PATTERN FORMATION IN STARFISH: ARM STUMPS, REGENERATION MODELS AND EVOLUTION
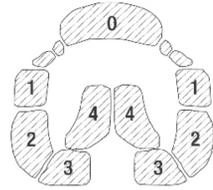
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SUMMARY

Positional information theory and regeneration models from amphibians, insects, and planarians are used to explain pattern formation in starfish.

BACKGROUND

Schematic cross section of the open wound of the starfish ray following amputation of an arm, emphasizing only the skeletal elements. The ambulacral groove is on the underside of the ray; the ray has bilateral symmetry through the vertical plane. The major skeletal plate series are: 0 carinals; 1 superomarginals; 2 inferomarginals; 3 adambulacral plates; 4 ambulacral plates.
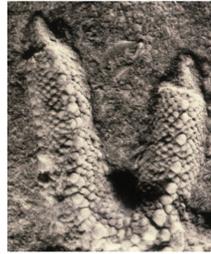
It is usual for starfish to regenerate an amputated arm. Middle Ordovician starfish *Promopalaeaster wilsonae* showing regeneration of two arms. [Bobcaygeon Formation, south Central Ontario; Royal Ontario Museum ROM 53320; Hotchkiss et al. 1997]

It is very unusual for the amputated arm of a starfish to not regenerate, but two examples of healed arm stumps from nature are described and analyzed here. A specimen of the Ordovician starfish *Urasterella ulrichi* with healed arm stumps that failed to regenerate the lost arm was described by Schuchert (1915:37).

THE PROBLEM: THESE ARM STUMPS FAILED TO REGENERATE AN ARM TIP

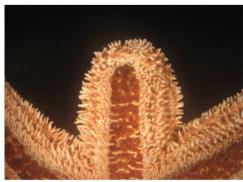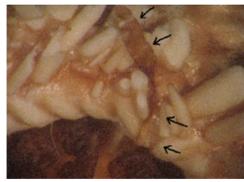Smithsonian Institution USNM 1111694   USNM 1111694   CONTROL: USNM 1111695

Order Valvatida. *Linckia laevigata* with healed stump and control specimen with regenerating arm tip; curio shop, Florida; probably from the Philippines.

Smithsonian Institution USNM 1112613   ARROWS: terminal tentacle

Order Forcipulatida. *Asterias rubens* with healed arm stump; Gulf of Maine; John H. Dearborn coll.

Description: The distal adambulacral plates of the two sides of the ambulacral groove arc and join together on the mid-line. In the open ambulacral groove (*Asterias*) the ambulacral plates seem to follow the arc of the adambulacral plates and to join together on the midline (not quite all in view, but fully implied by normal correspondence of ambulacral plates to adambulacral plates). The actinal intermediate plates adjacent to the adambulacral plates form smooth hairpin turns and merge from the two sides with only minor disruption (*Linckia*). The infero- and superomarginals of the two sides join smoothly (*Linckia*) and form the rounded terminus of the stump. Neither specimen has a terminal plate. A small terminal tentacle (without sucker) projects beyond the groove, emanating from where the left and right adambulacral plates arc and join (*Asterias*). The radial nerve is visible in the open ambulacral groove. Intradiscally and on the uninjured arms the plating is typical (*Asterias* and *Linckia*).

The comparative reference specimen (*Linckia*) lost an arm close to the disk and is now regenerating an arm. The regenerating arm and also the intact arms are forming new plates in a zone just proximal to the regenerated terminal plate. The plates that are forming are the ambulacral, adambulacral, inferomarginal and superomarginal plates, plus circa ten dorsolateral plates. The dorsolateral plates make an aboral pavement in which carinal plates are not separately differentiated (however three of five arms of the *Linckia* arm stump specimen have proximal carinal plates that undoubtedly formed behind the terminal plate at an earlier time).

BACKGROUND: POSITIONAL INFORMATION THEORY AND REGENERATION MODELS FROM AMPHIBIANS, INSECTS AND PLANARIANS
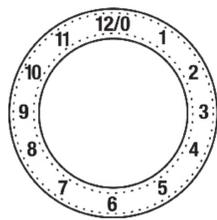
Positional information theory states that cells acquire information about their physical position from neighboring cells, or from chemical gradients, or other cues. In the *polar coordinate model* of pattern regulation positional information on the amputated cross section of a lateral appendage is represented by clock-face numbering: 1, 2, ..., 11, 12/0, with position values 0 and 12 being identical (French et al. 1976; Bryant et al. 1981). Excepting 12/0, no circumferential values are identical and this pattern of positional information is referred to as *asymmetrical*. Indeed, lateral appendages of insects, amphibians, etc., are asymmetrically differentiated (anterior ≠ posterior ≠ dorsal ≠ ventral).
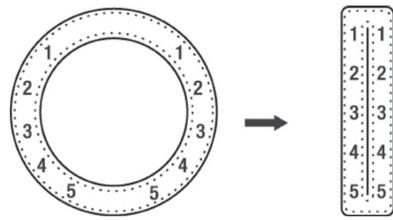
The polar coordinate model also allows structures to have *symmetrical* positional information. The symmetry hypothesis applies to symmetrically differentiated main body axis patterning such as transversely sectioned planarians (Saito et al. 2003) and *Drosophila* genital disks (Bryant & Hsei 1977).

Interaction of positional information at cut edges as they come together during wound healing determines the nature and extent of regeneration: injury creates gaps in positional information; wound healing then brings non-adjacent positional values into apposition; confrontation of positional values stimulates gap-filling intercalary regeneration. Using the example of clock-face numbering: if the numbers 6,7,8,9 are cut out from the clock face, circumferential wound closure brings position 5 and position 10 into apposition. Confrontation of position 5 and position 10 stimulates gap-filling intercalary regeneration and restores the missing values 6,7,8,9.

A special case of wound healing that can occur with symmetrical positional values is healing along the line of symmetry in a way that creates no positional value confrontations to stimulate intercalation, and in this case no growth or distalization is expected (Bryant et al. 1981): a permanent stump should form.

The *shortest intercalation rule* states that if two sets of gap filling values are possible, as on a clock face, then intercalation generates the shorter set. For example, when cells having the positional values 5 and 10 are placed next to each other, there are two possible gap filling sequences: 6, 7, 8, 9 and 4, 3, 2, 1, 12/0, 11. The shortest gap filling sequence is the one that intercalates.

The *complete circle rule for distal outgrowth* states that cells generated during circumferential intercalation at the regenerating tip of an appendage become distalized: they must adopt positional values that are more distal than those of the preexisting cells at the wound edge (Bryant et al. 1981).

The question arises: will repeated rounds of circumferential *intercalation with distalization* give an outgrowth which is both circumferentially and distally complete? Both regeneration experiments and graphical analysis of the polar coordinate model show that regeneration from wound surfaces with *asymmetrical* positional values is complete circumferentially and therefore complete distally, whereas regeneration from wound surfaces with *symmetrical* positional values is incomplete circumferentially and therefore incomplete distally (Bryant et al. 1981).
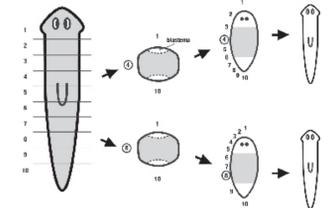

Schematic diagram of *intercalary model* of planarian regeneration along the anteroposterior (A-P) axis after amputation into 10 pieces (new model proposed by Agata et al. 2003, 2007). The anterior and posterior blastemas always have positional values 1 and 10, respectively, even though the body pieces have different original values. The blastemas produce anterior and posterior signals that interact with the body pieces to induce intercalary events and reorganize body regionality. Finally, stem cells located in the mesenchymal space start to differentiate appropriate cell types according to the newly acquired positional values.

The step of forming the signaling center is called *distalization*, and the new viewpoint of A-P regeneration is *distalization followed by intercalation*. This principle achieves anatomically complete distal regeneration and was proposed to apply to both symmetric and asymmetric patterns of positional values around the wound surface (Agata et al. 2007)

ANALYSIS
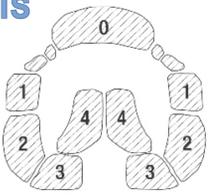
Failure of these starfish arm stumps to regenerate indicates that there were no positional value confrontations to stimulate gap-filling intercalation. Clock-face asymmetric positional information values can therefore be excluded, because under asymmetry all patterns of wound closure produce positional value confrontations that stimulate regeneration. Accordingly, the pattern of positional values in the wound must be symmetrical.
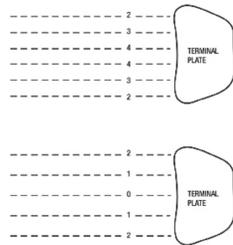
Deduced symmetrical pattern of positional values of ossicular series in the starfish arm. The arm grows by forming new plates in a zone just proximal to the terminal plate.
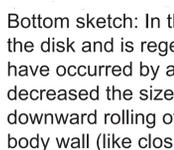
Top: under surface view. Bottom: upper surface view.
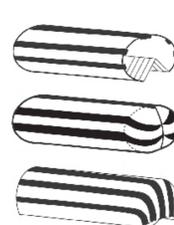
Top sketch: open wound.

Middle sketch: In the stumps that healed but did not regenerate, the arrangement of ossicles shows that wound closure proceeded by distal folding of the left and right sides (L-R wound closure; like closing a book).

Bottom sketch: In the specimen that lost an arm close to the disk and is regenerating, wound closure is inferred to have occurred by a combination of local contraction that decreased the size of the wound and dorso-ventral (D-V) downward rolling of epidermis over the cut edge of the body wall (like closing a roll-top desk).
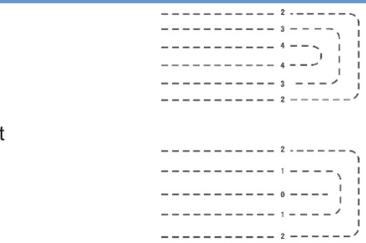
The loci of positional values following wound closure in the arm stumps. L-R wound closure brought homologous ossicular series into contact. Because there are no confrontations of non-adjacent positional values, healing proceeded without regeneration.

Top: under surface view. Bottom: upper surface view.

NEXT PROBLEM ...AND PROPOSED SOLUTION

The regenerating arm of the comparative reference specimen is not lacking any of the primary series of arm ossicles: it is *complete*. This is a dilemma because when the pattern of positional values on the circumference of the wound is *symmetrical* the polar coordinate model predicts circumferentially *incomplete* intercalation, and therefore distally *incomplete* outgrowth.

The solution: I propose that regeneration of the starfish arm is initiated by formation of a distalized signaling center that induces distal intercalary regeneration (the distalization followed by intercalation model of Agata et al. 2003, 2007, from studies on planarians). Further, I propose that distal intercalary gap-filling growth is the normal growth mechanism of the arm.

Discussion: According to this idea, regeneration reestablishes the normal growth mechanism of the starfish arm. There is always a positional gap between the last-formed section of arm and the distalized signaling center behind the terminal plate. Because every distally intercalated plate is like the previous plate of its series, the starfish arm is similar with itself throughout its length, the positional gap is never bridged, and growth is indeterminate.

The distalization and intercalary gap-filling mechanism applies individually to each primary plate series. Evidence is provided by a growth abnormality in which fusion of a terminal plate to a superomarginal plate stopped the intercalary addition of right-side superomarginal plates while all other plate series continued to intercalate (*Astropecten duplicatus*; USNM E12725).

FUTURE RESEARCH

Future research should try to produce, in the laboratory, starfish arm stumps like these. It is also worthwhile to search for more instances in nature. The possibility of intradiscal stumps is now evident and these can be looked for also (a candidate specimen is USNM 1113144 *Protoreaster nodosus*).

Future research should consider the evo-devo implications of these proposals. Distalization and intercalary gap-filling behind the terminal plate may help to explain the origins of novel plate series (such as ophiurid under arm plates and stenurid sublateral plates) without the necessity of ancestral primordia for such series. Likewise loss of plate series can have the same suddenness and explanation (one row of marginal plates instead of two). Once the organism possesses this signaling mechanism, then it would seem to be not too difficult for mutations to cause it to show up in more places than just behind the terminal plate. Thus, plate series that intercalate anywhere might have this type of origin (such as asteroid actinal intermediate plates, intermarginal plates, dorsolateral plates, etc.). The kaleidoscopic embryology concept of Dawkins (1996:232) can be used to comprehend the effect of intercalation-inducing mutations on the whole organism. Mosaic evolution and recurrent appearance/loss of intercalary plates within and between echinoderm lineages are expected under this proposal.

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