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A laboratory classroom exercise: cell migration in cutaneous wound healing and pigmentary pattern formation in the red-spotted newt

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The black-ringed red spots of the red-spotted newt, *Notophthalmus v. viridescens*, consist of a three-cell-type epidermal-dermal chromatophore pattern composed of epidermal erythrophores underlaid with dermal iridophores which are surrounded by a ring of dermal melanophores. Throughout the skin of the dorsum there are scattered epidermal melanophores and scattered dermal iridophores and melanophores (Forbes *et al.*, 1973).

In this open-ended laboratory exercise, students create full-thickness skin wounds on the dorsum of adult newts and follow early (24 h), and perhaps later(weeks to months), healing of the wounds. An interesting aspect of this system for short term observation is that the leading edge of the wound-healing epithelium, as well as epidermal melanophores, can be visualized with a dissecting microscope. The melanophores in the migrating epithelium serve as naturally marked cells. Students can observe clearly the complete re-epithelialization of a one-by-two mm rectangular wound in less than twenty-four hours. Furthermore, if the wound is made near one of the black-bordered red spots or between two closely spaced spots, the (epidermal) erythrophores of the spot(s) are carried into the wound epithelium allowing one to follow individual large bright red cells as wound epithelialization occurs (Zaccaria, unpub. obs.) (Figs. 1 and 2).

In long term observation, such migrated erythrophores attract (dermal) iridophores which can be seen individually to migrate into the wound area (Fig. 2), and to come to reside in the dermis directly beneath the erythrophores. This takes a few weeks and is a striking in vivo demonstration of directed cell migration.

Under even longer term observation, about two months, dermal melanophores migrate into the area of the wound and cluster around this epidermal-dermal erythrophore-iridophore association, thus creating a three-cell-type chromatophore pattern which resembles the appearance of the original black-bordered red spot (Zaccaria, 1977).

In an alternative procedure performed several days after the initial creation of a wound between two red spots, an erythrophore-

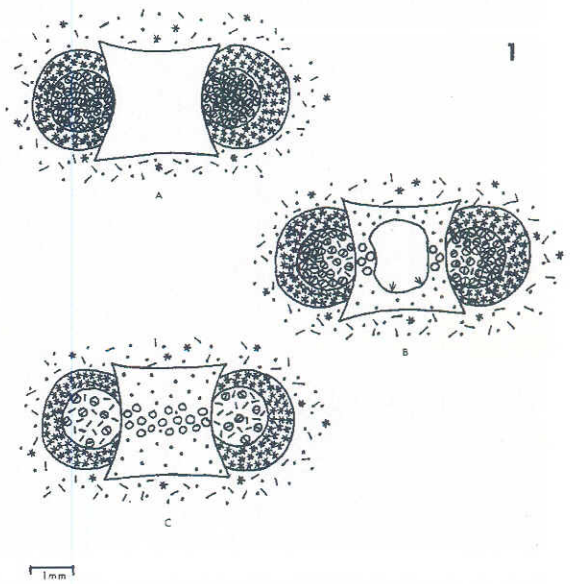

Fig. 1. Early epidermal migratory activity following excision of whole skin. A,B and C *represent 0, 9, and 48 h post excision, respectively. The clear area between the spots in* **A** *represents the wound (4 mm^2). *, dermal melanophore; •, epidermal melanophore; o, erythrophore; -, iridophore; θ, erythrophore(s) underlaid with iridophore(s); arrow, leading edge of migrating epidermis.*

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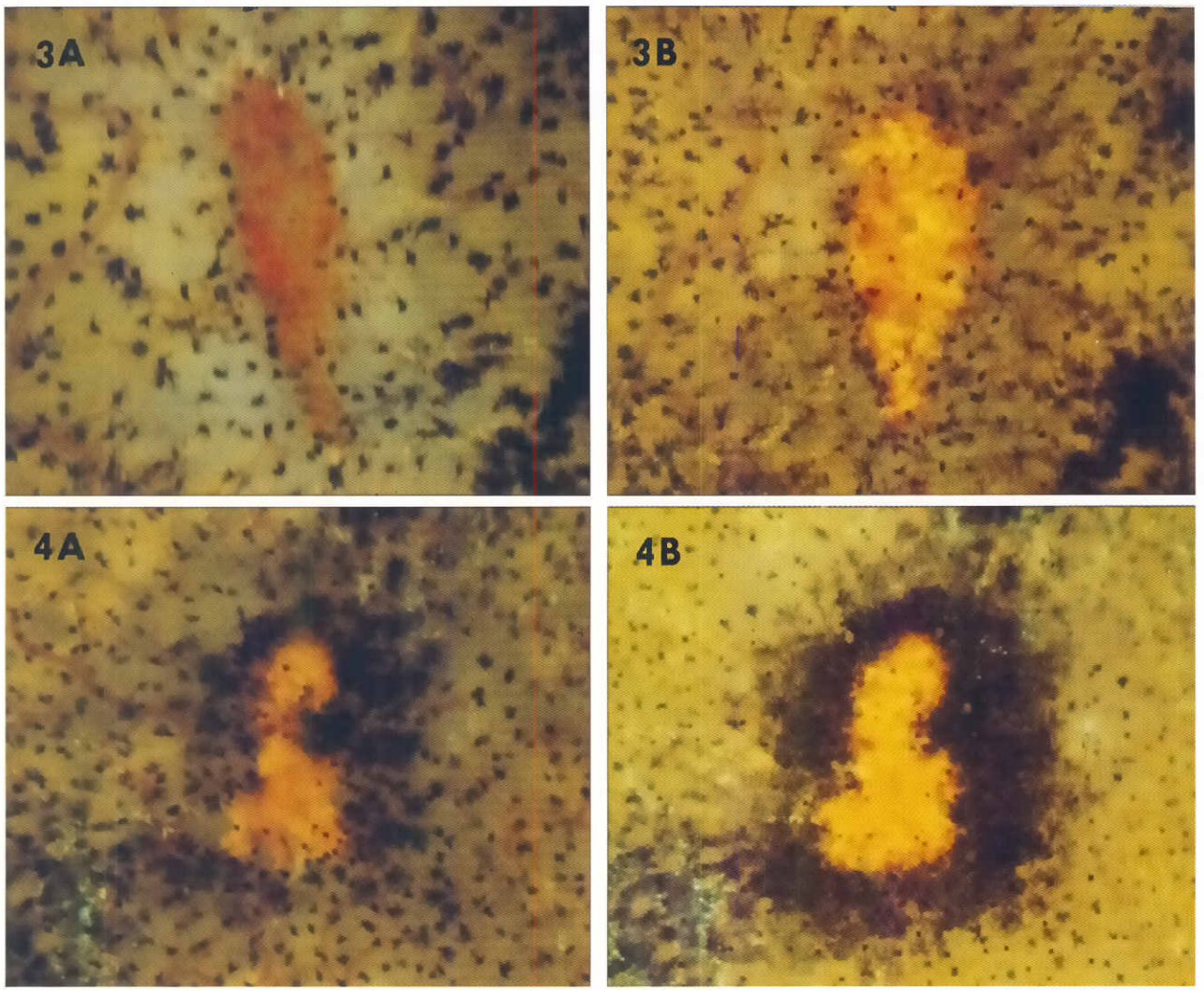
Fig. 2. Re-epithelialized wound between two red spots 14 days post excision. *Many individual erythrophores and epidermal melanophores are contained in the wound epithelium. Photographed using light of a high angle of incidence to reveal the reflective nature of the iridophores. Individual iridophores (at 2 and 4 o'clock of left spot and two at 9 o'clock of right spot) have begun to migrate into the wound.*



Figs. 3 and 4. Chromatophore interaction at two recipient sites of erythrophore-containing wound epithelium autografts showing (Fig. 3) *de novo* formation of an erythrophore-iridophore association and, (Fig. 4) further conversion of an erythrophore-iridophore association into a black-bordered red spot. (3A) *Erythrophore-containing autografted wound epithelium 16 days post grafting. The slightly pale appearance of the engrafted area distinguishes it from the surrounding intact skin. Epidermal (small, punctate) melanophores are scattered throughout the area of the graft. The reflective entities at the top and bottom of the mass of erythrophores are iridophores.* **(3B)** *Erythrophore-iridophore association at the same engrafted area as depicted in* **3A** *but at 41 days post grafting. The bright appearance is due to the aggregation of iridiphores which migrated individually from the surrounding dermis and came to lie beneath the mass of erythrophores.* **(4A)** *Accumulation of dermal melanophores about an erythrophore-iridophore complex which developed at another erythrophore-containing epithelium autograft site. Photographed at 48 days post grafting. Smaller epidermal melanophores remain scattered throughout the field of view.* **(4B)** *Same area as in* **4A**, *but at 72 days post grafting. Melanophores from the surrounding dermis have migrated into the graft and aggregated to form an essentially complete ring about the erythrophore-iridophore complex. This three-cell-type chromatophore pattern resembles that of an original red spot.*

containing piece of the wound epithelium is removed and autografted to a recipient wound site also on the dorsum but in an area heretofore devoid of red pigmentation. At that recipient site, if a minimum of eight to ten erythrophores remain viable in the transplanted epithelium , cellular interactions and migrations similar to those described above occur, resulting in the ectopic *de novo* formation of the three-cell-type chromatophore pattern of the red spot (O'Brien and Zaccaria, 1981) (Figs. 3 and 4).

References

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