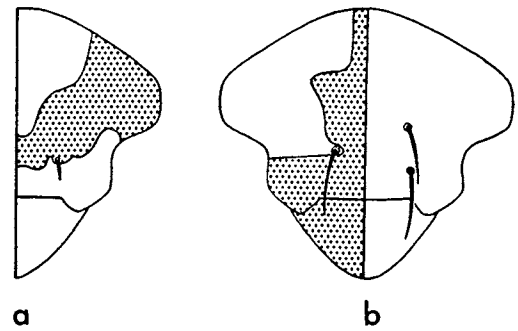


FIG. 10. MOSAIC INDIVIDUALS OF *Drosophila*

Shaded regions *ac*, white regions ac^+. a, showing a rudimentary bristle in *ac* tissue on one side of the thorax; b, differentiation in ac^+ tissue of a dorsocentral bristle at an abnormal site, the right half of the thorax consisting of ac^+ tissue only. (After Stern, 1956a.)

paratively stationary, is not understood. But, at least, the initial topographic likeness of male and female tissue is informative, for it indicates that the prepattern is identical in both the male and female cells.

One important fact emerging from the studies on triploid intersexes (Stern, 1957; Hannah and Stern, 1957), which possess fewer teeth than males, is that the response of intersexual cells to the sex-comb prepattern is lower than that of males cells, so that fewer cells respond to form teeth, but, whenever a cell does respond positively, it reacts in a fully male fashion and not in an intermediate degree. Thus, although intersexuality may cause a lower response in general, the response of a single intersexual cell to the developmental prepattern is all-or-none in type.

There are certain mutants in *Drosophila* that cause a repetition of structures. For instance, in *engrailed* (*en*), a secondary sex-comb is formed on the forelegs of males. It appears more or less as a mirror image of the normal primary sex-comb. The question therefore arises whether normal individuals possess a prepattern for an extra sex-comb. Using genetic mosaics, Tokunaga (1961) showed that when *en*/*en* tissue occupied the area of the secondary sex-comb on *en*/+ tarsi, differentiation of teeth occurred even when the homozygous area was small. It was concluded that *nonengrailed* as well as *engrailed* forelegs possess a prepattern for a secondary sex-comb and that phenotypic differences depend on the response of the genotypes.

The various taxonomically important forms of sex-comb in *Drosophila* species exhibit variation of the kind to be expected in the light of the processes discussed above (Stern, 1954a, 1954b). Very likely, the evolutionary process that diversified the sex-comb feature began as a response of mutated genes to pre-existing developmental prepatterns, and new prepatterns arose by mutation. There is reason to believe that homoeotic mutant genes produce their effects by remodelling the prepattern, for there are genes that not only produce extra sex-combs on the second and third legs, unlike normal flies that possess a sex-comb only on the foreleg, but also change the entire bristle pattern of the second and third legs into a pattern typical of the first (Hanna-Alava, 1958a, 1958b).

The study of genetic mosaics reveals that pattern differentiation may occur in two ways: different genotypes may either respond differently to an unvarying prepattern or they may create different prepatterns. In genetic mosaics these two processes appear to vary independently. The assumption that a prepattern exists is necessary to explain effects produced by genes at specific sites in patterns involving localized differentiation. The term prepattern signifies any kind of spatial difference in development and need not be mistaken for a morphogenetic field capable of organ differentiation with qualities of regulation, for a prepattern may or may not have morphogenetic consequences.

If we consider a prepattern as a spatial distribution of certain physicochemical factors, it is difficult to conceive how such a distribution could remain stable over a given period of time. Since developmental processes are dynamic in nature, pattern formation may be considered as a sequence of prepatterns and patterns in which every pattern acts as a prepattern for the next pattern till the final pattern is realized. Thus, a change occurring at any one of the steps in this hierarchy of events could modify the resulting pattern; such a change might be produced by either genetic or environmental factors. Taken in this light, bristle pattern appears to be an atypical case for two reasons: first, it remains stable over a fairly long time in development, and, second, it represents a final pattern in the sense that no other pattern becomes superimposed upon it. On the other hand, although the study of pattern genes beautifully demonstrates the invariable presence of a prepattern, it does not provide any information on the genesis of prepatterns.

PATTERN DIFFERENTIATION IN MORPHOGENETIC FIELDS

In insects with complete metamorphosis, such as Diptera, the future imaginal tissue is present during larval life in the form of imaginal buds or imaginal discs. These originate from the hypodermis of the embryo, which is a part of the ectoderm. During development various imaginal discs differentiate to form specific organs. The prospective significance of imaginal discs was studied by Stern (1940) and Zalokar (1943,

1947), while Birmingham (1942) demonstrated the boundaries of the tissue derived from the cephalic imaginal discs. The imaginal discs vary considerably in their response during development to a given concentration of hormone (Bodenstein, 1943, 1957).

It was thought for some time that long after the period of embryonic determination there is a second period when the imaginal discs are determined (Geigy, 1931; Lüscher, 1944; Gloor, 1947). However, the determination of imaginal discs is by no means fixed even during a late period of development. For instance, in the *Bar* mutant of *Drosophila* the fate of the eye imaginal discs can be modified by treating third instar larvae with temperature. An increase in temperature produces an increase in the number of eye facets, without involving an enlargement of the eye disc, at the expense of head ectoderm (Steinberg, 1941; Chevais, 1943). These findings suggest that the fate of imaginal discs can be altered during the last larval stage. Similar studies on eye imaginal discs (Bodenstein and Abdel-Malek, 1949) confirmed these findings. The labile character of the imaginal discs can also be demonstrated by subjecting third instar larvae to a heavy dose of X-rays. In this case, many of the imaginal cells are killed and those which remain produce duplicate organs or organs showing homoeosis — e.g., eyes located in the place of antennae or vice versa (Waddington, 1942b, c). In homoeotic mutants the imaginal discs also show plasticity of development comparable to the normal and ordinary mutant forms (Vogt, 1946a, 1946b, 1947). For instance, in individuals carrying the gene *aristopedia*, which causes part of the antennal bud to develop into a leg instead of an antenna, the amount of bud diverted into abnormal differentiation can be altered by treating the third instar larvae with high temperature. It is likely that the essential features of the imaginal discs are determined by influences from the mesoderm during the last larval stage, and, therefore, their determination in the embryo can only be of a preliminary kind (Shatoury, 1955).

An important feature of imaginal discs is their capacity of regulation both within themselves and between serial or opposite imaginal discs (Waddington, 1947, 1953a; Pantelouris and Waddington, 1955). If one of the two wing discs of a larva is removed mechanically, the remaining wing disc, in addition to forming a wing as it does normally, also gives rise to the wing of the disc that had been removed. On the other hand, in the *bithorax* mutant the haltere disc of the same side of the body is capable of performing the function of a wing disc that has been extirpated. Hadorn and his collaborators (1946–1963) have performed numerous experiments on *Drosophila* imaginal discs which have an important bearing on pattern formation, and these will be discussed in some detail.

The nature and degree of regulation in genital imaginal discs have been studied in great detail (Hadorn, 1963; Hadorn and Gloor, 1946; Hadorn, Bertani, and Gallera, 1949; Hadorn and Fritz, 1950; Hadorn and Chen, 1956; Ursprung, 1957, 1959, 1962; Lüönd, 1961; reviews: Hadorn, 1953, 1961; Ursprung, 1963). Genital imaginal discs of third instar larvae were cut into medial, paramedial, or transverse parts of different sizes. The fragments were then implanted into the body cavity of a host larva, which provides a natural medium for the implants with ample nutritive material and hormones. The fragments differentiated their imaginal structure simultaneously with the metamorphosing host.

The organ elements of the genital apparatus can be localized in the imaginal disc, and a schematic map can be designed (Fig. 11) to represent the positions of presumptive areas from which sperm pumps, claspers, anal plates, spermathecae, or vaginal plates can be derived. Different parts of the imaginal discs are only capable of differentiating their district-specific elements. Overlapping regulations do not occur, a fact which therefore suggests that the cell mass of a larval disc consists of a mosaic of districts with district-specific developmental potencies.

Each district in the imaginal disc behaves as an embryonic field (cf. Weiss, 1939) and is capable of full regulation in forming its district-specific organ element. If an imaginal disc is cut into three parts, it is capable of differentiating, for instance, into three pairs of anal plates instead of only one pair. These results suggest that parts of the field are endowed with the capacity of restoring the normal organization of an intact field, thus forming

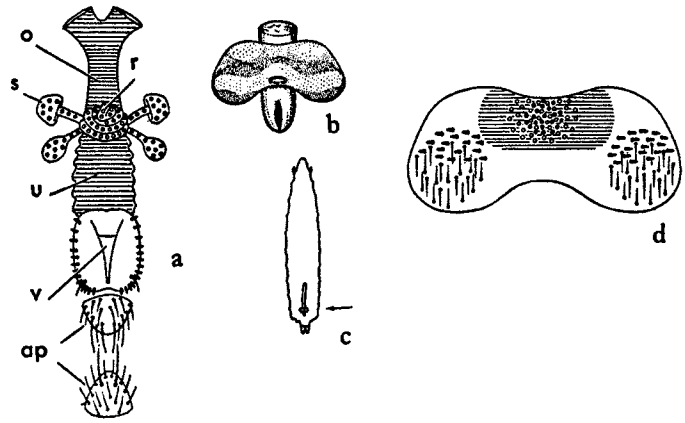


FIG. 11. THE DETERMINATION OF THE FEMALE GENITAL APPARATUS OF *Drosophila*

a, the genital organs of the adult: ap, anal plates; o, oviduct; r, receptaculum seminalis; s, spermatheca; u, uterus; v, vaginal plate. b, the genital disc, shown lying across the intestine. c, the position of the genital disc in the larva. d, the location of fields giving rise to adult genital apparatus, represented in a schematic map; the shading of areas in d corresponds to those of a. (After Hadorn and Gloor, 1946.)

field-specific structures. An important feature of the regulation of a field district is that it is accompanied by an increase in cell number; mitotic activity continues until the normal cell number characteristic of a whole district is achieved. Thus, the process involved in the regulation of field parts appears to lead to 'all-or-none effects.' However, a field is only able to regulate itself if a certain minimal mass of field material is present, or can be regenerated, before the onset of metamorphosis (Fig. 12).

The capacity of regulation in transplanted portions of a field is moreover influenced by the interval of time that elapses before the host larva enters metamorphosis. In younger hosts there is full regulation of field parts, largely owing to the availability of material and time required for extra cell divisions, but in older hosts the implants are forced to differentiate much earlier due to the steadily rising level of the hormones promoting metamorphosis. Disc fragments from older donors cannot regulate when transplanted into hosts of the same age, but full regulation can occur if pieces are transplanted into younger or older larva or even into an adult, provided there is a long lapse of time between the partition and the onset of metamorphosis. Thus, if pieces of a disc are transferred several times from one old larva to another, full regulation may occur.

Occasionally, an imaginal disc undergoes abnormal regulation in the sense that it forms structures atypical of its own species but typical of some related species. Thus, in Loosli's (1959) transplantation experiments on dorsal metathoracic discs of *Drosophila melanogaster,* a pattern of adventitious bristles appeared. In normal individuals of *Drosophila melanogaster* such a pattern does not exist, but it can be made to appear as a result of mechanical evocation, so the normal individuals must possess a latent, potential field of this nature. A pattern of metathoracic bristles comparable to the one arising in the transplanted imaginal discs of *Drosophila* exists in the Sepsidae. Possibly, then, during the labile period of the haltere imaginal discs, a regulative process comparable to that responsible for a homologous pattern in the Sepsidae occurs. These results can be interpreted in terms of the prepattern-competence concept of Stern (1954a, 1954b). According to this concept, changes in the competence of cells to respond to an unvarying prepattern may result in the expression of different phenotypes, provided a prepattern for such structures is present. In Loosli's experiments, the occurrence of adventitious bristles does not appear to have involved any change in the prepattern of the metathoracic discs. However, there are other cases in which imaginal discs may undergo a change of prepattern. For instance, if pieces of genital discs are cultured in adult larval hosts and are then retransplanted into metamorphosing hosts, the genital material

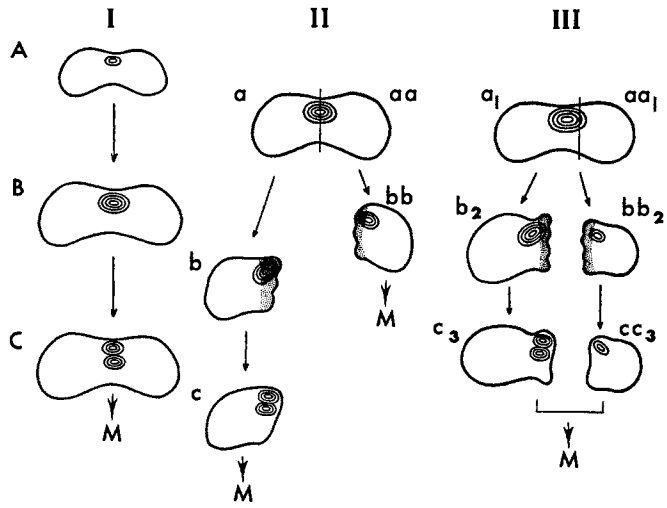


FIG. 12. A SCHEMATIC REPRESENTATION OF THE DETERMINATION OF THE SPERMATHECA FIELD IN THREE EXPERIMENTAL SERIES

I, the spermatheca field forming secondary fields during the larval stage under normal conditions; A, the location of the spermatheca field; B, the spermatheca field grows to a certain size necessary for its division into two secondary fields; M, onset of metamorphosis. II, in a series of experiments, the spermatheca field was cut medially into two halves, a and aa. The part (a) implanted into younger hosts differentiated as in IC, but the part (bb) implanted into older hosts did not differentiate into two secondary fields. III, in another series of experiments, the spermatheca field was divided into a larger part (a_1) and a smaller part (aa_1). The larger part showed normal regeneration, but the smaller part failed to regenerate into two subfields, an outcome suggesting that a minimal mass is required for the regeneration of secondary fields. (After Hadorn and Chen, 1956.)

gives rise to head structures — namely, antennae, aristae, orbital setae, ocelli, etc. (Hadorn, 1963). Similarly, wing parts can be obtained from eye-antenna discs (Schlaepfer, 1963). Such cases of homoeosis indicate a real change in the prepattern of imaginal discs.

In *Drosophila,* because of the highly specific arrangement of bristles and hairs on the body, insight into the pattern-forming processes could be gained by studying pattern formation in reaggregating cells that had been previously dissociated (Hadorn, Anders, and Ursprung, 1959; Usprung and Hadorn, 1962; Hadorn and Buck, 1963). Such experiments were first performed on vertebrates by Holtfreter (1939, 1943) and later by Moscona (1952), Weiss and Anders (1952), and numerous other experimental embryologists.

The imaginal discs in *Drosophila* can be partially dissociated by treating them with trypsin in vitro. If wing discs from different mutants and species are dissociated and mixed — *yellow melanogaster* with *ebony melanogaster,* or wild-type *pseudoobscura* with *yellow melanogaster* — and are then transplanted into the body cavities of normal *melanogaster* larvae, the resulting structures show both *yellow* and *ebony* (or wild-type) areas. The important fact is that these macromosaics show no sutures, an outcome implying that the cells derived from different wing discs can differentiate into uniform and regular structures. In the implants the patterns are composed of light and dark bristles — i.e., *yellow* vs. *ebony* or wild-type, which correspond to the normal bristle pattern on the wing margin. Thus, cells from different discs are capable of giving rise to uniform and integrated patterns ("Randborsten-Kombinate"). These results indicate that there is some kind of supercellular arranging ability in the morphogenetic field. There is also some evidence to suggest the presence of an organ-forming area in the wing discs, at the time of dissociation, for building the wing pattern. However, the dissociated cells of wing discs on transplantation do not always give rise to a normal pattern. In certain cases the bristles on the wing margin show abnormal patterns,

though in all such cases a pronounced longitudinal axis is exhibited. This result indicates that the pattern of the wing marginal bristles becomes reorganized under the influence of prevalent field conditions.

These studies give some information about pattern formation in morphogenetic fields through the dissociation of the cells of the imaginal discs and examination of the resulting pattern. However, a certain aspect of pattern formation in these experiments needs clarification. If one considers that a pattern is reformed in dissociated cells following cellular reaggregation, one would like to know by what mechanisms the reaggregation of the dissociated cells comes into being. Unfortunately, technical difficulties in the experiments employing tissue culture in vivo do not permit a direct analysis, and, consequently, cannot provide a picture of the process at work in cellular aggregation. However, it is possible that appropriate cells in dissociated discs during aggregation find each other by cell affinity. That would, however, entail considerable cell migration, which the imaginal cells do not reveal when they are cultured in vitro. This difference is quite probably due to the lack of refined techniques of tissue culture in insects, and, therefore, cell affinity as a mechanism of pattern restitution cannot be entirely ruled out. There is no doubt, however, that the dissociated cells after transplantation do undergo cellular reaggregation. If they do so without finding their exact original places in the pattern, the restitution of the pattern may represent origin of a prepattern de novo in the aggregated tissue such that cells derived from any region of the imaginal disc may form appropriate structures if located in the pattern-forming area. Futher investigations are needed to decide between these two possibilities.

An informative technique in the analysis of patterns (Kroeger, 1959b) is to combine mechanically tissues of different genetic constitutions or of different determinations — i.e., tissues which have a different developmental background. If the forewing and hindwing imaginal discs of *Ephestia* are mechanically combined and transplanted into a host, the mosaic tissue grows together to form a uniform complex. The two discs always form an integrated hinge consisting of hindwing and forewing with their typical sclerites. If different mutant tissues are combined and transplanted, they show similar features. The fact that the mosaic tissue always gives rise to integrated structures suggests that the imaginal discs first grow together to form a compound disc and later give rise to a prepattern for the hinged structures. The prepattern that gives rise to the forewing part of the hinge is identical to the one which forms the hindwing part of the hinge. Therefore, only when the final pattern is differentiated do the differences in determination begin to interact with the prepattern. The resulting pattern is the consequence of a differential response to an identical prepattern. The term 'determination' in this case denotes the competence of mosaic tissue to construct a wing hinge, while 'prepattern' signifies the site of differentiation which appears to be identical in each disc.

An important feature of a great many patterns is their bilateral symmetry. Numerous attempts have been made to explain the origin of pattern symmetry (Kroeger, 1958, 1959b, 1960a, b). If a wing imaginal disc is implanted into the body cavity of a younger host, a second wing imaginal disc, symmetrical to the first, is formed from it. Moreover, all malformations in the morphology of the hind sclerites of the original implants, arising as a result of accidental damage during transplantation, are exactly imitated in the new disc that sprouts from the cut portion of the implanted discs. When parts of the sclerite pattern are missing, this loss of structures proceeds in a strict sequence which commences at the axis of the symmetry of the respective system and progresses outward on both sides. The copying of malformations and loss of structures in a sequence from the medial parts to the lateral ones have led to the suggestion that, at a certain period of development, determination waves travel from the middle toward the sides. In certain cases such waves may travel only on one side. The waves responsible for lateral elements appear first, and those for central elements follow later.

Kroeger's hypothesis regarding the origin of bilaterally symmetrical structures has provoked considerable discussion (Waddington, 1962). It is rather difficult to accept Kroeger's concept of centrifugal pattern formation as a general

rule. Besides other difficulties, the main objection arises from the fact that all biological patterns do not arise in a centrifugal manner. In certain patterns the loss or inhibition of structures is more marked centrally than laterally, and this is just the opposite of what would be expected in a pattern that is determined centrifugally. For instance, if the development of an amphibian limb-bud is inhibited by nonspecific inhibiting agents, the inhibition of the 3rd and 4th digits is more persistent than that of the 1st, 2nd, and 5th digits (Tschumi, 1953). It must be admitted that at present we do not have any clue to the nature of processes involved in the genesis of pattern symmetry.

GENES, PRECURSORS, AND PREPATTERNS: A QUANTITATIVE APPROACH

The analysis of the development of patterns has largely confirmed the differential responsiveness of cells to an underlying invariant prepattern. The prepattern concept is attractive, but we are faced with several questions. How do the prepatterns arise? How can prepatterns be altered?

The prepattern can be pictured as the distribution of an inducing substance with regions of high and low concentration. The regions of high concentration occur at sites where structures later form. A process whereby such a distribution could arise has been suggested by Turing (1952). He considered the distribution in a morphogenetic field of two chemical substances, or "morphogens," together with an adequate supply of substrate from which they could be synthesized. These morphogens are assumed to be free to diffuse and to react with each other. Turing showed that, for certain values of the rates of reaction and diffusion, the initial homogeneous equilibrium is unstable; any disturbance of the equilibrium, for example by Brownian movement, will lead to the development of a standing wave of concentration of the morphogens. The actual pattern of peaks and valleys of concentration depends on the size and shape of the field and on the "chemical wave length" — that is, the preferred spacing between peaks, which in turn depends on the rates of reaction and diffusion.

Turing's suggestion provides a simple model of the process whereby a prepattern could arise. In cases in which a pattern is constant throughout a species, Turing's model is more satisfactory than the competitive model suggested by Wigglesworth (1959). According to Wigglesworth's model, the positions of the bristles will depend in part on which particular hypodermal cells happen by chance to be the first to differentiate, whereas Turing's mechanism could give rise to a pattern independent of the initial chance disturbance. Although a competitive mechanism could explain a series of structures whose only regularity is the approximately equal spacing between them, it lacks the precision that is required in producing patterns which are constant from individual to individual.

The arrangement of microchaetae and macrochaetae could be satisfactorily explained by similar mechanisms which differ only in the accuracy with which they are regulated. It can be shown that the positions of the microchaetae depend to a great extent on the shape of the field as a whole. Fig. 13a and b shows two solutions of Turing's equations in a uniform rectangular field. The dots correspond to peaks of concentration of one of the morphogens. The solutions differ only because slightly different reaction rates have been assumed. In Fig. 13c, d, and e, the arrangement of microchaetae on the sternites of the fourth abdominal segment of three individuals of

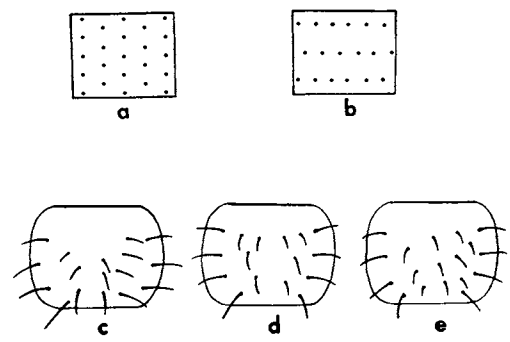


FIG. 13. COMPARISON OF SOLUTIONS OF TURING'S EQUATIONS AND THE ARRANGEMENT OF MICROCHAETAE IN *Drosophila*

a, b, solutions of Turing's equations in two dimensions; the dots represent peaks of concentration of a morphogen. c, d, e, the arrangement of microchaetae on the fourth abdominal sternite in three individuals of *Drosophila subobscura*. (After Maynard Smith and Sondhi, 1961.)

Drosophila subobscura is shown. In Fig. 13c, the microchaetae are somewhat regular; in 13d, rows of microchaetae are parallel to the boundary of the sternite; in 13e, the microchaetae are diagonally arranged. The resemblance between the arrangements in d and e and the theoretical distributions is striking. The important point to be made here, however, is that sternites with regular bristle patterns are common in flies with a low number of bristles, but an increase in the number of bristles on a sternite is usually accompanied by some loss of regularity. That is what would be expected in a Turing-type mechanism, which can give rise to a constant pattern only if the number of peaks is between 5 and 7. This is so because the number of structures formed will be the nearest integer to the ratio of the size of the field to the chemical wave length; therefore, the larger the number of structures which is to be kept constant, the smaller must be the coefficient of this ratio.

There is evidence that the arrangement of structures in animal and plant development may depend on the shape of the field as a whole. This is well demonstrated in the work of Sengel (1958) on the development of the feather papillae on the skin of the chick in vitro. If skin is removed from the dorsal regions of the embryo when the feather rudiments have just become visible, these rudiments disappear, and, later, new feather papillae develop in different positions, the first to appear forming a row along the center of the explant. Wardlaw (1953) has commented favorably on Turing's diffusion-reaction theory and has gathered evidence (Wardlaw, 1955a, 1955b, 1957, 1959) in support of it. Turing's two-morphogen model is, however, too simple to explain all the facts. As Waddington (1956b) has pointed out, it would predict that different patterns would arise if particular stages of differentiation occurred in embryos of different sizes, and this does not happen. But, in spite of this and other difficulties, Turing's suggestion of how a prepattern might arise is along the right lines.

Turing's model has been successfully applied in experiments on the expression of the pattern of ocelli and bristles in *Drosophila subobscura*. By using a major mutant gene, *ocelliless,* as a source of observable variation, an attempt has been made to deduce the nature of the pattern-forming processes and their genetic control by examining the range of variation in the end-product of these processes.

The number and arrangement of the head bristles and ocelli is uniform in the Drosophilidae, though several mutants altering the number, usually by removing one or more of these structures, are known. The sex-linked recessive mutant, *ocelliless,* in *Drosophila subobscura* removes the bristles and ocelli on top of the head. In the foundation population, homozygous for the mutant, a large number of flies lacked all the head bristles and ocelli; in the rest, varying combinations of bristles and ocelli were present. The ocelli in some of the flies were found to be displaced from their normal positions, and in certain cases their sizes were also affected. In a few flies the bristles were repeated unilaterally or bilaterally — that is, instead of a single normal bristle, additional bristles were found to lie close to the normal bristle on one or both sides. Since a positive correlation was found between the presence of bristles and of ocelli, the degree of expression of the mutant was measured by giving a unit 'score' for the presence of each of the structures studied. Starting from the original population, selection for a higher and for a lower number of structures was practiced for a number of generations.

Earlier experiments (Sondhi, 1960, 1961a) showed that selection for a higher number of structures was effective in increasing the mean number of ocelli and bristles to the wild-type level, although the selected population was still carrying the *ocelliless* gene. In the last selected generations the frequency of repeated bristles was found to be much greater than in the foundation population, and, occasionally, more than three ocelli were also seen. On the other hand, selection for a lower number of structures was effective in eliminating most of the structures in the center of the head, after which little progress was made. These experiments suggested that selection could either increase or decrease the competence of cells to respond to an unvarying prepattern present both in normal and *ocelliless* flies; or, to express the same idea in another way, selection could increase or decrease some chemical substance

(a precursor) that must be present in an adequate amount if structures are to be induced by an underlying prepattern.

If selection could increase or decrease the number of structures in the head pattern, it appeared logical to inquire whether or not the patterns themselves could be modified by selection. For such an experiment, however, a population was needed in which individuals with different types of patterns existed. Once again, the *ocelliless* mutant provided an opportunity to study this problem. In another series of experiments (Maynard Smith and Sondhi, 1960), selection for symmetrical and asymmetrical ocellar pattern was practiced on a population which was constructed by crossing the upward and downward selected lines. In the symmetrical line, only individuals with two posterior ocelli, but lacking the anterior ocellus, were used as parents. In the asymmetrical line, only flies with the anterior and the left posterior ocelli, but lacking the right posterior ocellus, were used as parents. In the symmetrical line the frequency of the selected phenotype increased from 14.75 per cent to 64.20 per cent, while the combined frequency of the asymmetrical phenotypes declined almost to zero. In other words, selection was effective in increasing the frequency of a symmetrical pattern in flies and in reducing in the same population the occurrence of asymmetrical structures. The response in the line selected for asymmetry was less striking. The distribution of phenotypes in the symmetrical and asymmetrical populations is shown in Fig. 14. An interesting feature of the symmetrical population was that the anterior ocellus, when present, was reduced in size (see Fig. 15b), often to a small speck, and was not displaced posteriorly. This fact suggests that selection produced a change in the ocellar pattern without changing the existing prepattern. The change in the ocellar pattern could have been produced by concentrating ocelli-bristle-forming substances, or some precursor, only in the posterior region of the head. The failure in producing an asymmetrical pattern of ocelli appears to be due to the absence of lateral gradients in the head of developing *Drosophila,* since the adult structure is symmetrical. Similar experiments on the microchaetae in *Drosophila* have been performed by numerous workers (Reeve and Robertson, 1954; Thoday, 1958; Reeve, 1960, 1961; for references to earlier works and a discussion of fluctuating asymmetry, see Van Valen, 1962).

The results in selecting for symmetrical and asymmetrical *ocelliless* populations can be explained if it is assumed that there is primarily an unvarying prepattern determining the posi-

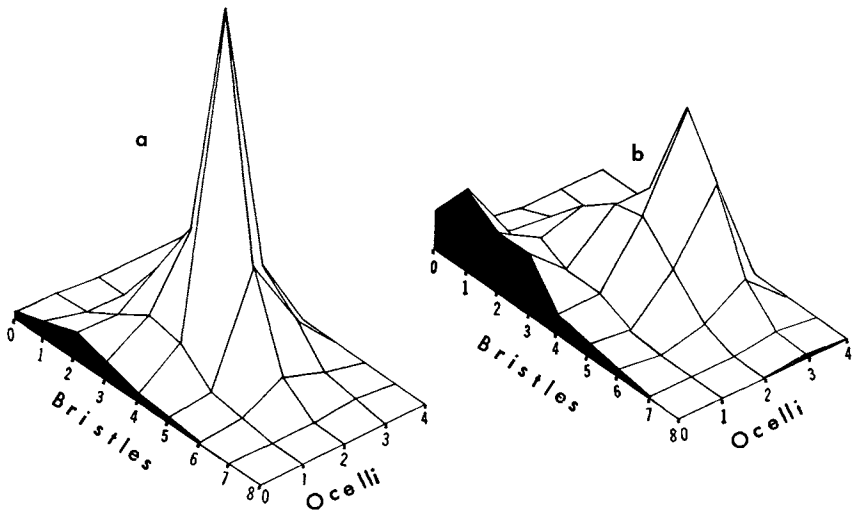


FIG. 14. FREQUENCY OF DISTRIBUTION OF PHENOTYPES IN THE SYMMETRICAL LINE (a) AND THE ASYMMETRICAL LINE (b)

Following selection for symmetry and asymmetry, respectively. (After Maynard Smith and Sondhi, 1960.)

tions of the ocelli and bristles and a varying amount of some common 'precursor' of bristles and ocelli. Since there exists in the foundation population a correlation (r=0.46) between the presence of bristles and of ocelli, and since both are derivatives of the hypodermis, a common precursor is postulated. It is supposed that there is a group of alleles which alters the expression of the *ocelliless* flies by altering the amount of this precursor, which is present in the required amount in the wild-type flies. Whether bristles or ocelli appear at these sites depends on whether the required amount of precursor is present, or, in Stern's terminology, whether the cells are competent to respond. For the sake of simplicity only three peaks of the prepattern (Figs. 15, 16) are shown, two of which represent the peaks which induce structures in the wild-type flies. The upper horizontal lines represent the threshold level which the prepattern must reach if it is to induce a

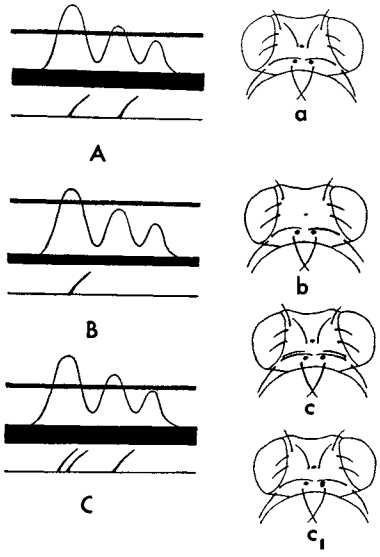


FIG. 15. THE ARRANGEMENT OF BRISTLES AND OCELLI IN *Drosophila subobscura*

a, the wild-type; b, c, c_1, individuals homozygus for the mutant *ocelliless;* b, showing the absence of certain bristles; c_1, c_2, showing the repetition of certain bristles and ocelli respectively. In the diagrams A-C on the left, the curved lines represent the prepattern, pictured as a varying concentration of an inducing substance; black areas represent the concentration of precursor; the upper horizontal line in each case represents the threshold level which the prepattern must reach if it is to induce a structure. (After Maynard Smith and Sondhi, 1961.)

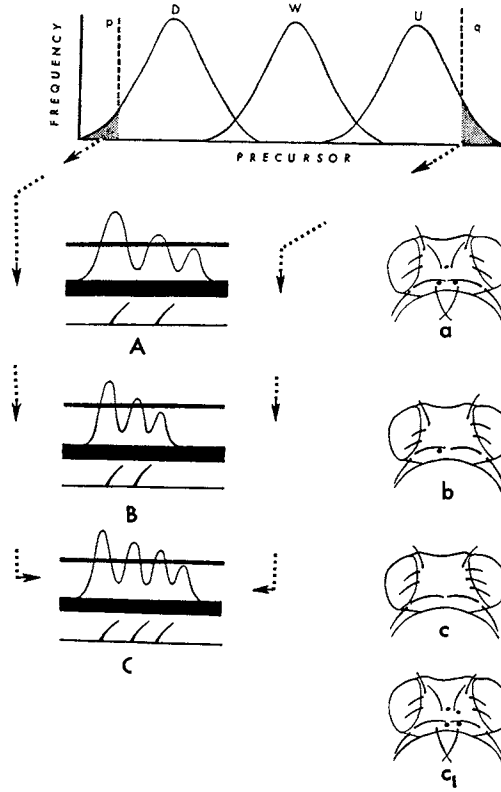


FIG. 16. THE FREQUENCY OF INDIVIDUALS WITH THE DIFFERENT AMOUNTS OF PRECURSORS IN THREE *ocelliless* POPULATIONS AND THE ARRANGEMENT OF BRISTLES AND OCELLI IN WILD-TYPE AND *ocelliless* *Drosophila subobscura*

Upper figure: in population D, the flies lack most of the bristles and ocelli; in population W, most of the individuals have wild-type phenotype; in population U, the individuals have a greater number of structures than in the wild-type; p and q represent the range of the precursor. Lower figures, the arrangement of bristles and ocelli in a, the wild-type; b, c, and c_1, individuals homozygous for the mutant *ocelliless*. In b, the bristles show homeomorphic distortion; in c and c_1, the bristles and ocelli respectively show non-homeomorphic distortion of structures arising at the two extremes of the mutant expression. See legend to Fig. 15. (Upper drawing, after Sondhi, 1962; lower drawings, after Maynard Smith and Sondhi, 1961.)

structure. The third, submerged peak of the prepattern indicates the possibility that in the wild-type flies there are peaks present to which wild-type tissue does not respond. On the basis of this morphogenetic model, which is an extension of that suggested by Stern (1954a, b) in the light of the mathematical considerations of Turing (1952), most of the experimental

results can be explained successfully (Maynard Smith and Sondhi, 1961; Sondhi, 1961b, 1962a, 1962b, 1963).

In cases in which structures normally present in wild-type flies are missing, the phenotypes can be explained on the basis of a low level of precursor substance (Fig. 15B). If the amount of the precursor is greater (Fig. 15C) than in the wild-type flies (Fig. 15A), it is possible that two structures might develop in response to a single peak of the prepattern. Such repetitions occur mainly in the populations which have been selected for an increased number of structures and, therefore, presumably for an increase in the amount of precursor.

These successes in explaining variation in adult structures in terms of varying responses to unchanging prepatterns carry with them the danger that the prepatterns may be regarded in a somewhat mystical light. So far none of these experiments has shown that the prepatterns can be changed; on the contrary, they have confirmed their constancy. Now, if all the constancies in development were to be explained in terms of pre-existing morphological constancies present at an earlier stage of development, this would lead by an infinite regression to a preformationist theory of embryology. The question now arises of whether there is any evidence of variation in the prepattern determining the positions of bristles and ocelli in *Drosophila*, and if so, of whether the variations are of the kind which would be expected if the prepatterns arise by the type of mechanism suggested by Turing.

The pattern which develops in a Turing-type process depends on the size and shape of the field in which the chemical reactions proceed and on the 'chemical wave length,' — that is, on the preferred spacing between peaks, a spacing which in turn depends upon the rates of reaction and diffusion. A small change in the ratio between the wave length and the size and shape of the field will lead to a distortion of the prepattern, but the number and general arrangement of peaks will remain the same. But if the ratio between field size and wave length changes to a greater degree, a changed pattern with different numbers of peaks will arise. Evidence that changes of this kind occur in the prepattern determining the positions of macrochaetae and ocelli will now be presented.

Changes in the prepattern of the macrochaetae and ocelli can be demonstrated in the *ocelliless* mutant, for, in certain populations of flies, changes in the positions of these structures cannot be explained easily on any other basis. Continuous selection for an increased number of ocelli and bristles in later experiments showed in some flies the presence of four to five ocelli, with distortion of the ocellar pattern — that is, the occurrence of extra structures was accompanied by a change in the positions of associated structures (Fig. $16c_1$). In the downward selected line, further selection for a reduced number of structures produced changes of two kinds. Fig. 16b shows a case in which, on the left side of the head, three large bristles on the margin of the eye are displaced posteriorly, as compared with the normally disposed bristles on the right side of the head. It is possible that a single bristle would develop in a position slightly displaced from the normal if the precursor were absent at the peak of the prepattern but existed a short distance away from it. In such cases, however, displacement of a bristle need not involve a change in the prepattern, but in this case all the three bristles on the lefthand margin of the eye were displaced in the same direction. This could only happen if the prepattern itself is distorted. Such a distortion has been termed a 'homoeomorphic distortion' — i.e., presence of a prepattern with the same number of peaks as usual but of a different shape.

In another illustration (Fig. 16c), four bristles are shown on the right margin of the eye. Here the anterior bristle is in its normal position, but the following three bristles, one more than normally present, show an irregular arrangement compared to that which is present in normal flies. Such a distortion has been termed a 'non-homoeomorphic distortion' — e.g., presence of a prepattern with a different number of peaks from the wild-type. The interest of this particular phenotype is that it shows an unusual change in the arrangement of the macrochaetae, one that can arise by Turing's mechanism. In terms of Turing's model, the ratio between the chemical wave length and the size of the field in which the waves are developing can vary within certain limits with-

out involving any change in the actual pattern formed but, ultimately, a threshold would be reached which would involve the appearance of an additional bristle or bristles and a respacing of other bristles in the field.

Prolonged experiments on increased and decreased expression of the *ocelliless* mutant (Sondhi, 1962a) have revealed a peculiar phenomenon of precursor-prepattern relationship. The important feature of the line selected for a lower number of structures was that the elimination of central structures increased the frequency of marginal bristles. The cases of nonhomoeomorphic distortion of marginal bristles were found to increase with an increase in the number of genes for lower score. On the other hand, an increase in the frequency of genes for a higher number of structures produced extra ocelli (Fig. $16c_1$) which showed nonhomoeomorphic distortion. Since such phenotypes were seen only at the two extremes (Fig. 16, top) of mutant expression, it is reasonable to suggest that the primary effect of the *ocelliless* gene is to alter the amount and distribution of the precursor and that this alteration in turn may change the prepattern. The importance of these findings lies in demonstrating a causal relationship between precursor and prepattern such that changes in the former can cause the otherwise unvarying prepattern to vary.

The change in a pattern can also be produced in a very different way, which may be the consequence of modified growth of the adjoining parts (Sondhi, in prep.). Such a case is witnessed in the downward selected line, where, as an extreme response to selection, the flies showed dorso-ventral depressions on the margins of the head. The head depressions were found to occur unilaterally as well as bilaterally, and they varied in expression. In the last generation of selection, these depressions were not restricted to the margins of the head but were found to spread over the entire surface of the head and to give it a hollowed-out appearance. In such cases the orientation of the marginal bristles was affected. The orientation of the bristles largely depended on the intensity of the head deformations. Since depressions might occur on one side or on both sides of the head, it could be seen that only that side of the head which carried the depression showed a change in bristle orientation. Moreover, in cases of severe head depressions the bristles were found to be bent at the base, a deformation which suggests that the change in the course of orientation was due to mechanical force applied during development. The changes in the orientation of bristles may be interpreted as secondary effects on the expression of the pattern (Sondhi, in prep.).

Stern (1954b) and Sturtevant (1961) have discussed the genetic mechanisms by which new patterns can arise. Loosli (1959) discovered that a new pattern which appeared when imaginal discs of *Drosophila melanogaster* were transplanted into host larvae existed in a closely related species. However, direct evidence on the evolution of structural patterns and on their genetic control is lacking. Continued selection experiments on the expression of the *ocelliless* population have demonstrated that a novel pattern can appear (Sondhi, 1961b, 1962a).

In experiments on the expression of the *ocelliless* mutant, a partial barrier preventing progress beyond the wild-type phenotype was encountered. The occurrence of such a barrier is in agreement with Waddington's concept of canalization, in which the canalizing selection during the past evolution of species insures that, in spite of minor variations, the same end result is achieved. However, the barrier, as judged by the distribution of phenotypes in the selected population, was not absolute. Rendel (1959), working with the mutant *scute* in *Drosophila melanogaster,* was able to obtain populations in which the flies had a greater number of bristles than are present in the wild-type. Therefore, an attempt was made to observe the effect of continued selection on the *ocelliless* population, with the view that an increase in the frequency of genes promoting a higher score might bring to expression new structures, though their appearance at specific sites could not be predicted. The results of these experiments showed that a novel pair of bristles would arise, always at a specific site and with a definite orientation. The frequency of such bristles, or 'neomorphs,' was found to increase with progressive selection. They were seen only in flies in which all the normal structures were present. A careful examination of flies closely related to *Drosophila* made available at

the British Museum of Natural History, London, revealed that a homologous pair of bristles does exist in a closely related family, the Aulacigasteridae. Such bristles were seen in *Aulacigaster leucopeza,* which also has a similar pattern of macrochaetae and ocelli.

There is some reason to believe that the occurrence of neomorphs is not attributable to the appearance of a new peak in the prepattern; if it were so, the neomorph would be associated with the displacement of existing peaks. Yet, in the flies in which the neomorphs occur, no change in the position of associated bristles is seen. Moreover, the presence of a homologous pair of bristles in a closely related family strongly suggests that the prepattern of these bristles is present in wild-type *Drosophila.* If so, the origin of neomorphs can be ascribed to an increase in the level of the precursor reaching the peak of the prepattern which lies submerged in wild-type individuals (Fig. 17).

The above analysis has provided some information on the nature of mechanisms at work in pattern formation. In these studies, by employing a mutant gene which causes variation in a constant pattern, an attempt has been made to deduce the nature of developmental processes and their genetic control by examining the range of adult variation. The limitations of such an analysis are obvious. Although the use of the methods of quantitative genetics in problems of development reveal to us the complex and integrated nature of developmental systems in terms of genes, little can be learned from such studies about the intermediate steps that are involved in the transition from gene to structure.

DISCUSSION AND CONCLUSIONS

In pattern formation we are mainly concerned with mechanisms by which order is established in developing systems. Since most of the visible patterns are expressions derived from an underlying primary pattern, it is the study of the latter that should provide a real clue to our problem. There have been two major attempts in this direction, one made by

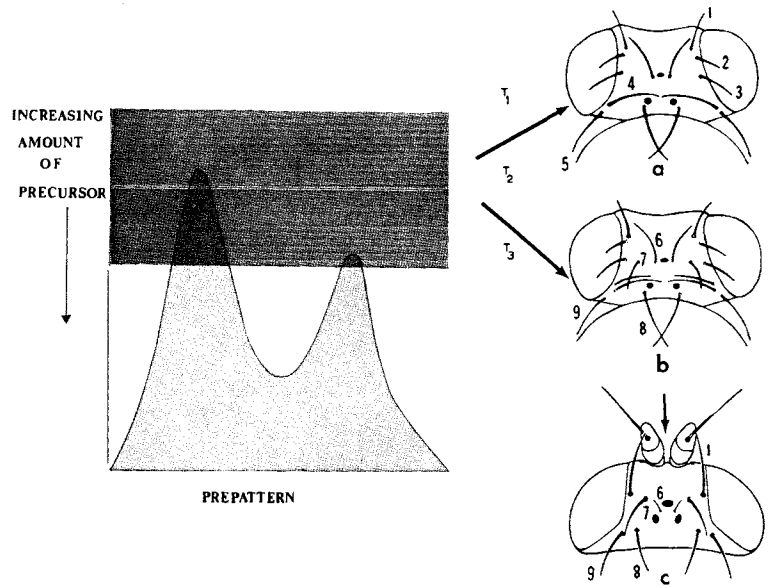


FIG. 17. MODEL TO EXPLAIN THE ORIGIN OF A NEOMORPH

Left, two peaks of the prepattern are shown. The larger one represents the peaks which induce structures in the wild-type flies, the smaller one represents the position of the neomorph. T_1, T_2, and T_3 represent three successively increasing levels of the precursor. If the amount of precursor falls between T_1 and T_2 wild-type structures are formed (a). Individuals with amounts of the precursor falling between T_2 and T_3 give rise to neomorphs and also in most of the cases show a repetition of structures (b). The pattern of macrochaetae and ocelli in *Aulacigaster leucopeza* is shown in c. 1–3, orbital setae; 4, vertical seta I; 5, vertical seta II; 6, ocellar seta; 7, neomorphic bristle; 8, post-vertical seta. (After Sondhi, 1962.)

Henke (1948) and the other by Turing (1952). Henke's attempts to explain the genesis of primary patterns in terms of simultaneous rhythms were unsatisfactory, for they failed to provide a suitable mechanism by which field centers or determination centers could come into being during development. On the other hand, Turing's suggested model does provide an explanation of the process by which constant patterns can arise. Although Turing's investigations were mainly concerned with mechanisms by which stationary waves during development come into being, he indicated that, with three or more morphogens, a mechanism was possible that could give rise to traveling waves. Unfortunately, no attempt has been made so far to develop this concept.

The development of a constant pattern can be explained in terms of two processes, one concerned with the formation of a prepattern which determines the positions at which structures are formed and the other which determines the amount of 'precursor' or competence (cf. Waddington, 1932) of cells to respond to an underlying prepattern. These two processes can vary independently of each other: there is enough experimental evidence to justify the distinction between them.

A considerable body of evidence suggests that comparable patterns, for instance, of sex-comb, bristles, or body segments may have identical prepatterns in unrelated species. An important feature of the prepatterns arising in a Turing-type mechanism is that comparable prepatterns may be produced by unrelated individuals with reaction systems of different genetic constitution. Differences between individuals, on the other hand, may arise because of genetically determined differences in competence between their cells to respond to an identical prepattern. Experiments on the wing hinges of *Ephestia kühniella* (Kroeger, 1959b) have shown that differences among serially homologous parts of the same individual may be determined by differential responsiveness to identical prepatterns. In this case, however, the differences of response are not genetically determined but arise in the course of differentiation.

If we consider development as a gradually changing reaction system, a prepattern at a particular stage would depend on the nature of substances entering the system and the conditions of the system, and these in turn would determine the ensuing pattern. In development the reaction system is continuously changing as growth proceeds. (For patterns of growth, see Huxley, 1932; Medawar, 1944; Weiss and Kavanau, 1957; D'Arcy Thompson, 1961). Each pattern acts as a prepattern for the next developmental stage till the final pattern is achieved. This concept is in harmony with the stepwise and epigenetic nature of development. However, a chemical basis of pattern formation advanced along these lines would be an oversimplification of the processes actually involved. Besides the chemical basis, the energy relations within the system (see Needham, 1942), and the biophysical properties of developing systems (see Weiss, 1950, 1961; D'Arcy Thompson, 1961) must be important factors.

The main difficulty which arises in a Turing-type mechanism, as Maynard Smith (1960) has pointed out, is that it cannot give rise to a constant pattern if the number of structures in the constant pattern is large. The simplest method of insuring the constancy of large numbers is by a process which has been termed 'multiplication.' The morphogenetic field is first divided by one patterning process into a small number of large regions and then subdivided by a second process into a larger number of smaller regions. It is therefore interesting that development of the genital disc (Ursprung, 1959) of *Drosophila* has a stepwise character of this kind. Since many of the developmental processes are stepwise in nature, it is likely that only processes of this kind can produce uniform results.

In patterns in which structures are arranged in a linear series, it is necessary that the two patterning processes should be separated so that one follows the other in time, but, if structures are arranged on a surface, another type of multiplicative process is possible without involving a time control. In this case two patterning processes can occur simultaneously but along different axes; one process can determine the number of 'rows,' and the second, the number of structures in each row. This model requires that the morphogenetic field should be initially anisotropic, whereas Turing supposed the field to be isotropic. Thus, in the cuticle of *Rhodnius,* on which the only structure visible

on the adult tergites is a series of transverse ripples, Locke (1959, 1960) has demonstrated the presence both of an antero-posterior gradient and of a side-to-side polarity. The arrangement of microchaetae on the sternites of *Drosophila* suggests that the field is isotropic, but there are reasons for supposing that, on the dorsal surface of the thorax, the rows and columns are separately determined by mechanisms which occur simultaneously but along different axes. The developmental analysis of the two-dimensional bristle pattern on the thorax of wild-type *Drosophila* and of a mutant form in which the rows of thoracic bristles are distorted is under investigation (Sondhi, in prep.).

In certain groups of segmented animals, there is no indication of multiplicative mechanisms, yet a large number of segments do show a great constancy of segment number within a species. The constancy of such periodic structures can be controlled by some kind of "chemical counting" (Maynard Smith, 1960), which would bring the series to an end when the required chemical constitution had been reached. This implies that, although various body segments may appear similar, they may be dissimilar in some characteristic individual property. There is a considerable body of evidence in favor of the concept of chemical counting. For instance, it may be recalled that apparently similar elements within the cuticular pattern of *Rhodnius* do show certain specificity, presumably of a chemical nature. An understanding of the process by which such specificity in apparently similar elements of a pattern comes into being should prove to be of great significance in understanding the genesis of patterns.

We have considered some of the possible mechanisms by which regular patterns can arise in development. Although some satisfaction may be derived from the general picture that has emerged from the present analysis, we do not have enough information about those factors that cause the breakdown of a uniform area during development into spatially arranged elements which give rise to the future pattern. Future studies may reveal more of the foundations on which patterns are laid down.

ACKNOWLEDGMENTS

I wish to express my sincere thanks to Dr. Ernst Hadorn and Dr. Curt Stern for their helpful comments in the preparation of the manuscript and to Dr. Nathan W. Shock for editorial advice. My deepest thanks are due to Mr. John Maynard Smith for providing a stimulus to the present study. The task of assembling information from papers written in German was shared by Gunthild Sondhi, to whom I am most grateful.

LIST OF LITERATURE

BATEMAN, K. G. 1959a. The genetic assimilation of the *dumpy* phenocopy. *J. Genet.*, 56: 341–349.

——. 1959b. The genetic assimilation of four venation phenocopies. *J. Genet.*, 56: 443–473.

BECKER, E. 1937. Die rotbraune Zeichnung der Wespennestmütter, eine durch mechanischen Reiz ausgelöste Pigmentablagerung in Liesegangschen Ringen. *Z. Vergl. Physiol.*, 24: 305–318.

BIRMINGHAM, L. 1942. Boundaries of differentiation of cephalic imaginal discs in *Drosophila*. *J. Exptl. Zool.*, 91: 345–363.

BODENSTEIN, D. 1943. Hormones and tissue competence in the development of *Drosophila*. *Biol. Bull.*, 84: 34–58.

——. 1957. Humoral dependence of growth and differentiation in insects. In *Recent Advances in Invertebrate Physiology*, 197–211. Univ. Oregon Publ., Eugene.

——, and A. ABDEL-MALEK. 1949. The induction of aristopedia by nitrogen mustard in *Drosophila virilis*. *J. Exptl. Zool.*, 111: 95–115.

BRAUN, W. 1936. Über das Zellteilungsmuster im Puppenflügel der Mehlmotte *Ephestia kühniella* Z. in seiner Beziehung zur Ausbildung des Zeichnungsmusters. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, 135: 494–520.

——. 1939. Contributions to the study of development of the wing-pattern in Lepidoptera. *Biol. Bull.*, 76: 226–240.

CASPARI, E. 1941. The morphology and development of the wing pattern of Lepidoptera. *Quart. Rev. Biol.*, 16: 249–273.

CHEVAIS, S. 1943. Déterminisme de la taille de l'oeil chez la mutant *Bar* de la Drosophile. *Bull. Biol.*, 77: 1–108.

CHILD, G. 1935. Phenogenetic studies on scute—1 of *Drosophila melanogaster*. I. The associations between bristles and the effects of genetic

modifiers and temperature. *Genetics,* 20: 109–126.

CLAUSEN, K. H. 1937. Kreuzungsanalyse des Zeichnungsmusters zweier Stämme von *Ephestia kühniella* Z. ("Siebenbürgen" and "Göttingen N VI"). *Z. Induktive Abstammungs–Vererbungslehre,* 72: 488–506.

DOBZHANSKY, T. 1929. The influence of the quantity and quality of chromosomal material on size of the cells in *Drosophila melanogaster. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 115: 363–379.

DUBININ, N. P. 1929. Allelomorphentreppen bei *Drosophila melanogaster. Biol. Zentr.,* 49: 328–339.

DUPONT-RAABE, M. 1957. Les mécanismes de l'adaptation chromatique chez les insects. *Arch. Zool. Exptl. Gén.,* 94 (fasc 2): 61–264.

FELDOTTO, W. 1933. Sensible Perioden des Flügelmusters bei *Ephestia kühniella* Zeller. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 128: 299–341.

FINCK, E. VON. 1938. Genetische Untersuchungen über die Schuppengrösse und Schuppenform bei der Mehlmotte *Ephestia kühniella* Z. *Z. Induktive Abstammungs–Vererbungslehre,* 74: 161–201.

FORD, E. B. 1937. Problems of heredity in the Lepidoptera. *Biol. Rev. Cambridge Phil. Soc.,* 12: 461–503.

GEIGY, R. 1931. Erzeugung rein imaginaler Defekte durch ultraviolette Eibestrahlung bei *Drosophila melanogaster. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 125: 406–447.

GEITLER, L. 1939–40. Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. *Chromosoma,* 1: 1–22.

——. 1954. Endomitose und endomitotische Polyploidisierung. *Protoplasmatologia,* VI, C: 1–89.

GLOOR, H. 1947. Phänokopie-Versuche mit Äther an *Drosophila. Rev. Suisse Zool.,* 54: 637–712.

GOLDSCHMIDT, R. 1920. *Die quantitativen Grundlagen von Vererbung und Artbildung.* 163 pp. Springer, Berlin.

——. 1923. Einige Materialien zur Theorie der abgestimmten Reaktionsgeschwindigkeiten. *Arch. mikroskop. Anat. u. Entwicklungsmech.,* 98: 292–313.

——. 1931. Die entwicklungsphysiologische Erklärung des Falls der sogenannten Treppenallelomorphe des Gens *scute* von *Drosophila. Biol. Zentr.,* 51: 507–526.

——. 1935a. Gen und Ausseneigenschaft. I. *Z. Induktive Abstammungs–Vererbungslehre;* 69: 38–69.

——. 1935b. Gen und Aussencharakter. III. *Biol. Zentr.,* 55: 535–554.

——. 1937. Gene and character. IV. *Univ. Calif. Publ. Zool.,* 41: 277–282.

——. 1938. *Physiological Genetics.* 375 pp. McGraw-Hill Book Co., New York.

HADORN, E. 1953. Regulation and differentiation within field-districts in imaginal discs of *Drosophila. J. Embryol. Exptl. Morphol.,* 1: 213–216.

——. 1961. *Developmental Genetics and Lethal Factors.* 355 pp. John Wiley & Sons, New York.

——. 1963. Differenzierungsleistungen wiederholt fragmentierter Teilstücke männlicher Genitalscheiben von *Drosophila melanogaster* nach Kultur *in vivo. Develop. Biol.,* 7: 617–629.

——, G. ANDERS, and H. URSPRUNG. 1959. Kombinate aus teilweise dissoziierten Imaginalscheiben verschiedener Mutanten und Arten von *Drosophila. J. Exptl. Zool.,* 142: 159–175.

——, G. BERTANI, and J. GALLERA. 1949. Regulationsfähigkeit und Feldorganisation der männlichen Genital-Imaginalscheibe von *Drosophila melanogaster. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 144: 31–70.

——, and D. BUCK. 1962. Über die Entwicklungsleistungen transplantierter Teilstücke von Flügel-Imaginalscheiben von *Drosophila melanogaster. Rev. Suisse Zool.,* 69: 302–310.

——, and P. S. CHEN. 1956. Die Feldorganisation der Spermatheken-Anlage bei *Drosophila melanogaster. Rev. Suisse Zool.,* 63: 268–277.

——, and W. FRITZ. 1950. Veränderungen am transplantierten weiblichen Geschlechtsapparat von *Drosophila melanogaster* nach Behandlung der Imaginalscheibe in Salzlösungen. *Rev. Suisse Zool.,* 57: 477–488.

——, and H. GLOOR. 1946. Transplantation zur Bestimmung des Anlagemusters in der weiblichen Genital-Imaginalscheibe von *Drosophila. Rev. Suisse Zool.,* 53: 495–501.

HANNAH-ALAVA, A. 1958a. Developmental genetics of the posterior legs in *Drosophila melanogaster. Genetics,* 43: 878–905.

——. 1958b. Morphology and chaetotaxy of the legs of *Drosophila melanogaster. J. Morphol.,* 103: 281–310.

——, and C. STERN. 1957. The sexcombs in males and intersexes of *Drosophila melanogaster. J. Exptl. Zool.,* 134: 533–556.

HENKE, K. 1928. Über die Variabilität des Flügelmusters bei *Larentia sordidata* F. und einigen anderen Schmetterlingen. *Z. Morphol. Oekol. Tiere,* 12: 240–282.

——. 1933. Untersuchungen an *Philosamia cynthia* Drury zur Entwicklungsphysiologie des Zeichnungsmusters auf dem Schmetterlingsflü-

gel. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, 128: 15–107.

——. 1935. Entwicklung und Bau tierischer Zeichnungsmuster. *Verhandl. Deut. Zool. Ges.*, 1935: 176–244.

——. 1936. Versuch einer vergleichenden Morphologie des Flügelmusters der Saturniden auf entwicklungsphysiologischer Grundlage. *Nova Acta Leopoldina, Halle,* N.F. 4 ,1, Nr. 18: 4–37.

——. 1943. Vergleichende und experimentelle Untersuchungen an *Lymantria* zur Musterbildung auf dem Schmetterlingsflügel. *Nachr. Akad. Wiss. Göttingen, Math.-Physik. Kl.*, 1–48.

——. 1946. Über die verschiedenen Zellteilungsvorgänge in der Entwicklung des beschuppten Flügelepithels der Mehlmotte *Ephestia kühniella* Z. *Biol. Zentr.*, 65: 120–135.

——. 1947. Einfache Grundvorgänge in der tierischen Entwicklung. I. *Naturwissenschaften,* 34: 149–157, 180–186.

——. 1948. Einfache Grundvorgänge in der tierischen Entwicklung. II. *Naturwissenschaften,* 35: 176–181, 203–211, 239–246.

——. 1951. Die Hauptformen der Gliederungsvorgänge in der Entwicklung des Insektenflügels. *Verhandl. Deut. Zool. Ges. Wilhelmshaven*: 42–62.

——. 1953a. Über Zelldifferenzierung im Integument der Insekten und ihre Bedingungen. *J. Embryol. Exptl. Morphol.*, 1: 217–226.

——. 1953b. Die Musterbildung der Versorgungssysteme im Insektenflügel. *Biol. Zentr.*, 72: 1–51.

——, and G. KRUSE. 1941. Über Feldgliederungsmuster bei Geometriden und Noctuiden und den Musterbauplan der Schmetterlinge im allgemeinen. *Nachr. Akad. Wiss. Göttingen, Math.-Physik. Kl.*, 138–196.

——, and I. MERTZ. 1941. Über die Kerngrössenunterschiede im Flügelepithel der Mehlmotte und ihre Beziehungen zur Grössendifferenzierung der Schuppen. *Biol. Zentr.*, 61: 40–63.

——, and H. J. POHLEY. 1952. Differentielle Zellteilungen und Polyploidie bei der Schuppenbildung der Mehlmotte *Ephestia kühniella* Z. *Z. Naturforsch.*, 7b: 65–79.

——, E. SCHATZ, and H. SCHWENK. 1946. Untersuchungen an *Drosophila* zur Entwicklungsphysiologie der Grösse und Form des Insektenflügels. *Nachr. Akad. Wiss. Göttingen, Math.-Physik. Kl.*, 5–7.

HINTON, H. E. 1958. On the nature and metamorphosis of the color pattern of *Thaumalea* (Diptera, Thaumaleiae). *J. Insect Physiol.*, 2: 249–260.

——. 1959. The function of chromatocytes in the Simuliidae, with notes on their behavior at the pupa-adult moult. *Quart. J. Microscop. Sci.*, 100: 65–71.

HOLTFRETER, J. 1939. Gewebeaffinität, ein Mittel der embryonalen Formbildung. *Arch. Exptl. Zellforsch.*, 23: 169–209.

——. 1943. Experimental studies on the development of the pronephros. *Rev. Can. Biol.*, 3: 220–249.

HOUSE, V. L. 1953a. The interaction of mutants affecting venation in *Drosophila melanogaster*. I. Interaction of hairless, engrailed and cubitus interruptus. *Genetics,* 38: 199–215.

——. 1953b. 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.

——. 1954. The interaction of mutants affecting venation in *Drosophila melanogaster*. III. The interaction at various temperatures of hairless, engrailed and cubitus interruptus. *J. Exptl. Zool.*, 127: 53–74.

——. 1955. The influence of temperature on gene expression at the cubitus interruptus locus in *Drosophila melanogaster*. *Genetics,* 40: 576.

HÜGEL, E. 1933. Über das genetische Verhalten der weissen Distalbinde und ihre genetischen Korrelationen zu anderen Merkmalen auf dem Vorderflügel der Mehlmotte *Ephestia kühniella* Zeller. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, 130: 202–242.

HUXLEY, J. H. 1932. *Problems of Relative Growth.* 276 pp. Lincoln Macveagh, Dial Press, New York.

IVES, P. T. 1939. The effects of high temperature on bristle frequencies in scute and wild-type males of *Drosophila melanogaster*. *Genetics,* 24: 315–331.

KÖHLER, W. 1932. Die Entwicklung der Flügel bei der Mehlmotte *Ephestia kühniella* Zeller, mit besonderer Berücksichtigung des Zeichnungsmusters. *Z. Morphol. Oekol. Tiere,* 24: 582–681.

——. 1940. Erbliche Ausfallserscheinungen und Regulationen am pupalen Flügeltracheensystem von *Ephestia kühniella* Z. *Biol. Zentr.*, 60: 348–367.

——, and W. FELDOTTO. 1936. Experimentelle Untersuchungen über die Modifikabilität der Flügelzeichnung, ihrer Systeme und Elemente in den sensiblen Perioden von *Vanessa urticae* L., nebst einigen Beobachtungen an *Vanessa io* L. *Arch. Julius Klaus Stift. Vererbungsforsch., Sozialanthropol. Rassenhyg.*, 10: 313–543.

——, and ——. 1937. Morphologische und experimentelle Untersuchungen über Farbe, Form and Struktur der Schuppe von *Vanessa urticae* und ihre gegenseitigen Beziehungen. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 136: 313–399.

KROEGER, H. 1958. Über Doppelbildungen in die Leibeshöhle verpflanzter Flügelimaginalscheiben von *Ephestia kühniella* Z. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 150: 401–424.

——. 1959a. The genetic control of genital morphology in *Drosophila. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 151: 301–322.

——. 1959b. Determinationsmosaike aus kombiniert implantierten Imaginalscheiben von *Ephestia kühniella* Z. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 151: 113–135.

——. 1960a. Hypo- and hyperdevelopment of the male genital apparatus and the *Bd-M* combination in *Drosophila melanogaster. J. Morphol.,* 107: 227–232.

——. 1960b. Die Entstehung von Form im morphogenetischen Feld. *Naturwissenschaften,* 47: 148–153.

KÜHN, A. 1926. Über die Änderung des Zeichnungsmusters von Schmetterlingen durch Temperaturreize und das Grundschema der Nymphalidenzeichnung. *Nachr. Ges. Wiss. Göttingen, Math.-Physik. Kl.,* 120–141.

——. 1937. Entwicklungsphysiologisch-genetische Ergebnisse an *Ephestia kühniella* Z. *Z. Induktive Abstammungs-Vererbungslehre,* 73: 419–455.

——. 1939. Über eine geschlechtsgekoppelte Mutation des Zeichnungsmusters (*dz*) bei *Ephestia kühniella* Z. *Biol. Zentr.,* 59: 347–357.

——. 1955. *Vorlesungen über Entwicklungsphysiologie.* 506 pp. Springer Verlag, Berlin-Göttingen-Heidelberg.

——, and M. VON ENGELHARDT. 1933. Über die Determination des Symmetriesystems auf dem Vorderflügel von *Ephestia kühniella* Z. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 130: 660–703.

——, and ——. 1936. Über die Determination des Flügelmusters bei *Abraxas grossulariata* L. *Nachr. Ges. Wiss. Göttingen, Math.-Phys. Kl., Nachr. Biol.,* 2: 171–199.

——, and K. HENKE. 1929–1936. Genetische und entwicklungsphysiologische Untersuchungen an der Mehlmotte *Ephestia kühniella* Zeller. *Abhandl. Ges. Wiss. Göttingen, Math.-Physik. Kl.,* N.F. 15: 1929 (I–VII); 1932 (VIII–XII); 1936 (XIII–XIV), 1–272.

LEES, A. D. 1941. Operations on the pupal wing of *Drosophila melanogaster. J. Genet.,* 42: 115–142.

——, and L. E. R. PICKEN. 1945. Shape in relation to fine structure in the bristles of *Drosophila melanogaster. Proc. Roy. Soc. (London), B,* 132: 396–423.

——, and C. H. WADDINGTON. 1942. The development of bristles in normal and some mutant types of *Drosophila melanogaster. Proc. Roy. Soc. (London), B,* 131: 87–110.

LIPP, C. 1953. Über Kernwachstum, Endomitosen und Funktionszyklen in den Trichogenen Zellen von *Corixa punctata* Illig. *Chromosoma,* 5: 454–486.

——. 1955. Beitrag zur somatischen Cytologie der Schmetterlinge. *Chromosoma,* 7: 1–13.

——. 1957. Die Bedeutung differentieller Zellteilungen bei der Entstehung des Schuppenmusters auf dem Flügel von *Pieris brassicae. Biol. Zentr.,* 76: 681–700.

LOCKE, M. 1959. The cuticular pattern in an insect *Rhodnius prolixus* Stål. *J. Exptl. Biol.,* 36: 459–478.

——. 1960. The cuticular pattern in an insect — the intersegmental membranes. *J. Exptl. Biol.,* 37: 398–406.

LOOSLI, R. 1959. Vergleich von Entwicklungspotenzen in normalen, transplantierten und mutierten Halteren-Imaginalscheiben von *Drosophila melanogaster. Develop. Biol.,* 1: 24–64.

LÜÖND, H. 1961. Untersuchungen zur Mustergliederung in fragmentierten Primordien des männlichen Geschlechtsapparates von *Drosophila séguyi. Develop. Biol.,* 3: 615–656.

LÜSCHER, M. 1944. Experimentelle Untersuchungen über die larvale und die imaginale Determination im Ei der Kleidermotte. *Rev. Suisse Zool.,* 51: 531–627.

MACDOWELL, E. C. 1915. Bristle inheritance in *Drosophila.* Extra bristles. *J. Exptl. Zool.,* 19: 61–98.

MAYNARD SMITH, J. 1960. Continuous, quantized and modal variation. *Proc. Roy. Soc., (London), B,* 152: 397–409.

——, and K. C. SONDHI. 1960. The genetics of a pattern. *Genetics,* 45: 1039–1050.

——, and ——. 1961. The arrangement of bristles in *Drosophila. J. Embryol. Exptl. Morphol.,* 9: 661–672.

MEDAWAR, P. B. 1944. The shape of the human being as a function of time. *Proc. Roy. Soc. (London), B,* 132: 133–141.

MOSCONA, A. 1952. Cell suspension from organ rudiments of chick embryos. *Exptl. Cell Res.,* 3: 535–539.

MULLER, H. J. 1932. Further studies on the nature and causes of gene mutations. *Proc. 6th Intern. Congr. Genet.*, 1: 213–255.

NEEDHAM, J. 1950. *Biochemistry and Morphogenesis.* 2nd ed., 787 pp. Cambridge, at the Univ. Press.

NEEL, J. V. 1940. The pattern of supernumerary macrochaetae in certain *Drosophila* mutants. *Genetics,* 25: 251–277.

——. 1941. Studies on the interaction of mutations affecting the chaetae of *Drosophila melanogaster.* I. The interaction of hairy, polychaetoid, and hairy wing. *Genetics,* 26: 52–68.

——. 1943. Studies on the interaction of mutations affecting the chaetae of *Drosophila melanogaster.* II. The relations of character expression to size in flies homozygous for polychaetoid, hairy, hairy wing and the combination of these factors. *Genetics,* 28: 49–68.

PANTELOURIS, E. M., and C. H. WADDINGTON. 1955. Regulation capacities of the wing- and haltere discs of wild-type and bithorax *Drosophila. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 147: 539–546.

PICKEN, L. E. R. 1949. Shape and molecular orientation in Lepidopteran scales. *Phil. Trans. Roy. Soc. (London), B,* 234: 1–28.

PIEPHO, H. 1955. Über die polare Orientierung der Bälge und Schuppen auf dem Schmetterlingsrumpf. *Biol. Zentr.,* 74: 467–474.

——, and W. MARCUS. 1957. Wirkungen richtender Faktoren bei der Bildung der Schuppen und Bälge des Schmetterlingsrumpfes. *Biol. Zentr.,* 76: 23–27.

PLUNKETT, C. R. 1926. The interactions of genetic and environmental factors in development. *J. Exptl. Zool.,* 46: 181–244.

POHLEY, H. J. 1953. Untersuchungen über differentielle Zellteilungen und somatische Mutationen am Schuppenkleid der Mehlmotte *Ephestia kühniella. Biol. Zentr.,* 72: 577–598.

REEVE, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila. Genet. Res., Cambridge,* 1: 151–172.

——. 1961. Modifying the sternopleural hair pattern in *Drosophila* by selection. *Genet. Res., Cambridge,* 2: 158–160.

——, and F. W. ROBERTSON. 1954. Studies on quantitative inheritance. VI. Sternite chaeta number in *Drosophila*: a metameric quantitative character. *Z. Induktive Abstammungs–Vererbungslehre,* 86: 269–288.

RENDEL, J. M. 1959. Canalization of the scute phenotype of *Drosophila. Evolution,* 13: 425–439.

SCHAARLOO, W. 1961. Temperature and mutant expression. *Experientia,* 17: 121–122.

——. 1962. The influence of selection and temperature on a mutant character (CI^4) in *Drosophila melanogaster. Arch. Neerl. Zool.,* 14: 431–512.

SCHATZ, E. 1951. Über die Formbildung der Flügel bei Hitzemodifikationen und Mutationen von *Drosophila melanogaster. Biol. Zentr.,* 70: 305–353.

SCHLAEPFER, TH. 1963. Der Einfluss des adulten Wirtsmilieus auf die Entwicklung von larvalen Augenantennen-Imaginalscheiben von *Drosophila melanogaster. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 154: 378–404.

SCHWANWITSCH, B. N. 1924. On the ground-plan of wing-pattern in Nymphalids and certain other families of the Rhopalocerous Lepidoptera. *Proc. Zool. Soc. London,* No. 34: 509–528.

——. 1925. On a remarkable dislocation of the components of the wing-pattern in a Satyride genus *Pierella. Entomologist,* 58: 266–269.

——. 1928. Pierellisation of stripes in the wing pattern of the genus *Rhaphicera* Btl. (Lepidoptera Satyridae. *Z. Morphol. Ökol. Tiere,* 11: 1–12.

——. 1929a. Evolution of the wing-pattern in palacarctic Satyridae. I. Genera *Satyrus* and *Oeneis. Z. Morphol. Ökol. Tiere,* 13: 559–654.

——. 1929b. Two schemes of the wing-pattern of butterflies. *Z. Morphol. Ökol. Tiere,* 14: 36–58.

——. 1931. Evolution of the wing pattern in palaearctic Satyridae. II. Genus *Melanargia. Z. Morphol. Ökol. Tiere,* 21: 316–408.

SCHWARTZ, V. 1937. Über einen geschlechtsgebundenen Letalfaktor bei *Ephestia kühniella. Biol. Zentr.,* 57: 347–354.

——. 1938. Über die Vererbung des Ausprägungsgrades der schwarzen Querbindenzeichnung auf dem Vorderflügel von *Ephestia kühniella. Z. Induktive Abstammungs–Vererbungslehre,* 74: 409–419.

——. 1953. Zur Phänogenese der Flügelzeichnung von *Plodia interpuntella. Z. Induktive Abstammungs–Vererbungslehre,* 85: 51–96.

SCHWENK, H. 1947. Untersuchungen über die Entwicklung der Borsten bei *Drosophila. Nachr. Ges. Wiss. Göttingen, Math.-Phys. Kl., Biol.,* N. F.: 14–15.

SENGEL, PH. 1958. Recherches experiméntales sur la différenciation des germes plumaires et du pigment de la peau de l'embryon de poulet en culture *in vitro. Ann. Sci. Nat.,* 20: 431–514.

SEREBROVSKY, A. S. 1930. Untersuchungen über Treppenallelomorphismus. IV. Transgenation

sc 6. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 149: 1–25.

SHATOURY, H. H. EL. 1955. Lethal "no-differentiation" and the development of the imaginal discs during the larval stages of *Drosophila. Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 147: 523–538.

SOBELS, F. H. 1952. Genetics and morphology of the genotype "Asymmetric" with special reference to its "Abnormal Abdomen" character (*Drosophila melanogaster*). *Genetica,* 26: 117–279.

——. 1954. Patterns of segmentation in the genotype *Abnormal Abdomen* of *Drosophila melanogaster. Proc. 9th Intern Congr. Genet.,* 2: 1159–1160.

SONDHI, K. C. 1960. Selection for a character with a bounded distribution of phenotypes in *Drosophila subobscura. J. Genet.,* 57: 197–227.

——. 1961a. Developmental barriers in a selection experiment. *Nature,* 189: 249–250.

——. 1961b. The evolution of a pattern in *Drosophila. Heredity,* 16: 523.

——. 1962a. The evolution of a pattern. *Evolution,* 16: 186–191.

——. 1962b. Patterns of gene pleiotropy in morphogenetic processes. *Science,* 137: 538–540.

——. 1963. Gene controlled processes in the genesis and evolution of animal patterns. *Proc. 16th Intern. Congr. Zool.,* 2: 216.

SONNEBORN, T. M., and G. H. BEALE. 1949. Influence des gènes, des plasmagènes et du milieu dans le déterminisme des charactères antigéniques chez *Paramecium aurelia* variété 4. In *"Unités biologiques douées de continuité génetique," Colloq. Intern. Centre Natl. Recherche Sci. (Paris),* 8: 25–36.

STEINBERG, A. G. 1941. A reconsideration of the mode of development of the Bar eye of *Drosophila melanogaster. Genetics,* 26: 325–346.

STERN, C. 1927. Der Einfluss der Temperatur auf die Ausbildung einer Flügelmutation bei *Drosophila melanogaster. Biol. Zentr.,* 47: 361–369.

——. 1938. The innervation of setae in *Drosophila. Genetics,* 23: 172–173.

——. 1940. The prospective significance of imaginal discs in *Drosophila. J. Morphol.,* 67: 107–122.

——. 1954a. Two or three bristles. *Am. Scientist,* 42: 213–247.

——. 1954b. Genes and developmental patterns. *Proc. 9th Intern. Congr. Genet.,* 1: 355–369.

——. 1956a. Genetic mechanisms in the localized initiation of differentiation. *Cold Spring Harbor Symp. Quant. Biol.,* 21: 375–382.

——. 1956b. The genetic control of developmental competence and morphogenetic tissue interactions in genetic mosaics. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 149: 1–25.

——. 1957. The role of genes in differentiation. *Proc. Intern. Genet. Symp. (Suppl. Vol. Cytologia)* 1956: 70–72.

——, and A. M. HANNAH. 1950. The sex-combs in gynanders of *Drosophila melanogaster. Port. Acta Biol., Ser. A* (R. B. Goldschmidt Vol.): 798–812.

——, and D. L. SWANSON. 1957. The control of the ocellar bristle by the scute locus in *Drosophila melanogaster. J. Fac. Sci. Hokkaido Univ., Ser.* VI, 13: 303–307.

STOSSBERG, M. 1937. Über die Entwicklung der Schmetterlingsschuppen. *Biol. Zentr.,* 57: 393–402.

——. 1938. Die Zellvorgänge bei der Entwicklung der Flügelschuppen von *Ephestia kühniella* Z. *Z. Morph. Ökol. Tiere,* 34: 173–206.

STROHL, J., and W. KÖHLER. 1934. Experimentelle Untersuchungen über die Entwicklungsphysiologie der Flügelzeichnung bei der Mehlmotte. *Verhandl. Schweiz. Naturforsch. Ges. Zürich,* 1934: 467–470.

STUBBE, A. E. 1938. Die Wirkungen verschiedener Reizung mit erhöhter Temperatur auf das Zeichnungsmuster zweier Stämme der Mehlmotte *Ephestia kühniella* Zeller. *Z. Induktive Abstammungs-Vererbungslehre,* 75: 233–255.

STURTEVANT, A. H. 1932. The use of mosaics in the study of developmental effects of genes. *Proc. 6th Intern. Congr. Genet.,* 1: 304–307.

——. 1961. The bristle pattern in *Drosophila. Science,* 134: 1436.

——, and J. SCHULTZ. 1931. The inadequacy of the sub-gene hypothesis of the nature of the *scute* allelomorphs of *Drosophila. Proc. Natl. Acad. Sci., U.S.,* 17: 265–270.

SÜFFERT, F. 1925. Geheime Gesetzmässigkeiten in der Zeichnung der Schmetterlinge. *Rev. Suisse Zool.,* 32: 107–111.

——. 1927. Zur vergleichenden Analyse der Schmetterlingszeichnung. *Biol. Zentr.,* 47: 385–413.

——. 1929. Morphologische Erscheinungsgruppen in der Flügelzeichnung der Schmetterlinge, insbesondere die Querbindenzeichnung. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 120: 229–383.

——. 1937. Die Geschichte der Bildungszellen im Puppenflügelepithel bei einem Tagschmetterling. *Biol. Zentr.,* 57: 615–628.

THODAY, J. M. 1958. Homeostasis in a selection experiment. *Heredity,* 12: 401–415.

THOMSON, D'ARCY W. 1961. *On Growth and Form.* 360 pp. Cambridge, at the Univ. Press.

TIMOFÉEFF-RESSOVSKY, N. W. 1931. Gerichtetes Variieren in der phänotypischen Manifestierung einiger Genovariationen von *Drosophila funebris. Naturwissenschaften,* 19: 493–497.

——, and H. A. TIMOFÉEFF-RESSOVSKY. 1934. Polare Schwankungen in der phänotypischen Manifestierung einiger Genmutationen bei *Drosophila. Z. Induktive Abstammungs-Vererbungslehre,* 67: 246–254.

TOKUNAGA, C. 1961. The differentiation of a secondary sex comb under the influence of the gene engrailed in *Drosophila melanogaster. Genetics,* 46: 157–176.

——. 1962. Cell lineage and differentiation on the male foreleg of *Drosophila melanogaster. Develop. Biol.,* 4: 489–516.

TSCHUMI, P. 1953. Ontogenetische Realisationsstufen der Extremitäten bei *Xenopus* und die Interpretation phylogenetischer Strahlenreduktionen bei Wirbeltieren. *Rev. Suisse Zool.,* 60: 496–506.

TURING, A. M. 1952. The chemical basis of morphogenesis. *Phil. Trans. Roy. Soc. London, B.* 237: 37–72.

TWITTY, V. C. 1945. The developmental analysis of specific pigment patterns. *J. Exptl. Zool.,* 100: 141–178.

URSPRUNG, H. 1957. Untersuchungen zum Anlagemuster der weiblichen Genitalscheibe von *Drosophila melanogaster* durch UV-Strahlenstich. *Rev. Suisse Zool.,* 64: 303–316.

——. 1959. Fragmentierungs- und Bestrahlungsversuche zur Bestimmung von Determinationszustand und Anlageplan der Genitalscheiben von *Drosophila melanogaster. Roux' Arch. Entwicklungsmech. Organ.,* 151: 504–558.

——. 1962. Einfluss des Wirtsalters auf die Entwicklungsleistung von Sagittalhälften männlicher Genitalscheiben von *Drosophila melanogaster. Develop. Biol.,* 4: 22–39.

——. 1963. Development and genetics of patterns. *Am. Zool.,* 3: 71–86.

——, and E. HADORN. 1962. Weitere Untersuchungen über Musterbildung in Kombinaten aus teilweise dissoziierten Flügel-Imaginalscheiben von *Drosophila melanogaster. Develop. Biol.,* 4: 40–66.

VAN VALEN, L. 1962. A study of fluctuating asymmetry. *Evolution,* 16: 125–142.

VOGT, M. 1946a. Zur labilen Determination der Imaginalscheiben von *Drosophila.* I. Verhalten verschiedenaltiger Imaginalanlagen bei operativer Defektsetzung. *Biol. Zentr.,* 65: 223–238.

——. 1946b. Zur labilen Determination der Imaginalscheiben von *Drosophila.* II. Die Umwandlung präsumptiven Fühlergewebes in Beingewebe. *Biol. Zentr.,* 65: 239–254.

——. 1947. Zur labilen Determination der Imaginalscheiben von *Drosophila.* III. Analyse der Manifestierungsbedingungen sowie der Wirkungsweise der zu Antennen- und Palpusverdoppelungen führenden Genmutation deformed-recessive-Luers (Dfd^{r-L}). *Biol. Zentr.,* 66: 81–105.

WADDINGTON, C. H. 1932. Experiments on the development of chick and duck embryos cultivated *in vitro. Phil. Trans. Roy. Soc. London, B,* 221: 179–230.

——. 1939. Preliminary notes on the development of the wings in normal and mutant strains of *Drosophila. Proc. Natl. Acad. Sci. U.S.,* 25: 299–307.

——. 1940. The genetic control of wing development in *Drosophila. J. Genet.,* 41: 75–139.

——. 1942a. The canalization of development and the inheritance of acquired characters. *Nature,* 150: 563–565.

——. 1942b. Growth and determination in the development of *Drosophila. Nature,* 149: 264–265.

——. 1942c. Some developmental effects of X-rays in *Drosophila. J. Exptl. Biol.,* 19: 101–117.

——. 1942d. The pupal contraction as an epigenetic crisis in *Drosophila. Proc. Zool. Soc. (London), A,* 111: 181–188.

——. 1943. The development of some "leg genes" in *Drosophila. J. Genet.,* 45: 29–43.

——. 1947. Regulation in bithorax "hemi-thorax." *D.I.S.* No. 21: 89.

——. 1948. The concept of equilibrium in embryology. *Folia Biotheoret.,* 3: 127–138.

——. 1950. The biological foundations of measurements of growth and form. *Proc. Roy. Soc. (London), B,* 137: 509–515.

——. 1953a. The interactions of some morphogenetic genes in *Drosophila. J. Genet.,* 51: 243–258.

——. 1953b. Genetic assimilation of an acquired character. *Evolution,* 7: 118–126.

——. 1955. On a case of quantitative variation on either side of the wild-type. *Z. Induktive Abstammungs-Vererbungslehre,* 87: 208–228.

——. 1956a. Genetic assimilation of the bithorax phenotype. *Evolution,* 10: 1–13.

——. 1956b. *Principles of Embryology.* 510 pp. George Allen & Unwin, London.

——. 1957a. The genetic basis of the "assimilated bithorax" stock. *J. Genet.*, 55: 241–245.

——. 1957b. *The Strategy of the Genes.* 262 pp. George Allen & Unwin, London.

——. 1960. Experiments on canalizing selection. *Genet. Res., Cambridge,* 1: 140–150.

——. 1961. Genetic-assimilation. *Advan. Genet.,* 10: 257–290.

——. 1962. *New Patterns in Genetics and Development.* 271 pp. Columbia University Press, New York.

WARDLAW, C. W. 1953. A commentary on Turing's diffusion reaction theory of morphogenesis. *New Phytol.,* 52: 40–47.

——. 1955a. Evidence relating to the diffusion-reaction theory of morphogenesis. *New Phytol.,* 54: 39–47.

——. 1955b. The chemical concept of organization in plants. *New Phytol.,* 54: 302–310.

——. 1957. The floral meristem as a reaction system. *Proc. Roy. Soc. Edinburgh, B,* 66: 394–408.

——. 1959. Methods in plant morphogenesis. *J. Linnean Soc., London, Botany,* 56: 154–160.

WEISS, P. 1925. Winkelmessungen am Schmetterlingsflügel. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 104: 409–420.

——. 1939. *Principles of Development.* 601 pp. Henry Holt & Co., New York.

——. 1950. Perspectives in the field of morphogenesis. *Quart. Rev. Biol.,* 25: 177–198.

——. 1961. Guiding principles in cell locomotion and cell aggregation. *Exptl. Cell Res., Suppl.* 8: 260–281.

——, and G. ANDRES. 1952. Experiments on the fate of embryonic cells (chick) disseminated by the vascular route. *J. Exptl. Zool.,* 121: 449–487.

——, and J. L. KAVANAU. 1957. A model of growth and growth control in mathematical terms. *J. Gen. Physiol.,* 41: 1–47.

WHITING, P. W. 1919. Genetic studies on the mediterranean flour moth *Ephestia kühniella* Zeller. *J. Exptl. Zool.,* 28: 413–441.

WIGGLESWORTH, V. B. 1940. Local and general factors in the development of "pattern" in *Rhodnius prolixus* (Hemiptera). *J. Exptl. Biol.,* 17: 180–200.

——. 1953. The origin of sensory neurones in an insect *Rhodnius prolixus* (Hemiptera). *Quart. J. Microscop. Sci.,* 94: 93–112.

——. 1959. *The Control of Growth and Form.* 140 pp. Cornell Univ. Press, Ithaca.

WULKOPF, H. 1936. Hitze- und Frostereize in ihrer Wirkung auf das Flügelmuster der Mehlmotte *Ephestia kühniella* Zeller. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.,* 134: 209–223.

ZALOKAR, M. 1943. L'ablation des disques imaginaux chez la larve de *Drosophile. Rev. Suisse Zool.,* 50: 232–237.

——. 1947. Anatomie du thorax de *Drosophila melanogaster. Rev. Suisse Zool.,* 54: 17–53.