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Butterfly wing patterns: how good a determining mechanism is the simple diffusion of a single morphogen?

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SUMMARY

The formation of the wing pigmentation patterns of three species of butterflies has been modelled using a mechanism based on a tripod of assumptions. First, that there may be morphogen sources in the foci of eyespots and morphogen sinks at some parts of the wing margin, all other cells being passive. Second, that the morphogen has a finite half life and diffuses simply and freely away from the sources throughout a wing of hexagonally packed cells. Third, that the overt pattern derives from cells interpreting the local morphogen concentration with respect to thresholds which determine scale colours. The final pattern thus follows lines of constant morphogen concentration and may, depending on the distribution of sources, comprise rings, curves or bands. With such a model, we have been able to compute stable patterns having the essential topology of the compound spots of *Tenaris domitilla*, the large rings of *Diaethria marchalii* and the pattern of eyespots, rings and asymmetric bands of *Ragadia minoa*. Quantitative analysis of the pattern-forming process shows that, with a biologically realistic diffusion constant ($\sim 5 \cdot 10^{-7}$ cm^2 sec^{-1}) and a morphogen half life less than 6h, the patterns form within ~12h over a wing of ~1000 cells in length. The limitations of the model are that the exact morphology of the eyespots and bands do not match precisely those of the original wings, that there are edge distortions and that optimal patterns may be critically dependent on the exact positions of sources and sinks. An explanation for part of the discrepancy is that we have assumed an adult wing shape and foci coordinates in modelling a process that took place earlier in development. Nevertheless, the limitations of the model argue against a mechanism based on a single morphogen operating *in vivo*. However, as the model can generate many features of butterfly wing patterns, it may be considered as a degenerate case of that mechanism.

INTRODUCTION

The wings of moths and butterflies exhibit a bewildering range of pigmentation patterns, but very little is known about the mechanisms by which these patterns are generated. Various types of experiment do, however, suggest that small areas on the wing blade act as foci from which pattern-determining influences emanate (Nijhout, 1978). The nature of these influences is unknown, but it has been suggested that molecules or 'morphogens' produced by

these foci diffuse through the epithelial cells of the presumptive wing; the relationship between cellular thresholds and the local morphogen concentration then determines scale colour (Nijhout, 1980a). Murray (1981) has shown that mechanisms of this form can generate aspects of the wing patterns of *Ephestia kühniella* and account for some of the abnormalities caused by microcautery of various regions of the pupal wing (see below). There has, however, been no detailed study examining the range of patterns that a diffusion mechanism can generate nor is it known whether this mechanism is capable of generating a wing pattern in the time that it takes *in vivo*.

In this paper, we first summarize experimental results on pattern formation in the wings of butterflies and moths. We then report on the extent to which a simple-diffusion model is capable of generating three butterfly wing patterns. We show that this mechanism can readily form the patterns over the pupal wing within the developmental time scale (1–3 days) and also that it can generate a wider range of patterns than might have been expected. In the discussion, we consider the extent to which the model is convincing and suggest experiments to test its predictions.

THE EXPERIMENTAL DATA

The wings of adult Lepidoptera develop from invaginated imaginal wing discs which evert in the last-instar larva, just before pupation. In this process, upper and lower wing surfaces first meet, except for haemolymph spaces (the primary lacunae which form the basis for the eventual pattern of wing veins), the pupal cuticle is then secreted and the animal finally moults into the pupa. During this pupal stage, cells of the wing epidermis divide, some differentiate into scale cells and the adult cuticle is secreted. It is the pigmentation of the cuticle of the individuals scales (and, in some cases, their detailed structure) which gives rise to the characteristic colour pattern of the wing (see Nijhout, 1984, for review).

There have been several attempts to classify lepidopteran wing patterns and to view each as a different partial expression of a basic 'groundplan' (see Nijhout, 1978 & 1984, for review). Süffert (1929) suggested that there are five classes of wing-pattern elements that may be present alone or in combination. Three of these elements are large *fields* or patches of uniform colour (Fig. 1A), repeating *ripples* or flecks of colour (Fig. 1B) and *stripes* of colour running along and/or between the wing veins (Fig. 1C). These patterns have not been extensively studied and, in this paper, we concentrate on the other two classes, *eyespots* (Fig. 1D) and *crossbands* (Fig. 1E).

An eyespot is a set of concentric (or elliptical) rings of colour, usually midway between two wing veins. The region of wing blade between two veins is referred to as a *cell*, and the eyespot may be restricted to one *cell* or may spread into neighbouring *cells* (Fig. 1D). Crossbands run between the anterior

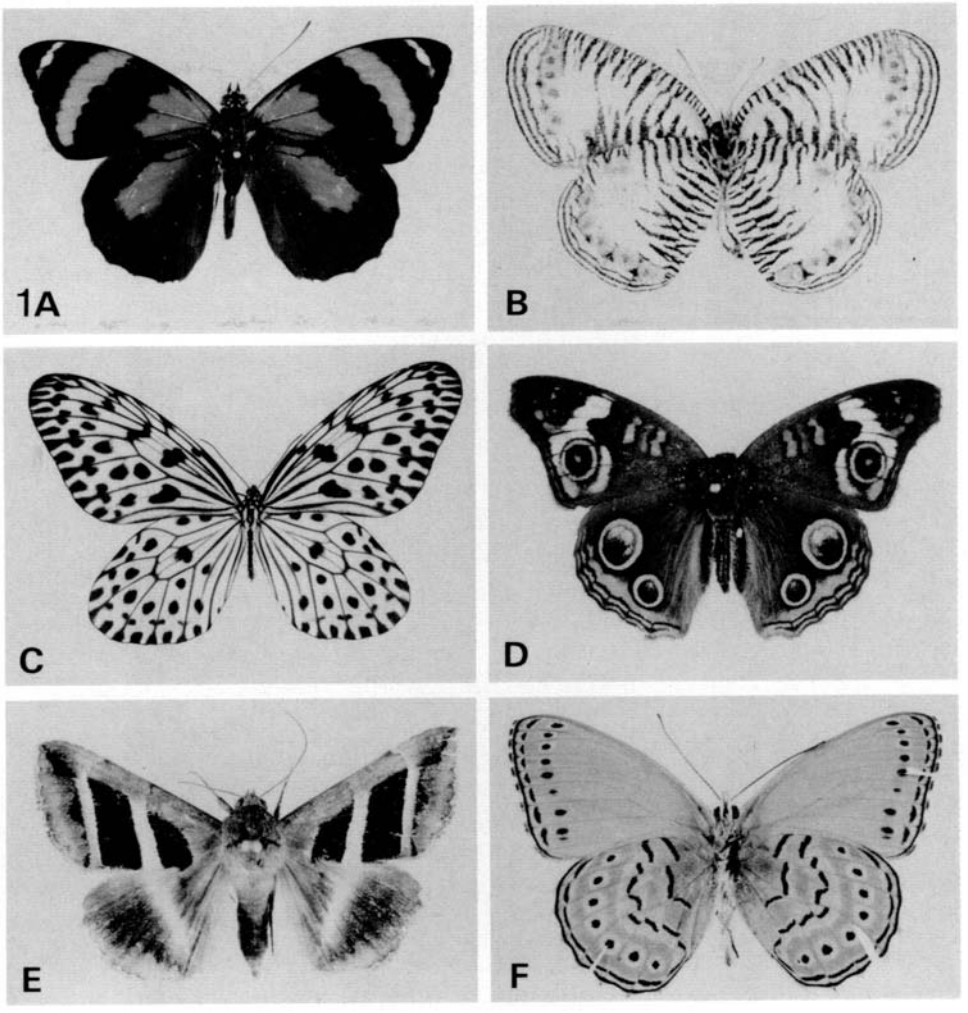

Fig. 1. The major features of lepidopteran wing patterns: (A) large *fields* of colours on the upper surfaces of both wings of *Catagramma sorama*; (B) repeating *ripples* (together with a row of eyespots) on the lower surfaces of both wings of *Physcaeneura pione*; (C) *stripes* over the veins (plus other markings) on the upper surfaces of both wings of *Idea malabarica;* (D) large *eyespots* on the upper surfaces of both wings of *Precis coenia*; (F) *crossbands* on the upper surface of the forewing of *Grammodes geometrica;* (F) the lower surface of the hind wing of *Sallya pechueli* has a pattern of crossbands and small eyespots. The more proximal crossbands show *dislocations* at *cell* borders.

and posterior margins of the wing. There are often several such parallel bands of different colours symmetrically arranged so that the more distal set may be regarded as a mirror image of the proximal one with the whole arrangement forming a *symmetry system*, often with eyespots on the line of symmetry. There may be more than one such arrangement on the wing, so that a central

symmetry system may have another, proximal or distal to it (Süffert, 1929; Nijhout, 1978). Frequently, the cross bands are not continuous, but appear broken in that a band in one *cell* is more proximal or distal than the corresponding band in the adjacent *cell;* this type of pattern is referred to as a *dislocation* (Fig. 1F).

Most experimental investigations of wing pattern formation have used either periods of heat shock or localized cautery of the pupal wing. Thus Kühn & von Engelhardt (1933) studied the symmetry system of crossbands in the flour moth, *Ephestia kühniella*. They found that a small burn made in the presumptive symmetry system of the wing during the first 36h after pupation caused a local effect: the band was deflected around the damaged area, towards the line of symmetry. A later cautery, made anywhere on the wing 36–66h after pupation, resulted in a global abnormality with the bands on both sides of the system being closer to the line of symmetry than normal. These results were interpreted as indicating that the position of the crossbands was set by a 'determination stream' originating from foci on the anterior and posterior wing margins and spreading across the wing during the period 36–66h after pupation. Kühn & von Engelhardt (1933) suggested that the stream would be deflected around any damaged area caused by a previous cautery and would be stopped in an intermediate position by cautery during its period of spreading. These and other such experiments (see Sondhi, 1963, and Nijhout, 1978) provide evidence that there are local foci that control the pattern and that pattern formation itself usually occurs shortly after pupation.

Definitive data on the role of foci in determining lepidopteran wing patterns come mainly from a recent, elegant study of the formation of the large eyespot on the forewing of *Precis coenia*[1] (Fig. 1D; Nijhout, 1980a). Following cautery of the presumptive centre of this eyespot during the first two days after pupation, Nijhout found that the earlier the cautery, the smaller the eventual eyespot. He concluded that this centre acts as a focus, possibly as the source of a morphogen that, over a period of about three days, might diffuse out to give a concentration profile that could be interpreted by wing cells to give the coloured rings of the eyespot. This view was supported by the results of the important experiment of grafting the presumptive centre to a different region of the wing: a small supernumerary eyespot formed in the surrounding host tissue. Nijhout (1978, 1980a) therefore suggested that the various patterns of eyespots and crossbands could result from the diffusion of morphogen from foci at various positions on the wing surface. This is the hypothesis that we test by simulation in the body of this paper.

[1]This genus of butterflies is also known as *Junonia* (Cowan, 1970).

THE MODEL

The simple-diffusion, single-morphogen model that is explored here has several components. These include the cellular environment, the properties of sources, sinks and intermediate cells, the type of diffusion and the choice of physical parameters.

The wing

The pupal wing was assumed to be the same shape as the adult wing, with the curves approximated by straight lines (see Fig. 2). The wing blade was modelled as an array of hexagonally-packed cells with its edges impermeable to morphogen diffusion. It was assumed that cell diameters were $\sim 15\mu$m (the exact value is not important for membrane-limited diffusion – see below) and that the length of the wing is ~1000 cells from the body to the wing tip. The figures are based on the measurements of Nijhout (1980b) on the pupal wing of *Precis coenia* as it was not possible to obtain the pupae of the butterflies whose patterns were simulated. A simulation on this scale would require that the wing would have $\sim 5 \cdot 10^5$ cells. This number is too high either for the computer memory or for reasonable computing times. A 1:4 linear reduction was therefore used and a typical wing for simulation had maximal dimensions of 240×160 cells (see below for implications).

The initial conditions

The centres or foci of eyespots were assumed to be morphogen sources and were placed on the wing at appropriate positions. In principle, each source could have a unique value; in practice, all maintained a morphogen concentration of ten arbitrary units, with the exception of a weak extra focus inserted so as to generate a pattern abnormality seen on a specimen of *Tenaris* (see Fig. 3). There are, to our knowledge, no experimental data that demand that there be morphogen sinks on the wing, at the edge or anywhere else. In order to simulate some patterns however, it was necessary to assume that they were present as a single line of cells at the appropriate margin and maintained a zero morphogen concentration. Note that the location of sources and sinks is taken as given; we do not deal here with what determines their origin or position on the early wing. All cells that are neither sources nor sinks were assumed passive: they allowed morphogen diffusion and assayed its concentration.

Kinetics

The morphogen was assumed to move through the wing epithelium by simple, membrane-limited diffusion. Here, morphogen movement across the cell membrane is much slower than that through the cell cytoplasms. This mode of diffusion is both plausible and easily computable (Bard & Lauder,

1974). One reason for its choice was the wish to find out whether this relatively slow mechanism would be fast enough to generate the pattern in the limited time available *in vivo* (see below), another was to use the simplest physical process. The morphogen was assumed to be lost exponentially with time either by simple decay or by a first-order biochemical degradation. Thus, the concentration of morphogen (C) at any time and place is given by the equation

$$\frac{\partial C}{\partial t} = \mu \nabla^2 C - \kappa C \tag{1}$$

Physical parameters

Relatively few parameters were required to specify the system. The most important of these were the morphogen diffusion constant (μ), the decay constant (k) and the threshold values. It turns out that, to a first approximation, the final optimal pattern is independent of μ and k (provided that the threshold values may be freely chosen); they primarily affect the time that it takes a pattern to form. To ensure that this was reasonably short, we chose the diffusion constant to be $5{\cdot}10^{-7}$, a value a little smaller than the largest value that Crick (1970) considered appropriate for biological systems. Values of the morphogen half life and thresholds were chosen by trial and error.

Scaling down the model wing required that the diffusion constant likewise be reduced. As diffusion constants depend on the square of the linear dimension, a 1:4 reduction in scale required a 1:16 reduction in the diffusion constant if times of pattern formation on a full-scale pupal wing were to be modelled. In all cases, therefore, we used a diffusion constant of $0{\cdot}3125{\cdot}10^{-7}$. Morphogen half lives ranged from 30min upwards.

Pattern stability and interpretation

As a simulation proceeds, the morphogen concentration builds up across the wing. If the half life is very short, a stable concentration pattern forms rapidly. If the morphogen half life is long, the regions between sources and sinks have a roughly linear gradient while other areas continue to accumulate morphogen. For regions a long way from a source, this accumulation is very slow and it is always possible to identify a time after which the pattern changes so slowly that it may be considered stable. A test to do this was therefore built into the program: a pattern was deemed stable if, over a period of one hour of pupal time, the concentration change in each of the $\sim 4{\cdot}10^4$ cells was less than 0·06 concentration units (<0·1% of the source value). When this test was satisfied, the simulation was stopped and cells allowed to interpret their local morphogen concentration as an instruction about the pigment colour that they should produce. It is assumed that the thresholds for interpretation are built in to the biochemical repertoire of each scale cell. The final pattern thus follows the lines of constant morphogen concentration over the wing.

Computing details

In order to generate a wing simulation, the following procedure was adopted. A tracing of a photograph of the wing was first made on a transparent plastic sheet and attached to graph paper. The wing dimensions and approximate foci positions were then measured and an idealized wing was then constructed (see Fig. 2). Foci and sinks were added and a trial simulation then run. The pattern was optimized by adjusting in turn the exact positions of foci, the decay constant and the thresholds values.

The program for solving the differential equations was written in Fortran 77 and run on a Vax 750. The effect of having hexagonal arrays of cells was to make the equations describing diffusion among the six nearest neighbours rather complicated. In a typical simulation, the position of the sources and sinks was first set up, the latter being regions of the boundary where the morphogen value was maintained as zero; diffusion was then allowed to proceed and the morphogen concentration in each cell calculated at successive time increments (6 sec). At 30 min intervals, the concentration of each cell was compared with its prior value; if the difference for every cell was below the test value, the simulation was terminated. The number of cells was such that it was only possible to appraise a simulation graphically. The data, stored as a matrix of numbers, were, in consequence, transformed to a hexagonal array and concentration contours plotted on a Versatec printer.

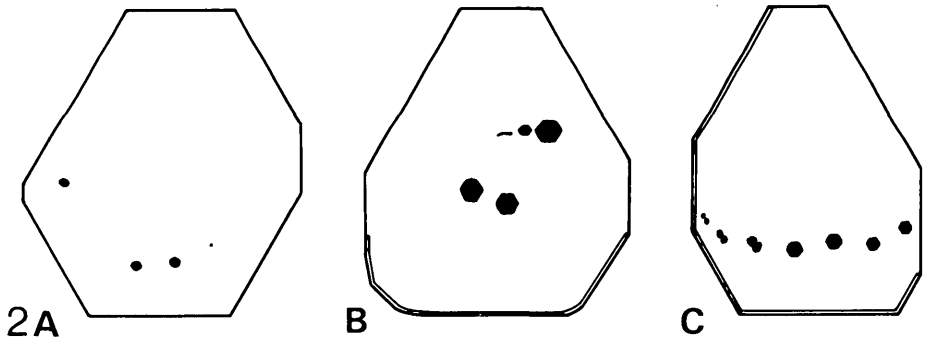

Fig. 2. The initial conditions for computer simulations of (A) *Tenaris domitilla,* (B) *Diaethria marchalii* and (C) *Ragadia minoa* (hind wing). Here, wings are outlined, central black blocks are morphogen foci and double lines at margins are morphogen sinks.

SIMULATION RESULTS

The butterflies

The three butterflies whose wing patterns were simulated were not randomly chosen: they all had well-defined foci, not too many discontinuities and no significant irregularities; each pattern also crossed *cell* borders; they were therefore patterns that were likely to be generatable by a simple-diffusion mechanism. The major feature of the first, *Tenaris*, can be explained by simple diffusion from sources, the second, *Diaethria*, and the third, *Ragadia*, require, in addition, a morphogen sink at part of the wing boundary. In this last butterfly, changes in the threshold values are further required to explain the differences between the fore and hind wings.

Butterfly 1 – Tenaris domitilla *(hind wings, lower surface)*

The pattern consists of three foci with concentric coloured rings (Fig. 3A). Two of these are close and the outer rings merge to form ellipses. The distal part of the wing is dark. In the example of the butterfly that was simulated, a small extra eyespot was present on the right wing adjacent to the normal double eyespot at the distal edge and overlapped it to the extent that the outer pigment ring was common to the three foci. The extra source was assumed to be of lower strength than those of the main foci because the central spot of the former is the colour of the first ring of the latter.

For this simulation, the normal wing was set up with three foci (Fig. 2A), each having a morphogen value 10·0. On the abnormal wing, a small additional focus was inserted and given a morphogen value 8·5 (see caption to Fig. 3 for other numerical and threshold details). With a decay constant of 0·0001 (morphogen half life of ~2h), the simulation reached equilibrium within ~6h.

The best computer simulation mimics the major features of the actual pattern (Figs. 3A, B). Thus, the appropriate number of rings was present around each focus and the distal region was marked as a separate domain from the proximal. Moreover, the outer rings of the complex foci overlapped to give the oval contours seen in the original wing. There are two obvious differences between the simulated and real pattern. First, there is a tendency for contours near the wing margin to extend towards that edge. Second, the spots are not as large as those on the original wing (using lower thresholds to increase spot size caused the outer rings to bow out towards the wing margins – see Fig. 3).

Butterfly 2 – Diaethria marchalii *(hind wing, lower surface)*

The basic pattern in this butterfly is more complex than that of the previous one in several ways. First, there are not only more foci but they have complex shapes; second, the dark rings are extremely large and the outer ones extend

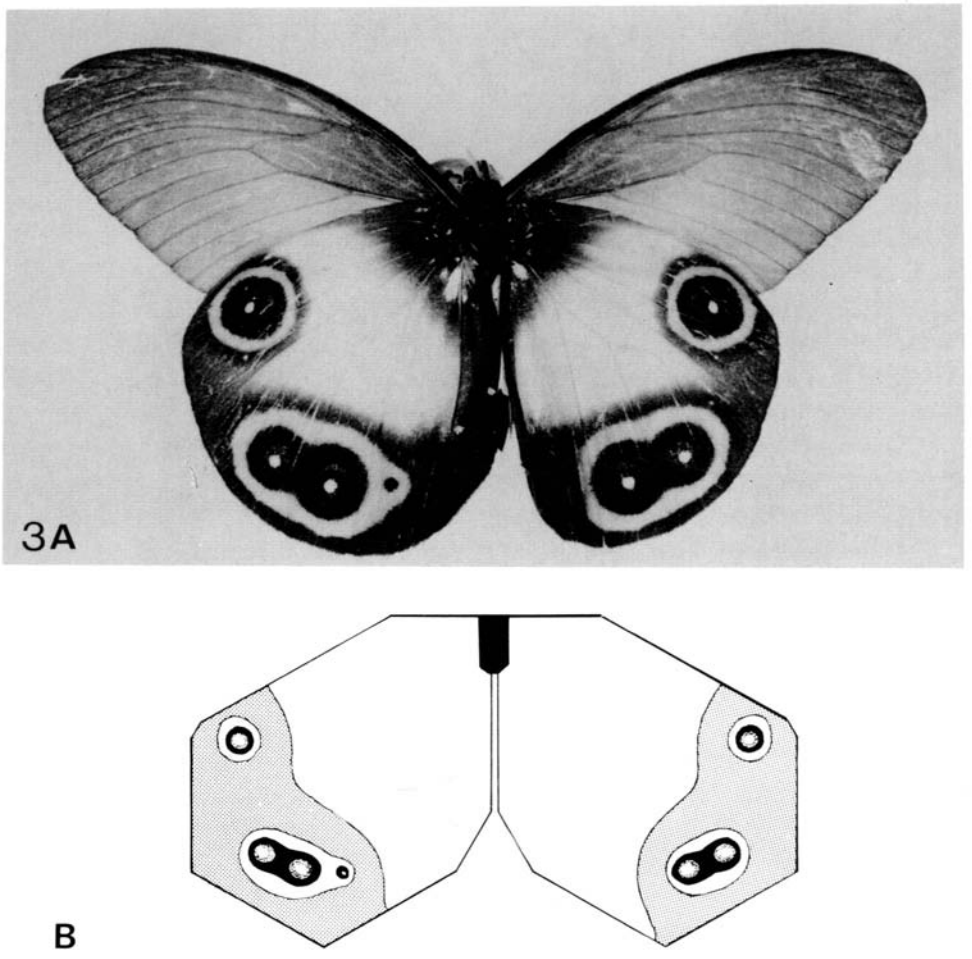

Fig. 3. The underside of the butterfly *Tenaris domitilla* (A) and computer simulations of its hind wings (B). For the latter, a decay constant of 0·0001 (half life: ~2h) was used; the morphogen values of foci were maintained at 10·0 and 8·5 respectively for the main foci and the minor focus on the left wing; there are no sinks. Threshold values are 10·0, 7·5, 6, 4·5 and 1·9; the simulation took ~9h to reach equilibrium. Note that the simulated eyespots are smaller than they should be and that the outer ring of the eyespots bows out towards the wing margins.

over the whole wing surface; third, there are asymmetries in the pattern in that there is an extra band on the distal edge and the banding pattern is replaced by a red area along the anterior margin. In addition, there is a small discontinuity

in the major bands near the meeting of the posterior and distal edges (Fig. 4).

Simulating this pattern required a slightly more sophisticated set of boundary conditions than those used for *Tenaris*. Other than minor changes to the shape of the wing and the foci, it was necessary to make the distal edge of the wing a morphogen sink. This was to ensure that morphogen values would be less here than at other edges and so enable an additional contour to be drawn for the distal band. A further difference was that the bands extend over larger distances here than do the spots on *Tenaris*, it was therefore necessary to allow either a much longer morphogen half life or much lower threshold values for the same diffusion constant. It turned out the best fit was obtained with a slightly shorter half life, but with much lower threshold values. Using the standard diffusion constant, the decay constant required was 0·0003 sec^{-1} (half life: 0·6h), the pattern not being sensitive to changes of 20% in this value. The pattern stabilised within about 2·5h of embryonic time.

The optimal simulation (Fig. 4) matches the major features of the pattern: the foci and the major bands are all present and in the appropriate places. The additional band on the distal edge is also clearly visible. The discrepancies between simulation and reality (Fig. 4) lie in the detailed morphology of the bands, the red area on the proximal anterior edge and in the inability of the

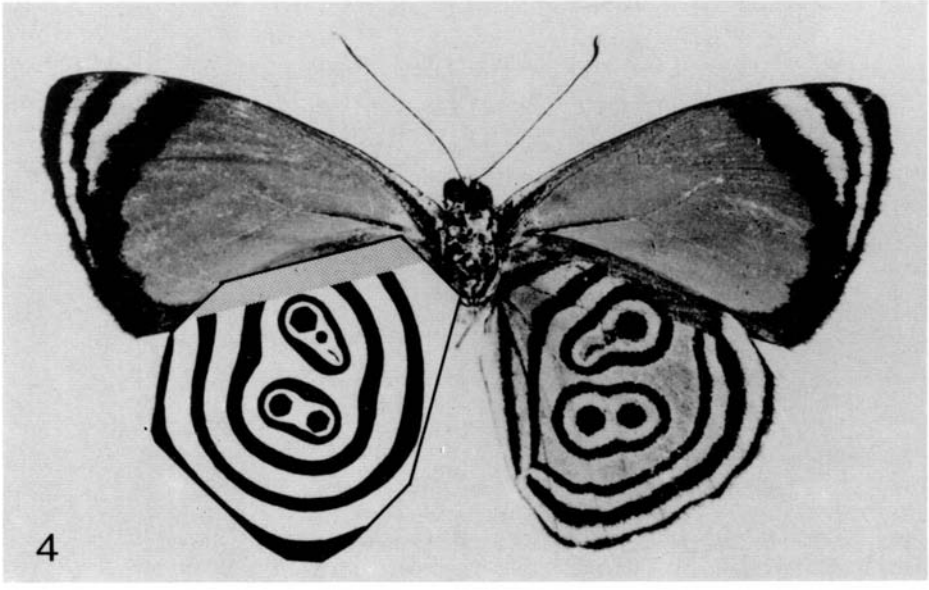

Fig. 4. The underside of the butterfly *Diaethria marchalii* with a computer simulation of a hind wing. For the latter, a decay constant of 0·0003 (half life: ~0·6h) was used; the morphogen values of the foci were maintained at 10·0 and the distal margin was maintained as a sink (see Fig. 2B). Threshold values are 10·0, 7, 4·9, 2, 1, 0·32, 0·18 and 0·04; the simulation took ~2·5h to reach equilibrium. Note that the minor dislocation in the posterior part of the real wing is not matched in the simulation and the anterior proximal band (stippled) is arbitrarily determined.

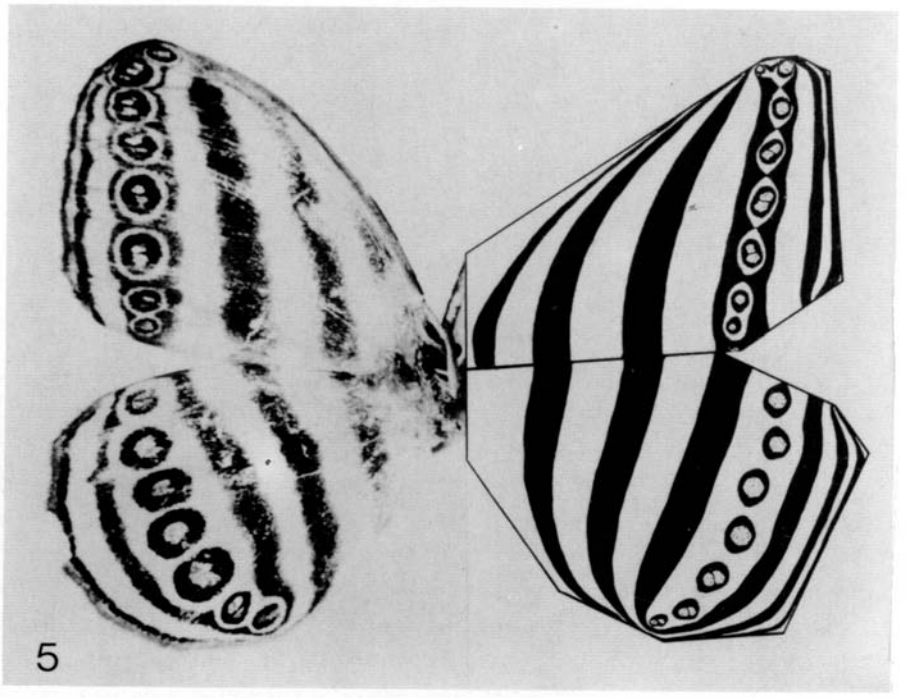

Fig. 5. The underside of the butterfly *Ragadia minoa* with computer simulations replacing one side. For these simulations, a decay constant of 0·00003 (half life: ~6h) was used; the morphogen values of the foci were maintained at 10·0 and the distal margins together with the anterior margin of the fore and the posterior margin of the hind wing were maintained as sinks (see Fig. 2C). Threshold values on the fore wing are 10·0, 8·3, 6·8, 3·1, 1·7, 0·75, 0·43, 0·25 and 0·2. Those on the hind wing are 10·0, 8·85, 6, 3·5, 1·8, 1, 0·42 and 0·21. The simulation took ~12h to reach equilibrium. Note that the eyespots are smaller in the simulations than in the original pattern and that the outer rings of simulation eyespots are slightly elliptical in the anterioposterior direction whereas those on the wing are, if anything, elliptical along the proximodistal axis.

simple model to explain the minor discontinuity in the bands where the posterior and distal edges meet.

Butterfly 3 Ragadia minoa *(fore and hind wings, lower surface)*

The pattern on the wings of this butterfly (Fig. 5) is a mixture of eyespots and bands. The bands are dark brown, the field pale brown and the foci silver. This butterfly is particularly interesting for several features. First, the fore and hind wings differ in that the foci of the fore wing are embedded in a dark band whereas those of the hind wing are not; second, there is a small proximal band on the fore wing which is not matched at the distal edge and which is also absent from the hind wing and, third, bands on one side run into the edge and

on the other curve around between the edge and the foci. It is also noteworthy that the distances from the foci to the most distant bands is very much greater than those in the previous two butterflies.

We consider first the hind wing with its balanced bands. For this simulation, the boundary conditions were similar to those of *Diaethria*, but with the difference that both the posterior and the distal margins were set up as morphogen sinks. This was to ensure that the bands curved around these edges rather than terminating at them. For this simulation, a long morphogen half life was required as the morphogen has to travel further here than in the other wings considered. It turned out that the value of the decay constant that gave the pattern closest to reality was 0·00003 (half life ~6·4h). With this value, the simulation stabilized in about 12·5h of real time (see legend to Fig. 5 for numerical details).

The resulting pattern displays the major features of the hind wing. There are seven foci, each surrounded by a dark ring, and three dark bands extending from the proximal posterior edge around the anterior edge and foci to extend back to the posterior edge. It is noteworthy that the thinning of the distal part of the bands is seen in both the original wing and the simulation; in the latter, it derives from contours having to be squeezed into the relatively narrow space between the foci and the distal sink. The morphogen concentration gradient between the foci and the distal edge (a sink) is roughly linear while that proximal to the foci approximates to an exponential decline.

There is one minor and one major discrepancy between simulation and reality. The lesser problem is that the shape of the distal part of the bands does not match exactly that on the original wing; the major discrepancy is that the rings around the foci are too small and flatten along the A–P rather than the P–D axis. Attempts to alter the threshold values to make the rings larger resulted in them merging. The significance of this discrepancy will be considered in the discussion.

The simulation for the fore wing is similar to that of the hind. The differences lie in the foci (they are more numerous and smaller and are embedded in a dark band), in the sinks (now on the anterior and distal edges), in the choice of thresholds (there is an extra proximal band) and in the shape of the wing. With the same diffusion and decay constant used for the fore wing, the pattern on the hind wing also stabilizes in about 12·5 h.

The simulation shows the essential topology of the original wing. The foci are embedded in a dark band and the number of outer bands on either side of the line of foci in the computed pattern matches that on the original wing. This is, at first sight, surprising as there are three proximal bands, but only two distal ones. Detailed examination of the concentration contours shows that there are, in fact, three distal bands, but that the outer two are so close (<1 cell diameter) that they effectively merge. The discrepancies between the simulation and the original patterns are the same as those for the hind wing.

There is one further point of interest in all the simulations that cannot be seen by simple inspection of the patterns shown in the figures: the pattern was highly sensitive to the relative positions of the foci. A change, for example, of one cell in the positions of the foci in the simulation of *Ragadia* (equivalent to ~4 cells on the original wing) caused eyespots to distort or merge (Fig. 6).

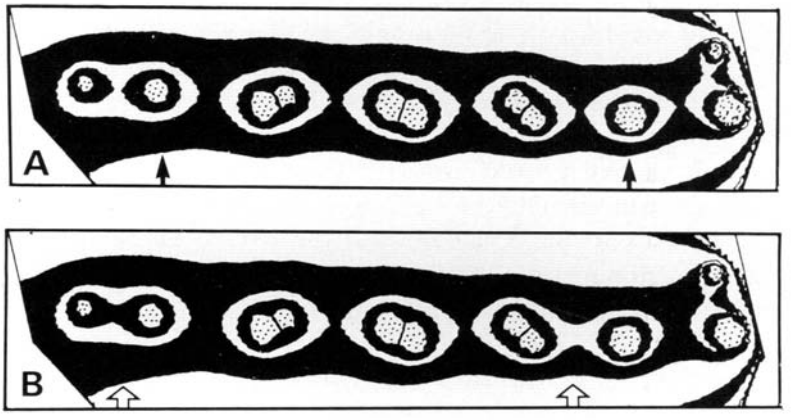

Fig. 6. A comparison of the simulation of line of eyespots of the fore wing of *Ragadia minoa* (A) with the simulation that results when two foci (A, solid arrows) are moved to the left by a single cell (B). This small movement causes the merging and the distortion of adjacent eyespots (hollow arrows).

DISCUSSION

The computer simulations show that it is possible to explain the formation of many of the pattern features of certain types of butterfly wing using a simple-diffusion, single-morphogen model. This model contains assumptions about the original pattern of sources and sinks on the wing, the physical mechanism itself and the mode of pattern interpretation. In the light of the discrepancies between the simulations and the original patterns, we examine here the extent to which these assumptions are valid and whether the model is relevant to other types of butterfly wing patterns. Finally, we consider how one might determine whether this or any other mechanism is actually used in the pupa. However, the first point that we raise is whether a diffusion-based mechanism is fast enough to account for the formation of a wing pattern *in vivo*.

The speed of pattern formation

The experimental evidence of Nijhout (1980a) suggests that the time between the start of focal activity and the final determination of the eyespot

pattern is about two to three days (the appearance of the pigment itself follows about three days later). The time taken for a pattern to form by diffusion over a domain varies as the square of its size and Crick (1970) has pointed out that it will take several hours for a linear gradient to form between a source and a sink ~70 cells apart. As most pattern formation events in embryos take place over domains of approximately this size (Wolpert, 1969), and a pupal wing is much larger than this, diffusion might be expected to be too slow a mechanism to generate the pattern here. Simulations show, however, that, using a reasonable diffusion constant, morphogen sources can generate a stable pattern over a wing blade in about 12h. This in turn means that a smaller diffusion constant and a concomitantly longer morphogen half life would be adequate to satisfy the temporal constraint. The reason for the unexpected speed of pattern formation in these simulations is that the morphogen is not required to form a linear gradient, merely to form a stable pattern that reaches the wing margins. Indeed, the time for a stable morphogen pattern to arise over the wing is determined as much by the decay constant of the molecule as by the formation of a linear gradient between a source and a sink. Thus, the simple diffusion of a morphogen from foci is fast enough to account for pattern formation in the butterfly wing. Murray (1981), discussing a rather different model (see below), has also concluded that diffusion can generate wing patterns in the time available.

Adequacy of a diffusion-based mechanism

Even if a diffusion-based mechanism is fast enough to form wing patterns, its plausibility must be judged by the quality of the simulated patterns. These demonstrate that, assuming only a wing with its sources and sinks already located, the basic topology of the original patterns is reproduced. It is worth noting that this reproduction of the basic pattern features comes from the arrangement of sources and sinks and is largely independent of wing shape (and hence of our straight-line approximations). Thus, our initial assumption that we could model the pupal wing shape by that of the adult is unlikely to lead to any major irregularities, even though shape changes between pupal and adult wings have been documented (Nijhout, 1980a). Any non-uniform changes will deform the pattern and could alter, say, the shape and thickness of bands, but not their arrangement.

There are, however, several ways in which the simulated patterns consistently fail to match the real ones. First, there is a tendency for contours in the simulations to be drawn out towards non-sink edges (see Fig. 3). This tendency derives from the nature of diffusion at such an edge: here, a hexagonally packed cell has only four nearest neighbours and no more-peripheral cells to act as a drain for morphogen which is diffusing in from central cells. Hence, there is a slight build up of morphogen at the edge which, in turn, leads to a distortion in the morphogen contours. In the real pupal

wing, in contrast, there can be continuity between the two surfaces of the wing and hence no distortion. In short, the edge distortion is likely to be relatively unimportant.

A second and more significant discrepancy between observation and simulation is the excessive pulling together of contours near adjacent foci (see Figs. 3, 5 & 6). Thus the eyespots of *Ragadia* and *Tenaris* in the best simulations are smaller than they ought to be and are elliptical rather than circular because the contours merge between adjacent foci far more readily in the simulations than in the actual patterns. The choice of simple, membrane-limited diffusion might be thought responsible for this discrepancy and, indeed, other forms of linear or non-linear diffusion mechanisms could have been chosen (see Bard, 1982). However, the essential effect of changing the details of morphogen movement through the tissue would have been to change the time taken for the pattern to form rather than to change the pattern itself. We have also assumed that the wing is isotropic so that diffusion occurs uniformly throughout the epidermal cell sheet and this may not be the case. During the formation of the eyespot pattern in *Precis coenia* (Fig. 1D), for example, the epidermal cell number approximately doubles and the uniform cell sheet develops parallel anterior–posterior rows of large scales, separated by small epidermal cells (Nijhout, 1980b); this could affect diffusion between cells. Even if diffusion did become anisotropic here however, there is no obvious way in which this change could have a significant effect on the final pattern.

It might be expected that the distortion of eyespots would be eliminated if morphogen could not move across the veins at *cell* borders. Neighbouring foci would then be unable to interact and adjacent eyespots should remain circular. However, close to such impermeable edges, the morphogen build up discussed earlier will still distort circular contours into ellipses (Fig. 7). Moreover, even though foci and their immediate rings may not merge, outer bands run continuously across *cell* borders and it is thus unrealistic to suppose that there can be no communication between adjacent *cells*. Indeed, a lack of any communication is likely to generate *dislocations* (see below).

Alternatively, it might seem possible that the decay kinetics of the morphogen is partially responsible for the discrepancies. Its mathematical formulation does not distinguish between an intrinsic instability and a cell-bound enzymic degradation. For the latter, it would in principle be possible to make the degradation rate concentration dependent in various ways. It does not, however, seem likely that such an alteration would have any major effect in the vicinity of the foci as diffusion would still smooth out any major changes so that, at the end of the simulation, the contours would barely alter. There is thus no simple way of altering the decay of the morphogen to improve the shape of the contours.

This analysis therefore suggests by default that the source of the discrepancies lies in the assumption that pattern formation *in vivo* can be modelled by

the direct interpretation of the concentration of a *single* diffusing morphogen. A satisfactory model must generate not only accurate patterns but also ones that are stable to small perturbations, a test failed by the model examined here. The most likely theoretical reason for this failure is that there is not enough information passed to each cell by it reading a single concentration value to generate a pattern that is not only complex but is also robust. In spite of these criticisms, it is clear that the single-morphogen mechanism is capable of generating the major features of a wide range of butterfly wing patterns. The model may therefore be considered as a degenerate formulation of the true mechanism.

There are various alternative mechanisms that could form the pattern on the pupal wing. While our model gives a pattern based on equilibrium concentrations of morphogen, Murray (1981) has examined a model where the predicted pattern depends on the transient activity of local sources producing a given amount of unstable diffusible morphogen. Wing cells then interpret the highest morphogen level that they have experienced while the morphogen has been spreading. With this mechanism, Murray has explored the effects of size and shape on the wing pattern of *Ephestia kühniella*. Assuming with Kühn & von Engelhardt (1933) that morphogen sources are located at the wing margins, he was able to simulate the abnormal patterns that followed early microcautery on the pupal wing. It is not clear what range or stability of patterns this mechanism can generate, but it is likely to be very similar to that of the equilibrium-concentration model.

More complicated models may readily be envisaged. Foci may act as a source of two or more diffusible molecules which may interact (see Meinhardt, 1982) or which may give different types of information to cells. Alternatively, pattern formation could be based on propagating waves (*e.g.* Turing, 1952; Goodwin & Cohen, 1969) spreading out from foci, or on a non-molecular mechanism such as one based on electrical fields. There could also be interactions between the overall mechanism and local properties in particular regions of the wing. Indeed, as many butterflies have specific pigment over veins (Fig. 1C), there may be specific cues in these regions. Thus, if thresholds are higher near veins than elsewhere, an elliptical contour could be interpreted as a circular eyespot. The implications of all these approaches remain to be elucidated.

Other butterfly patterns

The simple diffusion model which we have explored is capable of generating the major features of a range of wing patterns. This range includes eyespots around single foci, patterns of rings around multiple foci and a system of anterior-posterior crossbands around a line of foci. It further includes a symmetry system of bands with no eyespots (Fig. 1E), since there is no formal reason why threshold values should mark out the maximum morphogen

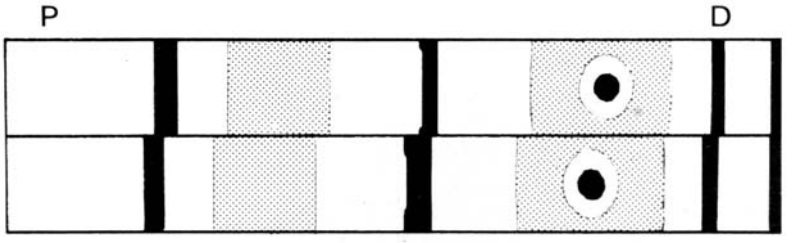

Fig. 7. If morphogen cannot diffuse across *cell* borders, *dislocations* may be generated (see Fig. 1F). Here, the two extended rectangles represent two such isolated *cells*, each of which has a major focus (morphogen concentration 10·0) near the distal (D) end and a lesser focus (morphogen concentration 5·0) near the proximal (P) end. Corresponding foci are, however, in slightly different positions in the two *cells*. The resulting pattern shows dislocations. Note that the central dark band represents a valley in the stable morphogen distribution between the two sources; its width is highly sensitive to the positions of the foci and the choice of threshold values (8·0, 5·8, 3, 1·55, 1·25 and 0·2). We emphasize that this simulation merely illustrates how dislocations may form; we do not suggest that the simple-diffusion model can account for all the details of the pattern of *Sallya pechueli* (Fig. 1F).

concentration. If there is more than one line of foci, more than one symmetry system of bands will form and, indeed, may interact (Fig. 7).

The model can also account for at least some types of dislocation (Fig. 1F) if the morphogen is unable to diffuse across the veins between neighbouring *cells*. If these *cells* are of different width or have the foci in slightly different positions, corresponding bands will be misaligned (Fig. 7). Wing patterns consisting of stripes overlying veins (Fig. 1C) could also be formed were veins, rather than local foci, the sources of morphogen (Murray, 1981).

Diffusion of morphogen cannot account for two of the pattern classes described by Süffert (1929): larger and often irregular patches of colour (Fig. 1A) and repeating ripples of colour (Fig. 1B). Furthermore, even some of the patterns broadly classed as *eyespots* or *bands* would be difficult, or even impossible, to generate with this model. In some species, there is a fine tracery of lines rather than a series of parallel, thick bands running across the wing surface. Such a thin line, often only a single scale wide, would require an unrealistically precise mechanism to interpret morphogen levels.

The simple model is also unable to explain such common, asymmetric repeating units as chevrons. It is, in principle, possible to generate such a chevron (or any other shape) from a regular concentration map formed by diffusion from a focus, provided that the interpretation ability of a scale-forming cell depends on its position in the wing *cell* (Nijhout, 1978). Setting up such position-dependent abilities does, however, require an extremely, even

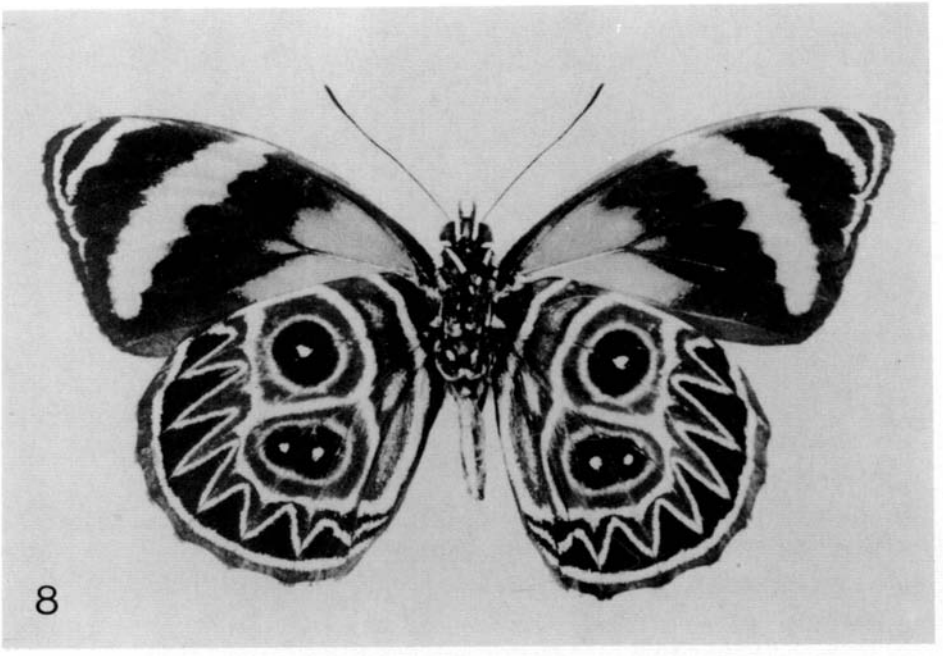

Fig. 8. The underside of the butterfly, *Catagramma sorama*, a member of the same tribe as *Diaethria marchalii*. Many of the features of the hind-wing pattern lend themselves to an explanation of the kind discussed in this paper (compare with Fig. 4). The linked chevrons do not.

unrealistically, complex mechanism: it has as initial conditions not just sources and sinks but a wing where every position within a *cell* has unique properties. Such a mechanism seems particularly implausible when the asymmetric elements are set in a pattern whose other elements seem not to require these complications (*e.g.* Fig. 8).

However, the inability of the simple-diffusion model to generate all classes of pattern does not imply that mechanisms of this type can play no role in pattern formation. In many butterflies, two classes of pattern are superimposed: in *Physcaeneura pione*, for example (Fig. 1B), there are both *ripples* and *eyespots* and these could be formed by separate mechanisms. In many cases, closely related butterflies have patterns which show strong similarities but differ in one feature. Thus, *Diaethria* (fig. 4) and *Catagramma* (Fig. 8) belong to the same tribe; but the pattern of the latter contains one feature, chevrons, which cannot be derived from a simple-diffusion mechanism. These comparisons provide further support for the view that a simple-diffusion model contains some but not all of the elements of the mechanism operating *in vivo*.

Testing a model

There have been very few experimental tests of the various components of the single-morphogen or any other model. The data of Nijhout (1980a) and

others have shown that the centres of eyespots are the source of some pattern-forming influence, but there is, as yet, no experimental evidence to indicate that there are sinks at some regions of wing margins. Their presence could be tested by excising parts of the pupal wing margin and examining the resulting pattern. The mode of morphogen spreading could be tested by inserting barriers or cutting out small areas of wing and comparing the predicted pattern obtained through simulation with that actually found. Ideally such experiments should be performed on a species with an uncomplicated eyespot or banding pattern for which the parameters of the model can be precisely formulated.

One prediction can be made to test the hypothesis that morphogen moves across the wing by simple diffusion. The kinetics of this mechanism show that the concentration drops off roughly exponentially with distance from the source, provided that there is no nearby sink, and that the gradient eventually becomes very shallow. This implies that the spatial accuracy of the threshold-reading mechanism will become increasingly less good and hence that the edges of bands near foci should be sharper than those a long way away. Examination of a single specimen of *Ragadia* suggests that this prediction is borne out.

The eventual validation of the class of pattern-formation mechanisms investigated here will depend on the identification of the 'morphogen' and its concentration dependent effect. The problems involved in so doing are formidable for this as well as for all other such developmental systems that have been studied. Nevertheless, there are certain advantages in conducting such experiments here. On the pupal butterfly wing, pattern formation occurs from identifiable foci, over a relatively long period of time, across a large two-dimensional sheet of accessible cells to give rise to a dramatic spatial pattern comprising a few, readily distinguishable states of cellular differentiation. Perhaps, as Nijhout (1980b) has pointed out, the wing is a promising but neglected system for investigating the molecular basis of pattern formation.

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