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NUPTIAL FEEDING IN THE FRESHWATER SNAIL *POMACEA CANALICULATA* (GASTROPODA: AMPULLARIIDAE)

Silvana Burela* & Pablo R. Martín

Laboratorio de Ecología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, (8000) Bahía Blanca, Argentina

ABSTRACT

The males of the freshwater snail *Pomacea canaliculata* have a copulatory apparatus consisting in a delicate penis running through a muscular channeled sheath bearing three different glands. The outer gland is the most conspicuous one and often secrets a sticky drop of mucus during copula. However, its function is enigmatic because it opens toward the exterior of the pallial cavity. During laboratory trials performed to register copula duration and mating behaviour, we observed that females of *P. canaliculata* eat this secretion with approximately two deglutition events on average during the copula. This behaviour can be interpreted as a nuptial feeding, possibly functioning as an additional male mating effort to entice the female to remain in copula for long periods. To our best knowledge, there are no previously registered cases of nuptial feeding in gastropods.

Key words: *Pomacea canaliculata,* copula duration, apple snail, nuptial feeding, mating effort, paternal investment.

INTRODUCTION

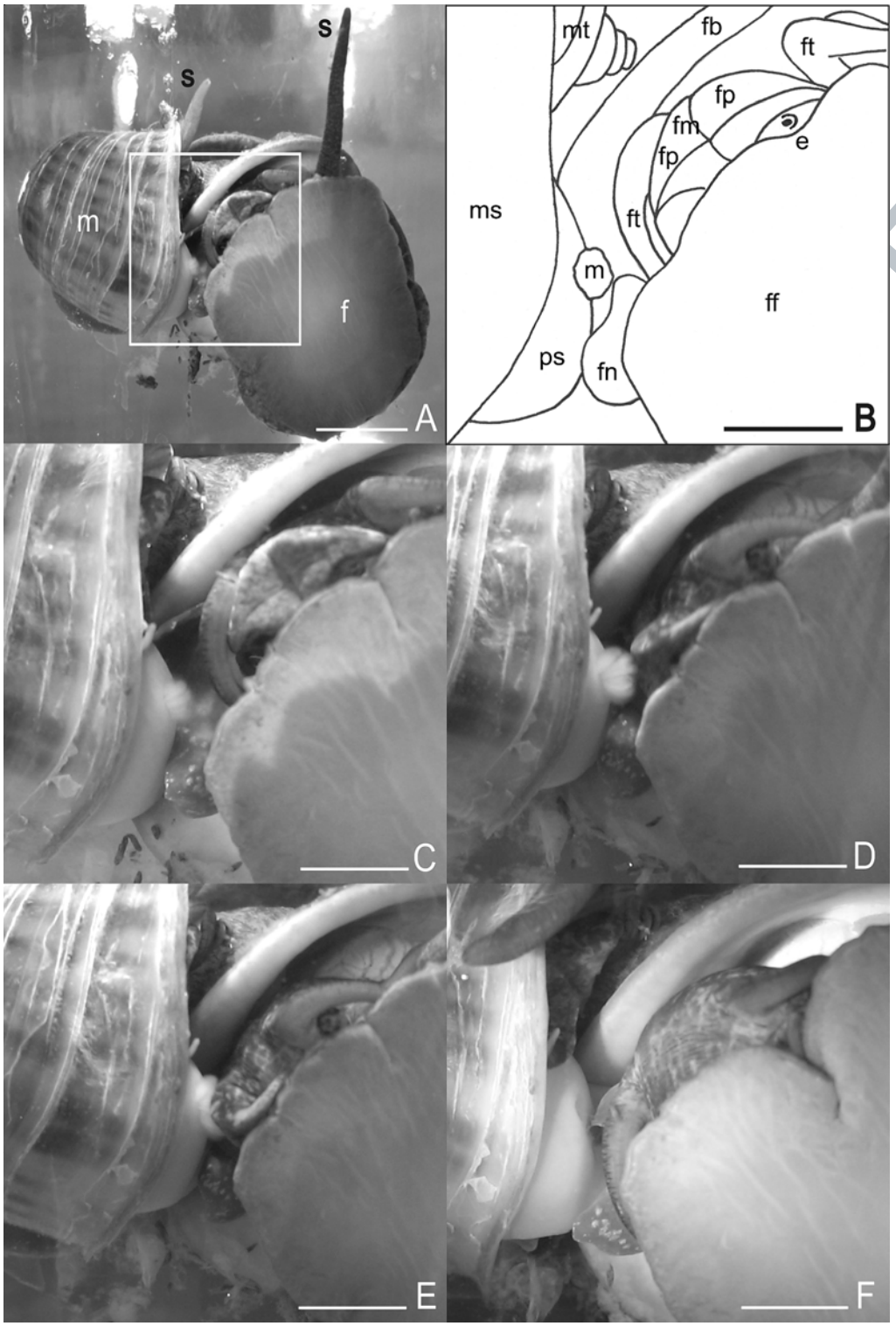
Pomacea canaliculata (Lamarck 1822) is a South American freshwater snail that like other members of the genus has become an invader, promoting ecosystem changes in natural wetlands (Carlsson et al., 2004) and a serious rice pest in Asia (Cowie, 2002) during the last two decades. This gastropod is dioecious, oviparous, and shows a long lasting copula (10–18 h) and a multiple mating behaviour, at least under laboratory conditions (Andrews, 1964; Albrecht et al., 1996).

Males have a complex copulatory apparatus that occupies the roof margin of the pallial cavity and consists of a muscular penis sheath with a deep channel on its inner face along which the penis runs during copula (Andrews, 1964; Gamarra-Luques et al., 2006). The penis sheath presents three macroscopic glands: the apical gland, the marginal gland (on the right margin of the channel), and the outer gland (on the basal portion). The outer sheath gland is the most bulky and conspicuous, showing an external slit-like opening slightly to the left of the medial sheath line. The secretion of the apical and marginal glands apparently acts in penis sheath adhesion and in the lubrication of the penis (Andrews, 1964), but the function of the outer sheath gland is enigmatic because it opens toward the exterior of the pallial cavity. During copula, the outer sheath gland often secrets a sticky drop of mucus (Andrews, 1964; Berthold, 1991). Andrews (1964) suggested that this secretion could act as a repellent for the males that remain near the mating couple. The aim of our paper is to report laboratory observations that clarify the functional role and the evolutive significance of this gland and its secretion in the mating behaviour of this snail.

METHODS

The experimental snails (males: 31–43 mm shell length, females: 41–49 mm) were brought to the laboratory in March 2005 from Curamalal stream, Buenos Aires Province, Argentina (37°14'31"S, 62°08'04"W), kept in a rearing room at 25 ± 2°C, under 14 h light/day and fed with lettuce *ad libitum*. Snails were sexed by the shape of the shell aperture and operculum (Estebenet et al., 2006). Laboratory trials were performed in 3 litre glass aquaria with water at 24–26°C and permanent

*Corresponding author: sburela@criba.edu.ar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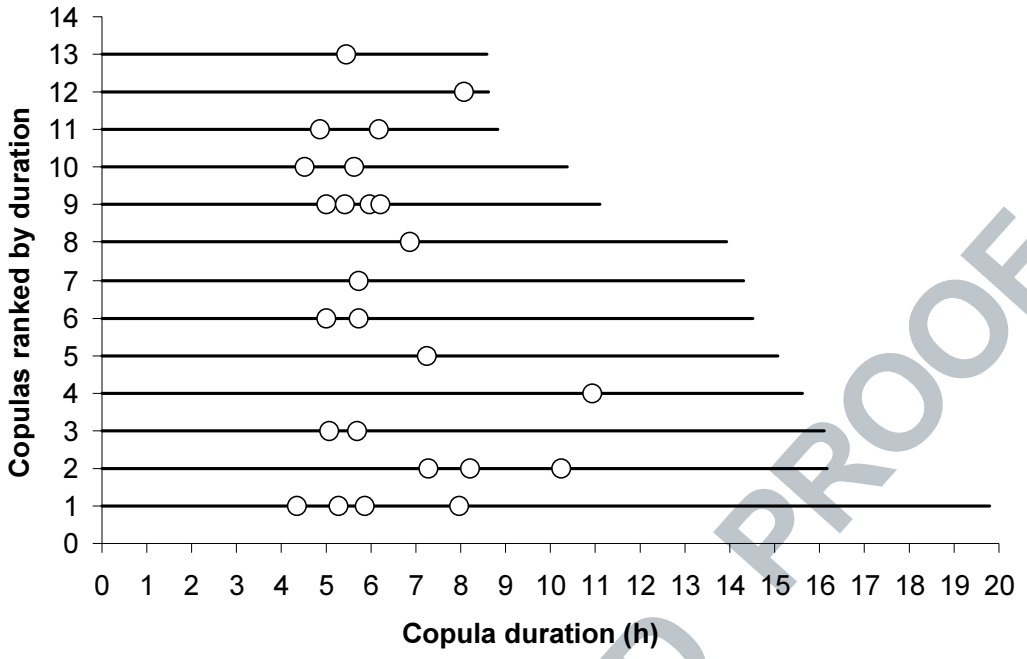

FIG. 2. Scheme showing the different copula duration and the deglutition events observed. Each bar represents one copula and its length the duration in hours. The circles indicate each mucus deglutition event.

illumination in order to observe the mating behaviour of isolated couples. The snails were observed continuously for 24 h in order to register the start, the end and any particular copula behaviour. During the copula the females remain mostly attached to or crawling on the aquarium walls, thus allowing the observation of the mating couple. We considered that the copula had begun when the male inserted the penis sheath into the female cavity, because it is seldom possible to observe the penis passing through the penis sheath channel (as described by Andrews, 1964).

RESULTS

During the courtship the male mounts the female adhering his foot to her last shell whorl, generally oriented toward the right side of her aperture (Fig. 1A). The male usually remains in this position up to 2 h. The female generally shakes her shell when the male adopts a closer position, unfolding his penis sheath to insert it into the female pallial cavity. If the female does not shake the male off, the copula starts. During most of the process both partners remain motionless with their heads par-

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FIG. 1. Different stages in a mucus deglutition event as seen through the wall of an aquarium (water surface is located towards the upper side of all pictures). A: Typical mating position (female on the right and male on the left) where it is possible to observe a mucus droplet released from the outer penis sheath gland opening; f, female; m, male; s, siphon; scale bar = 20 mm; B: Detailed scheme of the frame in A. fm, female mouth; ff, female foot; fb, female mantle border; ft, female tentacle; fn, female nucal lobule; fp, female palp; e, female eye; ms, male shell; mt, male left tentacle; ps, basal portion of the penis sheath; m, mucus droplet; scale bar = 10 mm; C: First moment in mucus droplet formation; scale bar = 10 mm; D: Detail showing the female turning her head towards the mucus droplet; scale bar = 10 mm; E: Detail showing the mucus deglutition; scale bar = 10 mm; F: End of deglutition event showing the depressed area of the outer sheath gland opening; scale bar = 10 mm.

tially withdrawn, commonly with their cephalic tentacles coiled (Figs. 1A, B). Females can move freely and feed during the copula, but males cannot and depend on the female's movements to go near the water surface to ventilate the aerial lung. The copula ends when the male withdraws his penis sheath, but then he often remains stuck onto the female's shell for around an hour longer with his penis sheath folded and motionless.

During the copula duration trials, we observed various males of *P. canaliculata* releasing a pale salmon coloured mucus from their outer sheath gland opening. Before the secretion begins, the outer sheath gland swells and its opening becomes evident as a depressed area. The secretion seems to be steadily extruded through the opening forming a mucus droplet (Fig. 1C) that is swallowed at irregular intervals by the female. The female turns round her head toward the gland opening (Fig. 1D), expanding her snout and picking up the droplet with her mouth (Fig. 1E) and slowly swallowing it, without the radular movements typical of grazing or ciliary feeding (Cazzaniga & Estebenet, 1984). After each deglutition event, it is possible to observe the depressed slit-like opening of the outer sheath gland before the accumulation of a new droplet (Fig. 1F). These observations of secretion and deglutition events were only possible when the male was loosely attached to the female.

Thirteen out of the 32 copulas observed in the laboratory showed at least one deglutition event, summarising 25 deglutition events (Fig. 2). We observed 1.9 ± 1.12 deglutition events per copula (mean ± S.D.), each of them lasting 5–15 seconds. The mean copula duration observed was 13.31 ± 3.5 h, and the first mucus deglutition was recorded approximately in the middle of the copula (6.18 ± 1.86 h). Out of a set of 56 copula (13 mentioned above and 43 additional copula trials) in which mucus secretion was observed, in only two cases it was not eaten by the female and the mucus formed a thick thread instead of a droplet.

DISCUSSION

Our observations on mating behaviour are in agreement with the descriptions of Andrews (1964) and Albrecht et al. (1996) for *P. canaliculata* from other geographical origins. The mucus release from the outer sheath gland seems to be common, because we have observed it in many mating couples, both in the laboratory and in the field. We believe that the deglutition of this secretion had been previously unnoticed because it is difficult to observe the exact moment in which it takes place. On one hand, the deglutition event occurs in a few seconds and, although the mucus droplet formation can last up to 25–30 minutes, it becomes visible only when it reaches a diameter of 2 mm or more. On the other hand, the deglutition events are often difficult to observe when the male is tightly adhered to the female and his head or the basal portion of the penis sheath are not visible. The female right nucal lobule often hides the basal portion of the penis sheath, blocking the outer sheath gland opening, but we do not know if in these cases it interferes with mucus secretion and deglutition or merely with its observation.

Andrews (1964) and Berthold (1991) had already observed the mucus release in laboratory copulas of *P. canaliculata*, but they never mentioned that the female feeds on it. Berthold (1991) also described the mucus secretion from the external sheath gland in males of *Marisa cornuarietis* (Linné, 1767) but he did not discuss anything about its function. Andrews (1964) reported the mucus release only when a group of males remains near the mating partners, suggesting that it could be acting as a repellent for prowler males. Now this explanation seems unlikely, because the mucus release occurs even in the absence of prowler males.

Nuptial feeding comprises any form of nutrient transference from the male to the female during or directly after courtship or copulation (Vahed, 1998), a definition suitable for the behaviour observed in *P. canaliculata*. Nuptial feeding often takes the form of food gifts, the nutrients being transferred in seminal fluids or spermatophores, as prey gifts captured or collected by the male or as somatic gifts (male's body or male glandular secretions) (Andersson, 1994; Vahed, 1998). To our best knowledge, there are no previously registered cases of this behaviour in gastropods. Love dart shooting in helicids has been considered as a calcium gift to the recipient partner, although currently it is interpreted as a case of reproductive manipulation (Pomiankowski & Reguera, 2001).

Two non-mutually exclusive hypotheses for the possible functions of nuptial feeding have been discussed (Vahed, 1998). In the first, it acts as a paternal investment, and the male increases his fitness by benefiting mainly his own offspring with the nutrients transferred. In

the second, it is part of the male mating effort acting in female attraction, female mating acceptance or in the lengthening of copula to maximise the sperm transference.

Pomacea canaliculata females are able to store the ejaculates in different pouches keeping the sperm fertile for up to 140 days (Estebenet & Martín, 2002). Sperm mixing takes place inside the seminal receptacle of *P. canaliculata* females, and double paternity occurs in a single spawn (Yusa, 2004). The sperm mixing and the possibility of cryptic female choice reduce the chances that a given male can benefit his own offspring through his nuptial gift. Although the mucopolysaccharides detected in the outer sheath gland of *P. canaliculata* (Andrews, 1964) and *M. cornuarietis* (Schulte-Oehlmann et al., 1994) could be nutritious and easily assimilated, the amount transferred is minimal, since each droplet is smaller and lighter than a single egg of *P. canaliculata*, and females can spawn 4,500 eggs on average during their lifetime (Estebenet & Martín, 2002). This makes less plausible the paternal investment explanation in *P. canaliculata*.

On the other hand, the number of eggs spawned by virgin females after a single copula is directly related to the copula duration (unpublished results). The nuptial feeding observed in *P. canaliculata* could be an additional mating effort to entice the female to remain in copula for long periods. Given the possibility of sperm mixing, this strategy could improve the competitive ability of a given male by increasing his sperm numbers relative to other's inside the female reproductive tract (sperm loading hypothesis, García-González & Gomendio, 2004).

Notwithstanding the advances in the knowledge of their reproductive anatomy (Berthold, 1991; Schulte-Oehlmann et al., 1994; Gamarra-Luques et al., 2006), for most ampullariid species details of mating behaviour are unknown. Albeit anatomically diverse, a presumably homologous outer sheath gland is present in all the Neotropical genera – *Pomacea, Marisa, Pomella, Asolene, Surinamia and Felipponea* – and in the Old World genus *Pila* (Berthold, 1991; Bieler, 1993). Probably the deglutition of the mucus from the outer sheath gland reported here for *P. canaliculata* is not an exception in the genus or the family, though a range of complexity in nuptial feeding behaviour can certainly be expected.

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