THE HAIR PATTERN DEVELOPED ON DISORIENTATED SKIN GRAFTED IN 1-DAY-OLD RATS

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Summary

The effect of skin disorientation on the development of hair pattern was studied by excising small pieces of skin from the mid-back of 1-day-old rats and returning them with altered orientation to their original bed.

A wide variety of regeneration patterns was obtained, and for grafts of equal size these depended on the direction and extent of the rotation. Markedly different results were obtained when smaller grafts were used.

As current theories of tension and differential growth offered no explanation for these results, it was postulated that processes involved in the determination of hair pattern in 1-day-old rats had not reached completion. Subsequent hair regeneration was subject to processes within the graft tending to maintain the original hair disposition and to processes in the skin surrounding the graft to impose on it normal behaviour for hair development, irrespective of the altered spatial relationships of the graft.

I. Introduction

A feature of mammalian hair patterns is their arrangement in tracts. This arrangement is constant for species and remains unchanged throughout the life of the individual. An examination of the hair tracts of an extensive collection of the young stages of marsupials (Boardman 1950, 1952) suggested the following hypotheses:

(1) The pilary system as a whole is a mosaic of hair fields each polarized by a divergent and a convergent centre. (The meaning of this will be made clear by reference to Figure 1 in which the hairs on the throat of the laboratory rat are shown conventionally charted (Fig. 1(*a*)) and also divided into their constituent fields (Fig. 1(*b*)). The small bilaterally paired gular fields are typical of the units which make up the pelt; each gular field is polarized by the centre of one of the two large centrifugal whorls at the base of the neck and by the centre of the small radial convergent field just in front of the interramal papilla. The tip of the snout acts as a divergent centre. Extremities of the limbs, ears, and tail are regarded as physiologically equivalent to convergent centres.)

(2) The divergent centre is a growth centre from which the initial growth wave of follicles takes origin. The convergent centre, apparently passive in pattern development, appears to be an inherited primary feature of the skin and not merely a consequence of the spatial relationships of divergent centres. Evidence has been submitted to support the proposal that both divergent and convergent centres are fundamentally similar in physiological significance, i.e. they are both growth centres.

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(3) The first-formed hairs of a developing pilary system lie always on a line connecting a divergent to a divergent centre and each hair has its free extremity directed towards the convergence.

(4) The geometrical pattern of follicle lines which run between a divergent centre and the convergences around it is considered as possibly due to activity at the centres having produced an ultrastructural organization of micellar (ultramicronic) lines which determines the basic arrangement of the hairs in rows.

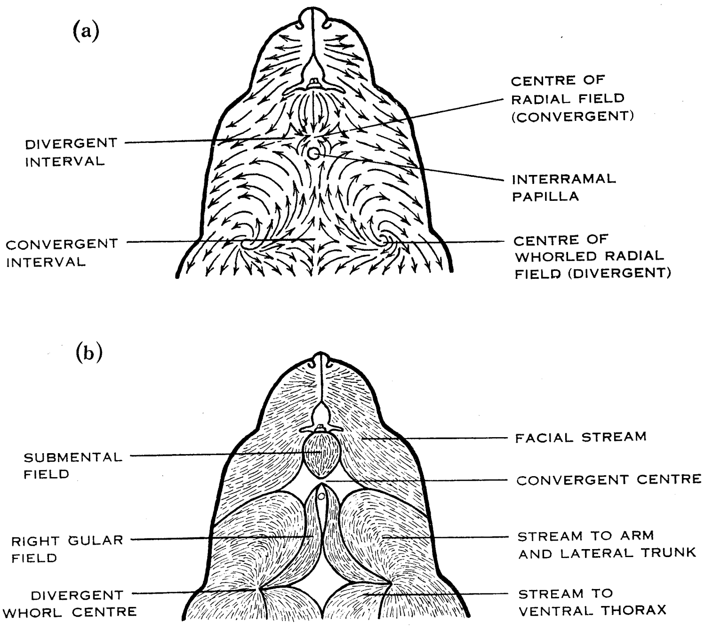

Fig. 1.—Hair tracts on the ventral aspect of the head and neck of the laboratory rat. (*a*) Normal bilaterally symmetrical arrangement. (*b*) An analysis of the region to illustrate the concept of polarized fields as units of tract pattern. The constituent fields are shown separated by heavy lines. Only two of the fields are complete in this view—the submental field polarized by a divergence on the chin and the interramal convergent centre, and the paired gular fields polarized each by a gular whorl and the interramal convergent centre. The intervals are exaggerated for clarity.

An initial experimental approach would be to alter the spatial relationships of areas of skin at the time during which the first hairs are being laid down, a procedure which is followed in the experiments recorded in this paper.

When mammalian skin is grafted, extensive regression occurs within the graft and the hair coat present at the time of the operation is lost. Subsequently the organization of the graft is restored and the hairs regenerated. If adult animals with a well-established pelage are used the new hairs conform to the original orientation plan irrespective of the direction shown on the surrounding undisturbed skin (Korschelt 1931; Durward and Rudall 1949; Billingham 1958). The experiments reported here show that this is not true when newly born rats are used for autograft operations involving skin disorientation. A wide variety of regeneration patterns are obtained. The purpose of the present work was to investigate this phenomenon.

Kiil (1949) has summarized the earlier work on skin grafting designed for study of the problem of hair slope and tract formation. Of several experimenters Trotter and Dawson (1932) met with the greatest measure of success, undoubtedly due to their use of the naked new-born rat as an experimental animal. Their technique consisted of the reversal of pieces of skin on the lateral lumbar region. Hair direction was modified for, of 36 survivors, 15 showed a ventro–caudad hair direction in regeneration and the remainder developed hairs having a mixed direction. These authors claimed that their results supported the idea that hair slope "is determined by the factors of tension effective at the time of development of the hair follicle". The two broad categories in their results are claimed to be correlated with healing by first and second intention respectively. With a slight change of operation site (mid-dorsal instead of lateral lumbar region) a repetition of this work is included in the experiments reported below.

II. Material and Methods

Litters of 1-day-old laboratory rats, mostly of the black-hooded and Irish strains were used. The technique employed was that of the fitted pinch graft (Billingham and Medawar 1951). Under light ether anaesthesia a small oval piece of skin was wholly severed from the mid-back and returned to the same location but with its orientation changed. Before removal the graft area was marked with indian ink to assist in accurate placing and subsequent checking for slip. In the 1-day-old rat the skin readily separates from the subcutaneous layers so that scraping or trimming of its dermal face is unnecessary. No attempt was made to disinfect the graft area prior to cutting, and freshly sterilized instruments were used for each animal. The graft was protected with a small square of sterile "Vaseline" gauze and secured with a single layer of $\frac{1}{2}$-in. adhesive plaster completely encircling the body. Dressings were removed after 6 days and not renewed; if taken off earlier the graft was too readily displaced and lost. At the same time 6 days is the limit that should be allowed for the retention of such dressings on a rapidly growing animal. Apart from some retardation of growth and occasional umbilical hernia which disappears shortly after removal of the bandages, the rats showed no adverse effects and rapidly recovered lost condition. With these methods infection was infrequent and the take of the graft was nearly 100%. As the mother is prone to nibble the dressings and frequently kills a part or the whole of her litter, losses from this source can be very high. Thus care in giving treated litters time to recover and to re-acquire the nest odour prior to the mother's return to the cage seemed to be a worth-while precaution. In the small-graft experiments, only the Sprague–Dawley strain was used and losses from interference by the mother were greatly reduced; these rats were much more gentle and less easily disturbed than the types used in the earlier experiments.

All rats operated on successfully were kept under observation for at least 100 days.

III. Experiments and Results

(a) *Experiment* 1: *Fitted Pinch Grafts Rotated through* 90°

Litters of black-hooded rats were used to take advantage of the longitudinal stripe of pigmented hairs on the mid-back which served as a marker confirming the orientation of the graft. The grafts were cut with an oval outline, 8–9 mm long and 6–7 mm wide. In one group of 33 animals the graft was rotated through 90° to the right (clockwise group), in the other group (27 animals) the graft was rotated the same amount to the left (counter-clockwise group).

When dressings were removed at the end of the sixth day the grafts were pink and clothed with short and mostly dark first-generation hairs, the development of which was terminated by the operation and which were in process of being ejected from the follicles. By the twelfth day most of these first-generation hairs had been lost (groups here and there may persist for a further day or two) and the scattered single hairs of the new growth were visible. By the eighteenth day the graft surface was thickly covered with hairs which showed a definite tract arrangement.

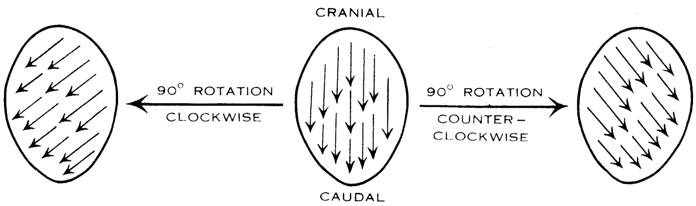

Fig. 2.—Experiment 1: 90° rotation. At centre is shown the hair disposition on the undisturbed skin of the graft area. To left and right the effects of rotation clockwise and counter-clockwise respectively are represented.

Within each of the two experimental groups the regenerated pelt showed remarkable uniformity in pattern. In the clockwise group the new covering was regularly disposed so that the individual hairs pointed caudally and to the left, inclination to the long axis of the body being about 45° (Plate 1, Fig. 2). Similar results were obtained for the counter-clockwise group except that the hairs pointed caudally and to the right (Plate 1, Fig. 1). The results are shown diagrammatically in Figure 2.

A control series was established for all experiments and consisted of 18 rats taken from the litters used in these experiments. The same techniques were used for removing the oval-shaped piece of skin, but after cutting, the graft was returned to its bed with the original orientation unchanged. The cycle of hair loss and regeneration followed the same sequence of timing as recorded for the 90°- and 180°-orientation groups. In no case did the regeneration pattern differ from the normal arrangement for the graft area, so much so, that the graft boundary early became impossible to define. In other words, grafts of this kind which maintain the original spatial relationships of the graft to the surrounding skin, reproduce the original head to tail hair flow.

(*b*) *Experiment* 2: *Fitted Pinch Grafts Rotated through* 180°

Rats of several strains were used as they became available. Methods used were identical with those for experiment 1, except that the graft was rotated through 180° before being returned to the same location.

Observations and analysis of the results were based on 67 grafts and each of the strains appeared to give similar results, but the number of animals used was not adequate to detect any variation from this source.

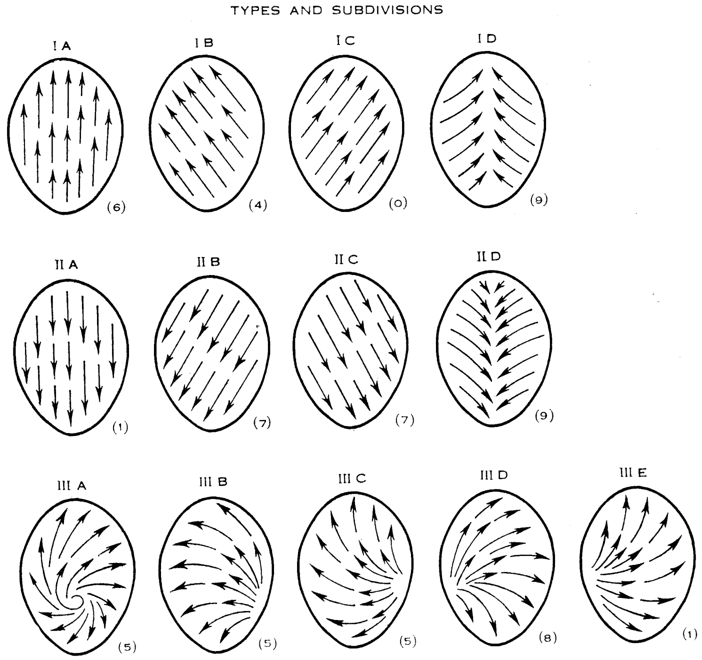

Fig. 3.—Experiment 2: 180° rotation. The figure shows the full range of the regenerate patterns which appeared and the classification adopted. The numerical results are given in brackets for the respective subdivisions.

In the regeneration processes the graft went through the same stages at the same times as recorded above for the 90°-rotation groups. However, the new hair growth did not appear in a predictable pattern as for the 90° groups. The graft acquired any one of a wide range of hair patterns (e.g. see Plate 2). For descriptive purposes these possibilities have been classified as three main types, each with subdivisions (Fig. 3). Detailed results of this experiment are also given in Figure 3. In type I, comprising 19 specimens, the general hair trend is towards the head. Type IB shows deviations of the hair stream to the left; type IC (deviation to the right, which presumably could occur) is not represented. In type ID, opposing hair streams meet on the long axis of the graft and produce a weak hair ridge there; in these examples a few hairs towards the caudal margin of the graft seem always to be directed caudally, thus forming a small convergent interval. Type II (24 specimens) is similar to type I and is likewise subdivided but has the hairs generally directed caudally; all four subdivisions of the type are represented. Type III, also with 24 specimens, differs from both of the other groups in having the hair pattern expressed as a divergent radial whorled system, the centre of which is always in the caudal half of the graft. The centre may occur on or near to the mid-line of the graft or on its right or left margin. With the whorl fully formed rotation is clockwise or counter-clockwise. It will be seen that types IIIB and IIID and types IIIC and IIIE constitute pairs of mirror images.

(*c*) *Experiment* 3: *Small Fitted Pinch Grafts Rotated through* 180°

To determine the effect of size of graft on the pattern of regenerated hair, the second experiment was repeated, but this time a graft about one-quarter of the area previously selected was used. The small grafts were, as with the previous experiments, cut to have an oval outline; the long axis measured 4–4·5 mm and the short axis 3–3·5 mm in length. The technique was identical with that employed in experiment 2.

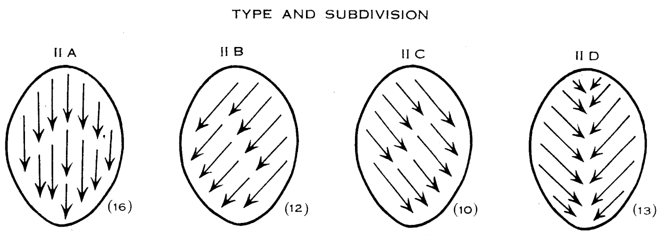

Fig. 4.—Experiment 3: small grafts with 180° rotation. All the patterns produced belong to type II (see Fig. 3). The numerical results are given in brackets.

Externally the graft showed the same cycle of changes as in the previous experiments. By the tenth day only scattered hairs of the original hair coat remained and by the sixteenth day the coat of new hairs was well established and their definitive pattern arrangement readily charted. However, the wide range of patterns recorded in the results of experiment 2 was not realized with the smaller graft. Fifty-one specimens survived and all showed a type II tract pattern, i.e. the hairs tended to be directed caudally. The detailed results are recorded in Figure 4.

(*d*) *Permanence of Regenerated Hair Patterns*

Regenerated hair patterns on the graft, once laid down, did not vary with age even though there were sharp discontinuities in relation to the surrounding normal skin. This is illustrated in Plate 1, Figures 2 and 3, in which hair patterns of a graft after 90° clockwise rotation are shown at weaning (21 days of age) and at 100 days respectively. This particular animal, together with others from both the 90° and 180° series, was kept under observation for 18 months. Their regenerated hair patterns were still distinguishable after this period.

IV. Interpretation of Experimental Results

(a) *Experiment* 1

In the experiments carried out, those in which fitted pinch grafts were rotated through 90° are unique in that the disposition of the regenerated pelt can be predicted for the individual. With clockwise rotation of the graft the new hair covering is regularly disposed so that the hairs point caudally and to the left; with counter-clockwise rotation the hairs come through aligned so that they point caudally and to the right (Fig. 2). An attempt at explanation is best commenced with this series.

Apart from their predictability the most significant feature of these results is that the regenerated pattern on the graft has the appearance of being a compromise between the pattern on the undisturbed skin and what the arrangement on the graft would have been (i.e. hairs transversely disposed) had the disorientation produced no alteration in hair direction. It would seem as though the new orientation of the hairs is a resultant of forces within the surrounding skin tending to maintain the normal head to tail flow and forces within the graft tending to produce a transverse direction. The fact that the disorientated skin regenerates a covering of hairs differing in direction of hair flow from normal undisturbed hair points to a retention of the plasticity in developmental potentialities which, as is well known, is later lost. That the disorientated piece in its new spatial relationships does not completely conform to influences imposed on it by the surrounding skin, influences which would give a head to tail flow in conformity with the immediate environment of the graft, becomes explicable if it be assumed that skin in this region is in process of becoming determined for the factors involved in pattern realization but that determination is not yet complete. In other words, the ultrastructural organization which is postulated as underlying the arrangement of hair in tracts is, in this experiment, re-orientated in accordance with the two influences to which the graft is subjected—a predisposition to retain hair flow towards its original posterior end and a predisposition to conform to cranio-caudal pattern tendencies imposed by the surrounding tissues. A similar situation has been encountered in experiments involving disorientation of organ rudiments (e.g. the ear) in amphibian embryos (Hall 1939).

(b) *Experiment* 2

The results of grafting with complete reversal differ notably from the 90°-rotation results in that the regenerated hair pattern on the graft is not predictable for the individual (cf. Fig. 3).

As in interpreting the results of experiment 1, the postulate is again made that the organization of the regenerated hair in the graft is subject to internal influences directed to the maintenance of the original hair pattern and external influences in the surrounding skin conditioning production of a coat with the hairs pointing caudally. With 180° rotation, however, this interplay of forces is such that the tendency to regenerate a hair covering caudally directed is in opposition to the tendency of the graft to regenerate a coat with the original flow. In the 90°-rotation experiment the two forces operative produced a result—45° inclination to the mid-longitudinal line, which would suggest that they are approximately equal in effect.

The three types produced (leaving aside for the moment the subgroups within each type) in this reversal experiment correspond to the three possibilities which might be realized when the opposing tendencies are approaching or are, in fact, in equilibrium. With effective excess of the graft's tendency to reproduce a coat like that which it originally possessed, type I would result, but with effective excess of the influence exerted by the surrounding tissues type II would be produced. With equilibrium between the opposed tendencies, or, excess of one or the other not at an effective level, the graft might be expected to organize itself round a new centre of activity as with type III patterns.

Hair direction in types I and II may be inclined to the right or left of the middle line, simulating the results of the 90° series. Types ID and IID have a median hair ridge due in all likelihood to the presence of a marginal convergence situated in front in type ID and behind in type IID. No explanation can be offered for the occurrence of these variants.

(c) *Experiment* 3

The size of the graft marks the difference between experiments 2 and 3. In experiment 3 grafts were only about one-quarter of the area of those used in experiment 2. All the grafts regenerated tract patterns classifiable as type II in which the hairs have a strong caudalwards component in their direction of pointing (Fig. 4). It should be remembered that such a hair direction is a reversal or near reversal of that exhibited by these skin pieces in their normal undisturbed position and presumably involves a change in polarity. The explanation again offered for type II patterns appearing in the results of this experiment is that the tendency of the surrounding skin to impose its caudally directed hair arrangement on the graft predominates over the tendency within the graft to produce a hair covering with the hairs pointing towards the head. The absence of types I and III is readily explained by the facility with which the normal polarity of small grafts is overruled in experiments of this kind. Again, as with experiment 2, the caudalwards flow of hairs shows variants for which no explanation can be given.

The occurrence of only type II forms in the results of this small-graft experiment strengthens the view put forward in discussing experiments 1 and 2 that the grafts are not completely determined with respect to the factors involved in producing hair arrangement in this region and so still respond in some degree to pattern tendencies in the skin surrounding the graft site.

V. Acknowledgments

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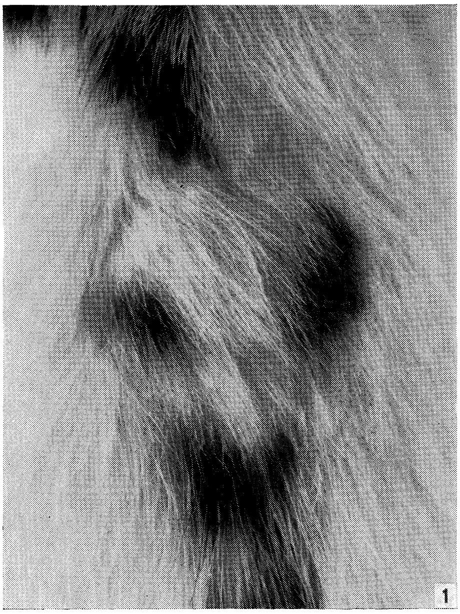
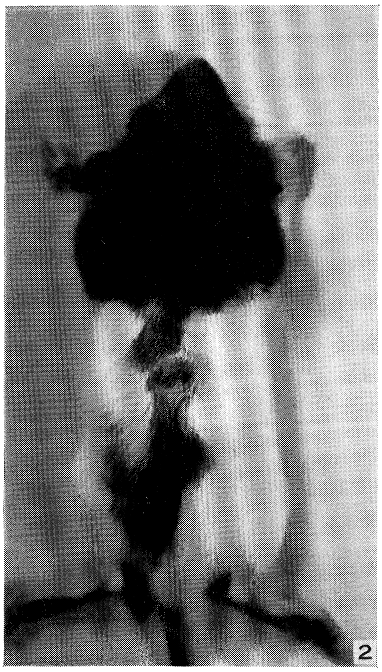
HAIR PATTERNS ON DISORIENTATED SKIN GRAFTS





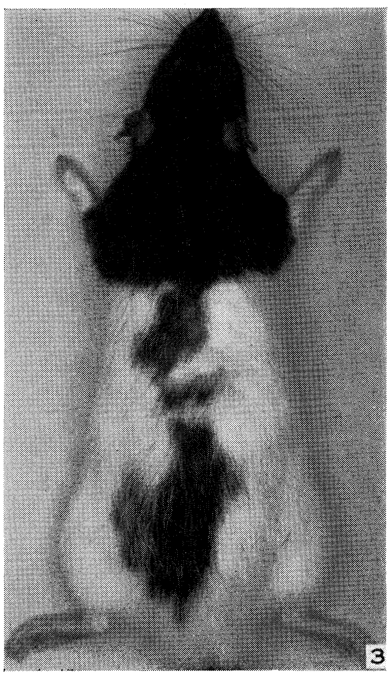
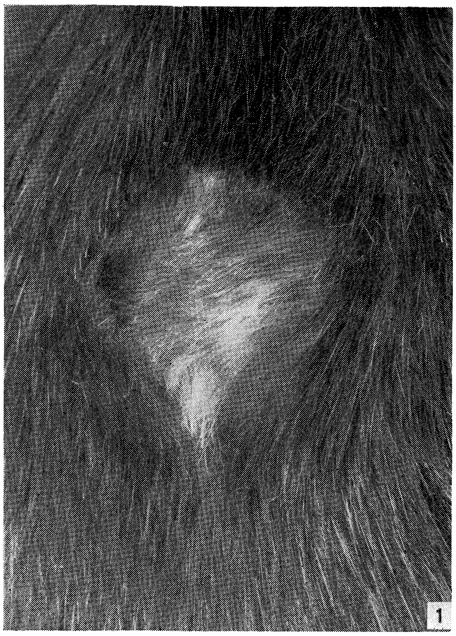


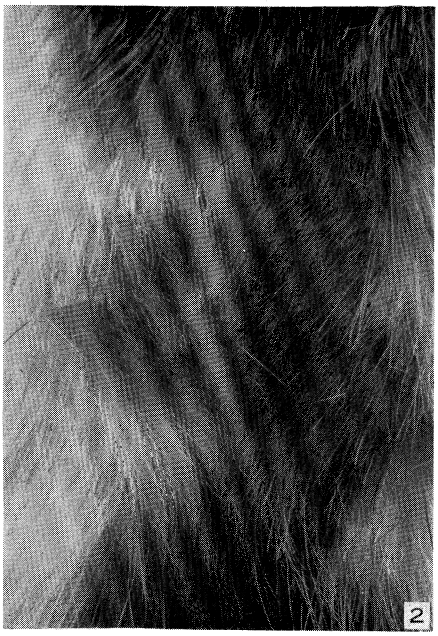
Fig. 1.—Regeneration of hair on a graft after counter-clockwise rotation through 90°. Photographed at 100 days. Fig. 2.—A graft rotated 90° clockwise as seen at 21 days. Fig. 3.—The same rat as shown in Plate 1, Figure 2, photographed at 100 days. The regenerated hair pattern on the graft is unchanged.

HAIR PATTERNS ON DISORIENTATED SKIN GRAFTS

II B



II D



III A

III D

Figs. 1–4.—Pattern types produced when grafts are rotated through 180° (expt. 2).