

The formation of the feather pattern in chick skin after a proportion of cells have been killed by X-irradiation

HAN-SUNG JUNG^{1*}, LEWIS WOLPERT¹ and DUNCAN DAVIDSON²

¹Department of Anatomy and Developmental Biology, University College London, London, United Kingdom and ²MRC Human Genetic Unit, Western General Hospital, Edinburgh, United Kingdom

ABSTRACT The formation of periodic patterns is of fundamental importance in embryonic development. One of the simplest and most frequently observed patterns is the maintenance of a minimum distance between neighbouring elements, for example between teeth, hair, feathers, digits etc. Theoretical models describing these phenomena have been proposed for feather patterning. However, there has been no detailed quantitative analysis of the relationship between cell population density and feather spacing. To define the relation between these quantities and specifically to test the prediction of a mathematical model, we have examined the formation of the feather pattern after varying proportions of the dermal cells have been killed by X-irradiation. It is known that the development of a feather primordium is normally associated with an increase in cell population density in the dermis. Using X-ray irradiation of the skin *in vivo* and *in vitro*, we show that the relation between cell population density and spacing of feather primordia indicates the importance of a threshold number of cells for feather patterning. Moreover, there is a *prima facie* case for supposing that X-rays act on feather spacing system, reducing the ability of dermal cells to prevent spreading of the pattern. Thus, X-irradiation may have a secondary effect on the spacing of primordia rather than, or as well as, affecting the mechanisms that determine their primary positions.

KEY WORDS: *feather buds, spacing pattern, pattern formation, X-irradiation*

Introduction

Spacing patterns are of fundamental importance in systems as varied as teeth, fingers and insect eyes where repeated structures develop at regular intervals. The chick skin develops as a regular pattern of feather buds and this provides a model system to study how spacing patterns develop. The mechanism by which the feather buds are spaced is not known, but analogy with other systems like the ommatidia of insect eye (Baker *et al.*, 1990) would suggest that lateral inhibition plays a key role during feather patterning. That is, a local signal from a developing bud inhibits formation of another bud in the immediate neighbourhood. Recently, the Notch/Delta receptor ligand pair has been implicated in the formation of the feather array (Crowe *et al.*, 1998; Viallet *et al.*, 1998).

A model for feather bud spacing proposed by Oster *et al.* (1983), is based on the idea that periodic patterns will form in response to mechanical instabilities created when several interacting forces in the tissue change during development (Harris *et al.*, 1984). These

forces are generated by the contractile properties of the cells and the physico-chemical properties of the matrix that they secrete. This model has been formulated mathematically, using variables that can be measured. Furthermore, the model predicts that cell population density influences the pattern formed.

The model predicts that feather spacing is a function of mesenchymal cell population density according to the equation:

$$\text{Spacing} = 2\pi [D_2 / rN]^{1/4} \quad [1]$$

where D_2 is a cell diffusion constant, and r and N are functions of the mitotic rate and cell population density respectively (Oster *et al.*, 1983). It is known that the development of a feather primordium is normally associated with an increase in cell population density in the dermis (Wessells, 1965). Indeed, artificially increasing the local cell density can lead to feather formation in normally non-feathered skin (Sengel, 1976). However, there has been no detailed quantitative analysis of the relationship between cell population density and feather spacing. To define the relation between

*Present and corresponding address for reprints: Developmental Biology Programme, Institute of Biotechnology, Viikki Biocentre 1, P.O. Box 56, FIN-00014, University of Helsinki, Finland. FAX: 358-9-708-59560. e-mail: hsjung@operoni.helsinki.fi

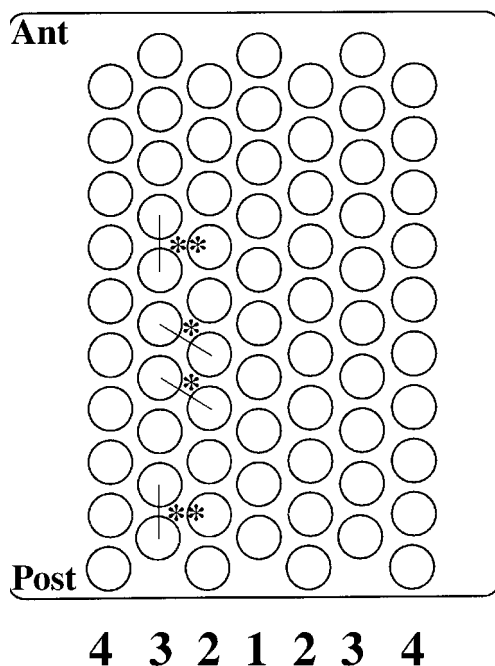


Fig. 1. Diagram of skin explanted from the dorsal pteryla to show numbering of feather rows. The first row (1) lies along the dorsal midline. Waves of feather formation sweep across the skin on either side of this to form subsequent rows. Primordium formation stops when these waves reach the edges of the dorsal pteryla. (*) Measurement of between feather rows and (***) measurement of within feather rows. Ant, Anterior; Post, Posterior.

these quantities and specifically to test the prediction of the model [1], we have examined the formation of the feather pattern after varying proportions of the dermal cells have been killed by X-irradiation. This method has previously been used to investigate the mechanism of pattern formation in the chick limb and it is clear from the results of Goff (1962) and Summerbell (1978) that the severity of skeletal anomalies seen in limbs which had been treated with doses of X-irradiation up to 1000 rads depends on the stage at which the embryo was irradiated (Wolpert *et al.*, 1979). Here we show, based on detailed analysis of the relation between cell population density and spacing of feather primordia, the importance of a threshold number of cells for feather patterning.

Results

Development of the normal feather pattern

The feather pattern develops in dorsal skin over a period of 2 days. Beginning at the midline, successive anterior-posterior rows of feather bud form to fill a well-defined region of the skin, the dorsal pteryla (Sengel, 1976). The first sign of feather formation is a local thickening of ectoderm, the epidermal placode, which is followed by a subsequent condensation of the underlying mesenchyme to form the feather bud (Wessells, 1965). An essentially normal, though often not perfectly regular, pattern develops in cultured skin over the same period (Davidson, 1983a,b). One notable anomaly of development in culture is the failure of the skin to expand: since feather positions form with approximately the same spacing as *in*

ovo, fewer than normal rows of feather primordia fill the pteryla (Davidson, 1983a).

Previous work has shown that, in skin with only one row of primordia, feather positions are established only in the next row: beyond this, the positions are not irreversibly determined under experimental conditions and may be as yet unspecified in normal development (Davidson, 1983a,b). In the present experiments, when skin was irradiated at the stage when one row, at most, was visible, we may assume that the positions of primordia in row 3 were not irreversibly determined (See Fig. 1 for an explanation of the numbering and the measurements of feather rows).

Feather pattern formation in X-irradiated skin

The number of rows of primordia formed (Table 1), the regularity of the pattern and the extent of morphogenesis in primordia that did form, was progressively reduced with increasing X-ray doses both *in vivo* and *in vitro*. After 550 rads, the patterns formed were nearly normal (Fig. 2B). After 625 rads discrete, well-spaced primordia formed, but in almost all specimens these were measurably more widely spaced than in the controls (Fig. 2C). Nevertheless, the pattern did fill the prospective pteryla (to within the width of one row of primordia) as judged by morphological identification of the edge of the prospective pteryla at the time of the experiments and by the distance from the midline to the last formed row as compared with controls. After 750 rads, visible development of feather primordia was only resumed after a lag of about 12 h following irradiation. Primordia subsequently formed were discrete, but more widely spaced than in the controls and the pattern was incomplete (Fig. 2D). About one fifth of cases formed primordia up to the edge of the prospective pteryla: the remainder formed only 2 rows. 1250 rads or higher rads prevented the formation of new primordia (not shown).

To quantify the spacing of primordia as a function of X-ray dose, each piece of skin was photographed one day and two days after irradiation and the distances between primordium centres measured on photographs, magnified x30. The results are shown in Table 2. The positions of primordia are determined one row in advance of the most recently-formed, visible row (Davidson, 1983a). Indeed, the midline row and row 2 of feather buds appear to develop with regular spacing in most cases. Therefore, we began by assaying the effects of X-irradiation on the spacing between rows 2 and 3 and between primordia within row 3. The mean distance between successive rows increases as a function of X-ray dose (Table 2A). We also, compiled an alternative data set containing the distances between all primordia in the explant that could be

TABLE 1
TOTAL NUMBER OF FEATHER BUDS ROWS FORMED
AFTER X-IRRADIATION

Dose (rads)	Number of embryos		by day 1 *		by day 2 *	
	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
control	18	10	3	3	5	4
550	12	n.a.	2	n.a.	5	n.a.
625	11	4	2	2	5	4
750	24	17	1	1	3	3
1250	7	4	0	0	0	0

*Rows of primordia observed under the dissecting microscope
n.a.: not available

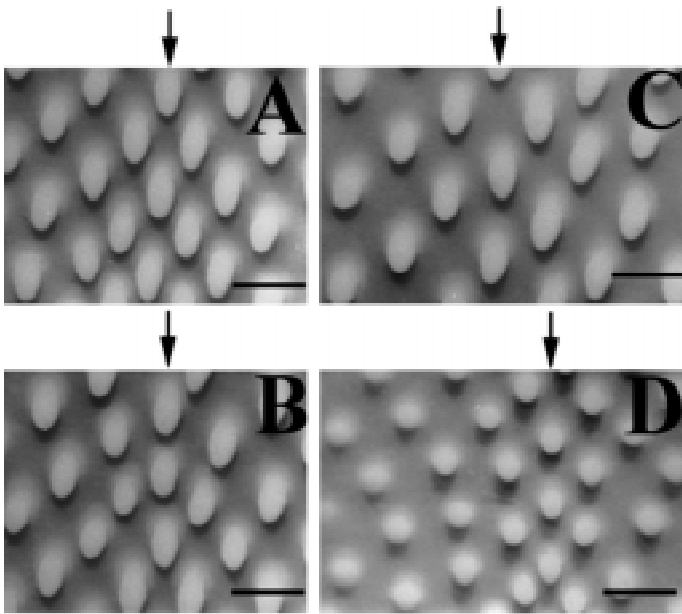


Fig. 2. Top view of explant skins. Individual skins are photographed 48 h after a single exposure to different levels of rads. (A) Control; (B) 550 rads; (C) 625 rads and (D) 750 rads. The regularity of the pattern and the extent of morphogenesis in primordia that did form, was progressively reduced with increasing X-ray doses. Arrow indicates row 1 in the midline. Bar in A and B, 300 μm ; in C and D, 400 μm .

reasonably judged to represent adjacent units in a pattern. This included the distances between primordia in the row 1: in at least some, and probably in the majority of the cases, the positions of primordia in this row would be determined at the time of irradiation. Nevertheless, the results clearly showed an increase in spacing between primordia in row 3 and between rows 2 and 3 approximately 48 h after exposure to 625 or 750 rads. Essentially the same effects of irradiation on spacing were found over the entire pattern (Table 2B). This observation suggests that the effects of irradiation are immediate and long lasting and, in addition, suggests that irradiation affects the spacing of already determined primordia.

Embryos were treated with the same range of X-ray doses *in vivo* and incubated for a further 48 h (Fig. 3). Embryos were transversely sectioned and the relative distance of primordia between the midline and lateral regions measured in the same way as for cultured skin. At the dose of 550 rads, the primordia in row 3 formed and the primordia were more widely spaced than in controls. After 625 rads, the primordia formed were discrete and showed retarded growth as compared to those formed after 550 rads. After 750 rads, although the primordia formed in the midline, the pattern was incomplete. It is interesting to note that although there were no detectable primordia formed after 1250 rads, epidermal placodes were found both in the midline and laterally.

Histology of X-irradiated skin

The histological structure of the dermis, particularly the deep layers, was more sensitive to disruption following irradiation than either the epidermis or the sub-dermal fibroblastic tissue. The disruption of dermal structure showed a steep dose-dependence in parallel with the effects of irradiation on pattern formation *in vitro* (Figs. 4 and 5). Transverse sections through skin fixed one day

after exposure to 550 rads showed only mild effects: the majority of dermal cells were intact and showed strong patterns of orientation similar to those seen in controls. Skin treated with 625 rads showed more marked effects on cell morphology with substantial numbers of pycnotic nuclei and an increase in the number of phagocytic cells. In skin fixed one day after exposure to 750 rads, many dermal cells bore gross signs of damage and those near the base of the dermis lacked the spindle-shaped morphology characteristic of the equivalent cells in controls. One day after exposure to 1250 rads, the dermis showed extensive damage, with very few cells of normal appearance.

The practical difficulties in estimating the number of cells surviving treatment should be noted. The major difficulty is in recognizing which cells are dead and which are alive. The data shown here are derived from counts of nuclear profiles with clearly visible nuclear boundaries and at least one nucleolus. Many of the nuclei counted in irradiated specimens had an abnormal reticular internal structure and an abnormally high proportion of nuclei were much more lightly stained than those in controls. It seems likely, therefore, that a proportion of cells counted in treated specimens, perhaps ten percent, were dead or dying. These counts were used to estimate cell density taking into account measured section thickness, measurements of cell length in the orthogonal plane, and applying the formula of Abercrombie (1946). Table 3 shows the proportion of dermal cells surviving 12 h, 24 h, and 48 h after a single exposure to 625 rads. (The data shown in Table 3 and Figure 6 refer to the dermis lateral to the most recently formed dermal condensation, where the next row in the feather pattern was about to form). Cell

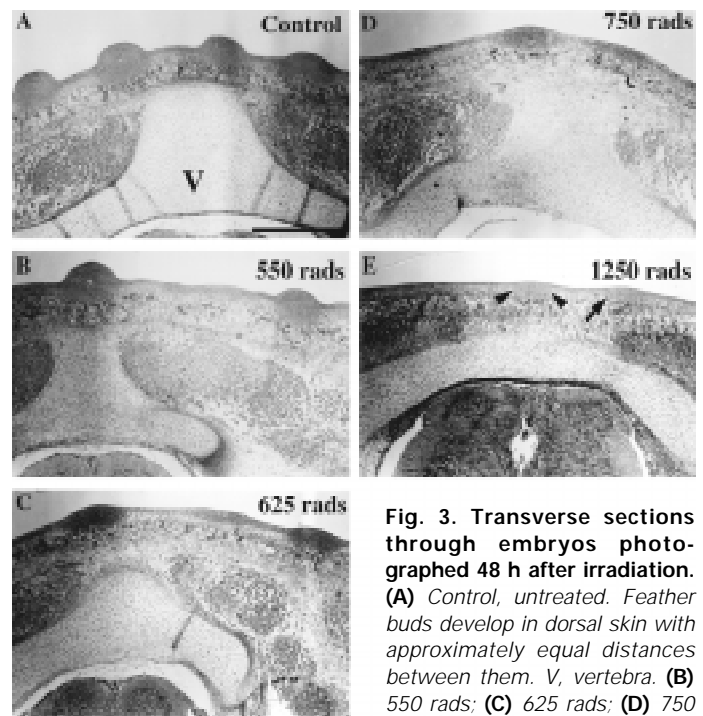


Fig. 3. Transverse sections through embryos photographed 48 h after irradiation. (A) Control, untreated. Feather buds develop in dorsal skin with approximately equal distances between them. V, vertebra. (B) 550 rads; (C) 625 rads; (D) 750 rads and (E) 1250 rads. Bar, 400 μm . The disruption effects of irradiation are evident and are proportional to dose. After 750 rads or higher, feather buds develop in poor condition. It is also interesting to note that although there is no feather bud in 1250 rads, epidermal placodes are formed in midline (arrowheads) and lateral row (arrow).

survival as a function of X-ray dose is also shown in Table 3 and in Figure 6. These data show the estimated number of cells surviving 24 h after a single exposure to X-rays when the formation of the feather pattern is about half-way to completion. The results showed that although a 5% higher cell population density was found after irradiation *in vivo* than *in vitro*, there is a striking decrease in the number of cells surviving irradiation in both systems.

In general, for the purpose of relating cell population densities after different treatments, we have compared skin fixed after the same time in culture (Fig. 6). However, since feather development in irradiated skin seems to lag behind controls, we have used the figures for 12 h culture in controls compared with 24 h culture in irradiated skins when attempting to relate cell population density to feather spacing, since this seems to reflect best the densities at the time when the equivalent rows of primordia were forming (Table 5).

Does the cell population density partly recover as a result of proliferation before the next row is formed? To answer this question, we have cultured irradiated and control, skin in the presence of colcemid for 7 h on the day following irradiation and counted the proportion of cells arrested in mitosis. The results are shown in Table 4. The rate of cell proliferation in treated skin is similar to that in controls. This result makes it unlikely that the cell population density would have recovered significantly relative to controls before new feather primordia formed.

Discussion

The results indicate that in irradiated skin, midline feather buds appear to develop with regular spacing in most cases. This is always consistent up to 750 rads since there are no primordia visible after 1250 rads. Second, the distance between all primordia showed a steep dose-dependence in parallel with the effects of irradiation. After 750 rads *in vivo* and *in vitro*, the distance between row 2 and row 3 increased by about 50% compared with controls. Third, there is about a 40% decrease in the cell population density

TABLE 2
DISTANCES BETWEEN FEATHER BUDS IN X-IRRADIATED SKIN

A. Pooled measurements within row 3 and between rows 2 and 3 at 2 days after treatment

Dose (rads)	Number of measurements		Distances between buds (μm) (S.D.)	
	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
control	202	156	282 (0.2)	280 (0.4)
550	53	n.a.	301 (0.4)	n.a.
625	50	42	315 (0.7)	340 (0.3)
750	48	37	380 (0.5)	400 (0.4)
1250	—	—	—	—

n.a. : not available

B. Pooled measurements within and between all rows at 2 days after treatment

Dose (rads)	Number of measurements		Distances between buds (μm) (S.D.)	
	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
control	224	426	280 (0.3)	270 (0.4)
550	108	n.a.	298 (0.5)	n.a.
625	99	113	310 (0.4)	340 (0.2)
750	128	203	382 (0.7)	400 (0.3)
1250	—	—	—	—

n.a. : not available

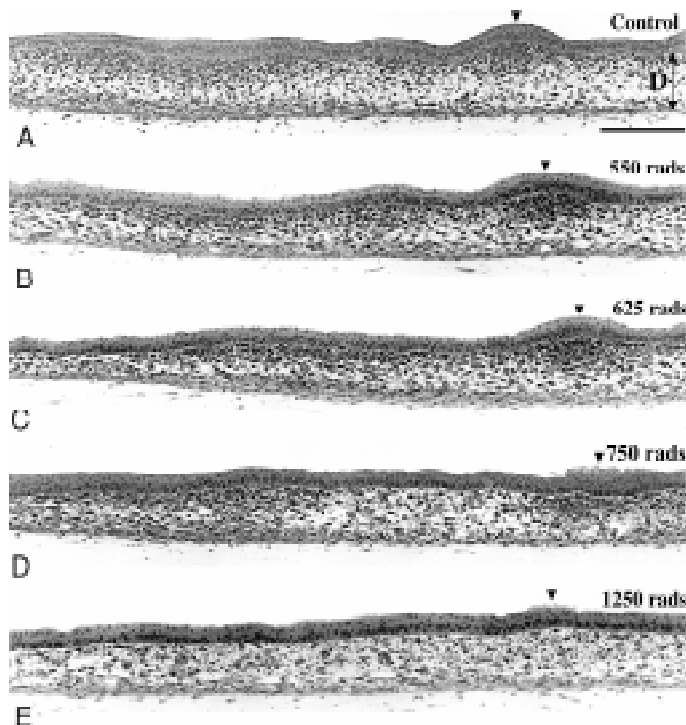


Fig. 4. Transverse sections through skin explants photographed 24 h after irradiation. (A) Control, untreated. (B) 550 rads; (C) 625 rads; (D) 750 rads. (E) 1250 rads. Bar, 200 μm . The disruptive effects of irradiation are evident and are proportional to dose. Although the sections will pass through successive rows differently in different specimens, the increase in spacing between primordia with increasing dose is evident. D, Dermis. Arrowheads indicate row 1 forming region.

after 750 rads and this is also a steep dose-dependence in parallel with the effects of irradiation.

Discrete feather primordia can form in skin where the dermal cell population density has been reduced by X-irradiation to approximately half that of controls (750 rads). This suggests that the mechanisms of primordium morphogenesis have some leeway in respect to the numbers, or population density, of cells in the dermis. It has been suggested (Sengel, 1976) that the formation of "dense dermis" during the maturation of the pteryla skin, a process that includes roughly a doubling of cell population density, is necessary for the development of feather primordia. If this is so, then by the time primordia form the critical requirement may be the prior deposition of extracellular matrix rather than the current number of cells.

The relation of feather spacing (S) to cell population density (D) is: $S = k\sqrt{D}$ (approximately). $k \approx 225$. S certainly falls between $k\sqrt{D}$ and $k^3\sqrt{D}$. (See Table 5 for the data on which this conclusion is based). Thus, the direction in which cell population density correlates with feather spacing is predicted by the Murray and Harris model (1983), but the quantitative relation predicted by this model is not born out by the present analysis. This conclusion does, however, rely on the supposition that cell population density in X-irradiated skin can be interpreted at face value. In particular, we have supposed the measured population density bears a constant relationship, in skin treated with different doses of radiation, to such factors as net cell traction that, in the model, are supposed to determine feather spacing. It is difficult to estimate the true cell

population density in irradiated skin. Moreover, it seems likely that both the direct effects of irradiation and the debris from dying cells will affect the ability of surviving cells to behave normally. The traction forces may therefore, be underestimated in skin treated with the highest dose of irradiation.

If feather spacing does depend on the numbers, or population density, of cells in the dermis then this clearly could reflect the involvement of several different cell properties. These include metabolism, for example, the synthesis or degradation of extracellular matrix, as well as morphogenetic, physical properties such as cell traction. It may be possible to distinguish broadly between these by specifically affecting the morphogenetic properties of cells by, for example, treating with antibodies to block cell adhesion molecules (Gallin *et al.*, 1986).

TABLE 4
DERMAL CELL PROLIFERATION IN IRRADIATED AND CONTROL SKIN

Experiment	% mitoses	
	Irradiated	Control
1.	15	15
2.	18	22

In each of two independent experiments, one piece of skin was irradiated with 625 rads and one other cultured without irradiation, as control. Colcemid was added to arrest mitoses 18 or 19 h after irradiation for 7 or 6.5 h respectively. Each value of % mitoses in the table was obtained by counting mitotic figures in just over 1000 cells.

One observation is difficult to reconcile with the notion that irradiation affects feather spacing by simply changing the cell density and thus modulating the normal spacing mechanism. This is the effect of irradiation on primordia in the midline. Previously, Davidson (1983a) investigated the time of determination of feather position by stretching the skin ahead of existing, visible rows of primordia and assaying the number of primordia formed in the stretched rows. If the same number formed as on the unstretched contralateral control tissue, the positions were considered to be determined; if a larger number formed in the stretched skin, this

TABLE 3
CELL POPULATION DENSITIES IN THE DERMIS OF X-IRRADIATED SKIN

Dose (rads)	Time (h)	Fix	Cells / 1000 μm^3 * (S.D.)	
			<i>in vivo</i>	<i>in vitro</i>
Control	12	GMA	2.7 (0.2)	2.4 (0.1)
Control	24	GMA	2.9 (0.6)	2.1 (0.3)
625	12	GMA	1.9 (0.3)	1.7 (0.3)
625	24	GMA	2.3 (0.5)	1.5 (0.3)
625	48	GMA	2.5 (0.3)	2.2 (0.2)
550	24	GMA	2.6 (0.7)	1.8 (0.7)
750	24	GMA	1.8 (0.7)	1.3 (0.9)
1250	24	GMA	1.0 (0.4)	0.7 (0.5)

* Cell population densities measured from cell counts per unit area, adjusted to cells per unit volume in sections 7.0 μm thick *in vivo* and 2.6 μm thick *in vitro* and corrected by the method of Abercrombie (1946) assuming that the cells are 5.7 μm long in the plane normal to the section. Fix, Fixation; GMA, Glutaraldehyde with cacodylate buffer and embedded in methacrylate.

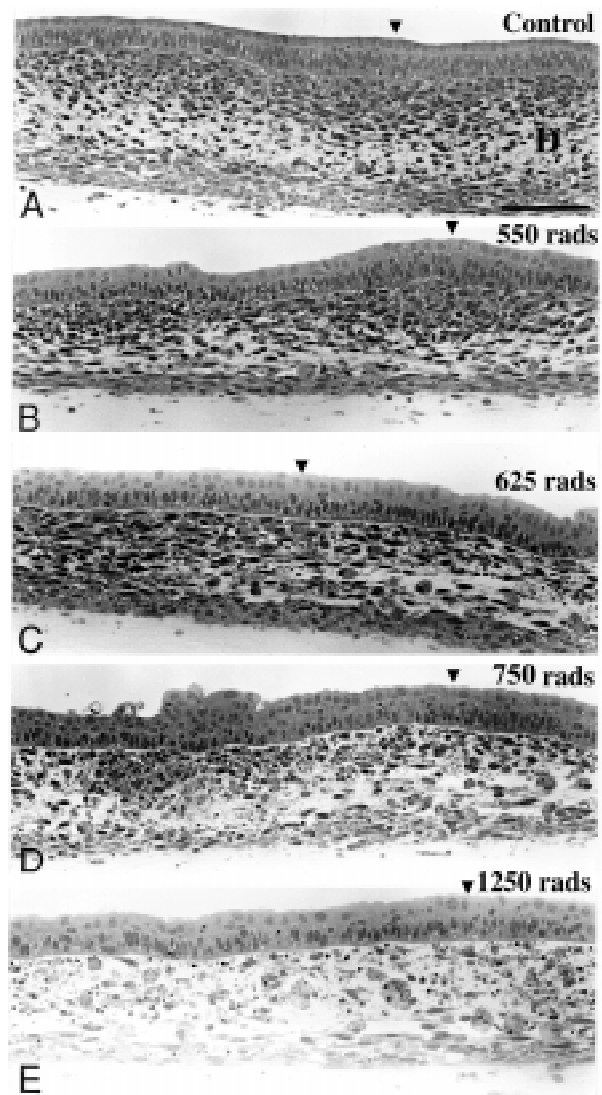


Fig. 5. Transverse sections through skin explants photographed 24 h after irradiation at higher magnification than in Figure 4. (A) Control, untreated. (B) 550 rads; (C) 625 rads; (D) 750 rads and (E) 1250 rads. Bar, 200 μm . The disruptive effects of irradiation are more clearly seen. Note, in particular that the range of dose given in these experiments gives the complete spectrum of effects from slight to complete disruption of the dermal structure. Note also the extent of damage in D (750 rads) which is around the maximum that can still support feather primordium formation. D, Dermis. Arrowheads indicate row 1 forming region.

was taken to indicate that the positions of primordia were as yet undetermined at the time of stretching. Although this experiment has not been carried out with the first row, data on primordia in row 2 suggest that they are determined (about 6 h) before they become visible. If we accept this value as indicating the normal time of irreversible determination of feather positions, then it is clear that in most cases, the positions of primordia in the midline were determined at the time of irradiation.

How then, has X-irradiation led to an increased spacing between primordia? One possibility is that even at this late stage in the development towards primordium formation, the normal spacing

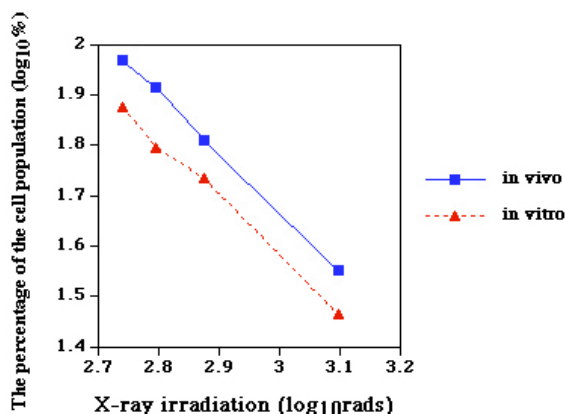


Fig. 6. Percentage of cells surviving after single treatment with X-rays as a function of dose. The cell population density (derived as described in Table 3) was calculated as a percentage of the cell population density in untreated skin. This value is plotted (on a log log scale) against the number of rads applied. Note that the relation is linear. Extrapolation suggests that treatment with less than about 500 rads *in vivo* and 450 rads *in vitro* would not kill any cells.

mechanism is vulnerable to changes in cell morphogenetic properties. Another possibility is that the spacing of primordia is only secondarily affected by X-irradiation. Two observations argue in favour of the second interpretation. First, during the course of a preliminary study, the positions of primordia were monitored over two days. We found that, although the mean distance between primordia remained constant, their relative positions did change. This indicates that primordia can move during development and suggests that there may be a mechanism that maintains their spacing over this time. Second, and more interestingly, detailed measurements show clearly that successive rows form at approximately equal distances from one another during normal development (D. Davidson, unpublished data). During the formation of the dorsal feather pattern *in vivo*, the skin expands laterally by a factor of two. In the final pattern, however, successive rows are roughly equally spaced and in fact, the more medial rows are closer together than the lateral rows. The most likely candidate for holding together the dermal elements of adjacent primordia are the arrays of cells and matrix that form between them. Indeed, these arrays are possibly a result of active and reactive forces in the dermis between condensations. Adhesions, dermal condensations and epidermal placodes (Davidson, 1984) may play a role in maintain-

TABLE 5
SUMMARY COMPARING CELL POPULATION DENSITY WITH
FEATHER SPACING

Dose (rads)	Cell population density (Cells / 1000 μm^3)		Spacing (μm)	
	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>
none	2.8 (24h)	2.4 (24h)	282	280
550	2.6 (24h)	1.8 (24h)	301	n.a.
625	2.3 (24h)	1.5 (24h)	315	340
750	1.8 (24h)	1.3 (24h)	380	400
1250	1.0 (24h)	0.7 (24h)	—	—

ing the integrity of the primordia in the face of the resultant shearing forces. There is at least a *prima facie* case for supposing that X-rays act on this system, thus reducing the ability of dermal cells to prevent spreading of the pattern. Thus, irradiation may have a secondary effect on primordium spacing rather than, or as well as, affecting the mechanisms that determine their primary positions.

Materials and Methods

Organ culture

Fertile white Leghorn chicken eggs from Polydon egg farm (Hertfordshire, UK) were incubated at $38 \pm 1^\circ\text{C}$ and then windowed and staged according to Hamburger and Hamilton (1951). The embryo was removed to a sterile dish where it was decapitated and membranes and debris were removed and the stage of the embryo assessed. The body was transferred to a saline-filled dissection dish. A rectangle was cut in the dorsal skin which encompassed the width of the spiral pterygia and length equivalent to about 11 feather buds in the initial row in the lumbo-sacral tracts. The rectangle of skin was peeled free using fine forceps and a tungsten needle, cutting attachments to underlying tissues with scissors to avoid strain in the skin. Under saline, the skin was laid flat on a piece of Isopore, $0.8 \mu\text{m}$ (Millipore). If necessary, the skin was gently adjusted to lie flat and undistorted using fine forceps. Allowing a minimum of distortion, the skin was moved to an organ culture dish with medium. F10 medium (Gibco) was used for culture of whole skin. This was supplemented with serum from new born calves (10%), glutamine (146.5 mg/ml), ascorbic acid (50 $\mu\text{g}/\text{ml}$) and antibiotics (20 $\mu\text{g}/\text{ml}$). Ascorbic acid was added to the medium to help preserve tissue integrity. 1.5 ml culture medium was added to each organ culture dish, and they were incubated at 38.5°C in a humidified atmosphere of 5% CO_2 in air.

X-ray irradiation

All the experiments were carried out at stage 29+ when three or four feather primordia were present in the midline. Explants were cultured for between one and three hours then X-irradiated with doses in the range of 250-2000 rads. The cultures were placed 43-50 cm from a Siemens X-Ray source operating at 250 Kv and fitted with a copper 0.2 mm filter. The applied dose was monitored with a probe lying beside the cultures. *In ovo* irradiation was carried out in the same way on windowed eggs on which were then incubated immediately after irradiation. These cultures and embryos were incubated for between 12 h and three days. The entire feather pattern takes about two days to develop in untreated, cultured skins (Davidson, 1983a). Development was assayed in culture under a dissecting microscope in 143 irradiated and 62 control skin explants and 54 embryos *in ovo*. The histological effects of irradiation were examined in skin fixed 12, 24 and 48h after treatment. For histology, skin was fixed overnight in 2.5% glutaraldehyde (buffer with cacodylate, 0.1M, 100 mOsm to pH 7.4) processed through graded alcohols and embedded in methacrylate (Davidson, 1983a). Sections 3 microns thick were stained with solochrome cyanin and examined under a Zeiss Universal microscope. Colcemid was used at concentration of $5.4 \times 10^{-6}\text{M}$ diluted in Dulbecco saline A.

Measurements of distances between feather buds

Measurements of distances between feather buds *in vivo* and *in vitro* were made from photographs taken under a dissecting microscope. The magnification of the photographs (x30) was calibrated against photographs of a graticule slide taken in the same experiment. Measurements of cell density were made in transverse histological sections under a microscope of approximately (x1125) magnification by counting nuclei and applying a correction factor to these nuclear counts using the methods of Abercrombie (1946).

Acknowledgements

We thank Prof. Cheryl Tickle and Dr. Jukka Jernvall for many helpful discussions and we are indebted to Dr. N.E. Gillies for his guidance on X ray irradiation.

References

- ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* 94: 239-247.
- BAKER, N.E., MLODZIK, M. and RUBIN, G.M. (1990). Spacing differentiation in the developing *Drosophila* eye: A fibrinogen-related lateral inhibitor encoded by *scabrous*. *Science* 250: 1370-1377.
- CROWE, D., HENRIQUE, D., ISH-HOROWICZ, D. and NISWANDER, L. (1998). A new role for Notch and delta in cell fate decisions: patterning the feather array. *Development* 125: 767-775.
- DAVIDSON, D. (1983a). The mechanism of feather pattern development in the chick. I. The time determination of feather position. *J. Embryol. Exp. Morphol.* 74: 245-259.
- DAVIDSON, D. (1983b). The mechanism of feather pattern development in the chick. II. Control of the sequence of pattern formation. *J. Embryol. Exp. Morphol.* 74: 261-273.
- DAVIDSON, D. (1984). Dermal cells form strong adhesions to the basement membrane during the development of feather primordia in chick skin. *J. Embryol. Exp. Morphol.* 84: 149-158.
- GALLIN, W.J., CHUONG, C.-M., FINKEL, L. and EDELMAN, G.M. (1986). Antibodies to liver cell adhesion molecule perturb inductive interactions and alter feather pattern and structure. *Proc. Natl. Acad. Sci. USA.* 83: 8235-8239.
- GOFF, R.A. (1962). The relation of developmental status of limb formation to X-radiation sensitivity in chick embryos. I. Gross study. *J. Exp. Zool.* 151: 177-200.
- HARRIS, A.K., STOPAK, D. and WARNER, P. (1984). Generation of spatially periodic patterns by a mechanical alternative to the Turing model. *J. Embryol. Exp. Morphol.* 80: 1-20.
- OSTER, G.F., MURRAY, J.D. and HARRIS, A.K. (1983). Mechanical aspects of mesenchymal morphogenesis. *J. Embryol. Exp. Morphol.* 78: 83-125.
- SENGEL, P. (1976). *Morphogenesis of Skin* (Eds. M. Abercrombie, D.R. Newth, and J.G. Torrey). Cambridge University Press, Cambridge.
- SUMMERBELL, D. (1978). Normal and experimental variations in proportions of skeleton of chick embryo wing. *Nature* 274: 472-473.
- VIALLET, J.P., PRIN, F., OLIVERA-MARTINEZ, I., HIRSINGER, E., POURQUIE, O. and DHOUAILLY, D. (1998). Chick Delta-1 gene expression and the formation of the feather primordia. *Mech. Dev.* 72: 159-168.
- WESSELLS, N.K. (1965). Morphology and proliferation during early feather development. *Dev. Biol.* 12: 131-153.
- WOLPERT, L., TICKLE, C., SAMPFORD, M. and LEWIS, J.H. (1979). The effect of cell killing by X-irradiation on pattern formation in the chick limb. *J. Embryol. Exp. Morphol.* 50: 175-198.

Received May 1998:

Accepted for publication: January 1999