Shifting the natural fission plane of *Holothuria atra* (Aspidochirotida, Holothuroidea, Echinodermata)

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Abstract

Fission stimulation involves the fission plane as the division area. In natural fission of Aspidochirote and Dendrochirotida, only a single fission plane is present. This paper presents successful assays of shifting the natural fission plane. The experiment was conducted in July 2007, when two stimulated areas were applied on each of 20 individuals of *Holothuria atra*. New tentacles and new anal pores started to appear in week 7 after division in anterior group (A) and posterior group (P), respectively. The middle group (M), which did not carry both mouth and anal complex, regenerated slightly slower than the A and P groups. Eleven individuals of the M group developed both tentacles and anal pore in week 9, and the number increased subsequently. When the experiment was terminated in week 12, the survival rate reached 96.67%. In induced fission, the natural fission plane was unlikely to be a definitive area of body division; therefore, in such experiments, the great potential of regeneration was expressed more than the phenomenon of induced asexual reproduction.

Introduction

The great potential of regeneration in sea cucumbers is demonstrated through the phenomena of evisceration and asexual reproduction by fission. In evisceration, the sea cucumbers expulse most of their internal organs due to physiological change or in response to varied external factors. Within a certain period of time the eviscerated sea cucumbers regrow new internal organs and live normally. The process of regrowing internal organs occurs in asexual reproduction as well.

Naturally, asexual reproduction through self-division results in posterior and anterior parts. Each fissiparous population tends to have their own fission plane located in various places of the, e.g. one third of the body length, anteriorly, in *Holothuria leucospilota* (Purwati 2004); 44% of the body length, anteriorly, in *H. atra* (Chao, Chen et al. 1993); or at the mid-body in *H. parvula* and *H. surinamensis* (Crozier 1917; Kille 1942). Each part resulting from fission develops into intact animal.

Fission stimulation, in which holothurians are induced to divide, has gotten more attention in the last two decades. The technique is relevant for overfished areas in which the population size no longer supports successful fertilisation. Successful fission stimulation doubles the initial number of individuals through a low-cost and low-technology procedure. The method can be used by coastal communities (Purwati and Dwiono 2008).

Fission stimulation has been proven fruitful in both fissiparous and non-fissiparous holothurians. Among those reporting on this field are Reichenbach et al. (1996), Purwati and Dwiono (2007), Laxminarayana (2006) and Razek et al. (2007). The experiments conducted mainly imitate the asexual reproduction occurring in sea cucumber natural habitats, with introduction of a single fission plane. The individuals resulting from fission show high rates of survival and rebuild new tentacles and anal pores in 2–3 months. In the case of induced fission on non-fissiparous holothurians such as *H. arenicola* and *Bohadschia marmorata*, expression of a great potential of regeneration seems more relevant than asexual reproductive capacity.

Up to now, only a single fission plane per individual was considered in induced fissions, the fission plane being similar to that occurring naturally. Learning from induced fission on non-fissiparous species, we deduced that the natural fission plane in fissiparous holothurians may not be the only area of division and that it may therefore be possible to shift fission planes and to multiply them. Thus, one individual could be induced to divide on more than one plane in a single event. This would be a breakthrough in terms of physiology and reproduction, and it would also benefit population enhancement efforts.

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Material and methods

We have previously reported successful fission stimulation on *H. atra*, in which we doubled two groups of 10 and 30 individuals in two separate experiments with survival rates 85% and 100% respectively (Purwati and Dwiono 2005, 2007; Dwiono et al. in press).

To prove that the position of natural fission plane can be shifted, in July to September 2007 we stimulated 20 individuals of *H. atra* at two positions: at 1/3 and at 2/3 of the body length starting from the anterior. The initial body weight of the stimulated specimens was 35–143 g. Similar procedures of inducement and rearing as those described in Purwati and Dwiono (2005) were followed. A rubber pipe used as inlet air of bicycle wheels (available in most areas of Indonesia) was used to tie each holothurian's body tightly. Each part obtained — A (anterior, mouth part), P (posterior, anal part) and M (middle part, with no mouth and anus) — was about the same length. During stimulation, the holothurians were kept in buckets with fresh sea water and slow aeration. No food was added.

After division, each of three parts (A, P and M) was kept in a bucket, then packed in a net and hung in the water column at about 7 m depth. This hanging method has proven to be effective (Dwiono unpublished report). The morphological appearance of the fission planes was observed weekly to monitor the reformation of tentacles and anal pores, in addition to the survival rate. Regeneration of the M individuals was observed at both fission planes. The experiment was terminated when most of the individuals redeveloped the anal pore and/or tentacles. By this time, feeding was necessary and rearing techniques had to be changed.

Regeneration intensity (R) was measured as follows (X indicates A, M or P individuals):

R(X)(expressed in %) = (number of regenerating individuals / total number of individuals) x 100%

Results

Failure occurred in the first week after fission, when one M individual did not survive. Among animals that survived, the wounds took three weeks to heal, during which the fission plane closed and showed principally a concave surface. Regeneration, which was characterised by a convex surface or a protrusion of the fission plane, started in week 3, 4 and 6 after division for groups A, P and M, respectively. Regeneration reached 100% in week 9 for A individuals and in week 7 for P individuals.

In A and P individuals, anal pore or tentacle reformation began after 7 to 9 weeks, while M individuals needed more time — between 8 and 11 weeks (Fig. 1). The recovery of the wound on the fission plane took longer in M individuals, while regeneration time was shorter.

New anal apertures in A individuals had not developed until week 7, concurrently with the appearance of new tentacles in P individuals. In week 8, one of M individuals regenerated anal pores, and another two grew tentacles. Among them, one individual had both new tentacles and an anal aperture.

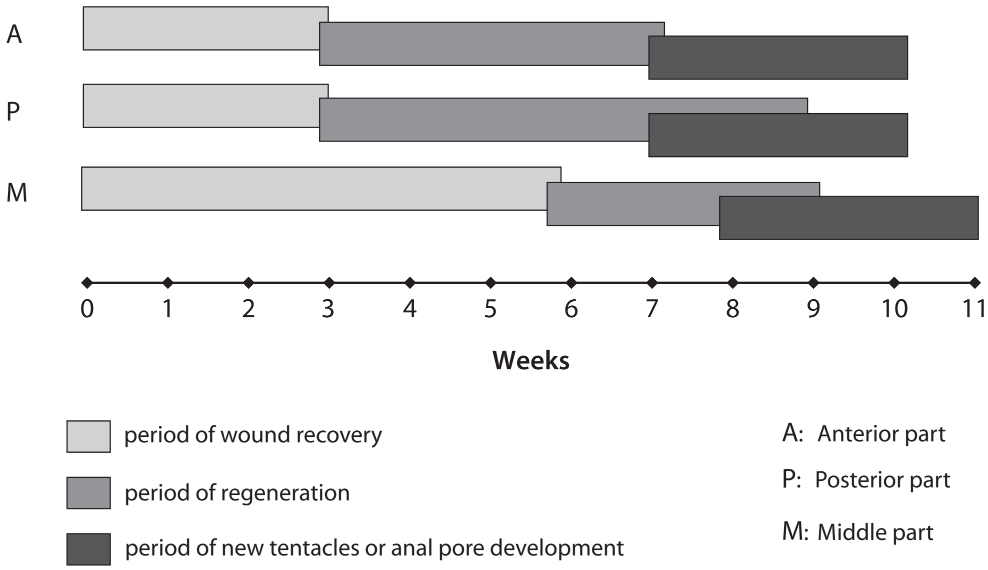

Figure 1. Regeneration intensity of A, P and M individuals.

Table 1. Time of appearance of new tentacles (t) and anal pore (a). The numbers in the table give the percentages of individuals.

Time (in weeks)	**7**		**8**		**9**		**10**		**11**	
	t	a	t	a	t	a	t	a	t	a
Individuals (%)										
Anterior	▨	10	▨	15	▨	30	▨	40	▨	85
Posterior	5	▨	35	▨	65	▨	95	▨		▨
Middle			10	5	45	15	50	30	85	55

In week 9, another 10 M individuals reformed both tentacles and anal pores, and another 6 grew new tentacles (Table 1). It seemed that tentacles grew slightly earlier than the anal pore. When the experiment was terminated after week 11, 29 out of 30 M individuals originated from 10 parents survived; the overall survival rate was 96.67%.

Discussion

Although echinoderms are not at the base of the animal phylogeny with groups in which self-division or other asexual reproduction (budding etc.) is very common (e.g. Porifera, Cnidaria), they share some characters with these basal groups such as asexual reproduction (Carneveli 2006). And even though natural fission demonstrates that there exists a single fission plane with one specific position in each fissiparous species, stimulated fission shows that the position of the natural fission plane may not be important.

Success of reformation of external alimentary organs may not be separated from internal body tissues. Mesentaries are tissues that keep the position of the anterior alimentary organs (oesophagus, stomach and the anterior part of small intestine) and connect them to the middorsal interadius. Hyman (1955) and Bai (1994) reported that the dorsal mesentery is responsible in the development of the missing digestive organs of individuals resulting from fission. Accordingly, redevelopment of the alimentary duct would occur as long as the applied fission stimulation and rearing procedures do not cause any damage to the mesentary tissue.

Nature must have its own strategy in having one single fission plane in asexual reproduction. During the process, there must be a guarantee that each of the parts resulting from fission will develop into a normal individual afterward; consequently, the aim of reproduction in maintaining and enhancing the population is fulfilled. Survival at the individual level in asexual reproduction by fission is higher than in sexual reproduction as there is no critical larva stage, no partner needed and a minimised predatory pressure (Purwati 2002; Purwati 2004). When natural fission results in more than two individuals, the middle parts, which carry neither mouth nor anal complex, are probably the weakest in terms of regenerating body parts as they need more energy and time to redevelop both anterior and posterior complexes. Based on this assumption, the success of fission inducement should be measured through the rate of survival and the time required to regrow into a complete individual. The present experiment demonstrated good results and the redevelopment of middle parts was not much different from that of the anterior and posterior parts.

Anterior and posterior individuals recovered in 7 weeks, which was similar to the experiment results of Purwati and Dwiono (2007), and relatively earlier compared to the individuals in one tie stimulation by Dwiono et al. (2008). Dwiono et al. (2008) measured drained body weight in their weekly measurement and this process mayhave stressed the animals and delayed the development of the missing organs. In the current experiment, disturbance on individuals was minimised.

As a comparison, regeneration period in *H. parvula* (excluding gonad regrowth) has been reported to be 3 weeks (Kille 1942) and reports from other experiments on the same species state that posterior individuals regenerate faster than anterior ones. The individuals start to feed after 2 months (Emson and Mladenov 1987). In *Stichopus chloronotus* and *Thelenota ananas*, feeding began after 3 and 5 to 7 months, respectively (Reichenbach and Holloway 1995).

When natural fission of *H. atra* and *H. leucospilota* is monitored (Chao et al. 1993; Conand et al. 1997; Purwati 2004), posterior individuals are more likely to stay in their habitat than anterior individuals. It is difficult to determine whether they are being washed away, being prayed upon, or are dying. In stimulated fission, both anterior and posterior individuals survive in great number, which may indicate that the loss of anterior individuals from their natural habitat in natural fission is caused by external factors.

Conclusion

There is no significant difficulty in producing two or three individuals with a survival rate of 90% to 100%. Fission at the laboratory protects the individuals from predatory and extreme environmental changes. In the same time, our experiment showed that every fission product possesses similar capability to become a complete individual. The feasibility of producing four or more individuals is probably a question of energy capacity required to regrow the missing organs, mainly for the middle parts. Survival also depends on the capability of mesentery tissue to act as totipotent tissue. This experiment proved that (*i*) the natural fission plane may no longer be important in fission inducement and can be manipulated, (*ii*) high regeneration capacity may be expected to work on such situations rather than the asexual reproductive process, even in fissiparous species, (*iii*) the induced fission technique can potentially be applied to non-fissiparous holothurian species in order to increase individual numbers.

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