Epigenetic control of pectoral fin web shape in *Lipophrys canevae* (Blenniidae, Teleostei)

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Summary. Between the lower pectoral fin rays of *L. canevae* the fin web shows a saw-like regression. Hypermorphic fin web growth can be induced by fin ray amputation distal to the site of fin web attachment, but not by injuring the fin web alone.
Key words. Teleosts; fin anatomy; development; form control; regeneration.

Lipophrys canevae is a small Mediterranean blenny, living in the surf zone of rocky shores[2]. As is the case for the pectoral fins of all species of the tribe Blenniini, the most ventral four to five fin rays differ from the rest of the fin by a number of characters which serve for clinging to precipitous substrates: 1) the rim of the fin web has a saw-like appearance, because it extends from the tip of a fin ray to the shaft of the upper of two neighbouring fin rays (fig. 1), 2) there is a connective tissue pad of the bony fin ray called the lepidotrichal cord (LC), and 3) there is a cuticular instead of a mucous epidermis[3]. This complex of characters is called 'fin hook'. In *L. canevae* the expression of these anatomical characters is spatially highly correlated: the strongest part of the LC and the cuticle are found on the edge of the fin ray which is left free because of the fin web regression. This correlation could be explained by a sustained epigenetic interaction between these three characters[4]. In particular it is assumed that the presence of LC and/or the cuticle inhibits fin web growth, which may help to maintain the fin web regression. To test this hypothesis the effect on fin web growth of removing most of the LC and the cuticular epidermis was studied.

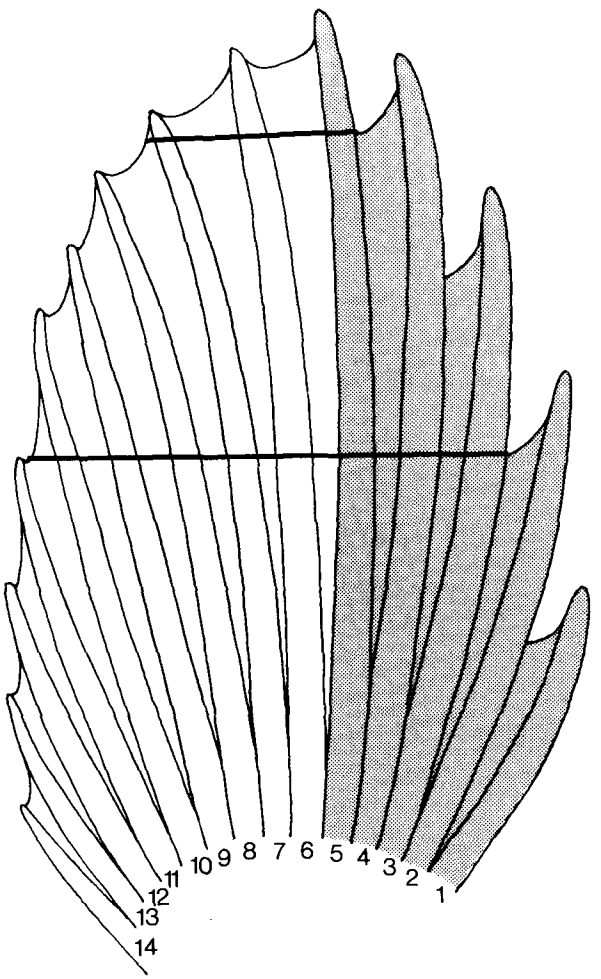

Figure 1. Drawing of the left pectoral fin of a blenny (by B. Y. Misof). The distal tips of the fin rays point to the top of the figure and the ventral rays are found to the right (same orientation as in fig. 2). The part of the fin containing the fin hook's typical characters is hatched. Note the asymmetric regression between the five most ventral fin rays. The dark lines indicate the levels of amputation in the two kinds of experiments performed (see text). The numbers at the bases of the fin rays are those used to identify fin rays in this study.

16 blennies from Rovinj (Yugoslavia) were used in the present study (standard length SL = 4.9 to 6.2 cm, mean = 5.6 cm; condition index after experiment CI = 1.28 to 1.8, mean = 1.54). Two types of experiments were performed. 1) In eight individuals the tip of the fifth fin ray was amputated just at the site of fin web attachment (= 78 to 86% of fin ray length, mean = 82%) (fig. 1). 2) Eight other individuals were likewise amputated at the third fin ray on the left side (= 60 to 78% of fin ray length, mean = 68%) (fig. 1). In addition, the fin web between the second and third fin rays was injured on the right side by two or three small incisions with microscissors to provide a mitogenic stimulus. All operations were performed under MS222 anesthesia. The fin ray length and the fin web attachment sites prior to amputation were measured. The animals survived for various periods between 14 and 40 days p.o., and were then killed by an overdose of MS222. The pectoral fins were removed and fixed under a glass slide in a petri-dish with 70% ethanol, and examined under a dissecting microscope.

Regenerative growth of the mutilated fin rays was observed in all experiments and was slightly faster after amputation of the third fin ray (0.071 $\pm$ 0.007 mm/day) than of the fifth fin ray (0.05 $\pm$ 0.003 mm/day). In all but one case of tip amputation of the fifth ray a hypermorphic growth of the fin web was recorded (max = 1 mm, mean = 0.5 mm, inferred from morphometric data). In

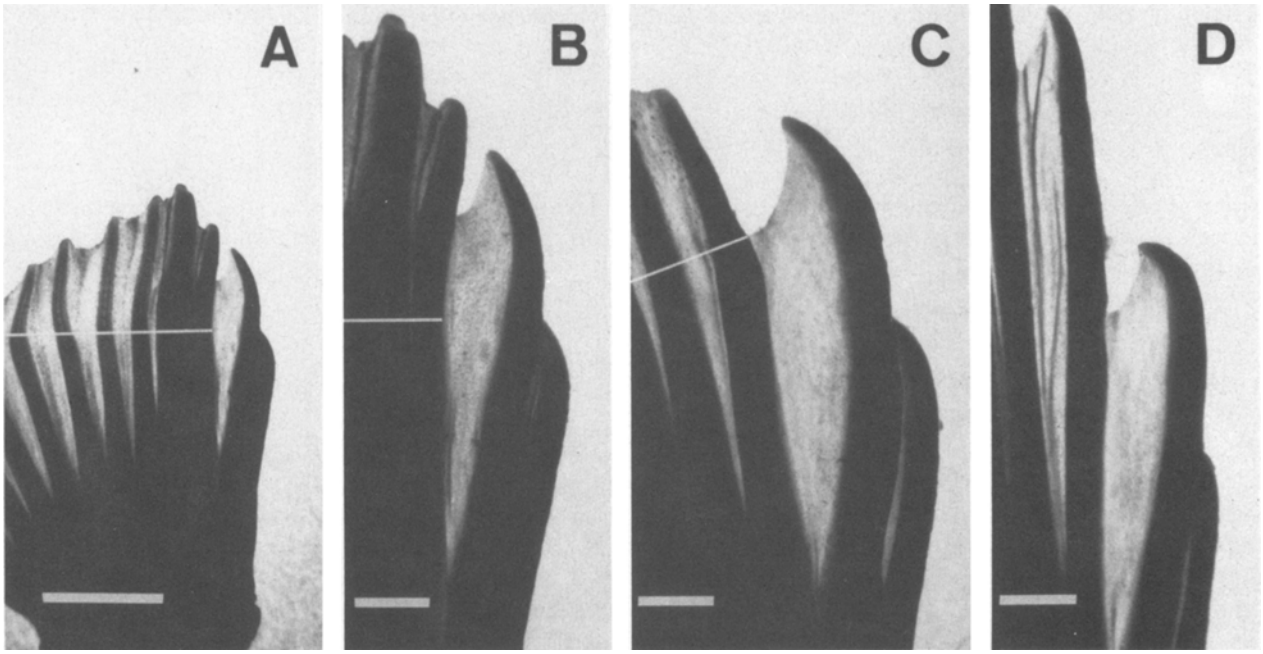

Figure 2. Left pectoral fin of *Lipophrys canevae* three weeks after amputation of the tips of the third and several following fin rays. *A* Overview of the left pectoral fin (bar = 3mm). The distal tip of the fin points to the top of the figure whereas the proximal part of the fin points to the bottom of the figure. the level of amputation is shown by the white line and can also be seen by the slight bend in the distal parts of the fin rays at the left of the figure. The fin web between the second and the third fin ray is spread. *B* Distal parts of the first, second and third fin ray from *A* at higher magnification (bar = 1mm). The level of amputation is indicated by a white line. Note that the fin web between the second and third ray extends distal to the level of amputation, although at the time of amputation the fin web was attached at the site of amputation. *C* As in *B* the first, second and third fin ray three weeks after amputation of the tip of the third and the following fin rays (bar = 1mm). In this case no hypermorphic growth of the fin web is recorded. *D* First, second and third fin ray of the fin which is contralateral to the one shown in *C* (bar = 1mm). The fin web was injured to provide a mitogenic stimulus, but no amputation was performed. According to morphometric data, the fin web does not extend beyond the original site of attachment.

the experiments with tip amputations of the third fin ray the site of amputation was easily recognized by a morphological discontinuity of the fin ray structure (fig. 2). Taking this as the point of reference, hypermorphic growth could be detected in five out of eight specimens (max = 1.4 mm, mean = 0.7 mm, excluding negative results) (fig. 2). On the contralateral side, where the fin web was injured but the fin ray tip had not been amputated, hypermorphic growth was observed only in one case out of eight.

As shown by the control experiments a mitogenic stimulus alone is not sufficient to induce hypermorphic growth. From histogenetic studies of blenny pectoral fin regeneration, it is known that regeneration of the fin hook's typical characters (lepidotrichal cord and cuticle) occurs after the regeneration of the bony fin rays [5]. Hence the hypermorphic growth of the fin web after fin ray tip amputation could be explained by the absence of LC and/or cuticular epidermis in the regenerating fin ray. This finding supports the hypothesis that the correlated expression of the fin hook's typical characters is maintained by a sustained epigenetic interaction between them [4].

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