Cah. Biol. Mar. (2008) 49 : 369-373

Position of arm loss and rate of arm regeneration by *Luidia clathrata* (Echinodermata: Asteroidea)

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Abstract: Three contiguous arms of six individuals of the asteroid *Luidia clathrata* were amputated. The initial mean lengths (± SE) of the two intact arms were 57.36 ± 2.13 and 57.38 ± 1.97 mm. The initial mean lengths of the amputated arm stumps were 12.18 ± 0.59 (proximal), 25.91 ± 1.67 (medial) and 42.25 ± 1.37 (distal) mm. Buds appeared on all amputated arms after approximately 8 days. After 54 days proximal, medial and distal regenerated arms had mean lengths (±SE) of 10.96 ± 0.90, 7.69 ± 0.61 and 3.99 ± 0.38 mm, mean dry weights (±SE) of 41.75 ± 6.79, 20.95 ± 3.18, 8.07 ± 1.13 mg, and mean amounts of organic matter (pooled samples) of 6, 3 and 1 mg, respectively. All three arm positions are statistically different from one another ($P < 0.001$). Appearance of arm buds is the first phase of arm regeneration and is independent of position of amputation (distance from disc). Growth of regenerating arms is the second phase and is dependent on position of amputation. Studies have reported rate of growth of intact arms and of arms regenerating from the disc also declines as the asymptotic length of the arm is reached. This suggests similar mechanisms of control of growth occur in all three situations, all depending on the relative position of the regenerating arm tip.

Résumé : *Position du point d'amputation et taux de régénération des bras chez* Luidia clathrata *(Echinodermata : Asteroidea)*. Nous avons amputé trois bras contigus de six *Luidia clathrata*. La longueur moyenne (± S.E.) des deux bras intacts était de 57,36 ± 2,13 et 57,38 ± 1,97 mm ; les moignons des trois bras amputés mesuraient 12,18 ± 0,59, 25,91 ± 1,67 et 42,25 ± 1,37 mm. Après 54 jours, les régénérats issus des amputations avaient, respectivement, une longueur de 10,96 ± 0,96, 7,69 ± 0,61 et 3,99 ± 0,38 mm, un poids sec de 41,75 ± 6,79, 20,95 ± 3,18, et 8,07 ± 1,13 mg, et une teneur en matière organique de 6, 3 et 1 mg. Les données relatives à la position des trois bras amputés sont toutes significatives ($P < 0,001$). Ces résultats confirment l'existence d'une relation entre vitesse de régénération et position du point d'amputation. La vitesse de croissance des bras intacts, comme celle des bras régénérant à partir du bord du disque, diminue en même temps que leur longueur asymptotique est approchée. Ceci suggère que des mécanismes de contrôle similaires s'appliquent dans les diverses situations de croissance/régénération et que leur expression dépend de la position relative de l'extrémité du bras en croissance.

Keywords: *Luidia* • Regeneration • Starfish • Asteroidea

Reçu le 20 août 2008 ; accepté après révision le 6 novembre 2008.
Received 20 August 2008; accepted in revised form 6 November 2008.

Introduction

The general relationship between position of body loss and rate of regeneration is known as Morgan's law (Moment, 1953). Thomas Hunt Morgan (1901) stated arm regeneration in starfish is more rapid from the base than at a more distal position. He based this on an observation by his student, Elizabeth King (1898). Her documentation was only a figure of an *Asterias vulgaris* (= *Asterias rubens* Linnaeus 1758) showing the difference in regenerating arm length according to the position of arm amputation and a statement she observed the same result in eleven other specimens. Other workers have given general accounts of this relation in starfish (Mead, 1899; Schapiro, 1914; Zirpolo, 1921; Edmonson, 1935) and brittlestars (Morgulis, 1909).

Moment (1953) noted early work on the relation between position of body loss and rate of regeneration was only qualitative. The relation between position of arm loss and rate of regeneration has been quantified for brittlestars (Dupont & Thorndyke, 2006; Clark et al., 2007) but it still is not well documented for starfish. This paper quantifies the relation between rate of regeneration and position of arm loss in *Luidia clathrata* (Say, 1825). The results indicate starfish would be a good model for studying regeneration and growth in single individuals. According to Morgulis (1909) advantages of using asterozoans as models in regeneration studies include the ability to operate on several arms simultaneously. This means the same animal with its five similar arms in crinoids, ophiuroids and asteroids can be used both for the experimental and the control conditions, variations incident to the use of different individuals being eliminated. Dupont & Thorndyke (2007) have emphasized the suitability of echinoderms as models for studies of molecular control of regeneration.

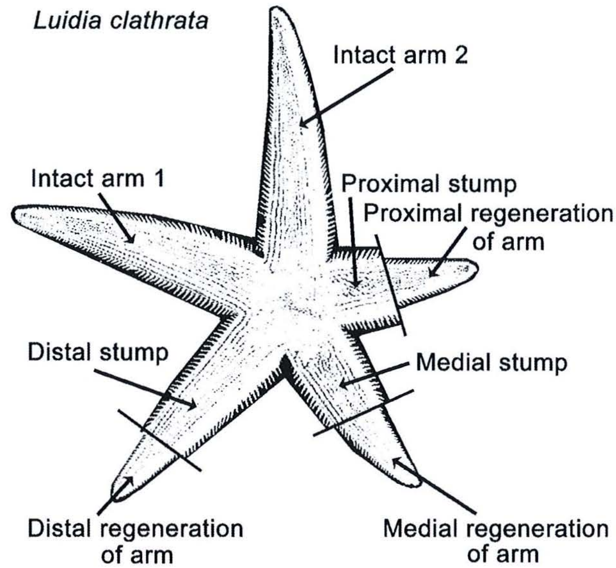

Figure 1. *Luidia clathrata*. Arms amputated at different positions.

Figure 1. *Luidia clathrata*. Bras amputés à différents niveaux.

Materials and Methods

Luidia clathrata were collected from Tampa Bay (27°58'0"N, 82°38'20"W) on 8 January 2007. They were maintained without food in the laboratory in aquarium containing filtered, recirculating sea water at 25 °C to allow them to adjust to laboratory conditions. On 16 February, three contiguous arms of six individuals were amputated near the disc (proximal), midway between the disc and arm tip (medial) and near the arm tip (distal) (Fig. 1). The lengths (from the disc) of both intact arms and stumps (distance from disc) of amputated arms were measured in triplicate with electronic calipers to the nearest 0.01 mm. Because the individuals did not differ significantly in size, these lengths indicate the amount of arm loss.

The experimental starfish were maintained without food in one aquarium until 5 April. Arm regeneration has been reported for starved *Asterias vulgaris* (King, 1898), *Asterias amurensis* (Lütken 1871) (Mikulich & Biryulina, 1970) and *Luidia clathrata* (Lawrence et al., 1986). Starvation eliminated possible individual variation in consumption of food that could increase variability in regeneration. One-half the seawater was replaced weekly.

Length, total dry weight and organic dry weight of regenerating arms were measured at the end of the 54 day period. Length of the regenerated arm is important in the function of the arm and the amount of production during regeneration is important in understanding the allocation of nutrients and energy during the process. The lengths of intact arms were measured as before. Lengths of regenerated arms were measured from point of amputation. The regenerated arms were amputated at the initial amputation point and dried (60 °C for 4 days). Because the amount of material was small, the samples were pooled and then ashed (500 °C for 4 hours) to obtain the percent organic matter. The percent organic matter was multiplied by mean dry weight to calculate the amount of organic matter.

The length and total dry weight data were analysed by randomized blocks ANOVA (each individual as a block) on ranked data (due to small sample size) and Tukey Multiple Comparison test with the level of significance set at 0.01. The data for organic matter obtained by pooling samples were not analysed statistically.

Results

The initial lengths of the two intact arms were similar to one another; while the initial arms and stumps of arms amputated at different distances were significantly different from one another (Table 1, Fig. 2A). Buds appeared on all amputated arms ca. 8 days after amputation. The lengths and dry weights organic matter of regenerated arms after 54 days showed an inverse relation to the length of the initial stump with all the three positions (proximal, medial, distal) significantly different from one another ($P < 0.001$) (Table 1, Figs 2B & 3). The amount of organic matter in regenerated arms also showed an inverse relation to the length of the initial stump.

Discussion

These quantitative observations with *Luidia clathrata* confirm qualitative reports of the relation between arm regeneration rate and position of arm loss in starfish. This difference in rate of arm growth according to position of amputation is like that reported for *L. clathrata* of growth of intact arms (Dehn, 1980) and of arms amputated near the disc (Lawrence & Ellwood, 1991; Pomory & Lares, 2000). In both of these latter two cases, arm growth was rapid initially and then decreased as the arm reached the asymptotic size. This suggests similar mechanisms of local control occur in all three cases, all depending on the relative position of the growing arm tip to the ultimate asymptotic size.

The position effect in regenerating arms of the same individual suggests local control rather than a systemic effect. Pomory & Lares (2000) reported growth of regenerating arms slowed when 40 to 50 per cent of the arm of *L. clathrata* had regenerated, the length at which gonads and pyloric caeca begin to appear. This is a reasonable explanation that implies a trade-off between energy allocation for somatic and gonadal or caecal growth.

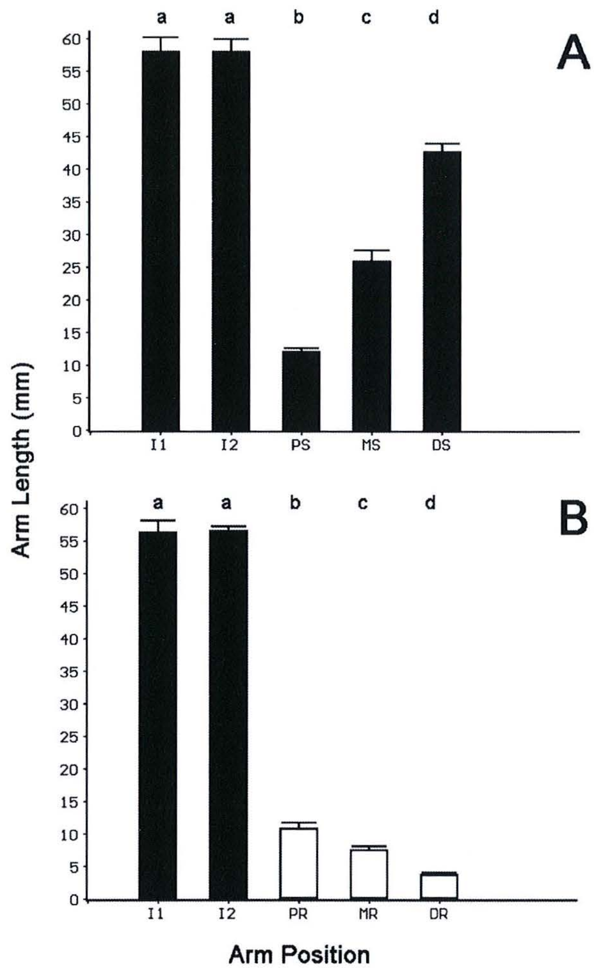

Figure 2. *Luidia clathrata*. **A.** Initial lengths (mean ± SE) of the two intact arms and arm stumps at beginning of the experiment. **B.** Final lengths (mean ± SE) of the two intact arms and regenerated arms at end of the experiment. I1, I2: intact arms, PS: proximal stump, MS: medial stump, DS: distal stump, PR: proximal arm regeneration, MR: medial arm regeneration, DR: distal arm regeneration. Different lower case letters (a-d) at top of graphs indicate significant differences based on Tukey test ($P < 0.01$).

Figure 2. *Luidia clathrata*. **A.** Longueurs initiales (moyenne ± SE) des bras intacts et des moignons des trois bras amputés. **B.** Longueurs finales (moyenne ± SE) des bras intacts et régénérés. I1, I2 : Bras intacts, PS : moignon proximal, MS : moignon médian, DS : moignon distal, PR : régénérat proximal, MR : régénérat médian, DR : régénérat distal. Les lettres (a-d) au-dessus indiquent les différences significatives (Tukey, $P < 0,01$).

Table 1. *Luidia clathrata*. *F* and *P* values from randomized blocks ANOVA on ranked data for comparisons of initial stump and intact arm lengths, final regenerated arm lengths, and final regenerated arm dry weights.

Tableau 1. *Luidia clathrata*. Valeurs du *F* de Fisher et probabilité associée *P*, ANOVA par blocs aléatoires sur les données ordonnées, comparaison sur la longueur des bras intacts, la longueur des bras régénérés et le poids sec des bras régénérés.

Comparison	*F*	*P*
Lengths of initial stumps and intact arms	$F_{4,36} = 264$	< 0.001
Lengths of final regenerated arms	$F_{2,10} = 122$	< 0.001
Dry weights of final regenerated arms	$F_{2,10} = 81.5$	< 0.001

However, the position effect we observed in the same individual occurred without any gonadal or caecal production.

Regeneration of the starfish arm has two phases. First is initiation of regeneration, a combination of morphallaxis

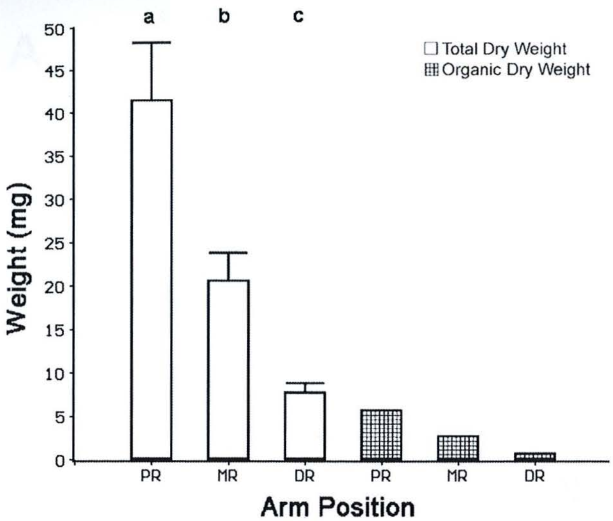

Figure 3. *Luidia clathrata*. Total (mean ± SE) and organic dry weights of regenerated arms at end of the experiment. PR: proximal arm regeneration, MR: medial arm regeneration, DR: distal arm regeneration. Different lower case letters (a-c) at top of graph indicate significant differences based on Tukey test ($P < 0.01$).

Figure 3. *Luidia clathrata*. Poids sec (moyenne ± SE) et poids de matière organique des bras régénérés à la fin de l'expérience. PR : régénérat proximal, MR : régénérat médian, DR : régénérat distal. Les lettres (a-c) au-dessus indiquent les différences significatives (Tukey, $P < 0{,}01$).

and epimorphosis (Bonasoro et al., 1998). This is not affected by position of arm loss in *Luidia clathrata*. The appearance of new arm tips in *L. clathrata* occurred at the same time after amputation regardless of the position of amputation. This confirms Schapiro's (1914) statement that the beginning stage of arm regeneration in starfish takes place at about the same time regardless of the position of arm loss.

Second is regeneration of the arm that results from the production of new ossicles behind the terminal plates of the arm tip. In contrast to the appearance of the arm tip, the rate of arm regeneration does depend on position of arm loss. King (1898) and Shapiro (1914) stated the arrangements of ambulacral ossicles in regenerating arms and normal arms are the same. Mann (1936) found this not completely correct because he observed the number of replacement ossicles at the regenerating arm tip decreased with increased distance of arm loss from the disc. He concluded the number depends on the size of the wound surface. Thus the rate of arm growth in starfish follows the same pattern in intact arms (small versus large individuals), in single arms regenerating from the disc and in multiple regenerating arms of an individual. The question of the mechanism responsible is of obvious interest.

Acknowledgments

We thank M. Jangoux, N.D. Holland and F.H.C. Hotchkiss for their assistance and the referees for their helpful comments.

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