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Disposable penis and its replenishment in a simultaneous hermaphrodite

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Although it is often thought that sexual selection is weaker in simultaneous hermaphrodites than in gonochorists, some simultaneous hermaphrodites exhibit bizarre mating behaviour. In the simultaneously hermaphroditic nudibranch *Chromodoris reticulata*, we found a peculiar mating behaviour, wherein the nudibranch autotomized its penis after each copulation and was able to copulate again within 24 h. To have sufficient length to be replenished for three copulations, the penis is compressed and spiralled internally. No other animal is known to repeatedly copulate using such 'disposable penes'. Entangled sperm masses were observed on the outer surface of the autotomized penis, which is equipped with many backward-pointed spines. There is a possibility that the nudibranch removes sperm already stored in a mating partner's sperm storage organ(s).

1. Introduction

Several hypotheses have been proposed regarding the evolutionary conditions promoting simultaneous hermaphroditism [1]. The most prevalent hypothesis postulates that low population density and low mobility favour simultaneous hermaphroditism, conditions where sperm competition may be of minor importance. However, hermaphrodites frequently live under high-density conditions. Furthermore, multiple mating is common in many species [2], indicating that sperm competition may be as important in hermaphrodites as it is in gonochorists. Consistent with this, many hermaphrodites have evolved sperm storage organs or specialized means of digesting excess sperm [3], and a cryptic choice may be performed by the 'sperm recipient (female role)' [4,5].

Because almost all nudibranchs (Gastropoda: Opisthobranchia) are simultaneous hermaphrodites whose female reproductive organs store allosperm donated from mating partners, there is a high potential for sperm competition. Nudibranchs perform both the 'male role' of donating sperm to a mating partner and the 'female role' of receiving sperm from the partner simultaneously during copulation. Nudibranchs generally have two sperm storage organs in the female reproductive system, a bursa copulatrix and a seminal receptacle, and they can digest allosperm in the bursa copulatrix (gametolytic organ) [6]. This enables the possibility of post-copulatory female choice or cryptic female choice. Among nudibranchs, the morphology of reproductive organs is extremely diversified, and the configuration and connection of each pouch differ among groups [6]. However, there have been few detailed studies of the relationship between mating behaviour and the morphology of reproductive organs, particularly from the perspective of sexual selection, and little is known about sperm competition in nudibranchs. In this study, we describe an extremely peculiar mating behaviour, wherein a nudibranch autotomizes its penis after copulation and can copulate again within a day, and we discuss the advantage of this behaviour in sexual selection.
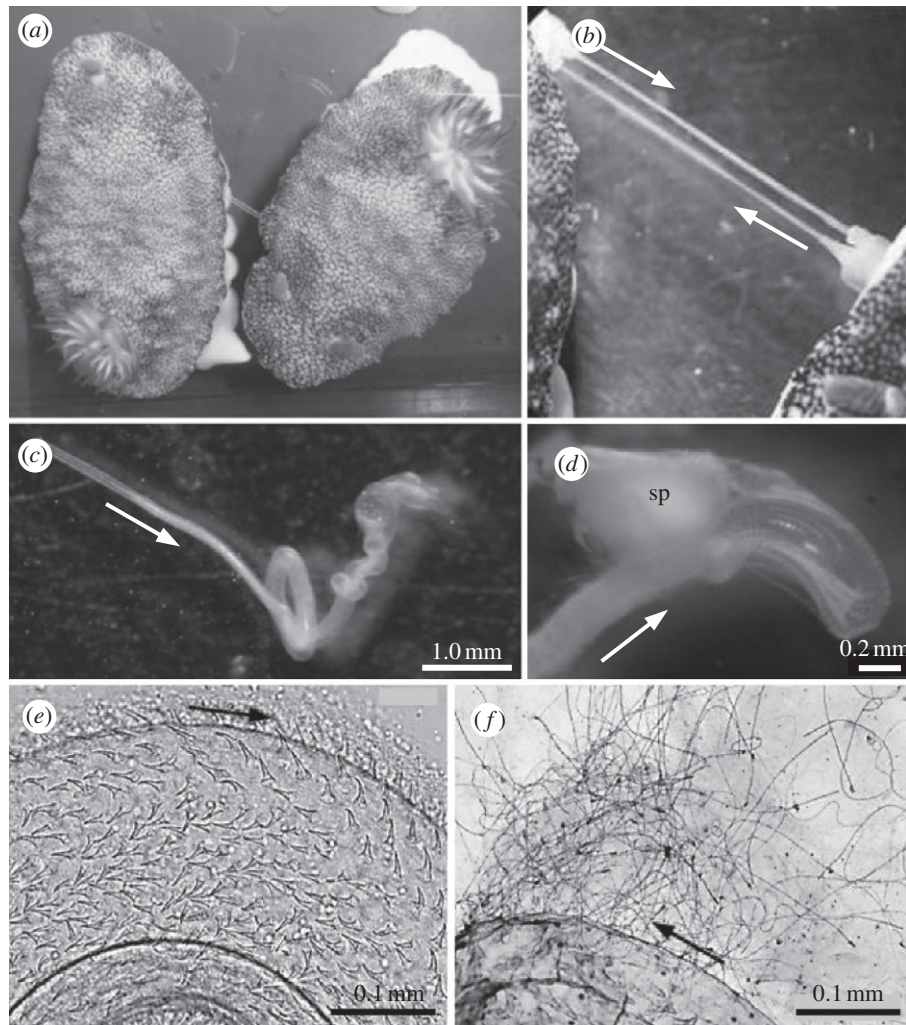
Figure 1. Mating and morphology of the penis in *Chromodoris reticulata*. (*a,b*) Two individuals reciprocally insert their penis into the partner's vagina. (*c,d*) Autotomized penes after copulation; the tip of the penis is swollen. A mass of sperm (sp) is attached to the thorny penis in (*d*). (*e*) Surface of the penis is equipped with innumerable backward-pointed spines. (*f*) Sperm are entangled in the spines. Autotomized penis stained with haematoxylin after being air-dried on a glass slide. Arrows show the direction of penis insertion.

2. Material and methods

(a) Subject animal and maintenance

Chromodoris reticulata is a non-selfing, simultaneously hermaphroditic sea slug (see the electronic supplementary material, figure S1). Both partners provide sperm during copulation. The animals were collected by scuba diving during their reproductive season, from April to June, in 2005, 2006, 2009 and 2010 in shallow coral reefs (2–7 m depth) in the vicinity of the Sesoko Station of the University of the Ryukyus, Okinawa, Japan (26°38′ N, 127°52′ E). Collected animals were maintained individually in perforated, small plastic cases sunk in a seawater tank.

(b) Experimental tanks (observations of mating behaviour)

For observations, we placed two individuals that had been isolated individually for over 24 h since collection in the experimental tank (27 × 9 × 6 cm; length, breadth, height). We observed the sequence of their mating behaviour and timed copulating duration (31 pairs). Furthermore, we set up matings between an individual, that had recently copulated during the experiment and another individual that had been isolated for 24 h or longer since collection or its last copulation, to determine the time interval between two copulations (108 pairs).

(c) Anatomical observations of reproductive organs

After behavioural observation, we observed the morphology of the male reproductive organs in cleared samples ($n = 7$). We histologically examined 8 μm-thick tissue sections of the vas deferens, composed of the penis and prostate gland, stained with Mayer's haematoxylin and eosin ($n = 5$). We measured the length and area of each part of the male reproductive organs on photographs, using image-editing software (NIS-Elements, Nikon, Tokyo).

3. Results

(a) Penis autotomy

We observed 31 reciprocal copulations. After each copulation, the penes were elongated and autotomized. In a typical case, two individuals encountered and touched each other with their genital orifices and then projected their copulatory apparatus after they separately crawled on the bottom and side of an experimental tank. After two individuals touched with the right side of their bodies, and adjusted the tip of the projected genitalia of the other, each inserted its penis into the partner's vagina and started copulation. After tens of seconds to a few minutes from the beginning of copulation, they pushed away the partner using the buccal bulb and separated with their penes stretched and elongated (figure 1*a,b*). When both individuals advanced further,

Table 1. Elapsed time from preceding copulation and the result of copulatory attempt. (Focal individuals copulated as males again after more than 21 h since previous copulation (χ^2-test, $\chi^2 = 102.598$, $p < 0.01$).)

elapsed time		unilateral		
t (hour)	failure	female role	male role	reciprocal
$0 \leq t < 3$	13	3	—	—
$3 \leq t < 6$	12	4	—	—
$6 \leq t < 9$	14	1	—	—
$9 \leq t < 12$	16	—	—	—
$12 \leq t < 15$	10	—	—	—
$15 \leq t < 18$	3	—	—	—
$18 \leq t < 21$	6	—	—	—
$21 \leq t < 24$	—	—	3	11
$24 \leq t < 27$	—	—	4	8

one individual removed its own penis from the partner's vagina. After a few minutes, the other individual also removed its penis and their copulation ended. After copulation, both individuals crawled with their elongated penes remaining outside the body. Finally, they autotomized and discarded their penes (figure 1c). The mean duration of copulation was 9.50 ± 5.05 min (mean $\pm$ s.d.; $n = 31$). In each observation of copulatory behaviour, *C. reticulata* exhibited penis elongation and autotomy. Penis autotomy occurred 20.57 ± 7.04 min (mean $\pm$ s.d.; $n = 9$) after unplugging the penis from the partner's vagina.

(b) Remating

Behavioural observations ($n = 108$) revealed that *C. reticulata* needed approximately 24 h after the previous copulation to copulate reciprocally. When we paired a focal individual that had copulated less than 9 h before with another individual that had been isolated for 24 h or longer since the last copulation, the focal animal never copulated reciprocally and only eight copulated unilaterally in the female role ($n = 47$; table 1). Focal animals copulated reciprocally ($n = 19$), or unilaterally in the male role ($n = 7$), 22.4 h after the first copulation or later. In one case, we observed three successive copulations each separated by approximately 24 h.

(c) Morphology of autotomized penes and reproductive organs

The surface of the autotomized penis was equipped with numerous backward-pointed spines (figure 1e). The tip of the autotomized penis was somewhat swollen (figure 1c,d), and sperm were entangled in the spines (figure 1f). In some cases, a sperm mass was attached to the penis (figure 1d).

When the vas deferens was fixed 24 h or later after copulation and observed in wholemount, the inner duct of the middle region of the penis was compressed and coiled loosely (figure 2; $n = 4$). Individuals that had been fixed immediately after copulation had no spiral part of the penis ($n = 3$). The average length of the internal penis, including the spiral part, in cleared samples was 30.7 ± 10.0 mm
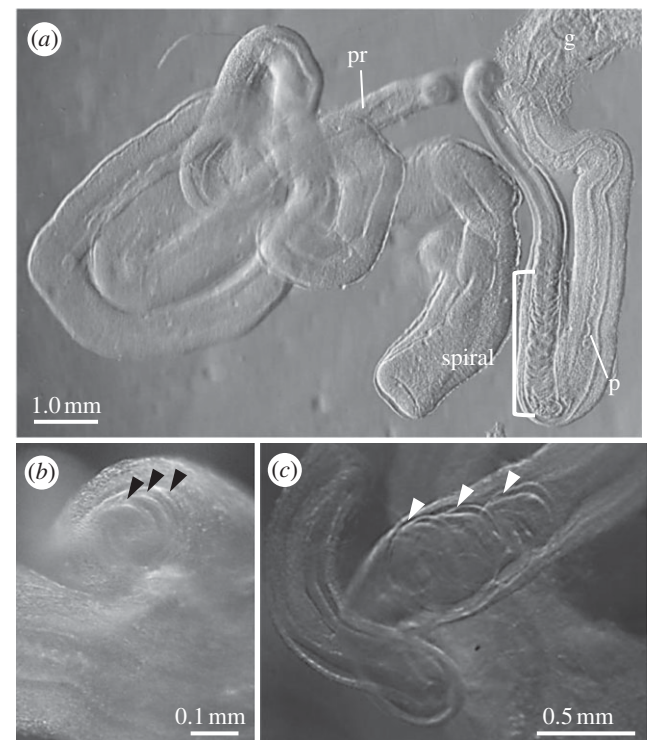

Figure 2. Vas deferens of an individual later than 24 h after copulation. (a) Compressed spiral structure is seen in the middle region of the inner duct of the penis (solid line). (b) An oblique view and (c) a longitudinal view of the spiral structure (arrowheads) are shown. g, genital orifice; p, penis; pr, prostate.

($n = 4$). The average length of an autotomized penis was 10.9 ± 1.5 mm ($n = 10$).

The arrangement, size and shape of the cell nuclei differed among the proximal spiral part of the internal penis, the distal part near the genital orifice of the internal penis, and the autotomized penis. Although the distal part and the autotomized penis had similar characteristics, the proximal part was different from the two regions. While the area of the nuclei significantly increased in order from the spiral part ($60.6 \pm 14.3\ \mu m^2$; average $\pm$ s.d., $n = 32$), to the distal part ($90.9 \pm 17.1\ \mu m^2$, $n = 31$) and autotomized penis ($131.7 \pm 30.5\ \mu m^2$, $n = 36$) ($p < 0.05$, Steel–Dwass test), the

cell density increased in reverse order (see the electronic supplementary material, figure S2).

4. Discussion

Although the penes of individuals that had been fixed and cleared at least 24 h since their last copulation had a spiral structure, those that had been fixed immediately after copulation did not. This suggests that the spiral structure is important in the autotomy and replenishment of the penis. On the basis of the average length of the internal penis, including the spiral structure, and that of the autotomized penis, *C. reticulata* penes were sufficiently long for at least three copulations. This corresponds with the observation of an individual that copulated three times over three consecutive days. The shapes and arrangements of cell nuclei were similar between the distal part of the penis and the autotomized penis, suggesting that only the distal part of the internal penis is available for copulation as the 'next penis'. The spiral part of the internal penis appears unready for immediate copulation. Since cell density in the penis decreased and nucleus size increased towards the distal part of the internal penis, we propose that the tissue at the spiral part of the penis is compressed and undifferentiated, gradually differentiating into the 'next penis'. When the distal part of the penis elongates to be used for copulation, part or all of the spiral structure seems to be uncoiled at the same time. After the penis is autotomized, it may need approximately a day for the spiral structure to be available for copulation.

The surface of the penis in *C. reticulata* was covered with numerous small, backward-pointed spines, and a large amount of sperm was entangled in the spines. One possibility is that *C. reticulata* performs sperm replacement using the thorny penis in the same manner as some damselflies, notably Zygoptera, such as *Calopteryx maculata* [7], which use a hook-shaped structure located at the tip of the male copulatory organ to remove the allosperm of previously copulating males already stored in a copulatory pouch [8]. Damselflies remove allosperm by voluntarily moving the hook-shaped copulatory organ. However, the penis in nudibranchs is not a muscular organ, as shown in the electronic supplementary material, figure S3, and thus it is impossible to move each spine voluntarily in *C. reticulata*. Another possibility is that excess sperm from the current donor was pushed out by the recipient and was attached to the penis. DNA analysis of the removed sperm is needed to verify its origin. The transformation and extension of the penis to insert deep into the copulatory pouch, and the development of backward-pointed spines, make it difficult for the penis to be withdrawn. This difficulty must be the essential reason for the particular penis autotomy.

Although various animals are known to autotomize parts of the body, only a few animals have been found to autotomize their penes or male reproductive appendages. For example, the hectocotylus of *Argonauta* [9] and the pedipalp of some orb-weaving spiders [10] are autotomized to facilitate transferring spermatophores. Furthermore, a periwinkle [11] sheds its penis after its reproductive season, probably to save the cost of maintenance. The reproductive organ is used differently in these animals compared with nudibranchs. Apophallation in *Ariolimax* [12] would instead have a common point that this land slug and *C. reticulata* sacrifice their penes under competitive conditions with rivals in reproduction. Different from *C. reticulata*, penis loss in *Ariolimax* is caused by the mating partner, and the land slug cannot regenerate its penis. *Chromodoris reticulata* may compensate for the short-term cost of decreased reproductive opportunities caused by the loss of a penis with the reproductive advantage gained by sperm displacement under severe sperm competition. They further minimize the loss of reproductive opportunities by preparing the 'next penis' using the spiral structure to shorten the interval until the next possible copulation. Thus, the 'disposable penis' in *C. reticulata* provides an example where sexual selection is as important for simultaneous hermaphrodites as it is for gonochorists.

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