Stages of mitosis that occur in the spores after tetrad formation—and which precede the production of an antheridial initial and a prothallial cell—include an interphase nucleus with nucleolus (Fig. 1A, arrow points to nucleus), early prophase where the nucleolus disappears and the chromatin condenses (Fig. 1, B and C), prophase with short, thick chromosomes (Fig. 1, D to G), metaphase (Fig. 1H), and late anaphase/telophase (Fig. 1I). Nearly 30 percent of the spores observed in a sampling of about 130 were in what we interpret to be interphase and nearly 60 percent were in prophase; the remainder observed resembled metaphase, anaphase, or telophase configurations. The described microspore features were abundant in several cone specimens of *L. schopfii*. With both light optics and electron microscopy, frequency, statistical data, and apparent sequence of mitotic stages, consistent size, configuration, proportion, and spatial arrangement within the spores, which conform well with such features in extant *Selaginella* microspores, compel us to propose that these structural features indeed represent nuclei and chromosomes rather than only condensed cytoplasmic remains as has been suggested by Knoll and Barghoorn (*18*) for cellular contents.

A second division within this material begins after the formation of the prothallial cell and antheridial initial (Fig. 1, J and K, compare with *Selaginella*, Fig. 1L). According to our interpretation of the fossil material, the interphase nucleus of the antheridial initial (Fig. 1J) goes into prophase (Fig. 1M) and produces two daughter cells, thereby forming a three-cell microgametophyte (Fig. 1N, compare with *Selaginella*, Fig. 1O). The prothallial cell becomes flattened and takes a position against the inner spore wall (arrow in Fig. 1J; Fig. 1, K and M). These early stages in microgametophyte development in trilete fossil lycopod spores are quite similar to those that occur in species of modern *Selaginella* microspores (*19*) (Fig. 1, L and O). It is significant that the microgametophyte generation of *L. schopfii* resembles *Selaginella* whereas megagametophyte development is like that of *Isoetes*. Perhaps *Isoetes* is a living relative of the Paleozoic arborescent lycopods. In addition, *Selaginella* may be related far more closely than previously thought.

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Pattern Formation by Cultured Human Epidermal Cells: Development of Curved Ridges Resembling Dermatoglyphs

Abstract. *In cultures made from disaggregated human epidermal cells, growth to a confluent cell layer is followed by the emergence of patterns resembling those of human dermatoglyphs. These patterns reflect intrinsic properties of keratinocytes. In vivo, only the epidermis of the volar surfaces forms patterns, but in culture, patterns are formed by epidermal cells from other sites as well. Patterns develop by a process of cell movement which first produces ridges and then curves the ridges into figures of increasing complexity, ultimately whorls.*

The skin of the palms and soles of primates becomes organized in embryonic life into patterns of ridges and grooves that are then maintained permanently. The patterns of the distal phalanges were designated by Galton as arches, loops, or whorls. At the conjunction of three adjacent ridge systems there is formed a triradiate structure converging to a single point (triradius). These features are the basis of classification of fingerprints for identification purposes. The patterns are thought to be the result of differential growth during embryonic life (*1*).

It is now possible to make serial cultivations of human epidermal keratinocytes under simple cell culture conditions (*2*, *3*). The epidermal cells depend for their multiplication on fibroblast products, and these products are supplied by including in the culture lethally irradiated fibroblasts (usually 3T3 cells, an established mouse fibroblast line). Epidermal growth factor (*4*) is added to each subculture beginning when the colonies are a few days old (*3*). Under these conditions, single epidermal cells give rise to colonies resembling a stratified squamous epithelium and consisting of multiplying and terminally differentiating cells (*5*). The colonies grow and eventually fuse to make a confluent layer (Fig. 1, 21 days). Some days later, numerous flattened cells begin to detach from the surface of the culture into the medium. These are terminally differentiated cells resembling the squames that become detached from the stratum corneum of intact skin, except that their nuclei have usually not yet been destroyed (*6*). At about this time, the cells remaining on the vessel surface begin to concentrate in thickened ridges, and by 30 to 40 days after inoculation, the ridges form patterns that can be described as arches, loops, and whorls. Transitional stages can also be seen.

Some well-developed patterns are shown in Fig. 2. Regions of organization are sometimes separated by triradials meeting at an angle of approximately 120°. Whorls are of either handedness. In some instances a whorl seems related to two triradii (Fig. 2B). These patterns contain features resembling those of the digital ridges of humans.

The development of epidermal ridges in embryonic life has been described (*7*, *8*). The process seems to begin with the formation of epidermal folds at around 4 months of gestation, perhaps at sites determined by nerves and blood vessels (*8*). While it has been suggested that the formation of ridges probably results from increased local proliferation in the underlying folds, this does not explain why the ridges should be curved into such ordered patterns as whorls. It has been

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postulated that somehow the (mesenchymal) volar pads determine the ridge pattern (*9*). The importance of the dermal connective tissue in determining other aspects of epidermal morphology has been clearly demonstrated (*10*). The possibility that epidermal cell movements might play a role does not seem to have been considered.

Human fibroblasts in confluent culture tend to orient parallel to each other and make curved bands of cells. Though they never make whorls, their patterns have a certain order (*11*). It could be imagined that cultured epidermal cells form patterns as a result of the influence of the fibroblasts. We have no evidence supporting this idea, and the following suggests that it is not correct:

1) Initially, our epidermal cell cultures contain numerous human fibroblasts, but

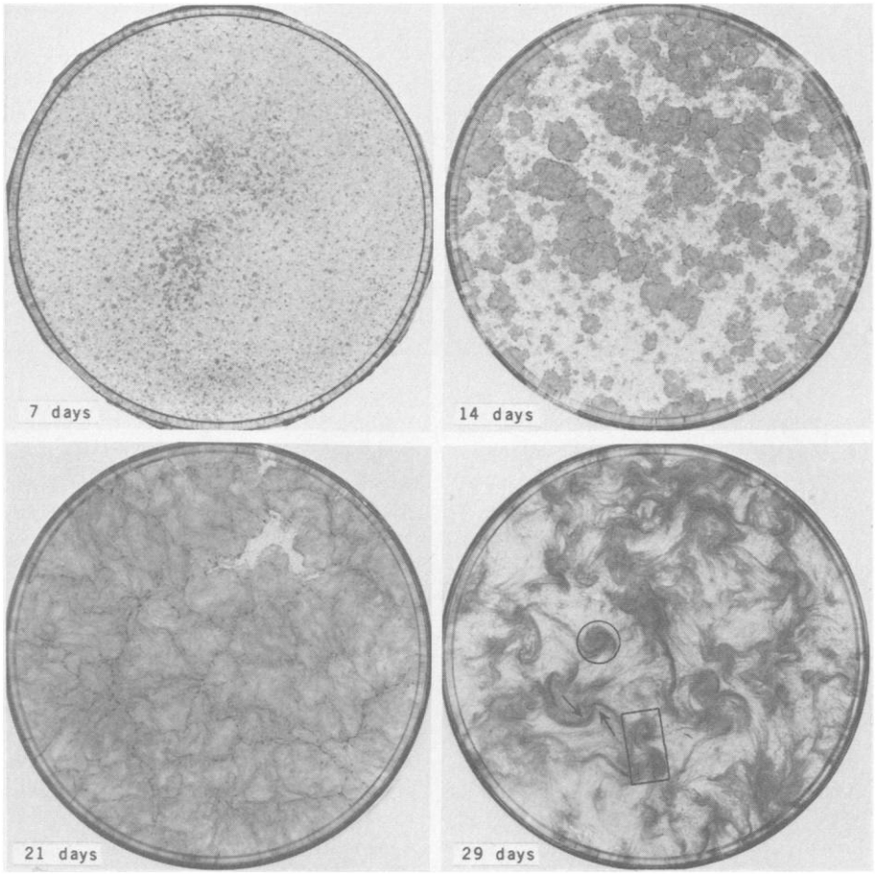
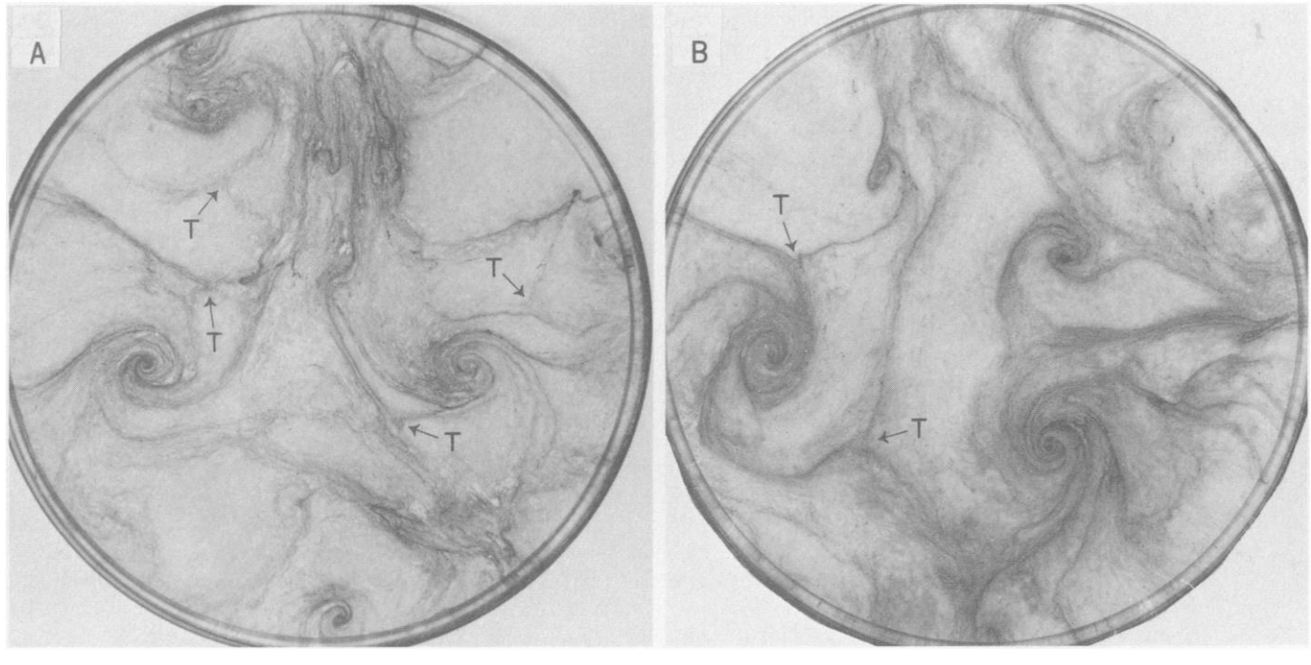


Fig. 1 (left). Growth of epidermal colonies and the development of ridge patterns. Petri dishes (50 mm in diameter) were inoculated with 2×10^5 keratinocytes of strain M, derived from the foreskin of human newborns (fifth serial transfer), and 4×10^5 lethally irradiated 3T3 cells. The medium (Dulbecco Vogt modification of Eagle's medium supplemented with 20 percent fetal calf serum and hydrocortisone, 0.4 μg/ml) was changed twice weekly. Epidermal growth factor (EGF) was added to 10 ng/ml, beginning 4 days after inoculation. For details of the culture conditions see (*2*). The cultures were fixed and stained with Rhodanile Blue at the times shown. The colonies, visible at 7 days, grow by excavating the 3T3 cells from the surface. By 21 days the colonies are confluent but no patterns have formed. Within a few more days, squame detachment becomes appreciable and patterns begin to form. A culture fixed at 29 days shows, among other features, a whorl (circle), a double whorl with openings to opposite sides (rectangle), and a transitional stage (arrows) that seems likely to lead to whorl formation. Development of ridges may proceed over a period of 2 to 4 weeks after a culture becomes confluent; then holes appear in the thinning background layer and the culture deteriorates. Fig. 2 (below). Advanced ridge patterns. Cultures were prepared as described in Fig. 1 with strain M (third serial transfer). The cultures were fixed and stained 35 days after inoculation. The photographs show numerous whorls of opposite handedness. The field is frequently divided into regions of different pattern by triradial lines meeting at close to 120° (*T*).



during the first two transfers their number is reduced to a very small proportion of the cells. This results from the inhibiting effect of the 3T3 cells on their growth (*2*), and the technique of removing them selectively with EDTA (*5*). Human fibroblasts are not completely absent from the cultures at the time they are used for pattern-forming experiments, but their number is sufficiently low (< 1 percent) that usually none are seen.

2) In studies of epidermal cell strain N, 26 well-isolated colonies (presumptive clones) were transferred to fresh dishes, allowed to grow, and observed for pattern formation. Only one culture was found to be contaminated with human fibroblast colonies. Fourteen isolates grew to confluence and were maintained for 40 days or more. Of these, ten developed one or more whorls and two had obvious pattern with less advanced organization.

3) Lethally irradiated 3T3 cells are always inoculated with the epidermal cells in order to support their multiplication and inhibit growth of human fibroblasts. Although some established lines of keratinocytes can be grown without 3T3 cells by using medium harvested from 3T3 cultures (*12*), we have not been able to grow normal human epidermal cells in this way sufficiently well to test their pattern-forming ability. The 3T3 cells do not orient nearly as much as human diploid fibroblasts, and especially after their lethal irradiation they show virtually no tendency to do so. By the time pattern formation begins, the remaining 3T3 cells have been mostly excavated from the dish surface and those remaining in the culture are confined to small clumps between confluent epidermal colonies. It is hardly plausible that the 3T3 cells could be the determinants of the human epidermal ridge pattern in culture.

The properties responsible for the pattern probably lie in the epidermal cells themselves. Time-lapse cinematography has shown that epidermal cells even in young colonies do not move as individuals, but rather flow together as a group (*5*). In a single colony there may be several moving groups that undergo displacements relative to each other. It may be assumed that the members of a moving group are bound by desmosomes, since these junctions are possessed in abundance by cultured epidermal cells (*2*).

The process of pattern formation in culture can be divided into two stages: first the formation of ridges, and second the curving of the ridges into whorls. This is shown by sequential photographs of two living cultures (Figs. 3 and 4). When the cultures first become confluent, detached 3T3 cells remain as small clumps at points between three confluent epidermal colonies (Fig. 3, 17 days). As time goes on, clumps are lost from the surface, but those remaining are useful in tracking the movements of the epidermal cells. About 1 week after a culture becomes confluent, ridges appear as the result of opposing movements of the cell masses.

At the front between two masses, a ridge may form from cells ejected from the deeper cell layers of one or both

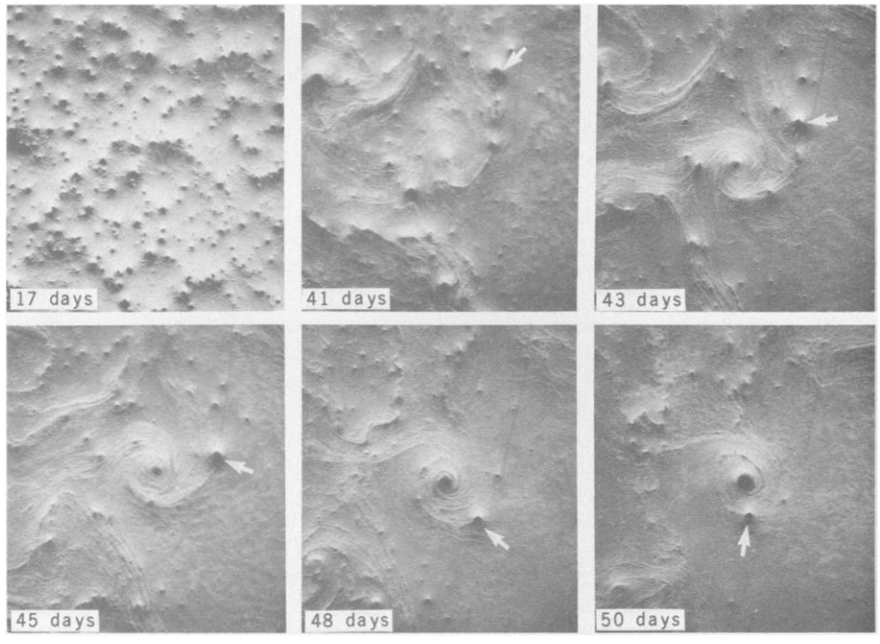


Fig. 3. The development of pattern. A living culture of strain N (fourth transfer) was photographed at intervals by Hofmann modulation contrast optics. The photographs show an identical region of the culture at approximately ×1.5 enlargement, at different times after inoculation (days). Arrows indicate movement of a clump of 3T3 cells as it is carried into the whorl by the movement of the epidermal cells.

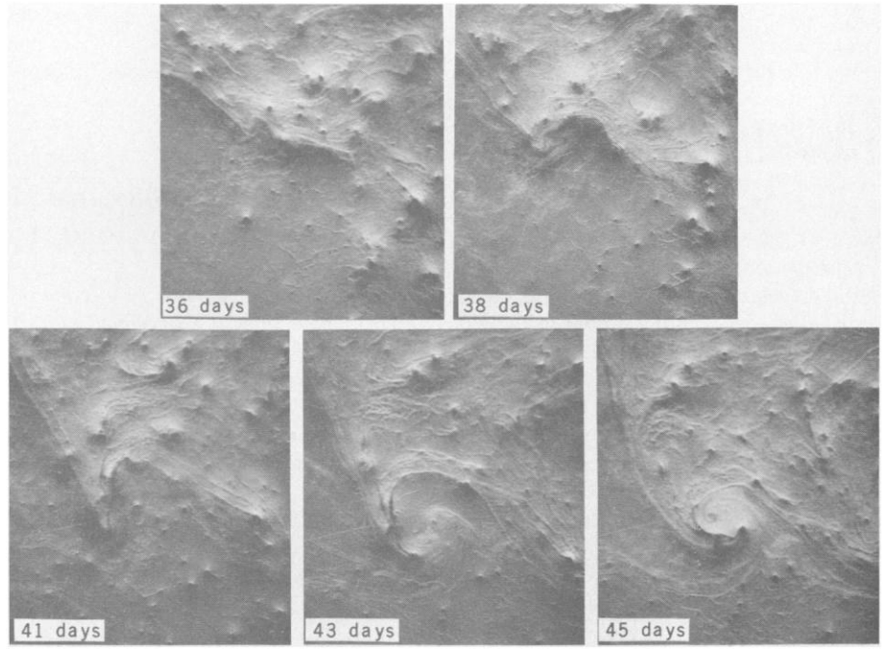


Fig. 4. The development of pattern. Another culture similar to that shown in Fig. 3. In this case, a structure resembling an arch is converted to a whorl. This whorl developed more slowly and more incompletely than usual, making it easier to observe the process.

masses; the shape of the ridge frequently suggests overriding of one mass by the other. In sequential photographs, short ridges may be seen to fuse into longer ones. Long ridges may undergo uniform lateral displacement, or only a local displacement, producing an arch. Sometimes an arched ridge curves into a whorl, suggesting that the resistance of the two sides of the arch were unequal. From the movement of the 3T3 clumps, epidermal cells at some distance from the center of a developing whorl can be seen to move toward its center. Though the photographs shown were usually taken at intervals of 2 to 3 days, displacements were very appreciable within 1 day.

All the cultures shown in Figs. 1 to 4 were grown in 50-mm dishes, and the whorls are of fairly uniform size; cultures grown to confluence in 33-mm dishes gave rise to whorls similar in other respects but of smaller size. It therefore seems that the size of a whorl is determined by the size of the field available for organization.

In the digits of embryos, the ridges form over epidermal folds that extend down into the dermis. These folds possess an expanded basal layer of proliferating cells which should generate more progeny per unit of skin surface than are produced between the folds, thus sustaining the form of the ridge. This is not possible in the cultures, since the basal cells are held in a flat layer by the surface of the dish. Vertical sections show that the ridges formed in culture consist mostly of differentiated cells resembling the upper layers of skin, while the basal layer of the ridge is very similar to that of the thin regions. Cell growth contributes to pattern formation, and omission of epidermal growth factor slows pattern formation appreciably; but unlike the situation in the digits, there may be no greater rate of proliferation under the ridges than elsewhere. On the other hand, the forces generating curvature of the ridges into whorls in culture should also be acting in the embryonic digits. Possible evolutionary relations between arches, loops, and whorls in the digits have been suggested (*1, 13*), but it seems to have been generally accepted that during embryogenesis the ridges first appear in their definitive pattern (*1, 9*). In view of the behavior of the cultured keratinocytes, this interpretation (or assumption) should be reexamined.

Although in the human, epidermal ridges are formed only on the volar surfaces of the hands and feet, the epidermal cells in our experiments originated from newborn foreskin. Even keratinocytes originating from corneal epithelium and the conjunctiva behave like epidermal cells in culture (*14*), and in the present experiments give rise to whorls very similar to those in Figs. 1 to 4 (*15*). There must therefore be some feature of the culture conditions which permits the keratinocytes to express properties similar to those they express in vivo in certain locations only. In the embryonic digits, the unusual amount of mesenchyme in the pads is probably essential for ridge formation (*9*), and might temporarily permit the ridges an unusual degree of mobility not found elsewhere on the body, but duplicated in the cultures.

On the basis of the idea that cell movement is an important factor in pattern formation in embryonic digits, it is possible to suggest an explanation for a curious influence of chromosome number on the pattern. Increasing numbers of X or Y chromosomes in the human diminish the total epidermal ridge count (*16*), even though the extra chromosomes are known to be largely or completely inactive genetically. It may be postulated that the bulkier nucleus resulting from the presence of the extra chromosomes would inhibit to a slight degree the cell movements and the distortion of cell shape necessary to produce curvature of the ridges, thus reducing the overall complexity of the patterns.

The degree of development of the patterns in culture varies in repeated trials and cannot be expected to equal in precision that of dermatoglyphs; even so, it may be that careful control of culture conditions would make it possible to distinguish some differences between the epidermal cells of individuals whose dermatoglyphs differ for genetic reasons. Any such differences would, of course, support the idea that the cell movements observed in culture are important in embryonic ridge formation.

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15. To our knowledge no cell type other than the keratinocyte gives rise to whorls in culture; however, see Elsdale and Wasoff (*11*). In a few trials, keratinocytes originating from nasopharyngeal epithelium, while resembling in many respects those of skin and conjunctiva, did not give rise to patterns in culture. This may be typical for keratinocytes arising from internal as opposed to external stratified squamous epithelia. This finding is in accord with the idea that though patterns may be influenced by mesenchymal cells, they are formed as a result of intrinsic properties of the keratinocytes.
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Preparation of Sarcolemmal Membrane from Myocardial Tissue Culture Monolayer by High-Velocity Gas Dissection

Abstract. *A high-velocity stream of nitrogen is used to simultaneously disrupt myocardial cells in monolayer culture and fractionate their sarcolemmal membranes. The membranes show a high degree of ultrastructural and enzymatic purity, with less than 1 percent intracellular residuum. They are produced in less than 1 second and remain as tightly adherent sheets on the surface on which the cells were grown. The cells are exposed to no agent other than nitrogen gas during the preparative procedure.*

A technique was previously developed that allows continuous, on-line measurement of isotopic exchange in neonatal rat heart cells in culture (*1*). The basis of the technique is growth of the cells on the surface of disks composed of scintillator plastic in such a way that the monolayer is attached directly to the isotopic detector. The approach has been used to evaluate the characteristics of calcium and potassium exchange in whole cultured cells with particular attention to the role of the sarcolemma and the surface coat–external lamina complex in these exchanges (*1–3*). It became clear that study of the isolated sarcolemmal complex

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