THE GENETIC CONTROL OF WING DEVELOPMENT IN *DROSOPHILA*

By C. H. WADDINGTON

California Institute of Technology, Pasadena, and Zoological and Strangeways Laboratories, Cambridge

(With Plates 2–5 and Fifteen Text-figures)

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I. THE PUPAL DEVELOPMENT OF THE NORMAL WING, WITH A NOTE ON THE DEVELOPMENT OF THE LEGS

INTRODUCTION

THE wings of insects have for some years past been favourite objects for experimental studies in morphogenesis, and in particular for investigations on the mode of action of genes during development. An essential preliminary for such studies is a thorough knowledge of the normal processes of development. In the last few years very complete descriptions of the pupal development of the wings have been given by Köhler (1932) and Behrends (1936) for *Ephestia kühniella*, by Schlüter (1933) for *Habro-*

bracon, by Kuntze (1936) for *Philosamia*, and by Hundertmark (1936) for *Tenebrio*. Important genetical and experimental-morphological investigations have been made in all these forms, but in none of them do we yet possess anything like the wealth of genetical material which is available in *Drosophila*, and it is clear that if this material is to be fully utilized, we shall require for that form also an account of development as careful as those which have just been mentioned. This is already in existence, as far as the larval stages are concerned, in the work of Auerbach (1936), who also provides some data on the pupal development; and some further remarks on normal wing development are to be found in Chen (1929) and in the papers of Goldschmidt (1935, 1937) which are mainly concerned with the development of mutants, while a general account of the pupal period has been given by Robertson (1936). In none of these accounts of pupal development, however, are the data sufficiently complete to enable one to evaluate the divergences from the normal which characterize the different mutant types. The purpose of this paper is to provide a normal table of wing development from the time when the blade of the wing becomes distinguishable to the adult condition. A preliminary account has already been published (Waddington, 1939).

MATERIAL AND METHODS

The greater part of the material was prepared during a stay, between January and April 1939, at the California Institute of Technology, Pasadena;[1] the remainder was collected in the Zoological Laboratory, Cambridge. In both places, the stock used was a wild race known as Oregon-R. Pupae were collected from the sides of the culture bottles and incubated until required at 25° C. The zero time, from which their age is measured, was taken as the time at which movement of the anterior spiracles was no longer possible. Fixation was usually by hot water, which, as Robertson noted, seems nearly as satisfactory as any more refined method, since most fixatives are unable to penetrate the chitinous sheaths in which the animal is enclosed. For certain points, however, it was necessary to use Carnoy and to tear the chitin so as to allow the fluid to reach the tissues.

The wings were investigated as whole mounts after staining in Delafield's haematoxylin, or as serial sections cut at 7μ.

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[1] I am glad to be able to express my gratitude to Prof. T. H. Morgan for the hospitality of his laboratory, and to the Rockefeller Foundation for the grant of a Travelling Fellowship which made my visit possible. I also owe a debt of gratitude to Professors Sturtevant and Dobzhansky for many interesting and helpful discussions.

THE DEVELOPMENTAL STAGES

It has been known since the investigations of Snodgrass (1924) on *Rhagoletis* that in Diptera the formation of the hard chitinous sheath, in which the animal becomes immobilized, is not necessarily a sign of pupation in the true sense. Robertson (1936) confirmed this for *Drosophila*, and showed that the animal inside the puparium undergoes a complete prepupal instar before pupation. The latter process does not occur till $11\frac{1}{2}$–12 hr. after the cessation of movement, and is announced by the comparatively sudden eversion of the head. Following Robertson, the animal, during the instar which separates puparium formation from pupation, will be called a prepupa.

v. Buddenbrock (1930) and Köhler (1932) have suggested dividing the period between two moults into a series of phases, which are repeated in regular sequence throughout the life of the animal. Following a moult, there is a resting stage (*Beharrungsphase*); a withdrawal and cell-division phase (*Chitinablösungs- und Zellteilungsphase*); a stretching and folding phase (*Streckungs- und Faltungsphase*); and a chitin-formation phase (*Chitinbildungsphase*) which is succeeded by the next moult. This system can be fairly easily applied to the *Drosophila* wing during the period with which we are concerned, and provides a simple classification into stages. We can first separate the two instars as the prepupal (*PP*) and the pupal (*P*); and within each instar we can number the phases mentioned above as 1, 2, 3 and 4; minor subdivisions of these phases can be indicated by the letters *a*, *b*, *c*, etc. The phases arrived at in this way can be identified without reference to the actual age of the animal, which is a great convenience in studying the processes of development in mutant stocks. At the same time, the developmental stage is of course very closely correlated with age. In my material the great variation in developmental stage attained by flies of the same age, to which attention was drawn by Goldschmidt, was not found, although there is a certain amount of variation, which becomes greater towards the end of pupal life.

Stages PP 1 and PP 2 (resting). 0–4 hr.

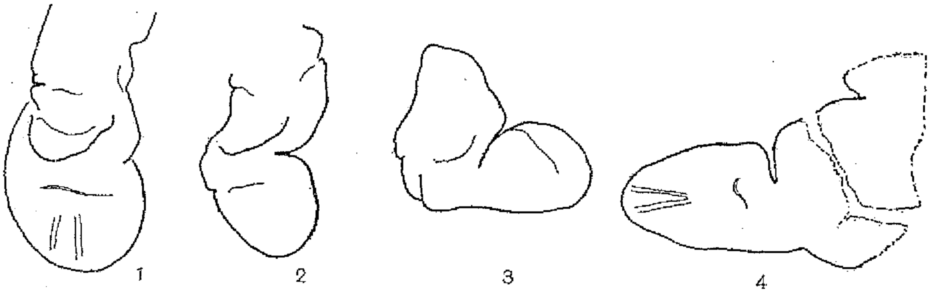
As far as the wing bud is concerned, there is no true resting stage at the beginning of the "pupal" period. In fact the evagination of the wing from the imaginal disk may begin during the end of the last larval instar, and continues throughout puparium formation and for the next few hours. The details of the process are of considerable importance in connexion with the mode of action of genes, and will therefore require rather full treatment.

Before eversion, the wing area is a thickened region on the ventral side of the dorsal mesothoracic imaginal bud. The two buds lie near the dorsal surface of the larva, with their blunt ends pointing posteriorly, while in the anterior they are drawn out into a stalk which connects them with the hypodermis. This stalk is extremely thin and, as Robertson points out, is difficult to find in sections; but it is fairly easy to see in whole mounts, and the connexion with the hypodermis is not broken before puparium formation. The eversion of the wing starts by a folding of the thickened area upwards, that is to say into the cavity of the bud. The wing region is thereby formed into a hollow sac (Pl. 2, fig. 1). This is directed posteriorly along the length of the bud, and it elongates in this direction for some time, during which period the thin dorsal wall of the imaginal bud degenerates and disappears, thus leaving the wing free.

The wing fold is at first broad, its upper, dorsal, surface being smoothly rounded, while the lower one is wrinkled parallel to the position of folding. At the lower, more posterior edge of the opening which leads into the fold, there is also a thickened region, which Auerbach has described as the marginal ridge. On the upper surface, the smooth wing area ends anteriorly in two deep transverse folds which meet at a fairly obtuse angle; farther anteriorly than this the epithelium of the bud is corrugated by a complex series of transverse folds. All these folds are difficult to show in photographs or drawings, since they lie at different focuses.

The eversion of the wing is not a simple folding of an already formed plate, since there are some other changes in shape involved. The primary phase may be likened to the protrusion of the human tongue; as the wing fold elongates, it narrows from side to side and becomes more nearly cylindrical. The analogy is, however, not exact, since it fails to take into account another change which is proceeding at the same time. The whole bud, as has been said, lies more or less in the long axis of the larva, and the eversion of the wing at first proceeds in this direction. Very soon, however, the wing blade is bent laterally, so that it eventually assumes a direction at right angles to its original position, running from the dorsal surface down towards the ventral, with its length lying in a plane transverse to the length of the whole animal (Text-fig. 1). This bending was noticed by Auerbach, who thought that it was probably due to a more rapid transverse growth of the original broad and shallow wing fold. This is not the case. The bending is really due to a rotation of the fold about its base. The point is of some importance, since, if Auerbach's account was correct, the length of the final wing would appear as the breadth of the first wing fold, and vice versa. According to the present account, on the other hand, the axis of the adult wing is in the early wing fold parallel to the long axis of the larva. On the other hand, the fact that the early wing fold narrows and becomes more cylindrical as it elongates, shows that the relation of adult shapes to shapes of the fold will not be simple; in the earliest wing fold the future long axis has been telescoped up.

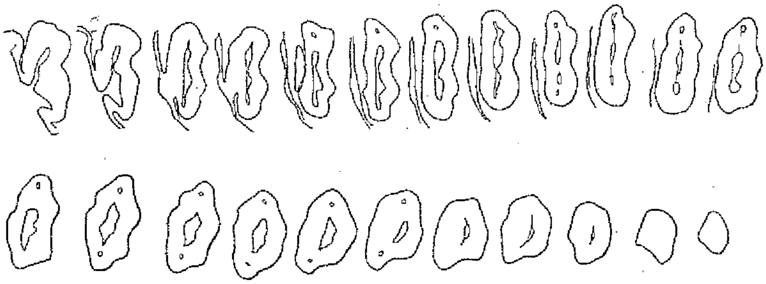
The reason for the confident statement that the bending is really a rotation can be found in a study of the earliest signs of the veins. The histological structure of these will be more fully described in the next stage, when they are better defined. But the very earliest signs of them can be seen in the partially everted wing, just before the time of the bending. At that time two fine pale lines may sometimes be made out running longitudinally; these are the traces of the two branches of the central lacuna which will be described in the prepupal wing. The fact that they run longitudinally before the bending shows that the final long axis is already longitudinally disposed at this stage. During the bending, their direction can be seen to alter in conformity with that of the wing as a whole, so that they always run along the length of the wing blade (Text-fig. 1; cf. Text-fig. 9).



Text-fig. 1. The evagination of the wing in stages PP 1 and PP 2. The long axis of the larva runs along the length of the page, with the head at the top. The buds were drawn by transmitted light, as transparent objects. 1, ventral view of left bud, showing traces of veins; 2, dorsal view of right bud, showing early stage of bending of wing fold; 3, dorsal view of right bud, wing bent further; 4, dorsal view of left bud, wing fold elongated and showing traces of veins.

At this stage the wing fold is usually still hollow, and the future veins are represented only by slightly wider spaces between the two surfaces. Corresponding to these wider spaces are rather feeble longitudinal folds on the outer, dorsal, surface (Text-fig. 2). We shall find reason later, in the discussion of the action of some genes, to believe that these folds are not by any means so casual as one might think, but are on the contrary fairly rigidly determined and incapable of regulation in conformity to the size of the wing fold on which they lie.

The developmental processes which have just been summarized are rather rapid, and are completed in about 4 hours after puparium formation. There is considerable variation in developmental stage of different animals of the same age during this early period, and this is only to be expected, since the condition of the bud at puparium formation is sometimes more advanced and sometimes less so.

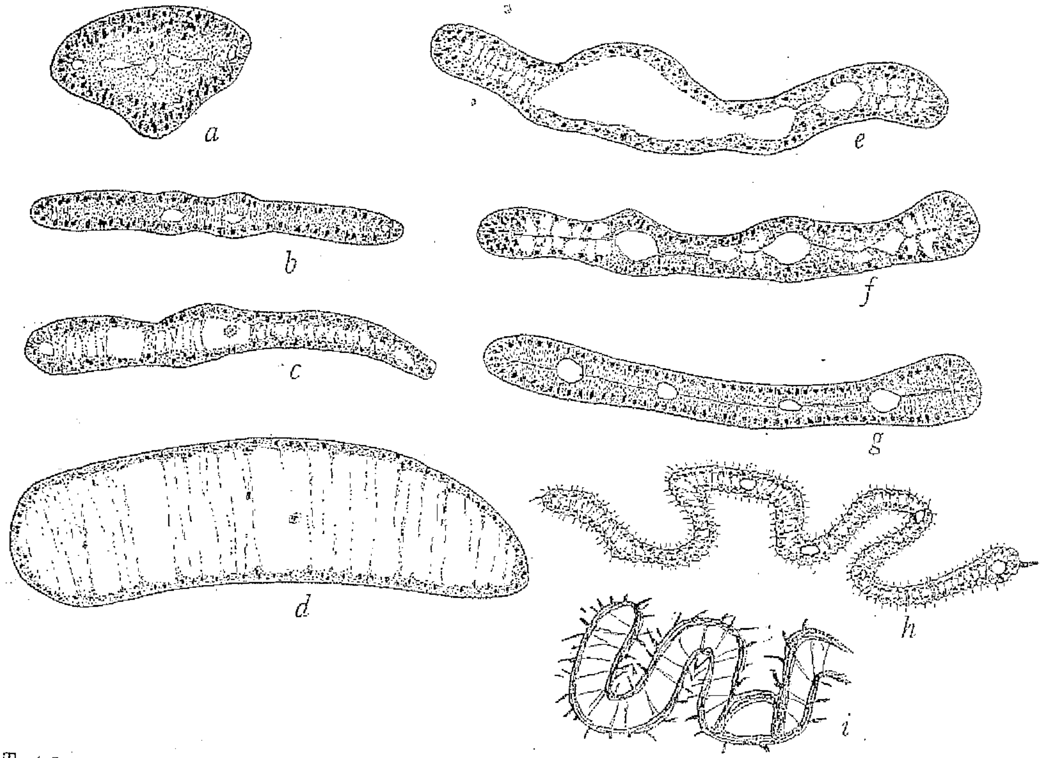


Text-fig. 2. Serial sections of the wing fold in stage PP 1, showing the feeble ridges on the dorsal surface and their connexion with the veins. Dorsal surface to right. The series runs from top left to the right, like letterpress in a book.

Stage PP 2 (*withdrawal and cell division*). 4–6 *hr.*

We may date the second stage of the prepupal period from the time when the wing has completely escaped from the peripodial membrane and is lying vertically along the side of the animal. The larval hypodermis may still be covering it at this time, but it is rapidly degenerating and soon disappears.

In whole mounts the wing appears as a solid, rather cylindrical block of tissue, about three times as long as it is broad (Pl. 2, fig. 2). In sections it can be seen that this solidity is not at all deceptive (Text-fig. 3*a*). The two surfaces of the fold come together as they are invaginated into the interior of the imaginal bud, so that the inner cavity of the wing fold is almost obliterated. There are, however, certain lines along which the two surfaces do not come completely together, and thus hollow spaces are left. These constitute the first system of blood lacunae. They are disposed somewhat differently to the second (pupal) blood lacuna system from which the adult veins develop. There are, first, two marginal veins, along the anterior and posterior margins of the fold; that along the posterior margin, which has no analogue in the adult wing, is the thicker of the two. One main lacuna runs down the centre of the wing, dividing at about the middle of the wing into two branches, both of which continue to the wing tip. These seem to correspond to the third and fourth longitudinal veins (medius and cubitus) of the adult. There is also a lacuna running in a curve just posterior to these two, and corresponding to the fifth vein; it joins the posterior marginal at a wide triangular-shaped junction. There is no trace of anything to correspond to the second longitudinal (radius), nor can any cross-veins be made out (cf. Pl. 2, figs. 3–5).



Text-fig. 3. Semi-diagrammatic sections of the wing. *a*, stage PP 2; *b*, stage PP 3; *c*, beginning stage PP 4; *d*, end stage PP 4; *e*, stage P 2*b*; *f*, end stage P 2*c*; *g*, stage P 2*d*; *h*, stage P 3; *i*, stage P 4 just before emergence.

The course of the blood lacunae is difficult to follow in whole mounts of this stage, owing to the thickness of the wing epithelia. These consist of high cylindrical cells, with the nuclei near the outer surface, although they are not arranged at any definite layer. The bases of the cells are drawn out as long protoplasmic cylinders. In some places a central membrane may be formed where the two surfaces are in contact, but it only persists for a short time and has disappeared by the next stage. In this respect conditions are intermediate between those in *Tenebrio*, which has a central membrane, and *Habrobracon*, which has not.

Stage PP 3 (*stretching and folding*). 6–9 *hr.*

During the early part of this stage the wing begins to rotate again about its base, so as to bring its long axis once more nearly parallel to that of the animal as a whole. Histologically, the most important change is that the thick, solid wing blade becomes rapidly transformed into a thin plate of considerably larger area (Pl. 2, figs. 3, 4). The process seems to occur very quickly, since it is in the early part of this stage, more than in any other part of early development, that individual variation between animals of the same age is most noticeable. The thinning of the plate is mainly due to rearrangement of the cells. The epithelia, instead of being composed of high cylindrical cells, are formed of a single layer of quite flat cells, which are thin enough for the whole mounts to be fairly transparent (Text-fig. 3 *b*). The blood lacuna system is therefore much easier to distinguish. The sudden thinning of the wing, which initiates the stage, causes a considerable expansion in area, and this continues throughout the remainder of the stage.

As regards the wing, this stage is predominantly one of stretching, and there is comparatively little sign of any folding of the wing surfaces. This folding is, however, very well seen in the legs.

It is during this stage that one can, for the first time, see the nerve and tracheole which extend into the wing. They enter near the anterior margin, where the central vein fuses with the anterior marginal, and the main branch runs down the central vein to about the point where it forks; a smaller branch runs forward a short distance along the anterior marginal vein. The two structures persist throughout the subsequent development, and presumably form a basis about which the veins L 1 and L 3 of the pupal wing are laid down. The other pupal veins, however, are not provided with either nerves or tracheoles, and must develop independently of them.

Stage PP 4 (*chitin formation*). 9–12 *hr.*

The stage of chitin formation is not sharply distinguished from that of stretching. The wing begins to secrete a chitinous sheath at about 6 hr. after puparium formation, but this sheath is at first not only extremely thin, but is also clearly extensible, since it grows as the wing expands. In fixed pupae it can fairly easily be removed from the wing, as is necessary if proper staining is to be achieved. The chitin which is deposited in the present stage is thicker and more abundant, and becomes more difficult to remove without damage to the underlying epithelium. The expansion in area which was described for the last stage also continues throughout the present one, and even beyond. The distinction between the stages is, however, not entirely artificial, since the mechanism of the expansion is somewhat different in the two periods.

In whole mounts of this stage the wing is seen to be not only increasing in size, but also to become more transparent, while the system of blood lacunae becomes more difficult to make out (Pl. 2, figs. 5, 6). In sections it is seen that the cells of the wing surfaces are still increasing in surface area and decreasing in thickness (Text-fig. 3*c*). This does not apply to the wing as a whole. Indeed, it now begins an increase in thickness which, in the next stage, attains remarkable dimensions. This is achieved by the appearance of spaces between the basal ends of the cells. It is now impossible to discern any central membrane; the bases of the cells appear to be drawn out into long thin processes which stretch from one surface of the wing to the other, and the main system of blood lacunae can only be distinguished as rather larger and more continuous spaces extending through the spongy interior of the wing. A somewhat similar phenomenon was described by Hundertmark in *Tenebrio* and by Schlüter in *Habrobracon*, except that in the former case a middle membrane was always present.

At about the middle of this stage, at about 11 hr. after puparium formation, the wing has a very characteristic appearance (Pl. 2, fig. 6). Its central parts are still fairly flat, although somewhat swollen; the blood lacunae can be made out without much difficulty, particularly the two branches of the median lacuna. But at the edges, and in the region of the tip, the wing is difficult to stain completely in whole mounts, in which these peripheral portions usually have a somewhat washed out and fuzzy appearance. In sections it can be seen that the central part of the wing is considerably inflated, the two surfaces being only connected by processes in the manner described above, while the peripheral regions are still flat, with the two surfaces in contact. Moreover, at the wing tip and along the edges there are thick deposits of chitin closely adherent to the epithelia. It is probably a combination of the comparatively close texture of the interior and the thick cuticular covering which makes these regions less easy to stain than the central part.

The superficial appearance of a whole mount of this stage might be taken to suggest that the peripheral parts of the wing were degenerating and were about to disappear. A process of this kind is known to occur in the peripheral regions of the wings of certain Lepidoptera (Sueffert, 1929). But it is extremely unlikely that any such event occurs in *Drosophila* at this stage. No trace of recently shed tissue can be found within the chitinous sheath, although the circulation in these regions must be exceedingly inefficient at removing debris. It should be remarked that the marginal degeneration postulated by Goldschmidt in certain mutant *Drosophila* wings is supposed to occur at a later stage (in P 2). It is discussed in a later section. Here it may be said that the only appearances during normal development which could possibly be interpreted as a degeneration are those which occur during the present stage and which have just been discussed.

Stage P 1 *(resting).* 12–18 *hr.*

This stage begins with the eversion of the head, the time from which the true pupal period is usually reckoned.

At the end of the previous stage, the wing is already a fairly thick sac, with thin walls which enclose a loose spongy interior (Pl. 2, figs. 7, 9). The growth in thickness of the wing continues probably until about 15 hr., that is to say somewhat after the eversion of the head. At this time the wing may be almost circular in cross-section, but it is almost completely empty of tissue (Text-fig. 3 *d*). The surface membranes are very thin and tightly stretched, lying closely apposed to the chitinous cuticle. Within the sac one can at first trace the elongated basal processes of the cells stretching across from one surface to the other. At the height of the expansion, however, it seems that most of these processes are broken. A few can usually be made out in the distal and proximal regions of the wing, but in the central region there is no sign of them. Their absence is, however, difficult to prove conclusively, since they may be so thin as to be almost invisible.

In those regions where they can be definitely seen, one often finds a cell whose base is drawn out to a point, as though attached to a fibre running across the wing, although no fibre may be visible. In the central regions of the wing, however, even these indications of possible fibres are lacking, and there seems little doubt that the connexion between the two surfaces is entirely broken. The process of inflation thus goes much farther in *Drosophila* than in *Tenebrio* or *Habrobracon,* where it is also a noticeable feature of this phase of development. Whether this is generally so in the Diptera has not been determined.

Stage P 2 (withdrawal and cell division). 18–45 *hr.*

During this phase the wing contracts again to a thin blade, which lies freely within the large sac of pupal cuticle. As the contraction occurs, the definitive blood sinuses appear, and from these the adult veins are directly formed. Since the veins are one of the most important features of the wing, from the point of view of an attack on the mode of action of genes during development, it is necessary to consider their formation in detail, and it therefore appears advisable to divide this stage into substages.

Substage P 2a (Pl. 2, fig. 8). The first phase in the contraction of the wing from the inflated bag of the last stage occurs when the two wing surfaces begin to come together at the tip and around the margin. In these regions, at least at the tip, it is probable that even at the stage of fullest expansion some connecting fibres between the upper and lower surfaces have persisted. The contraction may well be initiated by the shortening of these fibres. But this can scarcely be all that is involved, since as the two surfaces come together a well-marked central membrane is formed. The appearances suggest that as the two surfaces approach the cells put out basal processes, and that when these meet they form a central membrane between them.

The stage P 2*a* is considered to be complete when the two surfaces have made contact all round the margin of the wing, which then has the appearance of a thin-walled sac with a thick ridge round it, along the margin. This occurs at about 19 hr.

Substage P 2b (Pl. 2, fig. 10, Text-fig. 3*e*). In this stage the veins begin to appear. The first to be visible are two veins at the tip of the wing, where the contraction is taking place most rapidly. They correspond to L 3 and L 4 of the adult wing. Very shortly afterwards the contraction, which is progressing fast, particularly round the margins, begins to be noticeable towards the root of the wing, where it cuts off and delimits a large space from which the bases of the veins will eventually form. At the end of stage P 2*b*, however, this space is still undivided; it is a cavity fairly sharply outlined by regions in which the two surfaces are in contact; immediately distal to it, in the centre of the wing, is an uncontracted region, while again at the tip of the wing, contact between the surfaces has been attained except in the region of the two veins L 3 and L 4 mentioned above. This condition is reached at about 20 hr.

Substage P 2c (Pl. 2, figs. 11–13, Text-fig. 3*f*). During this stage the contraction spreads from the distal and proximal regions, and also from the margins inwards, and obliterates the central vesicle which has just been described. In the process the remaining veins appear. The final disappearance of the central vesicle takes place in the region of the posterior cross-vein, which can in fact be considered merely as a last vestige of it. The anterior cross-vein is at first indicated merely by the nearness of veins L 3 and L 4. The veins are at this time much wider than they are in later stages, and the anterior cross-vein appears at the place where L 3 and L 4 were in contact with one another, becoming a definite vein in its own right as these two become narrower and therefore would tend to lose contact with one another.

At the end of this process all the veins are present except for the radius, L 2. The intervein material is still spongy in texture, since there are fairly large spaces between the basal process of the cells. In the wing epithelia, cell division is proceeding more actively, and is particularly rapid immediately above and below the lacunae. The cells of the vein surfaces thus become crowded together, and assume a high columnar form, with rather deeply staining cytoplasm, whereas over the rest of the surface they are still flat and drawn out beneath in the processes which extend to the central membrane. This stage is reached at about 24 hr.

During the later part of this stage the radius (L 2) is formed. In the manner of its appearance it differs from all the other veins, since it is not formed directly as a space left when the upper and lower surfaces are coming together. On the contrary, the region between L 3 and the anterior margin is at first filled with spongy tissue, constituted by the basal processes of the cells as was described above. The radius begins to appear as an extension of the large cavity which has persisted at the base of the wing since stage P 2*b*. It appears that along the line of this vein the spaces between the basal processes enlarge and fuse together to form a continuous channel, while above and below this the epithelium thickens to form the typical vein surface. It is likely that a similar process also starts at the point where the vein will meet the marginal vein, and progresses basipetally from there; but the greater part of the vein is certainly formed by extension from the base towards the tip. The process appears to be somewhat irregular, both in the exact stage at which it begins and in the speed with which it is carried out, since in different specimens showing half-formed radii, the width of the other veins is rather variable. The process is, however, usually complete by about 28 hr., and at that time all the veins of the adult are laid down.

The formation of the radius by a means different to that of the other veins does not appear to have been described in any other form.

A word should be said as to the formation of the marginal vein. It is first necessary to point out that no vein appears in the posterior margin at this time; the large sinus which was found here in the prepupal wing has no representative in the pupa. Along the anterior margin there is, from the very beginning of the contraction, some sign of a vein in the sense that the tissue there is markedly thickened. But there is at first no lumen, and this only appears gradually, at first as a narrow discontinuous tube which later becomes continuous. It remains, however, much narrower than the other veins until the later part of the next stage.

Substage P 2d (Pl. 2, figs. 14, 15, Text-fig. 3*g*). During this stage the veins become narrower, and at the same time the epithelia become more solid. The stage is probably, in fact, one of cell division, which seems to spread from the vein surfaces over the entire wing blade. The spongy texture of the intervein regions disappears, and the small spaces between the basal processes are lost. The whole epithelium acquires much the same appearance as that over the vein surfaces, except that in the intervein regions the cells are higher. The central membrane persists, and is in fact very obvious. The wing, which had contracted somewhat in area during the foregoing stages, expands again slightly.

The stage is a long one; it closes at about 45 hr., by which time the wing is a flat plate, complete in nearly every detail. The hairs, the first traces of which can be made out at about 30 hr., are by now present over the whole surface, but are still merely thin protoplasmic processes. This is the last stage before the adult at which the wing can be easily investigated, and it may be called the definitive wing stage.

Stage P 3 (*stretching and folding*). 45–60 *hr.* (Pl. 2, fig. 16; Text-fig. 3*h*)

At the end of the definitive wing stage the epithelia of the two wing surfaces are quite dense, there being no spaces between the bases of the cells, which remain plump and well filled with cytoplasm right down to the central membrane. After about 45 hr. the wing begins once more to expand, much as it did in stage PP 3. Spaces appear near the bases of the cells, and gradually enlarge. Immediately the spaces become noticeable, the central membrane disappears, and the central region of the wing is occupied by protoplasmic processes extending from one wing surface to the other.

A similar disappearance of the central membrane during the corresponding stage was noted by Schlüter in *Habrobracon*. In that form it was accompanied by a migration of cells, or at least of nuclei, from the surface epithelia into the interior of the wing. This migration does not occur to any great extent in *Drosophila* at this stage, though there may be a few nuclei which do not lie at the surface. The cells of the epithelia, however, become interdigitated with one another, so that the surface is no longer smooth but is made up of alternately protruding and retreating cells.

The expansion of the wing at this stage is clearly dependent on the swelling of the cells; it appears that the rate of cell division is not markedly increased, and it may in fact have ceased entirely. The swelling may plausibly be attributed to an imbibition of water. If pupal wings are dissected out in tap water or distilled water at any stage during pupal life, except perhaps during the stage of maximum expansion in stage PP 4, they very rapidly expand in area; in physiological salt solution this does not occur. This is certainly an osmotic swelling, and the expansion which normally occurs during development is probably of the same kind.

Since the wings are expanding within the limited space available inside the pupal chitin, they become creased and folded. The folds follow a definite pattern, which, however, has not been made out in detail.

Stage P 4 (*chitin formation*). 60–96 *hr.* (Text-fig. 3*i*)

At some point during the folding and expansion, the deposition of the adult chitin begins. The exact time at which this first becomes noticeable has not been ascertained, but it is in the region of 60 hr.

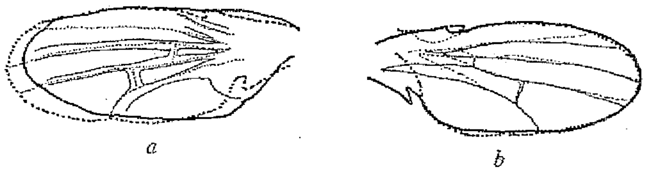
In the pupa just before emergence, the wings are heavily chitinized, and the greater part of the cytoplasmic substance of the cells has disappeared. The two surfaces are connected by rather coarse fibres, which run from side to side with no sign of a central membrane. The chitin is equally thick on the dorsal and ventral surfaces, but is considerably thickened round the veins.

After emergence the wing is unfolded and flattened partly by movements of the legs and partly, it appears, by the internal pressure of the body fluids. The latter presumably withdraw into the body as the wing dries out.

CHANGES IN THE SHAPE OF THE WING DURING THE PUPAL PERIOD

It is during the pupal period that the wing assumes its final adult shape. The changes involved are somewhat complex. During the early part of the period, the thick inflated wing becomes a flat plate. The contraction is not only in thickness, but also in area. The magnitude of the contraction can be best estimated by reference to the chitinous pupal sheath, which attains its final form during the period when the wing is most fully inflated. By the end of stage P 2*c*, the wing is already fairly thin and flat, but it lies loosely within the pupal sheath. When both the wing and the sheath are flattened out under a cover-slip, the relative areas are about 100 : 70.

It is clear that by stage P 3 the wing has already started to expand in area again, and it is probable that the change from a contraction to an expansion occurs some time during the later phases of stage P 2. But it is difficult to determine this point at all exactly, nor is it, for any purposes at present in sight, very important to do so. More interest attaches to the question of relative changes in shape of different regions of the wing. If one compares a wing of stage P 2*c* with one from the end of stage P 2 or the very beginning of stage P 3 (Text-fig. 4*a*), it will be seen that the most important change which has taken place is a relatively greater contraction of the proximal region. In fact, it seems that the proximal region continues to contract in absolute size even after the time at which the distal regions begin to expand; one of the most noticeable effects of this is that the posterior cross-vein becomes farther removed from the margin. The contraction of the proximal region is certainly connected with the narrowing of the veins, which continues right through stage P 2 and even beyond. Since the proximal region has a much higher proportion of vein material than more distal parts, it is to be expected that it will contract for a longer time and to a greater extent. Its relative contraction in fact continues throughout the expansion period of P 3 and P 4, since if early P 3 wings are compared with adult ones (Text-fig. 4*b*), the most noticeable difference in their properties is the greater relative breadth of the proximal region of the former.



Text-fig. 4. Changes in shape of the pupal wing. *a*, wing of stage P 2 *c*, in full lines, superposed on one of the end of stage P 2*d* (dotted lines), note the relative contraction of the proximal part during this interval; *b*, wing of late stage P 2*d* (full lines) superposed on an adult wing reduced to the same size (dotted lines), note contraction of basal part of wing.

During the contraction the outline of the wing undergoes certain rather subtle changes which bring it into the adult shape. There is a certain pointing of the tip; and as the proximal region contracts, the region posterior to L 5 becomes well rounded, and L 5 itself is bent into a sharper and more tense-looking curve. These changes are, as we shall see, probably dependent on the same forces as those which are responsible for the distortion of the wing in mutant types such as dumpy.

THE DEVELOPMENT OF THE LEGS

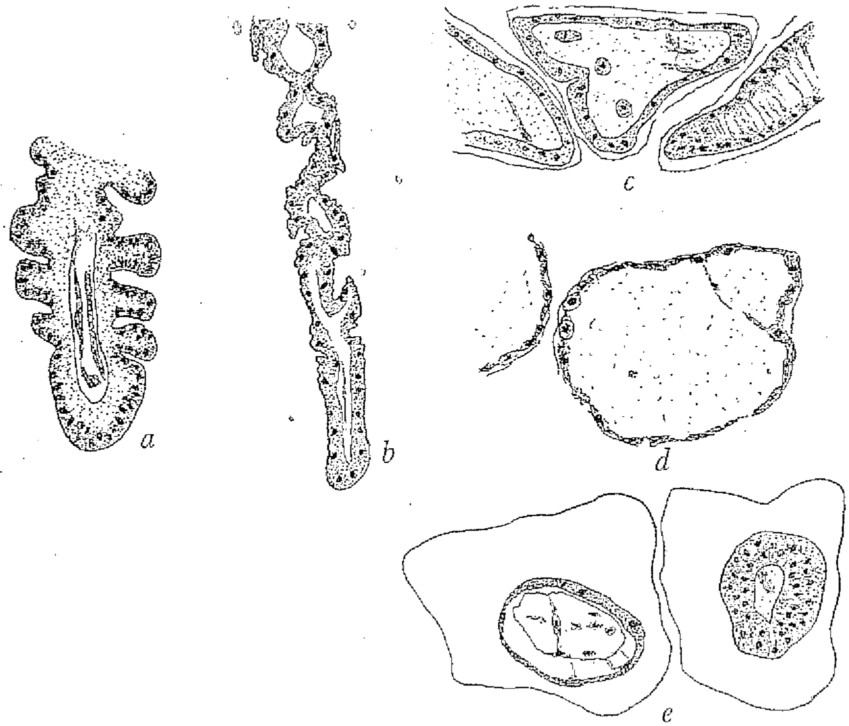
The development of the legs in the larval period, and their eversion from the peripodial sacs in the early prepupa, have been fully described by Auerbach, and nothing need be added to her account. After puparium formation the legs undergo changes which illustrate very well the succession of two instars, and exhibit particularly clearly the division of the prepupal instar into phases of resting; withdrawal and growth; stretching and folding; and chitin formation.

The leg becomes free from the peripodial sac in the withdrawal stage PP 2. At this time the segmentation is very clear in whole mounts (Pl. 3, fig. 13). In sections the leg is seen to be composed of a rather thick epithelium, with the nuclei lying near the surface. A vein and a nerve run down to the tip of each leg (Text-fig. 5*a*).

In the following stage, PP 3, the legs undergo great extension. At the same time they become highly folded, showing this feature much more markedly than do the wings (Pl. 3, fig. 14; Text-fig. 5*b*). The numerous creases and folds obscure the segmentation, which can no longer be made out. In the following stages, PP 4 and P 1, the folded leg is expanded and blown out in a way similar to that described for the wings. The legs are at this time very difficult to prepare in whole mounts, since the surface is closely invested by the chitinous covering, which is difficult to remove without injury, and which prevents proper staining. In sections of the fully inflated stage, the epithelia are very thin, and the cells only just retain contact with one another. No sign of the segmentation can be made out (Pl. 3, figs. 15, 16; Text-figs. 5*c*, 5*d*).

In stage P 2 the legs begin to contract again from their inflated form. The contraction starts at the distal end and progresses proximally, and as it proceeds the epithelium becomes thicker (Text-fig. 5*e*). It appears that a basement membrane is formed, but this cannot be made out satisfactorily. The joints between the segments appear as slight foldings (Pl. 3, fig. 17), which gradually cut more and more deeply into the shaft of the leg. The definitive leg stage, at which all the adult structures can be recognized, is reached at about the same time as the definitive wing stage (Pl. 3, fig. 18). Subsequent to this the legs remain more or less unchanged, except for the deposition of the chitin of the adult instar.

There is no folding comparable to that seen in the wing in the stages P 3 and P 4.



Text-fig. 5. Semi-diagrammatic sections of legs. *a*, stage PP 2, longitudinal section of tarsal region, showing the folds which correspond to the tarsal joints; *b*, longitudinal section of the tarsal part during stage PP 3; *c*, transverse section of tarsus of third leg near the wing tip at the beginning of stage PP 4; *d*, transverse section of tarsus of third leg near the wing tip during stage P 1, at the time of maximum inflation; *e*, transverse sections of tarsi of second and third legs near the wing tip during stage P 2*b*; the section cuts the distal joint of the second leg (on the right), and a more proximal joint of the third leg.

Discussion

This section of the paper is intended only as a straightforward description of the development of the wings, and the many interesting bearings of the data which have been presented on questions of genic action are reserved for later discussion.

Comparing the wing development in *Drosophila* with that of other insects, perhaps the most remarkable features which it shows are the extreme inflation of the wing at the end of the prepupal period, and the transitory nature of the central membrane. Of the other insects whose wing development has been fully described, *Tenebrio* is the one which most nearly approaches *Drosophila* in the degree of inflation, but even it is far behind. Moreover, *Tenebrio* possesses a central membrane throughout the prepupal period, and this persists during the expansion, although the cells of the two surfaces may pull apart, so that the single central membrane breaks down into two basement membranes which are not quite in contact with one another. The complete disappearance of the central membrane has been noted in *Tenebrio,* but only in the region of the posterior edge of the hind-wing, towards the end of the chitin-formation stage of the pupa (stage P 4). In *Habrobracon,* however, the central membrane is more transitory, and conditions in this respect approach those in *Drosophila.*

It is perhaps worth drawing attention to the fact that the adult veins develop directly out of blood lacunae, and that these in their turn are not dependent on the presence of tracheae or tracheoles, but are anatomical entities in their own right.

SUMMARY

Development after puparium formation comprises the prepupal and pupal instars. Each of these can be divided into four phases: resting, withdrawal and cell division, stretching and folding, and chitin formation. Applying this scheme to wing development, the events can be summarized as follows:

First prepupal stage PP 1, 0–4 *hr.* The wing is everted by being pushed dorsally through the peripodial sac. It is thick and fairly narrow and hollow, the prepupal veins being represented by ridges on the dorsal surface which are reflected by wider spaces within the cavity of the wing. At first its long axis is in the long axis of the larva, but it soon turns to lie at right angles to this, in the transverse plane of the larva.

Second prepupal stage, PP 2, 4–6 *hr.* The wing becomes somewhat thinner and flatter. The prepupal venation becomes easily visible; it consists of a main longitudinal vein which branches at about the middle of the wing, a posterior vein following the course of the adult L 5, and veins along the anterior and posterior margins.

Third prepupal stage, PP 3, 6–9 *hr.* The thinning of the wing continues rapidly, and the wing rotates again so as to lie more or less along the long axis of the larva. There is comparatively little folding of the wing, but this is marked in the legs. The vein and tracheole have entered the wing by this stage.

Fourth prepupal stage, PP 4, 9–12 *hr.* The wing expands in thickness by an inflation which forces the two surfaces apart. The bases of the epithelial cells are drawn out in long thin processes, and the venation becomes obscured.

First pupal stage, P 1, 12–18 *hr.* The inflation of the wing persists, or even increases. Most of the basal processes probably break, leaving the two wing surfaces unconnected.

Second pupal stage, P 2, 18–45 *hr.* The wing contracts again to a flat plate. Owing to the importance of the sequence of events, the stage may be subdivided as follows:

P 2*a*, 18–19 *hr.* The wing contracts along the margin, becoming a hollow sac with a thickened seam.

P 2*b*, 19–20 *hr.* Contraction spreads from the distal end, and tips of two longitudinal veins (L 3 and L 4) make their appearance. Contraction also starts proximally, leaving an uncontracted "central vesicle" in the middle of the wing. As the wing surfaces come in contact, the cells of the epithelia put out basal processes which unite to form a central membrane. The cavities between the basal processes give the contracted parts of the wing a spongy appearance.

P 2*c*, 20–28 *hr.* The rest of the venation appears. L 3, L 4 and L 5 appear as persisting cavities as the two surfaces come together. L 2 is formed by the coalescence of cavities, so that it seems to develop out of a spongy region. The anterior cross-vein is at this stage only a place where L 3 and L 4 unite; it gradually acquires a separate existence as these two become narrower. The posterior cross-vein is the last remnant of the central vesicle. The anterior marginal is formed gradually in the thickened anterior margin.

P 2*d*, 28–45 *hr.* The veins become narrower, and the epithelia more compact, so that the spaces between the basal processes disappear, and the spongy appearance is lost.

Third pupal stage, P 3, 45–60 *hr.* The wing expands by an expansion of the cells, and it also becomes folded. The central membrane disappears, the epithelia being connected by comparatively few coarse fibres.

Fourth pupal stage, P 4, 60 *hr. to emergence.* Chitin is deposited, and the living material degenerates and disappears.

The most remarkable feature of this development, compared with that in other insects, is probably the great exaggeration of the inflation of the wing at about the time of pupation.

II. DEVELOPMENT OF SOME MUTANT TYPES

Introduction

There are few objects as well suited for the study of the genetic control of morphological development as are the wings of *Drosophila*. Not only is their structure rather simple when compared to that of most animal organs, but we possess a very large number of genes which affect it. With such a wealth of material one, may hope, first, to analyse the morphogenetic process into its constituent phases, and secondly to determine the ways in which these developmental processes can be modified by gene substitutions. Ultimately, one must aim at determining the chemical mechanisms by which the gene effects are produced. But in asking this last question, one is raising the general problem of the chemical basis of morphology, a problem which lies at the root of the whole phenomenon of animal form, and finally of animal function also. The solutions of it which can at present be offered are, as is well known, of an extremely tentative and general kind, and one can hardly expect to be able to resolve at one blow the two problems of the chemical nature of morphogenesis and of gene action. The data which are presented in this paper are offered merely as an approach to these two questions, which, although it does not yet carry us all the way to a solution, nevertheless seems to hold out some prospect of advance.

We shall find that the three most important elements in the wing, from the developmental point of view, are the epithelial sac, the veins, and the margin. It might at first sight seem sensible to study successively the genes which affect these different elements; but in fact this would be a misplaced simplification. The three are not separate and independent entities, but are bound together in a continual interplay of causal relations. It has therefore seemed better to approach the matter extensively rather than intensively, and to investigate a number of genes. This means, however, that the potential field of study is very large. One has, awaiting investigation, not only the numerous individual genes, but also their combinations, a full study of which will occupy a very considerable time. The present account, therefore, although it deals with some matters in fair detail, is to be taken as in some measure an interim report, since the conclusions which are drawn in it may have later to be modified in accordance with investigations which can already be envisaged, and are, in part, already in progress.

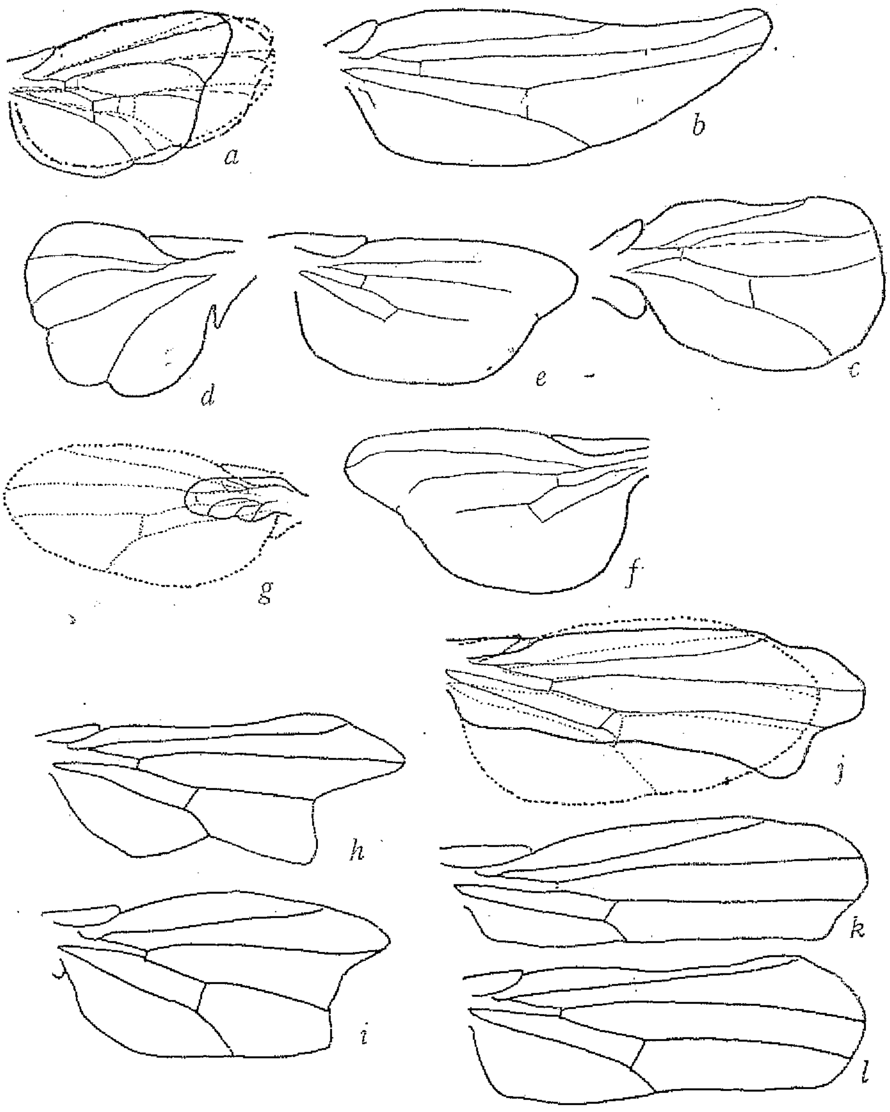
Nearly all the genes studied were in *Drosophila melanogaster*, and are kept in the stocks at Pasadena. The histological treatment was as recorded in the previous part of this paper. A preliminary notice of some of the results was published in Waddington (1939).

THE DUMPY PHENOMENON

One of the most characteristic mutant wing shapes is that produced by the dumpy allelomorphs; a similar effect is caused by spade and possibly by other mutants which have not been examined in this study. Dumpy wings are shorter than normal, and the distal end is characteristically curved inwards to give a concave margin. The effect can be found in a series of grades, the weakest of which is merely a slight flattening of the tip of the wing (Text-fig. 6*a*).

Goldschmidt (1937) and Auerbach have both studied the development of dumpy wings, and have pointed out that it is quite normal in early stages, the characteristic shape only developing fairly late in pupal life. In more precise terms, the shape is normal up to the inflated bag stage, PP 4 and P 1, and the dumpy effect becomes noticeable during the contraction stages of P 2. Auerbach offered no explanation of the gradual alteration to the dumpy shape, but Goldschmidt suggested that there is a degeneration of subepidermal tissue at the wing tip, which leads to a collapse of the margin there. At one place he speaks of a degeneration affecting the mesoderm only. The data on the histology of wing development, which were not available to Goldschmidt, make it clear that this explanation is not satisfactory as it stands, since there is in fact no subepidermal or mesodermal tissue which could degenerate in this way. Further, a glance at a dumpy wing shows that the effect is by no means a localized one; not only is the wing margin bent inwards at the tip, but the proximal part of the wing is actually wider than normal, and the whole set of veins is distorted.

If one bears in mind the developmental processes which are known to be proceeding at the time when the dumpy shape becomes apparent, the explanation of the dumpy phenomenon immediately presents itself. At that time the wing is contracting from an inflated sac to a flat plate of considerably smaller area, and the veins are appearing as lines of more coherent epithelium lining hollow canals. The dumpy effect is due simply to a disproportion between the forces due to the contracting wing epithelia and those due to the veins, which as they develop gradually give support to the wing blade. This immediately accounts for the general and unlocalized changes characteristic of dumpy, such as the widening of the basal part of the wing. Moreover, it enables one to see the connexion between dumpy and another type of wing, that caused by Blade



Text-fig. 6. The dumpy phenomenon. *a*, various grades of dumpy (dotted = normal wing reduced to same size, dashed = weak dumpy, full line = strong one); *b*, Blade, usual type; *c*, Blade, dumpy type, from same fly as fig. *b*; *d*, dumpy-cut-6; *e*, dumpy-veinlet, note the sharp angle between the two parts of L 3; *f*, dumpy-veinlet, L 3 curved so as to meet its tip smoothly; *g*, vestigial (full line) superposed on wild-type (dotted) reduced to equivalent size, note the spreading of *vg* veins; *h*, vestigial-notched, wing of elongated type, note acute angle between L 2 and L 5; *i*, vestigial-notched, wing of more rounded shape, angle between L 2 and L 5 normal; *j*, an elongated Beadex-C wing (full lines) superposed on equivalent normal (dotted) to show squeezing together of longitudinal veins; *k*, Beadex-C; *l*, Lyra, note squeezing together of longitudinal veins.

in *D. pseudo-obscura*, which at first sight seems completely opposite to dumpy, but which must be in some way connected with it, since the Blade factor occasionally produces dumpy effects (Text-fig. 6*c*). In Blade the wing is long and drawn out towards the tip, but like dumpy has a complete marginal vein (Text-fig. 6*b*). The effect here could be due to an increased tension of the contracting wing sac in the transverse plane, whereas in dumpy the tension seems to be abnormally great in the longitudinal plane of the wing. The development of Blade fits in with such a supposition to the extent that here again the wing is normal in shape until the contraction stages begin (Pl. 4, figs. 5–8).

As has been mentioned, flies carrying Blade occasionally have dumpy wings. This seems to be due to slight environmental effects, since flies have been seen which had one wing Blade and the other dumpy in type. It is probable therefore that as the contraction begins, the decision as to whether the tension shall develop in the longitudinal or the transverse planes turns on very minor alterations in conditions. One can easily understand that once the wing has begun to elongate, as in Blade, or to shorten, as in dumpy, the tendency to go on changing in the same way would increase cumulatively.

There are no data which would allow one to discover the crucial conditions which decide between the Blade and dumpy results of increased epithelial tension. One might suggest that the elasticity of the anterior marginal thickening, which later becomes vein L 1, was the decisive factor, since there is evidence that the contraction of the sac is checked by the developing veins. This can be seen in several ways. For instance, in the compound of dumpy with cut, the cut gene causes the partial disappearance of the anterior margin, and it is found that the wing becomes even more rounded than is normal in dumpy, and that the rounding of the tip of the wing begins proximal to the distal end of L 2, exactly as though the contracting sac had been able more easily to bend the weakened margin (Text-fig. 6*d*). Again, many dumpy wings are ill formed, and appear crumpled. On examination it will be found that in these wings the veins are too long for the membrane between them; it is as if the contraction had acted a little later than usually, and was unable to cause the necessary shortening of the veins.

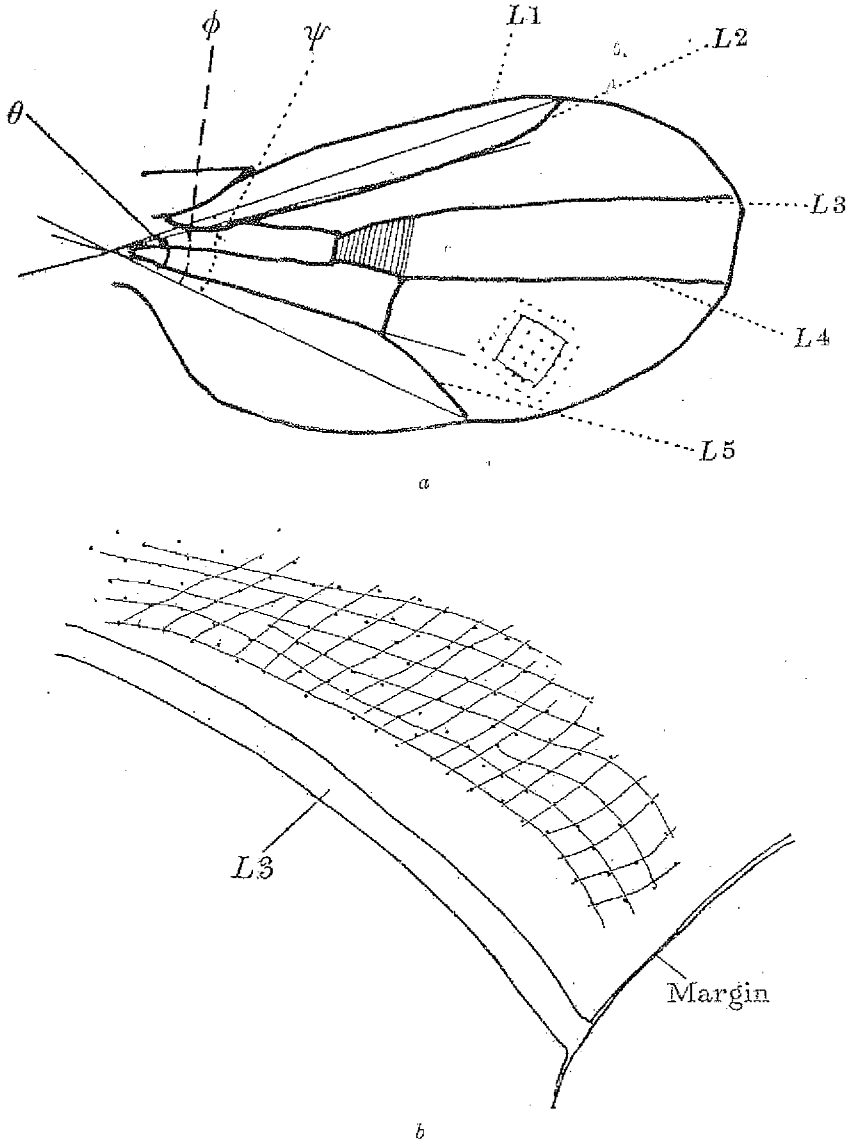
This raises another point. We have spoken of the dumpy effect as produced by the contraction of the epithelium. But a general distortion of the wing might also be brought about if the veins had a specific tendency to assume a certain pattern, and in doing so dragged the epithelia after them. The fact that the tendency in dumpy is for the veins to be too long, and the epithelia too small in area, shows, however, that the contracted shape of dumpy cannot be due to the shortening of the veins. Comparable data for the Blade type, in which one would expect the tendency to be for the veins to be too short, are not to hand, since Blade-type wings are usually well formed.

The only other agent which one might imagine could produce a generalized effect such as the dumpy wing would be a specific distortion of the margin, reacting on the rest of the wing surface. The possibility of this can be to some extent tested by making up the compound of dumpy and veinlet. The latter gene causes the disappearance of the distal ends of the longitudinal veins, so that the margin becomes connected with the rest of the wing only through the thin epithelia instead of through the fairly thick veins as well. In this compound the dumpy shape is perfectly developed, and it seems difficult to believe that a contraction of the margin, by squeezing up the epithelium, has caused the distortions of the vein system; the epithelium may reasonably be supposed to possess considerable tensile strength but hardly any resistance to compressive stresses (Text-figs. 6*c*, 6*b*).

As will be shown in a later section, the distal tips of the longitudinal veins are originally present in veinlet in stage P 2*b*, but very soon disappear. This circumstance provides an explanation for an interesting phenomenon, which, like the malformed dumpy wings mentioned above, seems to show that the onset of the tension in dumpy wings may be shifted in relation to the time of appearance of the veins. In some $dp^{02}ve$ wings the tip of L 3, as far as it remains, is bent downwards as it would be in a normal dumpy; one can make out on the margin of the wing the place where the vein should meet it, and one can see that the missing part of the vein forms a smooth curve, just as though it had originally been present, and was distorted by the tension (Text-fig. 6*f*). In other specimens, however, the vein L 3 ends abruptly, with no downward curvature (Text-fig. 6*e*). This suggests two conclusions: first, that the tip of the vein had already disappeared before the tension had developed sufficiently to cause much distortion; and secondly, as a corollary, that the tension is more effective when acting on a closed system of veins, such as is provided by L 3 and the margin when they are united, than when acting on an open system, such as that of L 3 ending blindly in the epithelium. This latter deduction echoes the earlier suggestion that it is difficult to believe that the distortion of the veins is produced by stresses transmitted entirely through the epithelia.

The fact that a contraction of the epithelia is involved in the development of dumpy is obvious from mere inspection. Now Dobzhansky (1929) showed some time ago that in the adult wing each membrane is only one cell thick, and each cell carries a single hair. By counting the number of hairs in a given area, it is therefore possible to determine the average area of the cells. As would be expected, the area is smaller in dumpy wings than in normal ones. In fairly extreme dumpies the number of hairs per unit area in the region of the posterior cross-vein is about 1·8–1·9 times as great as it is in normal wings. It varies according to the grade of dumpy and according to the region of the wing. In the wings which were measured to get the figures just quoted, the reduction in the area of the cells just accounted for the reduction in the total area of the wing, as compared with normal. Although, as has been said, the reduction in cell size is not uniform, and the ratio given above would therefore not hold accurately over the whole surface, this variation would only give rise to a second-order correction, and the general agreement in cell size and wing size can be taken as strong evidence that in the development of dumpy there is no actual loss of cells; the effect is a deformation and not a degeneration and disappearance of part of the structure.

The method of counting the hairs is particularly interesting in connexion with the question of whether, during the distortion of the wing, the veins are actually shifted relative to the epithelium, or whether, on the other hand, the veins and epithelium are merely distorted as a coherent structure. The places where one might look for a possible effect of this kind are chiefly at the distal end of the space between L 2 and L 3, and in the region of the cross-veins, since it is in these two regions that the distortion is most noticeable. The latter region is the more favourable to exact study. It is a noticeable feature of dumpy that the distance between the intercepts of the cross-veins on L 4 is considerably shortened, not only absolutely, but also relatively. Thus if one measures along L 4 from the junction of L 4 and L 5 to the anterior cross-vein, from there to the posterior cross-vein, and from the posterior cross-vein to the margin, and expresses the lengths as percentages of the total length (Text-fig. 7), the figures for normal wings are about 30 : 20 : 50, whereas for fairly strong dumpies the figures are about 30 : 15 : 55. This shortening of the inter-cross-vein distance does not, however, fully describe the changes in shape, since there is also a considerable spreading out of the veins. Thus if one draws a line along the main direction of L 2 and one along the proximal part of L 5, the angle (θ in Text-fig. 7) between them is about 29° in wild-type wings, and about 36° in moderately



Text-fig. 7. *a*, wing of dachs, to show various dimensions measured. θ = angle between main directions of L 2 and L 5, ϕ = angle between L 2 and tip of L 5, ψ = angle between tips of L 2 and L 5. The shaded area is the "area bounded by L 4, the anterior cross-vein, L 5 and a prolongation of the posterior cross-vein". At the bottom right some hairs are drawn (at a magnified scale) to show the roughly linear arrangement, which forms parallelograms whose length and breadth were investigated in narrow and broad. *b*, hair distribution in dumpy, just anterior to L 3, showing the bending of the lines of hairs as they approach the margin.

dumpy wings. A greater difference is found if one measures the angle between the direction of L 2 and a line joining the intersection of the previous two lines to the marginal end of L 5 (ϕ in Text-fig. 7); in this case the angle for wild type is about 38° and for dumpy about 47°. The spreading effect is in fact more marked distally than it is proximally.

A similar spreading of the veins can be found in other mutants which show a shortened inter-cross-vein distance, such as dachs, approximated, etc., and this will be discussed later. In the case of dumpy we are dealing, as we have seen, with the deformation of a moderately elastic system of linear members, the veins. It is clear that the shortening of the inter-cross-vein distance and the spreading of the veins are causally connected as two aspects of the general deformation. The question arises as to whether such a deformation of a coherent structure is all that has occurred or whether the posterior cross-vein has actually shifted its position along L 5 and L 4, so as to approach the anterior cross-vein. If the vein system has merely been deformed as a coherent material structure the number of cells included in various regions between the veins should be the same in dumpy as it is in wild type, whereas if the veins slide along one another the number of cells should differ.

This is not a very easy point to determine. If one takes a large area of the wing, such as that included between the anterior cross-vein, L 4, L 5 and the margin, the area of the cells is not constant throughout it in such a type as dumpy, in which the deformation has a differential effect. On the other hand, there are no smaller areas which are at the same time considerably deformed and also strictly bounded. As a compromise one may consider an area bounded as follows: by the anterior cross-vein, L 3, L 4 and a line prolonging the posterior cross-vein to cut L 3 (cf. Text-fig. 7). The relative areas of these regions in a wild type and a dumpy wing which were measured were 1452 : 650 (i.e. nearly 22 : 10). In the same regions the relative areas of the cells were 81 : 45. The relative number of hairs in the two regions was therefore 1452/81 : 660/45, that is 100 : 82. In another case the figures came to 100 : 69. This denotes a reduction in number of cells of about 25 % in the dumpy. One can visualize what is involved if one imagines that, taking the region as it is in the wild type, the cells are reduced in area to the dumpy size, and the region is then deformed to the shape it has in dumpy, and that finally the posterior cross-vein is slid in towards the base of the wing sufficiently to reduce the number of cells in the region in question by 25 %. Now since the prolongation of the posterior cross-vein is longer than the anterior cross-vein, a reduction in cell number of 25 % will be achieved if the posterior cross-vein moves somewhat less than 25 % of the distance between it and the anterior cross-vein. In fact, the amount of slide which is indicated is at most 20 % of the final dumpy inter-cross-vein distance. Another way of putting this is to say that the calculation indicates that before any sliding takes place, the normal inter-cross-vein distance would be shortened by 40 % of its length, owing to the deformation, and that sliding of the posterior cross-vein appears to be responsible for shortening it a further 10 %. Whether this result is really significant is somewhat doubtful; the limits of the areas to be compared are bound to be somewhat vague, since they are bounded by definite veins on only three sides. If, however, one accepts them at their face value, one should note that the sliding covers a length which is not much more than four times the width of the cross-vein in the adult wing, and would certainly be included within the width of the cross-vein when this is first formed in stage P 2. One may conclude that if any shifting of the cross-vein occurs, it goes on within the broad lacuna from which the adult vein develops, and is very definitely subsidiary to the main effect, which is a deformation. We shall see later (p. 119), however, that shifting of the cross-vein can occur at least in the early part of stage P 2: but perhaps this is before the dumpy tension is fully developed.

Owing to the absence of areas which can be delimited even as crudely as the one we have just considered, it is not possible to discuss the deformation of the submarginal cell (between L 2 and L 3) in a similar way. There is, however, another question which can be investigated in this region, which is not without interest, particularly in connexion with considerations which will be discussed later in relation to mutants such as broad, lanceolate, narrow, etc. The distribution of hairs can give us information not only as to the area of the cells but also as to their arrangement, and one may enquire how far this is affected by the distortion of the epithelium. Do the cells retain their connexions with one another, so that the deformation is entirely by stretching and compression of them, or do they slide over one another into a new arrangement?

A glance at the distribution of the hairs answers the question, at least partly in favour of the latter possibility. The hairs on the wing are arranged in rough rows. If no sliding took place, rows of hairs which normally continue out beyond the end of L 2 towards the tip of the normal wing would in dumpy have to be curved round, since, in that form, the wing tip does not extend beyond the end of L 2. It is clear that no such exaggerated curvature is found. On the other hand, the distribution of the hairs still gives some evidence of strain, as can easily be seen if the hairs are joined up as symmetrically as possible in groups of four to make little diamonds; these are more extended from anterior to posterior in the more distal regions, where the deformation of the submarginal cell as a whole is greatest, and this is evidence that the cells themselves have been deformed here. We find, then, that the deformation of the epithelium takes place partly by sliding of the cells over one another and partly by stretching of the cells. One cannot determine, from the data available, what is the relative importance of these two processes in producing the force required to deform the veins.

The dumpy-Blade phenomenon in other mutants

The distortions of dumpy and Blade may, as we have seen, be attributed to abnormal relations between the contracting wing epithelia and the resistance of the veins. In both of these cases the contracting force of the epithelia is abnormally strong. It is interesting to enquire whether there is any sign of a similar interplay of forces in normal development, or whether the epithelia and veins are there so perfectly adjusted that they contract to exactly the same extent. It has been pointed out, in the description of normal development, that there are some changes in shape of the wing blade during the relevant stages (P 2), and these can presumably be at least partly attributed to forces of the kind which become abnormal in dumpy and Blade. The morphological changes involved are subtle and not very easily defined; there is some pointing of the distal tip of the wing, reminiscent of that seen in Blade, and probably correlated with the increasing strength of the anterior marginal vein; and there is a broadening of the proximal region posterior to L 5, correlated with the pinching together of the region which will form the wing articulation.

One is more impressed with the reality of these changes if one compares a normal wing with that of Delta-6, for instance. Regarding for the moment only the general shape, and paying no attention to the thickening of the veins, there is no doubt that the Delta wing has a peculiarly lax, slack appearance, which is exactly what one would expect if the epithelial tension had been abnormally low.

Very definite evidence that a dumpy-Blade process is involved in the development of all types of wings is provided by some of the scalloped wing types. It is clear that if large pieces of the wing are removed, while the pattern of veins remains unaltered, the balance between epithelium and vein will be altered. This is exactly what happens in some of the scalloped types, and the occurrence of a distortion in these types shows that the epithelial tension and vein resistance are in fact present there just as they are in dumpy and Blade.

Since in these types the wing margin is not entire, the distortion must be judged from measurements which relate only to the veins and have no reference to the margin. The best index of this kind is the angle between the main directions of L 2 and L 5 (θ). In the wings in which pieces are removed from the anterior and posterior margins, it is found that as the wing becomes more narrow and elongated, this angle becomes smaller. Thus in a normal wing it is about 29–30°; in Lyra, in which both anterior and posterior margins are removed, it is reduced to about 25–26°; in Bx^{G} it may be reduced further, to 22° (Text-fig. 6 *j*, *k*, *l*). Study of the figures of some of the other narrow scalloped wings will show that in very many of them there is a tendency for the angle θ to be smaller, that is to say, for the narrowness of the wing to be exaggerated. While I am not prepared to say that the reduction in the angle is quantitatively proportional to the narrowing which has been previously produced by the scalloping, it is clear that there is a real correlation between the shape of the wing before contraction and the kind of distortion which is produced. This comes out very well in wings such as those of vestigial-nipped, in which the position of the scalloping is somewhat variable (Text-fig. 6 *h*, *i*). In some wings which have lost only the distal end the angle may be normal or even slightly larger than normal, while in others with longitudinal scalloping the usual reduction in the angle occurs. In Xasta a deep notch is removed from the tip of the wing, which is thus somewhat squarer than normal; and correlated with this the angle θ is increased to about 33–36°. In vestigal only the base of the wing is represented, and, owing to the peculiar conditions of contraction of this region, its shape cannot be directly compared with that of a more nearly complete wing. It behaves, however, as one would expect a broad rounded wing to behave; that is to say, the veins are somewhat splayed out instead of being squeezed together. The best indication of this is the wide angle between L 3 and L 4, the anterior cross-vein being, when present, considerably longer than normal and running along the margin (Text-fig. 6*g*).

The Blade-like elongation of already narrow wings, and the corresponding dumpy-like broadening of rounded wings, seem reasonable enough mechanically. Clearly we do not know enough to draw any detailed deductions from the phenomena, but in general terms they seem to indicate that the epithelial tension increases with increasing curvature, and is strongest along the line of maximum curvature. Thus in the narrow wings, the radius of curvature in the transverse plane must be relatively shorter, in the inflated stage, than it is in wider wings. This consideration may also explain why Blade wings can develop in such very different ways even in the same fly; if the wing once starts to elongate, it must thereby become narrower, and its radius of curvature in the transverse plane shorter, so that the tendency for narrowing and elongation will be increased, whereas of course if it starts by becoming broader and shorter, an exactly opposite set of phenomena will occur which will tend to reinforce the dumpy effect.

Again, the suggestion that the epithelial force may be related to the curvature of the surface suggests the possibility that abnormal tensions may be produced in particular regions of the wing in which the shape has been altered. The conditions for such abnormal local forces are given in certain of the scalloped types, where pieces of the wing may be apparently bitten away. We do indeed find in these wings local differences in the contraction; that is to say, a wing which is more or less cylindrical in the inflated stage may contract down to a blade with quite an irregular margin. The changes of shape which would be produced during the inflation and contraction of the wing would, however, depend in a complicated way on the elastic properties of the epithelium, and the radii of curvature in different planes, and no useful purpose would be served by discussing various possibilities at this stage.

Summary

In dumpy and similar mutants the entire wing is distorted as compared with normal, as can be seen from the venation, particularly by the widening of the angle between L 2 and L 5 and by the shortening of the inter-cross-vein distance. During development the distortion appears during the contraction from the inflated stage. It involves an abnormally great contraction, since the area of the wing is reduced in comparison with normal. The area of the cells is correspondingly reduced, so that no cells are lost by degeneration.

The contraction is not dependent on the margin, since it is operative in scalloped types from which the margin is absent. Nor is it dependent on the veins, since these may be too long for the epithelia instead of vice versa; moreover, the contraction occurs normally in dumpy-veinlet wings in which the connexion between the veins and the margin is broken. On the other hand, all the facts are consistent with the hypothesis that the contraction is primarily due to the epithelia.

The distribution of hairs shows that the contraction occurs partly by the sliding of cells relative to one another and partly by the elastic deformation of the cells.

The developing veins act as stiffening rods within the contracting epithelia. They can be altered in length, and the whole system can be splayed out or squeezed together; they are only slightly, if at all, shifted in relation to the cells in the region of the posterior cross-vein. The stage of maximum contraction can occur at different times in relation to the stiffening of the veins; thus sometimes the veins are already too stiff to be adequately deformed, and remain too long for the epithelium. Similarly in dumpy-veinlet wings the distal end of L 2 is sometimes affected by the contraction and sometimes not.

An increased contraction of the epithelium can also lead to an elongation of the wing, as in Blade (of *D. pseudo-obscura*). The choice between a Blade type or a dumpy type of contraction may perhaps be related to the degree of elasticity of L 1 at the crucial stage; in any case the choice rests on slight variations in conditions, since both types of wing may occur on the same individual.

Elongation by a contractive mechanism occurs in all wings which are abnormally long in shape at the time of the contraction, for instance, in some of the scalloped types such as Lyra, Beadex, etc. Abnormally short types, such as Xasta and vestigial, undergo dumpy-like shortening.

It is probable that the tension developed in the contracting epithelium is related to the radius of curvature of the partly inflated wing sac.

SCALLOPING GENES

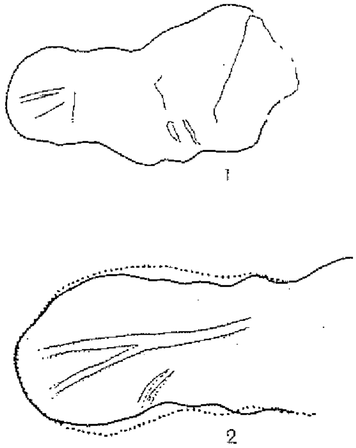
A very considerable number of genes are known which produce wings which look, in a rough way, much like normal wings from which pieces have been mechanically removed, as it might be with a pair of scissors. One need only mention the alleles of vestigial, cut. Beadex, Lyra, scalloped, etc. The development of mutants of this type has been studied by Chen (1929), Auerbach (1936), and particularly by Goldschmidt (1935, 1937). None of these authors, however, followed the pupal development right through in a continuous series, and it is doubtful whether the interpretations which have been put on the appearances which have been noticed can be allowed to stand.

The author who has discussed the development of these mutants most thoroughly is Goldschmidt. His fundamental point is that, if one examines the development of the slighter grades of notching, one finds that the wing is normal and complete at an early stage, and that the notches are produced by a later degeneration, which causes the disappearance of certain material from the margin. If a series of different grades are studied it will be found that not only does the degeneration extend farther in from the margin, but also that it starts earlier, so that in extreme scalloping the effect is supposed to be visible at the time of pupation. The latter point had already been stated by Chen for vestigial and was confirmed by Auerbach for vestigial and Pointedoid. In these extreme forms, however, little has been made out as to the mechanism of the effect; the evidence that we are dealing with a degeneration comes from a study of the less extreme forms, and this evidence must now be examined.

When one compares the photographs published by Goldschmidt with the developmental stages described and figured above it is clear that the earliest stage which he figures is that of the inflated bag (PP 4 and P 1). The series of wings in which the notches can be seen to appear belong to the successive phases of stage P 2. Now it has been shown that during this stage the normal wing undergoes a contraction by which the hollow inflated sac of stage P 1 becomes a flat wing-blade. As soon as this is realized, a new interpretation for the gradual appearance of the notches becomes possible and requires investigation. One must consider whether the progressive incision of the notch is not due merely to the deflation of an already notched sac. It is a well-known phenomenon that if one inflates an irregularly shaped elastic bag (such, for instance, as a toy rubber balloon in the form of a doll), the irregularities of the surface disappear as the bag becomes more inflated, and appear again as it collapses.

An examination of certain favourable cases makes it clear that this phenomenon is at any rate concerned in the wing development. For instance, the stock Xasta has a wing from which a large distal area is missing (Pl. 4, fig. 4). In bag stages this lack can be discerned, by the flatness of the distal end of the sac (Pl. 4, fig. 3), but it is by no means so marked as it becomes in the adult, and a series of stages can be found paralleling the phenomena described by Goldschmidt in other mutants. When, however, one examines the prepupal wings, from stages earlier than the bag stage, one finds a very marked distal notch (Pl. 4, fig. 2). Clearly the notching becomes less obvious in the inflated stage, and the appearance of the wing in that stage is not a safe guide to the actual state of affairs.

Xasta causes a very marked change in the shape of the general outline of the wing, and is therefore particularly easy to detect even in the tiny prepupal wings. The lower grades of distal notching could not be expected to be so obvious, and it is extremely likely that they could be detected, if at all, only by refined statistical analysis of exactly comparable stages. Similarly, the more usual marginal scallopings which cause comparatively slight changes in general outline will not be very easy to detect. Probably the slightest which has been as yet detected in the prepupal wing is cut-6 (Pl. 4, figs. 9–13). This causes the loss of parts of the anterior and posterior margins, and a certain pointing of the wing tip. Goldschmidt considered it one of the strongest scallopings which remained normal up to his "early pupal stage", that is to say up to our bag stage. Actually, however, it is narrower than normal in the bag stage, although the distal end is well rounded. Moreover, it is somewhat narrower than comparable normal wings even in prepupal stages. Here again then the observed appearance of the scalloping in the contraction stages is at least to some extent deceptive, since a certain amount of this scalloping must be present earlier. A similar prepupal scalloping can also be seen in other mutants of the same type, such as Lyra (Text-fig. 8), and is, of course, more obvious in the still more extreme forms such as Bx^J, etc., in which it has also been noticed by Goldschmidt.



Text-fig. 8. Lyra. 1, outline of stage P 2; 2, stage P 3 (full lines) superposed on normal (dotted lines) of same stage.

With the demonstration that the deflation accounts for some of the apparent degeneration, it becomes natural to ask whether it does not in fact account for it all. Is there in fact any actual degeneration subsequent to the inflated stage? No histological demonstration of it was offered by Goldschmidt, although he drew attention to the appearance of the edge of the wing in the scalloped region in whole mounts. However, one must remember that the wing is a fairly thick object at this time, and that in looking at the edge of a whole mount one is taking a tangential view of a fold. The breadth of the dark band which runs along the edge will therefore be very dependent on the exact conditions of the fold and degree of inflation. If, as has been suggested above, the wing actually has an irregular shape, and if these irregularities are now appearing after being obliterated by the inflation, we should expect that the conditions would be different at different points along the edge, and that the marginal dark band would vary in width. Even at the highest magnifications I have not seen any positive evidence of degeneration in whole mounts, and an examination of sections leads to the same conclusion. It might be, however, that the histological picture of degeneration was not easy to recognize, and we must therefore devote further consideration to the indirect evidence of degeneration, which provided the basis for Goldschmidt's hypothesis. This evidence is perhaps most striking in the case of vestigial itself. From Goldschmidt's figures, two main points emerge. In early stages one can see a slight knob on the tip of the wing which seems to be on the point of being broken away and lost; and again one notes that in late stages the wing rudiment occupies a smaller proportion of the enveloping chitinous sac than normal. Both these appearances suggest a degeneration (Pl. 4, figs. 15–17).

The terminal knob is an extremely regular feature of vestigial wings. It is most marked in the inflation stages, and at that time it is often connected only by a narrow bridge with the rest of the wing. The chitinous sheath here cuts deep into the outline, and it certainly looks plausible to suggest that the whole knob may be as it were strangulated during the contraction, and torn loose. However, an extensive search has failed to reveal any traces of such cast-off knobs; and it seems certain that if they are in fact cast off they should be found, since there is no circulation within the wing sheath which might remove the remnants of them. Again, it is noticeable that in later stages, when one would have to imagine that the casting-off had already occurred, the wing still shows a slight terminal bulge, although this is much less sharply constricted off than the earlier knob. This suggests rather that the knob is later withdrawn more or less into the main body of the wing.

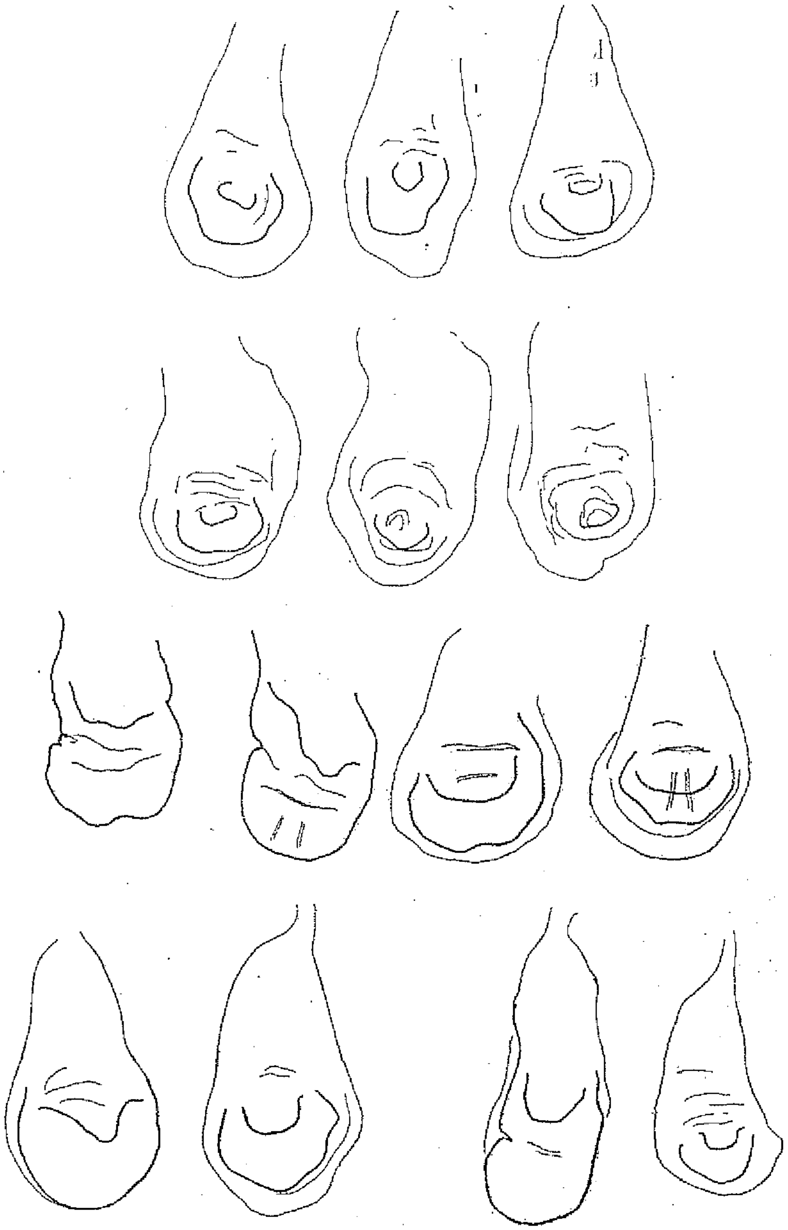
My own preparations again confirm the second point of Goldschmidt's figures, namely, that the late vestigial wing occupies an unusually small proportion of the pupal sheath. Before one can evaluate this fact, there are two main points to be borne in mind. The first is that the degree of contraction of the wing is not the same in all regions. As was shown earlier, the base of the wing contracts much more than the tip. Now the vestigial wing comprises only the most basal part of the normal wing, and is made up very largely of vein tissue which in the adult is much smaller celled than is the membrane. It would therefore be expected to contract much more than would a complete wing, and therefore to bear a different relation to the pupal sheath.

The second point is also concerned with the degree of contraction of the wing. The veins of the vestigial wing can easily be identified with those of the normal wing, and if the patterns are compared it is clear that the vestigial is deformed, in much the same way as dumpy is deformed. Thus the central vein of vestigial must be L 3, and in fact it bears the characteristic chorotonal organs. Moreover, this vein is often prolonged posteriorly round the margin (where it should perhaps be regarded as representing the anterior cross-vein. It is clear that the whole vein system has been to some extent spread out, and with the example of dumpy in mind one naturally thinks of an abnormal contraction as the cause of this. The suggestion is confirmed if one examines the size of the cells of the intervein membrane in vestigial. It is considerably less than in wild type, typical figures being 4·6, 4·54 and 4·3 hairs per unit area, as compared with a figure for wild-type wings of about 2·8. Thus the area of the vestigial cells is only about 60 % that of normal cells.

We have therefore two well-attested and definite reasons why the late vestigial wing should occupy an abnormally small space inside the pupal sheath. Again the question arises whether it is necessary to postulate any degeneration of the margin in addition. Unfortunately it is extremely difficult to give any quantitative estimate of the extent of the contraction of the proximal region of the wing in a longitudinal direction. In the transverse direction, however, the contraction from the fully inflated stage to the definite wing stage in which the wing articulation is complete is about from 100 to 30 for the most basal regions. The additional contraction by a "dumpy" phenomenon, which we have seen to occur in vestigial, would bring this down to a contraction from 100 to about 18. The maximum transverse contraction even of the main blade of a vestigial wing is of the order of 100 to 40. There seems no reason, therefore, to postulate any additional minifying effect such as a marginal degeneration.

The foregoing discussion can be applied, *mutatis mutandis*, to the development of other mutants of the scalloped type. It is only in the extreme types of scalloping, in which only the basal region of wing is retained, that the excessive contraction of this region during pupal development plays a part in simulating degeneration. In the less extreme forms this appearance is brought about partly by the tendency for re-entrant angles to be smoothed out in the inflated stage, as we saw in Xasta, and partly by the occurrence of the dumpy phenomenon. The latter is particularly marked in wings which deviate largely from the crude overall shape of a normal wing. In the elongated types such as Beadex, for instance, the elongation is exaggerated in the adult, as is shown by the acute angle between the longitudinal veins. In broader types, which more nearly approach the normal shape, the effect is less noticeable (see previous section).

It appears then that there is no necessity to attempt to explain the phenomena of pupal development of scalloped wings by postulating a marginal degeneration. Before this hypothesis can be rejected, however, it is desirable to replace it by some other which can equally well account for the remarkable shape of the adult wings. The general impression given by a scalloped wing—that it is a more or less normal wing from which pieces are missing—is really very surprising. One is bound to conclude that the scalloping takes place after the main determination of the veins, since even if deformations of the vein system, or possibly minor shifts such as that of the posterior cross-vein, may be considered possible, it is clear that major readjustments of the longitudinal veins to the new wing shape do not occur. Now in the extreme types of scalloping the first effect on wing shape is noticeable very early, even before the pupal period. Goldschmidt and Auerbach have both pointed out that in vestigial, for instance, the region of the imaginal bud which becomes invaginated to form the wing fold is smaller than normal from its very first appearance (Pl. 4, fig. 18; Text-fig. 9). This can also be shown to be true for several other mutants. Thus in Lyra and cut-6, the invagination is from the very first narrower than in wild type, and in Xasta the tip of the invaginating fold is truncated (Pl. 4, fig. 1; Text-fig. 9). At this early stage, the abnormalities in the shapes of the mutant wings are very much less obvious than they later become. But, in describing the normal development, it was shown that as the wing fold is everted, it is also protruded and stretched (p. 78), and it is clear that, if one tries to project the later wing back on to the early wing fold, one would have to imagine it squeezed up longitudinally; one is reminded of the map of presumptive areas of the amphibian gastrula, in which the presumptive notochord, for instance, occupies a much broader and shorter area than it does later



Text-fig. 9. Imaginal buds. Upper row, Beadex-J; second row, vestigial; third row, Xasta; fourth row, two normals on left and two vestigial-nipped on right. Note narrow wing-fold in Beadex-J and vestigial-nipped, small fold in vestigial, fold of normal breadth with indented tip in Xasta.

on. It is only natural that the peculiarities of the mutant shapes are less obvious in this highly condensed stage.

Since the wing shape is already altered at this time, and since the characteristic feature of these mutants is that the venation is not adjusted to the altered shapes, it is clear that the course of the veins is already determined at the time when the invagination takes place. That is to say, the veins are already determined at the time when the folding brings their upper and lower surfaces together. But we have just seen that the folding is not always in the same position; in Beadex the fold is narrow, in Xasta broad and so on. If one supposes that both the upper and the lower surfaces of the veins are equally determined, it is inevitable that with these variations in folding the two surfaces would not always be brought into perfect apposition, so that one would expect to find parts of veins on the upper surface dissociated from those on the lower. This never occurs, either in the scalloped types or in any others, such as dumpy, in which the veins develop under considerable tension (bloated is a possible exception, but here there is no doubt that the veins have been secondarily disrupted, see p. 129). One must conclude that only one surface of the venation is determined, either the upper or the lower, and that this then impresses its own pattern on the other surface by some sort of inductive mechanism.

Moreover, if it is, for instance, the upper surface which is determined, and if, by some change in the shape of the folding, tissue which is normally part of the upper surface is brought on to the lower surface, it is clear that it behaves like lower-surface material, that is to say, its own veins do not develop but it can form veins if called upon to do so by the upper surface.

It is not easy to see any other alternative to these conclusions, but the idea of a determination of one surface, an inductive relation between the two surfaces, and the inability of the determined veins to continue their development if they arrive on the wrong surface, may appear somewhat fantastic and unlikely. Fortunately, one can point to a visible feature of the wing bud which would apparently exactly fulfil the requirements. It was pointed out in discussing normal development (p. 80) that in the earliest stage of their appearance the veins are represented by wider spaces corresponding to weak ridges on the upper surface of the wing fold. If one supposes that these ridges are, by the very fact of being ridges, thereby determined as vein surface, everything falls into place; they are originally on one surface only (the upper); they do have a differential relation to the lower surface, by failing to come in contact