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The effect of scale changes on repetitive patterns: influence of 6-aminonicotinamide on feather germ number

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Abstract. There are many examples of patterned developing systems which are size invariant: if the total size of the system is reduced, then the pattern responds by changing its scale in such a way that the number of pattern elements remains constant. This phenomenon is one of the bases which underlay the formulation of the concept of positional information, one of the great unifying ideas in developmental biology. However, there are less common examples of patterns which are size dependent. In these, alterations of overall size lead to a reduction in pattern elements. Such size-dependent patterns are therefore of theoretical interest. Here we describe how the number of feather germs along the wing bud of the developing chick embryo responds to shortening of the limb, and consider the implications of these observations.
Key words. Size-invariant; size-dependent; feather germ; chick; embryo; wing.

The influence of scale on systems in which patterns are developing is of profound theoretical interest[1]. Two classes of outcome may be obtained. In many instances, size reductions do not affect the overall pattern: pattern regulation is obtained either by morphallactic or epimorphic regulation. This phenomenon was one of the bases underlying the concept of positional information elucidated by Wolpert[2]. Less commonly, however, patterns show size dependence, in that a reduction in size brings about a concomitant decrease in the number of pattern elements, each of which is approximately of normal size. Examples of this are reviewed by Held[1]. A further example is the number of feather germs along the caudal margin of the wing bud of the developing chick of *Gallus gallus*, the domestic hen. This was first observed and briefly described by one of us in a previous paper[3]. In this paper, we examine the nature of the relationship between wing size and feather germ number in chick embryos, and discuss its implications for development and pattern formation.

Feather germ numbers vary with the size of the animal. In fully displayed wings in the Bell-Pettigrew Museum of the University of St. Andrews, these range from 17 (the sum of the primary and secondary remiges) for the greenfinch, to 24 for the sea eagle. The wings themselves are of course much more disproportionate in size than is represented by these numbers, and the greenfinch in consequence has much smaller feathers than the eagle. It is possible, though by no means certain, that variations in feather germ number may have adaptive value.

The nicotinamide analogues 6-aminonicotinamide (6-AN) and 3-acetylpyridine (3-AP) are teratogens for the developing chick embryo, with marked effects on limb morphology (see ref. 3 for details). Treatment with 6-AN reduces the wet weight of embryos in proportion to the dose and time of treatment, and markedly reduces limb length while limb width is much less affected. The underlying basis for these effects is not entirely clear. However, at the histological level, 6-AN causes some leakage of blood from blood vessels and death of the central cells in the elongating cartilage elements of the limb. This last observation may result from the competitive exclusion of nicotinamide in areas poorly supplied by metabolites. Perhaps in consequence of this damage to the rapidly elongating parts of the elements, the cartilaginous models of the long bones are considerably shortened, although their pattern is otherwise normal in form and orientation. Feather germs are normal in appearance, but their number is affected by the treatment, and this is possibly a consequence of the reduction in limb length.

Here we investigate the effect of 6-AN administration over the period in which the feather germs are being formed, in order to explore the relationship between limb length and feather germ number, and from the results draw conclusions as to the likely underlying mechanisms.

Methods

1 g 6-AN (Sigma) was dissolved in 20 µl of 0.5 molar HCl, and further diluted with distilled water to a stock concentration of 400 µg ml^{-1}. Fertile hens eggs (Muirfield Hatcheries, Kinross, Scotland) were incubated with the blunt end up for periods of between 3 and 8 d at 39 °C, and then windowed in this position through the air space. The shell membrane was peeled back, and 50 µl of the stock solution (representing 20 µg

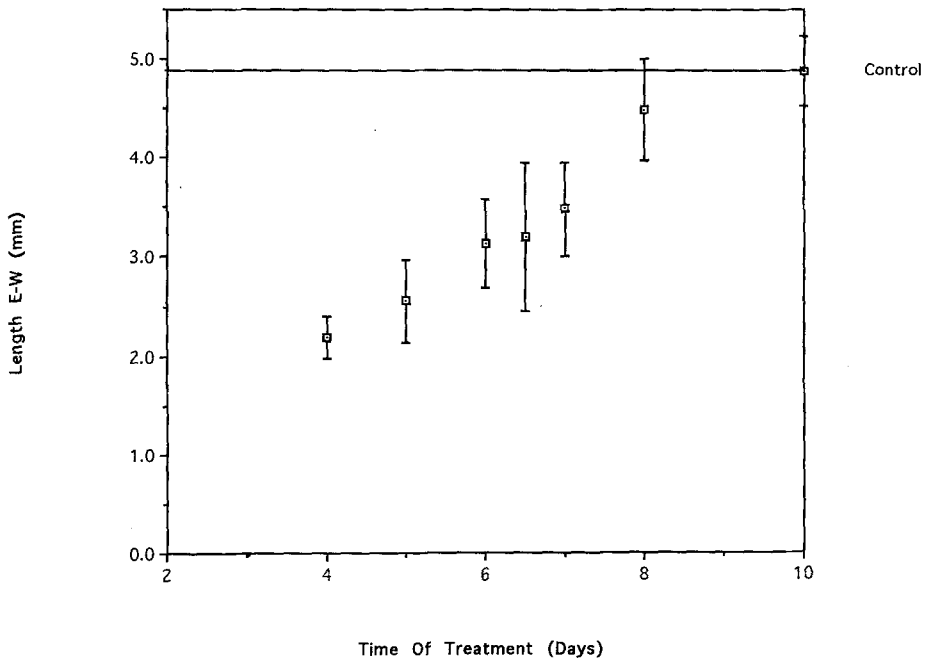

Figure 1. Effect on treatment times on limb length.

of 6-AN) was placed on the embryonic membranes above the embryo, using a Gilson pipette. Embryos were sacrified after 10 d total incubation, and were removed to Dulbecco's phosphate buffered saline for examination and measurement.

Examination was carried out using a Wild M8 dissecting microscope. The number of secondary remiges (feather germs associated with the ulna) was counted as those germs lying between the angle at the elbow and the wrist. After various trials, length was determined with the aid of a photographically reduced scale held with watchmakers forceps against the limb. This method was found to provide accuracy of ± 1 unit, each unit being approximately 160 µm. It was noted that in some instances treatment before 8 d brought about the occurrence of incomplete rows of germs. These cases were noted separately.

Results

Administration of 6-AN reduced wing lengths compared to control limbs at all treatment times between 4 and 8 d (fig. 1, table) No embryos survived treatment at 3 d. The degree of length reduction was inversely proportional to the time of treatment. All reductions were statistically significant by t-test ($p < 0.05$ or better) except for embryos treated at 8 d.

The number of feather germs between elbow and wrist also varied with treatment time in a similar manner to limb length (fig. 2). 6-AN administration at 4, 5, 6, 6.5 and 7 d gave significant differences from control values (by t-test; $p < 0.05$ or better). This figure demonstrates that there is indeed a significant decrease in feather germ number in limbs treated with 6-AN during early development. Figure 3 represents the resulting relationship between wing length and feather germ number. It

Time treated (days)	Feather germs (M ± S.D)	Length (mm) (M ± S.D)	No. embryos treated	No. embryos survived
3.0	-	-	13	0
4.0	7.25 ± 1.09	2.190 ± 0.22	23	3
5.0	9.63 ± 1.09	2.560 ± 0.42	19	15
6.0	10.75 ± 1.66	3.130 ± 0.45	13	9
6.5	10.94 ± 1.21	3.200 ± 0.75	13	12
7.0	11.73 ± 1.03	3.480 ± 0.47	14	12
8.0	12.88 ± 0.95	4.490 ± 0.52	12	12
Control	13.36 ± 0.81	4.880 ± 0.36	18	13

All eggs were treated with 20 µg of 6 AN in 50 µl of water.

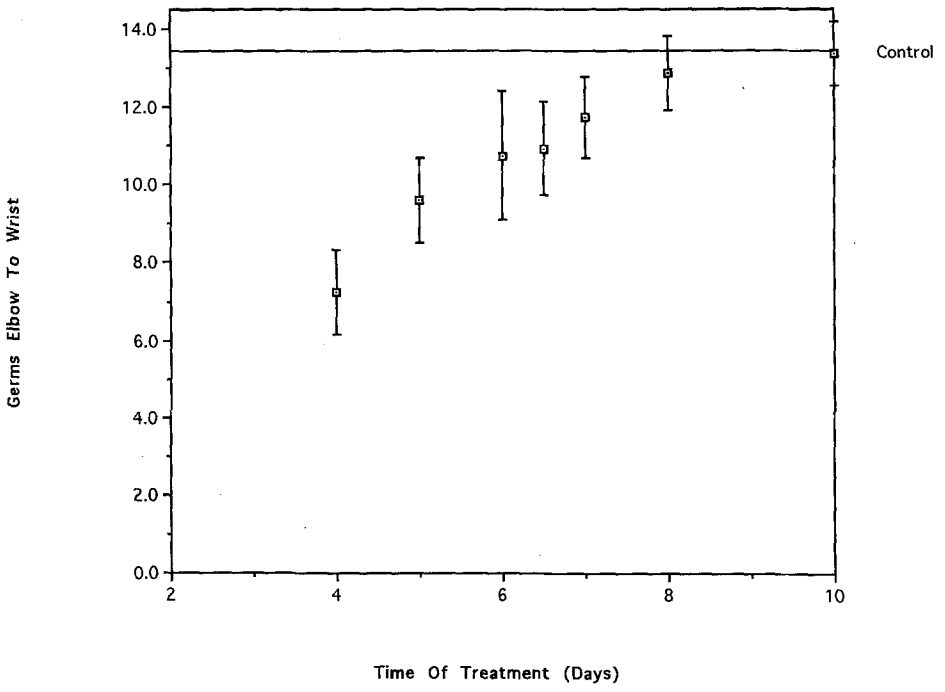

Figure 2. Feather germ numbers after treatment with 6-AN.

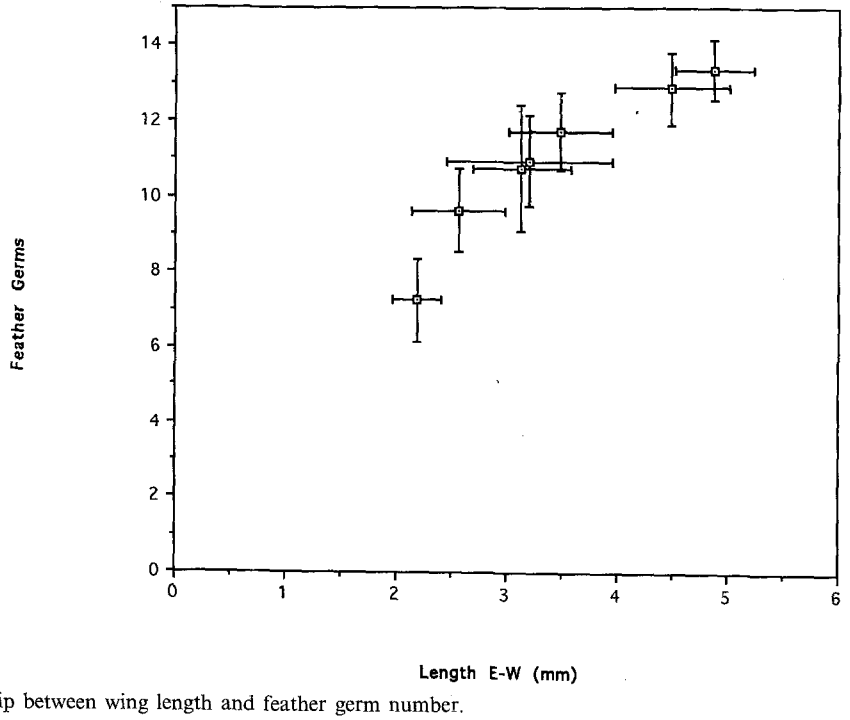

Figure 3. Relationship between wing length and feather germ number.

can be seen that there is a relationship between limb length and feather germ number, such that as limb length decreases, so does feather germ number, although this relationship may not be directly linear. Additionally, with earlier treatment, increasing numbers of embryos displayed limbs with incomplete rows of feather germs along the limb margin. In 10-day control embryos, feather germs are generally present all along the ulnar margin to the wrist and beyond. This information is presented in figure 4.

Discussion

Feather germ pattern on the trunk develops in a regular triangular array that spreads sequentially from the dorsal mid-line across the skin, forming row upon row of primordia. Although the limb has a more complex

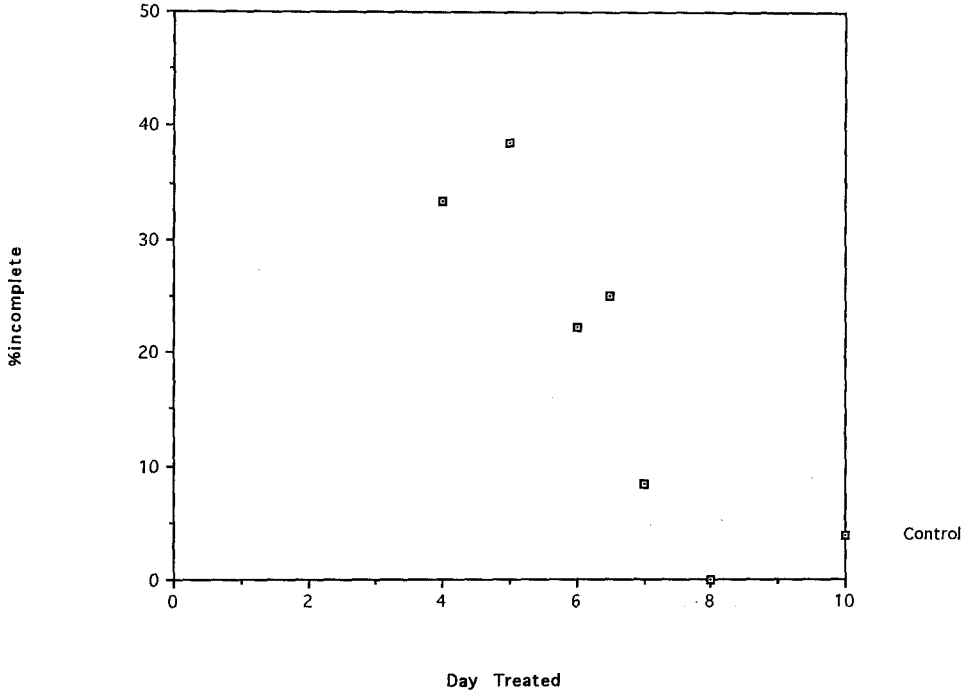

Figure 4. Percentage of wing buds showing incomplete rows of feather germs elbow to wrist.

morphology, limb feather germ formation appears to preceed in a similar manner. Quantification of the feather germs along the caudal margin is merely a convenient measure of this process. The mechanism of generation of pattern has been suggested to be by a template pattern by which deposition of feather sites in one row governs the positions in the next[4]. This would predict a decrease of germ number if limb length were reduced. Alternatively pattern could be determined by a gradient of positional information predicting a maintenance of germ number on length reduction. The data presented in figure 3 clearly show germ number declining proportionally as limb length decreases, supporting a sequential induction mechanism.

Preliminary observations had led to the suggestion that there was a critically sensitive time of development over which germ number may specifically be affected[3]. No evidence for such a critical period can be found in the present results, although an upper time limit after which 6-AN cannot affect germ number along the limb can be identified. Following 8 d, further treatment has little effect on either limb length or germ number. This accurately corresponds with the histological appearance of epidermal placodes in this region but is after the dermis condenses[5]. This indicates that dermal condensation does not indicate the irrevocable positional fate of a feather germ.

No lower limit on any critical period could be found, and germ number was still significantly affected at 4 d of incubation. Such effects cannot be direct since the earliest signs of germs anywhere is at Hamburger and Hamilton stage 29 (ref. 9) and determination of position for germs has been shown to be only one or two rows in advance of established germs[7]. Thus a response at these early stages must be due to the effect of the teratogen on limb length.

We also observed increased occurrence of incomplete rows along the wing as limb length decreased. This may indicate that limbs respond to overall size rather than directly to the passage of time as a signal to initiate the onset of germ formation. Such effects are unlikely to be due to metabolic inhibition in the germ since there is near complete cessation of DNA synthesis and mitosis in vivo[8] (although this is not seen during in vitro observations of chick skin cultures[7]).

These results are consistent with sequential induction of feather germ elements. This is not a pre-pattern model, as alternatives to positional information models are sometimes categorised, but is instead an interactive model, in which the number of germs is always appropriate to the space available. This technique therefore offers a valuable tool to explore theories of pattern formation during development.

These results also suggest that in species showing different numbers of feathers in the adult, these differences are achieved by differences in the time of onset of germ formation, and this can be studied experimentally.

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