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Extreme autotomy and whole-body regeneration in photosynthetic sea slugs

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Autotomy, the voluntary shedding of a body part, is common to distantly-related animals such as arthropods, gastropods, asteroids, amphibians, and lizards[1,2]. Autotomy is generally followed by regeneration of shed terminal body parts, such as appendages or tails. Here, we identify a new type of extreme autotomy in two species of sacoglossan sea slug (Mollusca: Gastropoda). Surprisingly, they shed the main body, including the whole heart, and regenerated a new body. In contrast, the shed body did not regenerate the head. These sacoglossans can incorporate chloroplasts from algal food into their cells to utilise for photosynthesis (kleptoplasty[3]), and we propose that this unique characteristic may facilitate survival after autotomy and subsequent regeneration.

Five in 15 (33%) laboratory-bred individuals of a morphotype of *Elysia* cf. *marginata* species complex (see Supplemental Experimental Procedures), and a field-collected individual of another morphotype of *Elysia* cf. *marginata*, autotomised at their neck position during lifetime (Figure 1 and Methods S1E). One individual even autotomised twice. In all cases, the pericardium region, including the heart, was left completely in the autotomised body (Figure 1A). Furthermore, the shed body was highly likely to contain the kidney, intestine, and most of the reproductive organs[4]. The head, separated from the heart and body, moved autonomously, even immediately after autotomy (Figure 1B and Video S1). The wound closure of the head was completed within a day, and the heads of relatively young (226–336 days after hatching; N = 3) individuals started to feed on algae within a few hours. Surprisingly, they started regeneration of the heart within 7 days (Figure 1B) and completed regeneration of the whole body within ~20 days (Figure 1C,D). The heads of older individuals (480–520 days after hatching; N = 2) did not feed and died in 10 days. The bodies moved, reacting to tactile stimuli, for several days to months (Methods S1E). However, none of the bodies regenerated the head. The bodies gradually shrank and became pale, apparently from losing chloroplasts, and eventually decomposed. The beating of the heart was visible just before the body decomposed.

Likewise, among 82 individuals of *Elysia atroviridis* that were field-collected and parasitised by the copepod *Arthurius* sp. (Figure 1J), three (4%)

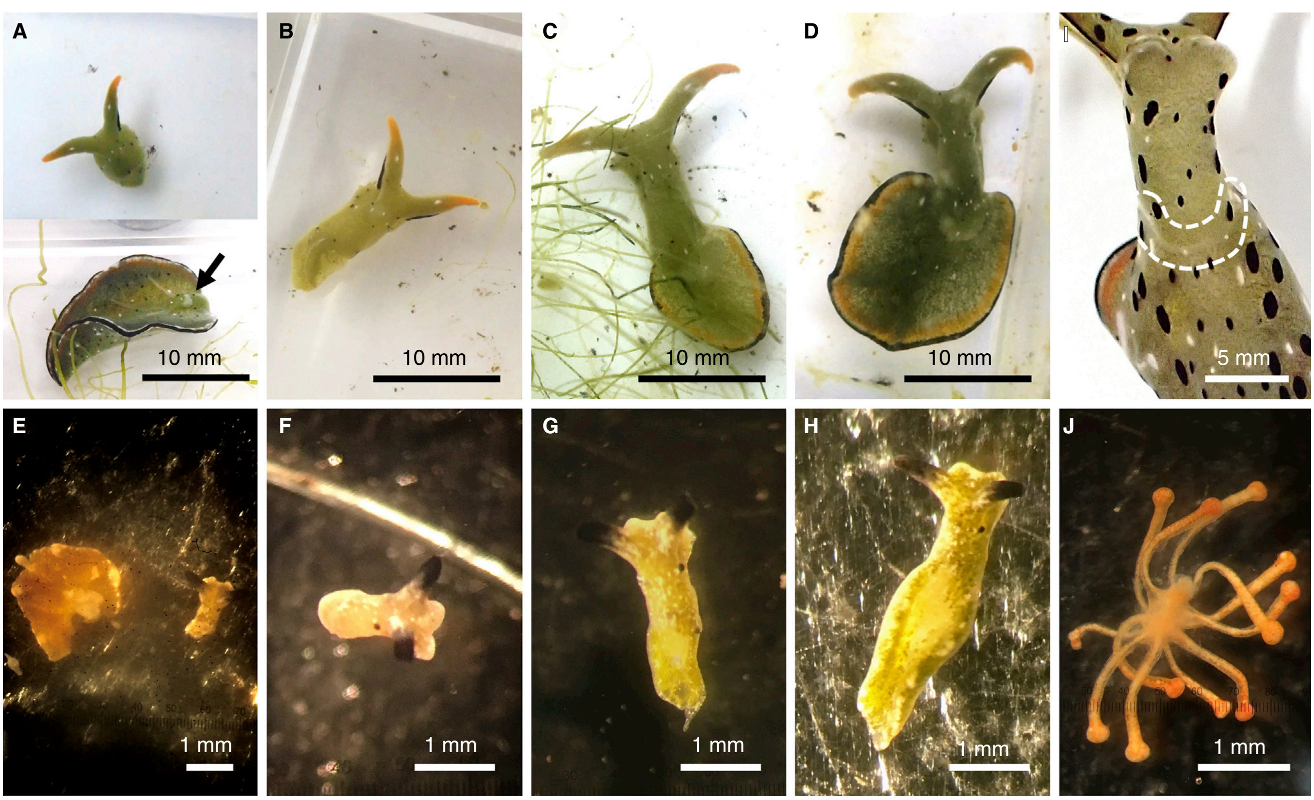

Figure 1. Autotomy and regeneration of body in *Elysia* cf. *marginata* (A–D) and *Elysia atroviridis* (E–H).
(A) Head and body of *Elysia* cf. *marginata* (individual no. 1) just after autotomy (day 0), with the pericardium (heart) remaining in body section (arrow). (B) day 7, (C) day 14, (D) day 22, showing whole-body regeneration. (E) Head and body of *Elysia atroviridis* (individual no. 1) just after autotomy (day 0). (F) day 3, (G) day 14, and (H) day 28. (I) Healthy individual of *Elysia* cf. *marginata* with a ‘breakage plane’ at their neck (dotted line). (J) *Arthurius* sp., the internal parasitic copepod of *Elysia atroviridis*.

autotomised at their neck position (Figure 1E and Methods S1E). Two of them regenerated their bodies within a week (Figure 1F–H). The shed bodies moved, reacting to stimuli as in *E.* cf. *marginata*, and by dissection it was confirmed that they contained the parasite. In addition, other 39/82 (48%) individuals gradually lost part of their bodies, presumably by autolysis, without autotomy during rearing, and discharged the copepods. Among them, 13 (33%) regenerated the lost body part, but the remaining died without regeneration. In contrast, none of 64 parasite-free individuals collected simultaneously autotomised any body part.

These two sacoglossan 'species' have a transverse groove at their neck, which appears to be a predetermined 'breakage plane' (Figure 1I). We gently tied this part using a fine nylon string in six individuals of *Elysia* cf. *marginata* (Methods S1A–E). Five of them autotomised at the tied position within a day (Methods S1E). The tied position of the remaining individual shifted slightly lower, and it autotomised at the neck 9 days after treatment. Their autotomised bodies represented 80–85% of the total weight. In contrast, imitated predator attacks, like pinching of the head and cutting of the parapodia (lateral extensions), did not induce autotomy (see Supplemental Experimental Procedures).

Both *Elysia* cf. *marginata* and *E. atroviridis* shed the main body, including the heart. Some other sea slugs also autotomise, but they shed minor body parts such as tails, parapodia, or dorsal papillae[1]. Other invertebrates (e.g., cnidarians, planarians, and asteroids) can regenerate their main body following division. Also, some amphibians are known to have a high regeneration capacity, including tails, limbs, eyes, and even the heart ventricle[5]. However, autotomy in this study is remarkable in that animals with complex body plans can survive even if they lose the main body, including the heart, and subsequently regenerate the whole lost area. The reason why the head can survive without the heart and other important organs is unclear. We have succeeded in a complete rearing of *Elysia* cf. *marginata* for multiple generations — thus, they can be used as a model system for studying autotomy and regeneration of the body.

The function of this large-scale autotomy remains unclear at present. Most animals, including some sacoglossans[1,2], are considered to autotomise to escape predation. However, adult sacoglossans generally have few predators due to their cryptic coloration and presence of toxic chemicals incorporated from their food[3,6] (but see Nakano *et al.*[7]). Our experiments also showed that autotomy of *E.* cf. *marginata* took several hours, which is not effective to avoid predation, and that imitated predator attacks did not induce autotomy. Therefore, their autotomy is unlikely to function as predation escape. We suggest that removing internal parasites is a likely function of autotomy, at least in *E. atroviridis*. All autotomised individuals (and autolysed ones as well) had a parasite, and after regeneration they became parasite-free. The parasites occupy most of the main body of *E. atroviridis* and strongly inhibit its reproduction during lifetime (data not shown). Thus, removing such parasites by autotomy likely enhances the host's reproductive success. *Elysia* cf. *marginata* did not appear to have parasites in our culture, but this species complex has another congeneric parasite[8]. Such 'parasitic autotomy' has been shown in earthworms to counteract a protozoan parasite[9]. Alternatively, but not mutually exclusive to this idea, autotomy by the sacoglossans may function to escape from being tangled in algae or to remove accumulated toxic chemicals.

The body shedding of the sacoglossans appears to, firstly, have a defensive function (to eliminate endoparasites); secondly, occur by an intrinsic mechanism; and thirdly, be a controlled loss. All of these are conditions often used to distinguish autotomy from other losses of body parts[1]. Why these sacoglossans can regenerate their body even if they lose most organs remains unclear, but we suspect involvement of kleptoplasty. In *Elysia*, a highly branched digestive gland is spread over the majority of its body surface, including the head, and the gland is lined by cells that maintain ingested algal chloroplasts[4]. Thus, these sacoglossans can obtain energy for survival and regeneration from photosynthesis by kleptoplasts, even when they cannot digest food[10]. Further investigation of autotomy in these and other sacoglossans will elucidate its function, mechanism, and evolutionary origin.

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures, methods file, and one video and can be found with this article online at https://doi.org/10.1016/j.cub.2021.01.014.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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