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PRELIMINARY NOTES ON THE DEVELOPMENT OF THE WINGS IN NORMAL AND MUTANT STRAINS OF DROSOPHILA

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Many mutations are known which affect the structure of the wings in *Drosophila*, and that organ therefore appears favorable for investigations on the developmental effects of genes.

Technique.—Pupae were fixed in boiling water, which seems as satisfactory as any more refined fixative, owing to the impermeability of the pupal chitin. The wings were dissected off and stained in Delafield's haematoxylin, and examined both as whole mounts and sectioned at 7 μ. The series of normal wings were accurately timed at 25°C.; the others were not timed, but the developmental series were constructed by comparison with the normal stages. Timing of some of the mutant types will be undertaken in the near future. The great variability in developmental timing noted by Goldschmidt was not found in this material.

The Normal Development.—In the mature larva and newly formed pupa, the wing is constituted by a fold in the epithelium of the dorsal mesothoracic disc. The epithelium in this region is thick, with elongated cells, the nuclei of which lie mainly towards the outer surface, not, however, forming a single row. The wing fold breaks through the outer surface of the disc three or four hours after pupation, and soon becomes exposed on the surface of the body. In cross-sections, the thickness from exterior (eventually dorsal) to interior (ventral) is almost as great as the width from anterior to posterior. Even at this stage, the two layers of the fold have come together, nearly obliterating the cavity. The remnants of the cavity constitute the prepupal veins, which can be made out in whole mounts and more clearly in sections, as early as 6 hours after pupation. This venation is not altogether identical with that of the adult; there is a vein along each edge of the wing, in the angles of the fold; that along the anterior margin is somewhat small and intermittent, while the posterior one is larger. One

main vein runs down the center of the wing, dividing slightly distal to the middle into two branches, which correspond to those known in the adult as $L3$ and $L4$; both these extend to the tip of the wing. Posterior to them, $L5$ takes a course similar to that which it follows in the adult, and joins the posterior marginal vein. No cross-veins have been made out with certainty.

The wing very soon becomes flatter (broader and thinner) by the rearrangement and multiplication of its cells; this must occur rather rapidly, since wings of the same age may be in different stages of the process, which may be complete at $6^1/_4$ hours. Except over the veins, the boundary between the upper and lower surfaces of the wing seems to disappear entirely, and no central membrane, such as that described by Hundertmark in *Tenebrio*, for instance, can be seen. After about the eighth hour, the wing begins to swell by the accumulation of fluid between the two surfaces, which are gradually forced apart. During this process the external surfaces consist of a thin cuboidal epithelium, in which lie the nuclei, while the basal parts of the cells are pulled out into thin filaments stretching across the wing from the dorsal to the central side. The process continues until the wing is a thin, tensely inflated bag. It is at its height at about 17 hours, and at this time it is not clear, from the sections, whether the basal filaments which connect the two surfaces of the wing are still persisting, or whether they have all been broken. Undoubtedly most of them have disappeared, but the experimental evidence described later is difficult to understand unless one can suppose that some have persisted.

After the stage of maximum expansion, the wing begins to contract again to a flat plate. Elongated processes appear at the bases of the cells on both surfaces, and these come together so that the two surfaces are once more joined; at this time, a central membrane is clearly visible between the upper and lower surface. As the process proceeds, the veins appear, and the exact sequence of events is therefore of great importance for an understanding of the effects of genes on venation patterns. At present, the following details seem fairly well established. The approximation of the two wing surfaces begins peripherally along the margins, and progresses inwards. Before it has affected the whole wing the hollow at the basal part of the wing begins to assume a definite form, and from it the coming together of the surfaces progresses forward; thus at one stage (about 20 hours) the veins may be present both in the tip and the base of the wing, while the middle region and the extreme base are still hollow, with a considerable space between the upper and lower surfaces; the hollow space in the middle of the wing will be called the central vesicle.

The veins which appear during this approximation of the surfaces are at first quite broad and gradually become narrower. They are, essentially, those which were present in the prepupa, $L3$, $L4$ and $L5$. The prepupal

anterior and posterior marginal veins are at first absent. The posterior cross-vein can be regarded as the last vestige of the central vesicle; it is at first quite extensive and gradually becomes narrower as the vesicle disappears. The anterior cross-vein appears to be formed from an anastomosis of $L3$ and $L4$, which develops into a definite vein as these two become narrower.

The approximation of the wing surfaces takes place quite rapidly, and at about 23 hours the wing, which at 17 hours was a bloated sac, has become a fairly thin blade. During the same period, there is a considerable contraction in the area of the wing viewed from the dorsal side. In whole mounts the main part of the wing has a somewhat speckled texture, which is due to the presence of numerous small lacunae between the fibrous basal ends of the cells; only at the margins and on the veins themselves is the texture more continuous, and this is caused both by the absence of fibres running across the vein-lacunae and by a greater condensation of the superficial cells, which here form a thicker epithelium with more cylindrical cells. During the next 16 hours or so, the main changes consist in (1) a gradual expansion in area, (2) an extension of the thicker epithelium to cover the entire wing and (3) the formation of veins $L1$ and $L2$. $L1$ appears as a narrow discontinuous tube within the thickness of the anterior margin; it gradually becomes wider and continuous. $L2$ can first be seen just after the disappearance of the central vesicle. At first it looks like a protrusion from the large hollow at the base of the wing, and it appears to elongate distally until it meets the just-formed $L1$. In sections it is seen to form by a fusion of the small lacunae, with a disappearance of the basal cell processes; at the same time, or perhaps even slightly earlier, its course is marked out by the thickening of the surface cells into the higher type of epithelium mentioned above.

By about 40 hours the wing is fully formed, with all its veins. It consists of a flat plate, each surface of which is an epithelium, only one cell thick, with the nuclei lying near the surface. The basal processes of the cells are not very long and those from the two surfaces are apparently fused to form a well-marked central membrane. Each cell is beginning to form a hair. This will be called the definitive wing stage.

Shortly after this stage, the wing begins to become folded. The folding is accompanied by a great increase in area and a certain inflation of the wing, which forces the surfaces apart, stretching the basal processes. The central membrane immediately disappears. At the time of emergence, the two surfaces of the wing are fairly widely separated, but strong fibres, developed from the basal processes, run from one surface to the other. During the expansion of the wing in the young imago, these fibres persist while the spaces between them become filled with fluid. After the final

contraction and drying out of the wing, the internal structures become completely obliterated.

It is apparent from this description that the wing, during pupal development, passes through two cycles of expansion, inflation and contraction; in general outlines the process is rather similar to that described in *Tenebrio* by Hundertmark,[1] with the exception that in *Drosophila* the appearance of a central membrane is ephemeral and perhaps deceptive.

Determination.—An understanding of the mode of action of wing genes requires a knowledge not only of the changing appearances of the wing but also of the underlying processes which cause them. The first step in acquiring this knowledge is to discover the time at which the various developmental processes become determined. Only rather little information about this is yet available.

We may first ask whether the appearance of the definitive venation from the inflated stage is dependent on the proper sequence of events during the contraction. Investigation of this point has been made by puncturing the inflated wing and expressing most of the contained fluid; wings treated in this way proceeded with their development and formed perfectly normal adult wings. It is clear that the question posed above must be answered in the negative. Probably the most plausible hypothesis is to suppose that even in the fully inflated stage the upper and lower surfaces are still connected by some fibres which control the way in which the approximation of the surfaces takes place.

Defect experiments on earlier stages can be made by injuring the wing with a glass needle. If injuries are made to the imaginal disc immediately after pupation, the wing may completely fail to be everted; even so it develops a histologically normal structure, though one cannot make out when the structure is complete in the sense of containing all regions of the wing. In other specimens in which eversion has occurred, the final expansion of the wing from the folded stage may not take place, and again it is difficult to trace the complete structure. One can make out, however, that parts of the margin may be missing, and this can also be clearly seen in the few operated wings which have expanded normally. One must conclude that complete regulation to form a new wing margin does not normally occur when defects are made as early as the onset of pupation.

Gene Effects.—Some twenty-four genes have been studied in sufficient detail to be mentioned here. The study relates only to the first apparent effects of these genes on the phenotype; it must be emphasized that the fundamental changes produced by the genes may occur a considerable time before they first become visible, and can only be revealed by studies on the determination of the developmental processes. The present data are to be regarded as indications of where it is profitable to look for the determinative effects of the genes.

The gene effects investigated can be classified in five groups: (1) effects on wing size and shape, (2) breakages of veins, (3) additions of veins, (4) productions of blisters, (5) curvature of the wings.

Wing Size and Shape.—(1*a*). The wings of flies homozygous for dusky (*dy*) and miniature (*m*) are smaller than normal, but unaltered in shape. Dobzhansky[2] has shown that the individual cells of the adult wing are smaller in area than normal but equally numerous. The area of the wing is found to be abnormally small throughout development, at least from the 8-hour stage and probably earlier; the area of the cells has not yet been measured earlier than the definitive wing stage, but at that time they are already smaller than normal, both in area and in thickness. The small size of miniature and dusky wings is therefore not due, as might have been thought, to a lesser expansion of the cells during the latter part of pupal life, but is initiated at an earlier stage.

(1*b*). Broad (*br*) and lanceolate-2 (ll^2) change the shape of the wings, which in the former are broader and in the latter longer and narrower than normal; the total area is little affected. The characteristic shape of broad can be seen in the prepupal wing immediately after eversion, and persists throughout development; that of lanceolate-2 is visible in the early contraction stages (c. 23 hours) but has not been detected with certainty earlier.

(1*c*). The dumpy (*dp*) wing is smaller than normal, and has a quite abnormal shape, the whole tip of the wing being broad, forming a gentle curve concave distally. In development, the wing is smaller than normal from the time of eversion, but is normally shaped until the end of the inflated stage. The characteristic shape is assumed during the contraction and approximation of the surfaces. In the adult the cells are smaller than normal in area. The mutant Blade in *Drosophila pseudoöbscura* usually causes the wing to be narrower and considerably longer than normal, but occasionally wings of the dumpy type are found, and in some individuals the two wings belong to different types. The earliest stages of this mutant have not been examined, but the wing is quite normal in the inflated stage, and is apparently pulled out of shape in the contraction; it seems that the two types of wing represent two different ways in which the abnormal contraction may occur. In the adult the cells of the dumpy type wings are smaller than those of the Blade type, even when wings of the same individual are compared. Possibly the cell area is affected by the type and amount of contraction, but the developmental phenomena relating to cell size have not been followed.

(1*d*). Several mutants are known in which the wing appears quite normal except that portions seem to have been cut off and removed. Goldschmidt[3] has explained the phenomenon by the hypothesis that, after the wing is fully formed, a degenerative process occurs which removes the ap-

parently missing portions. His evidence is based on whole mounts of pupal wings; he claims that in fairly mild cases the wing can be seen to be quite normal at an early stage and to become progressively more abnormal, whereas if the gene produces a stronger effect the degenerative changes begin earlier and the wing is abnormal from the earliest stages. In Goldschmidt's material, the developmental stage reached at a given age seems to have been very variable and the ages which he gives do not accurately identify the stages figured. An examination of his figures, however, shows that the earliest stages he used corresponded to what I have called the inflated stage. Now at this time the wing is tensely blown out into a hollow sack, and minor irregularities in its outline may disappear owing to the internal pressure. That this actually occurs can be seen in the stock Xasta, which has a deep notch at the tip of the wing; this notch is obviously present in the prepupal wing, but becomes less obvious in the fully inflated stage, only to appear again even more strongly during the contraction. The same remarks apply to Lyra (*Ly*) which produces a wing from which parts of the anterior and posterior margins seem to be missing. It appeared possible, therefore, that the appearance of a degeneration is illusory, and is simulated by the normal contraction occurring in a wing which already lacks certain portions. A careful examination was therefore made of the wings of cut-6 (*ct-6*), one of the most strongly affected cases which Goldschmidt claims to be normal in the "earliest" (i.e., inflated) stage. It is true that in the inflated stage and earlier, the wing outline is smoothly oval. But the inflated bag is somewhat narrower than normal, and the same is true for all stages back to the just-everted wing. The evidence for degeneration therefore falls to the ground, and one can conclude that the missing parts of the wing are already missing at the time of eversion. Since we have seen that the wing margin, at least, is already determined at this time, it is possible to entertain the hypothesis that the genes of this group act by altering the position of the wing fold relative to an already determined pattern of wing veins on the surface of the imaginal bud.

The lack of convincing evidence for degeneration in the mutants just considered makes it unnecessary to assume that the altered shape of dumpy wings is due to a degeneration occurring inside the wing margin, as Goldschmidt suggested.

It should be noted that the temperature-effective period for the production of vestigial-like wings as phenocopies and for alterations of vestigial wings falls during the latter part of larval life, which is the time when the folding of the wing out of the imaginal disc is occurring. The effective period for alteration in normal wings is later, in early pupal life. The mechanisms of the two effects are probably quite different, and it may be possible to demonstrate this by investigation of the cell sizes in adult wings.

Breakages of Veins.—The following genes were studied: *Radius in-*

completus (*ri*). This was studied in *D. simulans*. In the adult *L*2 is missing from near the middle of the wing almost to the marginal vein; a small trace of it may occur distally attached to the marginal vein or this may be absent. During development, *L*2, which it will be remembered is formed by the fusion of lacunae after the appearance of the other veins, simply fails to extend, the lacunae becoming gradually filled up by the condensation of the wing without ever fusing into a continuous channel. The distal piece, if present, is probably formed centripetally, starting from the marginal vein and extending inwards.

Tilt (*tt*) causes a gap in *L*3. This vein is normal in the prepupal stage. At the very earliest appearance of the definitive veins, the vein appears to extend through the gap, but it disappears there almost immediately, and the region acquires the mottled texture of the loosely porous submarginal cell lying anterior to it. At a slightly later stage, the looser texture of the submarginal cell has the appearance of spilling through the gap in *L*3 into the more condensed region between *L*3 and *L*4. It is possible that this gene has some similarities with balloon, which also seems to slow up the condensation of the submarginal cell: Thus a tilt mutation in *D. affinis* often causes the appearance of extra veins in the submarginal cell.

Veinlet (*ve*) causes a lack of the distal ends of *L*3, *L*4 and *L*5. The prepupal venation is normal, and *L*3 and *L*4 extend to the tip of the wing in the earliest contraction stages (*L*5 has not been traced at this time). The ends of *L*3 and *L*4 disappear fairly soon, probably by the formation of cell processes connecting the two surfaces of the wing. At about a 30-hour stage in normal wings, the surface epithelia are thicker, with more closely packed cells, immediately above the veins than in the intermediate regions. In *ve* the strands of thickened epithelium continue beyond the ends of the lumina in *L*3 and *L*4.

It is possible that the parts which fail in *ve* are those normally formed centripetally, while the intact parts are those which form from the central vesicle, but this is not yet certain.

In *cubitus interruptus* (*ci*) *L*4 is broken distal to the posterior cross-vein, and *L*5 proximal to it; in both cases short fragments of vein may be present. Here the effect can already be seen in the prepupal venation. In the 7- or 8-hour stage only very vague traces of *L*4 can be found, and somewhat more definite traces of *L*5; this was confirmed in sections. The pupal venation has its characteristic pattern from its earliest appearance during the contraction. In another allelomorph, ci^{w}, the abnormality (total lack of *L*4 distal to the cross-vein) can also be seen from the earliest contraction stages. The prepupal condition has not been investigated.

Crossveinless (*cv*) causes the absence of both anterior and posterior crossveins. In the *cv ct-6* compound, something like the posterior cross-vein can be seen in early contraction stages, when it is still more or less part of the

central vesicle. It soon disappears. The anterior cross-vein is at first no more than an anastomosis of $L3$ and $L4$. In *cv* it disappears as these veins become narrower in about the 28–36 hour period.

The Formation of Extra Veins.—There appear to be two main mechanisms by which extra veins are formed: (1) a precocious and exaggerated condensation of the surface epithelium, to give a thicker, more closely packed layer, in which supernumerary luminae appear and over which extra chitin is secreted; (2) a failure of condensation of the basal regions of the cells, which retain a porous texture, with many small luminae, right up to the stage at which the wing begins to fold, giving eventually thin chitinization with weak, straggly veins. The first process, exaggerated growth of the epithelium, occurs characteristically in the second posterior cell (i.e., posterior to $L4$, distal to the posterior cross-vein) and along the margin particularly at the tips of $L2$, $L3$ and $L4$. The strongest example I have seen is in net (*net*), where the thickening of the anterior margin and the second posterior cell is so great as to give the cross-section the appearance of an elongated dumbbell. The condensed regions seem to exert a tension on the rest of the wing, since the comparatively uncondensed cell between $L3$ and $L4$ may be stretched in the middle region, so that the tips of $L3$ and $L4$ are bent towards one another. The formation of extra veins within the thickened regions reminds one strongly of the formation of $L1$ along the anterior margin in normal development, since this region is normally somewhat thickened. Probably the position in which the lumen occurs is dependent on the thickness of the overlying tissue, since in the exaggerated thickenings along the margin and at the ends of $L2$, $L3$ and $L4$ it occurs some distance in from the edge, giving "deltas," such as those found in the mutant of that name, which acts very similarly (Dl^6 was examined). In plexus (*px*) and blistered[2] (bs^2) conditions are very similar to those in net, though less extreme, and there is some indication of a lack of condensation in the submarginal cell, which is more marked still in Dl^6, in which the adult shows a markedly thickened $L2$. This lack of condensation has been most strongly seen in balloon (*ba*). It gives rise to a weak chitinization, though in other stocks a blister may be formed here, presumably by a failure of the final unfolding and drying out of the wing. Balloon also shows some degree of thickening of the second posterior cell, and extra veins appear here; by comparison with net and plexus, the thickening hardly seems considerable enough to account for these, and some other mechanism may be involved.

In all the above, the prepupal venation is normal.

Formation of Blisters, etc.—In these cases the adult wings show more or less extensive blisters, which may remain filled with fluid or may eventually dry out; sometimes the whole wing is affected, in which case its expansion from the folded condition which it has in the late pupa may completely

fail. Some of the genes concerned seem to have no effect until the actual time of unfolding; examples are Wrinkled (*W*) and vesiculated (*vs*). Probably they act by causing the breakage of some of the fibres which normally hold the two surfaces of the wing together during the unfolding. The gene bloated (*blo*) produces a somewhat similar abnormality, but here the effect can clearly be seen in the definitive wing, though the prepupal wing is normal. The contraction from the inflated stage is very incompletely performed, and at the time the wing starts to fold it is still quite loose-textured and porous, and contains many extra pieces of vein, while the normal veins are wide and irregularly formed. There are also cysts derived from the surface epithelia, which form small hollow balls within the thickness of the wing; they eventually differentiate into spherical nodules of typical wing membrane, with the hairs pointing inwards. At the time of dissection it was noted that the *blo* pupae seemed to be particularly full of body fluid; it may be that all the abnormalities of the wings can be explained by a greater internal pressure, which prevents the normal contraction and may even cause the rupture of the wing surface, thus setting free the patches of tissue which form the cysts.

The mutation blot (*blt*) causes a faulty unfolding of the wing, but this is only a secondary effect. It actually produces a partial twinning, portions of a second wing being formed in the region proximal and posterior to *L*5 (the third posterior cell). The twin is a mirror image of the normal wing, mirrored in the plane of *L*5, but only rarely is it complete enough to include any of the stiff bristles characteristic of the anterior margin. Its venation is irregular, and not yet fully made out. The alula does not appear to be involved. The effect is highly variable, with a low penetrance.

Wing Curvature.—Studies have been made of the definitive stages of Curly (*Cy*), in which the wing is usually bent upwards, and of curved (*c*) in which it is usually bent downwards. In both cases there is no trace of curvature in the definitive stage. Probably the genes affect only the drying out of the young imaginal wing, though it is possible that studies on cell size will reveal earlier effects.

[1] Hundertmark, A., "Die Entwicklung der Flügel des Mehlkäfers Tenebrio Mollitor," *Zeits. Morph. Oekel. Tiere.*, **30**, 506 (1936).

[2] Dobzhansky, Th., "The Influence of the Quantity and Quality of Chromosomal Material on the Size of the Cells in Drosophila melanogaster," *Arch. Entwmech.* **115**, 363 (1929).

[3] Goldschmidt, R., "Gen und Aussencharakter. III," *Biol. Zbl.*, **55**, 535–554 (1935); "Gene and Character. IV," *Univ. Cal. Pub. Zool.* **41**, 277–282 (1937).