The distribution of melanocytes in the dorsal coats of a series of chimaeric mice

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SUMMARY

Chimaeras, made by aggregating two embryos from the randomly-bred Q-strain, and X-inactivation mosaics both have similar, balanced distributions of pigmentation in the dorsal coat, while (C57BL x C3H)F₁ ↔ Recessive chimaeras tend to have an unbalanced pigment distribution, with a higher proportion of F₁ pigmentation in the posterior than in the anterior part of the coat. This observation can be explained if differences in the timing of the migration of the two melanocyte populations from the neural crest cause anterior–posterior differences in selection pressures.

INTRODUCTION

The melanocytes responsible for coat pigmentation provided one of the first genetic markers in mouse aggregation chimaeras. Mintz (1967) suggested, in an early paper, that 'the initial melanoblast types seem first to take up alternating rather than random positions' in two longitudinal mid-dorsal chains. A much larger study by Mintz led her to retract her earlier claim for a non-random distribution (see Mintz, 1971), and Mintz's revised interpretation of the origin of coat melanocytes from two chains of seventeen melanoblast clones, randomly distributed along the length of the neural crest, is now widely accepted. Very similar patterns of coat pigmentation are seen in many chimaeras, and also in X-inactivation mosaics heterozygous for Cattanach's flecked translocation (Cattanach, Wolfe & Lyon, 1972; Cattanach, 1974).

The present study represents an analysis of the distribution of the pigmentation in the dorsal coat of (C57BL x C3H)F₁ ↔ Recessive chimaeras and other groups of chimaeras and mosaics.

MATERIALS AND METHODS

(a) Mice

Four groups of chimaeric or mosaic mice with two distinguishable melanocyte populations (pigmented and cream or albino) were used in this study. Eight-cell morulae from pigmented and unpigmented stocks were aggregated to produce chimaeric mice (McLaren & Bowman, 1969). Pigmented stocks were

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either (C57BL/McL♀ × C3H/BiMcL♂)F₁ or pigmented members of a closed, random-bred stock of Q-strain mice. Unpigmented stocks were either albino members of the Q-strain or from the multiple recessive strain used by McLaren & Bowman (1969) and here designated 'Recessive'. The X-inactivation mosaics were all *flecked* mice, heterozygous for Cattanach’s translocation (Cattanach, 1961), and were all of the duplication, *Dp(7;X)Ct* form.

(b) Analysis of dorsal coat

Mice were killed by cervical dislocation and skinned following a ventral, medial incision. The pelts were preserved by rubbing with a mixture of boracic acid and Keating's insecticide powder and pinned on cork boards for two weeks.

Patches of roughly uniform pigmentation were traced on a 6 in. × 8 in. photograph of each pelt, with reference to the original. The pigmentation was subjectively classified on a five-point scale: (A) almost entirely unpigmented; (B) predominantly unpigmented; (C) approximately equal proportions of pigmented and unpigmented hairs; (D) predominantly pigmented; (E) almost entirely pigmented. To avoid complications from the *tanoid* gene (*atd*), which is segregating in the Recessive stock, only the dorsal coat was considered. A line was drawn from the tip of the nose, mid-way between the ears, to the base of the tail at the rump. This line was divided into four equal lengths, numbered 1–4 from the anterior, and areas to the left and right of this mid-line were marked off by drawing parallel lines either side, at a distance equal to one-quarter of the width of the pelt at the centre. In this way the dorsal coat was divided into eight areas, as shown in Fig. 1. The area of each patch of pigmentation was measured from the photograph, using a planimeter, and the percentage of pigmentation calculated as:

\[
\frac{\left(\frac{1}{3}B + \frac{1}{3}C + \frac{1}{3}D + E\right)}{A + B + C + D + E} \times 100\%.
\]

This percentage was calculated separately for the entire dorsal coat, each of the four anterior-posterior regions (summing over both sides), and the two sides (summing over the four regions). The deviation of each of these six areas from the whole dorsal coat was calculated in order to remove variation in total pigmentation between the chimaeras and allow comparisons between regions, or sides.

(c) Electrophoretic analysis

The proportions of the two component populations in samples of gut and abdominal wall were subjectively estimated to the nearest 10% by starch gel electrophoresis of glucose phosphate isomerase (GPI), as described elsewhere (West, 1975). Samples taken from different parts of the gut and abdominal wall were compared to determine whether anterior–posterior trends occurred in these organs. (The samples of gut show only the two homopolymer isozyme bands, whereas the abdominal wall samples show three bands: *aa, ab* and *bb*).
Fig. 1. Pelt of (C57BL × C3H)F1 Recessive chimaera X33, showing extreme imbalance in anterior–posterior pigment distribution. Below: drawing of pelt showing the eight regions of the dorsal coat analysed.
RESULTS

In agreement with Cattanach et al. (1972), the coats of the mosaic group appear similar to the chimaeras made by aggregating embryos of the random-bred Q-strain, and some animals in each group show dorsal and ventral mid-line effects.

The estimates of percentage pigmentation for the (C57BL x C3H)F₁ → Recessive chimaeras are in good agreement with independent estimates by Grünberg & McLaren (1972) on the same animals. The regional deviation from the whole dorsal coat estimate, for each of the four anterior-posterior regions (summing over left and right sides), is shown for each of the four groups in Fig. 2. Fig. 2C suggests that the proportion of F₁ pigmentation is higher in the posterior than in the anterior half of the coat of (C57BL x C3H)F₁ → Recessive chimaeras.

Comparisons between left and right sides in the four groups of mice, using Student's t-test, show no significant differences. Similar comparisons between the two regions anterior and posterior to the mid-point (regions 2 and 3) show a significant difference only for the group of 21 (C57BL × C3H)F₁ → Recessive chimaeras (t₁₀ = 6.69; P < 0.001). Analyses of variance show differences among the four regions in both this chimaeric group (F₁₀₈₀ = 32.85; P < 0.001) and the group of 30 mosaics (F₁₁₆ = 7.72; P < 0.01). Further analyses using the method of Sokal & Rohlf (1969) show a significant regression only for the chimaeric group (F₁² = 21.06; P < 0.05), although this is significantly different from a linear regression (F₀² = 4.27; P < 0.05).

The results show that the pigment distribution in (C57BL x C3H)F₁ → Recessive chimaeras is clearly unbalanced, while in the other three groups the distribution is more nearly balanced between the four anterior-posterior regions.

Electrophoretic analysis failed to reveal any anterior-posterior imbalance in the gut or abdominal wall.

DISCUSSION

The slightly higher proportion of pigmentation in the rump (region 4) of the mosaics may be due to the subjective classification method. The proportion of pigmentation is likely to be overestimated when pigmented and unpigmented populations are more thoroughly mixed and Cattanach (1974) suggests that cell mingling is greater in the rump region. The progressive darkening of white areas in mosaics, observed by Cattanach & Isaacson (1965), seems unlikely to account for the slight pigment imbalance seen here as the darkening would be expected to accompany the waves of hair growth in an anterior to posterior progression.

The more marked anterior-posterior imbalance in pigment distribution in (C57BL x C3H)F₁ → Recessive chimaeras suggests different selection pressures in different regions of the dorsal coat. A similar unbalanced distribution in the
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Fig. 2. Deviation of percentage of pigment, in the four anterior–posterior regions of the dorsal coat, from the whole dorsal coat. (See Fig. 1 for regions considered.) Four groups of mice were studied: A, thirty mosaics; B, six pigmented-\(Q\) <-> unpigmented-\(Q\) chimaeras; C, twenty-one \((C57BL \times C3H)F_1\) <-> Recessive chimaeras; D, eight pigmented-\(Q\) <-> Recessive chimaeras.
skeletal system of C3H ↔ C57BL/6 chimaeras has been explained on the basis of an anterior–posterior temporal gradient (Moore & Mintz, 1972). These authors suggest that the apparent spatial gradient of strain types along the axial skeleton may 'reflect an underlying temporal gradient in which the C57BL/6 genotype has an early advantage that is lost, with time, to the C3H genotype'.

The melanocytes migrate laterally from the neural crest and colonize the skin and hair in an anterior–posterior sequence (Rawles, 1947). If the timing of the initiation of melanocyte migration is cell-autonomous, Moore & Mintz's temporal-gradient concept is compatible with the present results from the dorsal-coat pigmentation. If the migration of the melanocytes from the anterior part of the neural crest is initiated earlier in Recessive melanocytes, these would be at an advantage and each Recessive clone could colonize a large area of skin and hair. However, if the anterior to posterior sequence of migration occurs more quickly in (C57BL × C3H)F₁ melanocytes, the development of this population may overtake the Recessive melanocytes and they may start to migrate from the posterior part of the neural crest earlier than the Recessive population and so have a selective advantage in this region.

Moore & Mintz (1972) suggest that C57BL/6JNlcR embryos appear to be initially larger, but, by day 13, C3H/HeNlcR embryos have caught up or overtaken them. Studies on preimplantation embryos (McLaren & Bowman, 1973) suggest a delay in the initiation of cleavage of C3H/BiMcL and (C3H♀ × C57BL♂)F₁ embryos compared to C57BL/McL and (C57BL♀ × C3H♂)F₁ embryos, which is probably due to a delay in the fertilization of C3H/BiMcL eggs (Nicol & McLaren, 1974). Differences in developmental timing clearly occur between strains, although no data are available for the melanocyte migration patterns in Recessive or (C57BL × C3H)F₁ embryos.

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REFERENCES

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