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Love will tear us apart: traumatic mating through consumption of body parts in a sea slug

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There is growing evidence that "traumatic mating," a copulation that involves wounding the partner's tegument (Lange et al. 2013), is a widespread phenomenon. Traumatic mating may have evolved associated with several distinct fitness benefits, from physical anchorage during mating to fertilization advantages (Lange et al. 2013). Currently, two hypotheses explain how traumatic mating may lead to fertilization advantages. First, the trauma inflicted during mating may be "collateral," a by-product of another strategy associated with reproductive success (e.g., Morrow et al. 2003). For example, the injection of secretions that manipulate the partner's physiology generally involves piercing by sharp structures (e.g., love darts in snails; Chase and Blanchard 2006). Second, the trauma itself may be adaptive (Johnstone and Keller 2000, Morrow et al. 2003). For instance, the wound would induce a refractory period in the partner, which would avoid further mating and, thus, further damage. This scenario would benefit the sperm donor by increasing its short-term fertilization success because the number of subsequent sperm competitors would be reduced (Michiels 1998, Johnstone and Keller 2000). While there is increasing empirical evidence for collateral harm in traumatic mating (e.g., Hotzy and Arnqvist 2009), adaptive harm has been supported on theoretical grounds only (e.g., Morrow et al. 2003).

Although also present in gonochorists, traumatic mating is far more prevalent in hermaphrodites (Michiels 1998, Lange et al. 2013), which may accept further copulations even when traumatic mating is costly (Michiels and Koene 2006). Most sea slugs are simultaneous hermaphrodites, i.e., individuals that produce functional male and female gametes simultaneously (Michiels 1998). While studying the reproductive behavior of sea slugs in the coast of São Paulo state, Brazil, during May–June 2016, we found specimens of *Phidiana lynceus* Bergh, 1867 (Fig. 1a), an "eolid" nudibranch with a narrow body and numerous finger-like dorsal projections called cerata (Fig. 1a). When two individuals were placed together in a Petri dish with seawater, we were astonished by their aggressive behavior, which at first seemed agonistic (Fig. 1a, b). However, at the end of the interaction, both individuals had a pack of sperm near their genital pores, a clear evidence of reciprocal mating. To analyze this behavior, pairs of possible sexual partners were randomly selected from the 10 specimens collected and recorded under a stereomicroscope equipped with a high-definition video camera.

All encounters ($N = 5$) resulted in mating with reciprocal sperm transfer and rather consistent behavioral pattern (i.e., except where noted, the described behavioral components were observed in all matings). First, one partner (hereafter called "follower") followed and contacted the other using its oral tentacles. Then, the second turned to the follower and made contact using the oral tentacles. Although it was difficult to observe the precise moment when the penises were everted due to the ceratal movement, a few seconds after the follower has made contact its penis was already everted (Video S1: 00:00:13). Subsequently, they aligned the right side of each other (Fig. 1a), allowing contact between their gonopores. Once they approached, they started to pluck and ingest cerata from each other (Fig. 1a, b; Video S1: 00:00:24; 00:01:12), although sometimes the cerata were only ruptured and their contents sucked through the wound (Video S1: 00:01:47). Histology revealed the ingested cerata within the stomach of the specimens (Fig. 1c; Appendix S1: Fig. S1). Both partners directed their cerata toward each other in an apparent reaction to cerata consumption (Video S1: 00:01:00). Nevertheless, they maintained their mating alignment, and started to move in a circle, while simultaneously attempting to ingest cerata from their partner and repel these attacks by directing their own cerata (which have stinging organelles at their tip) against their partner. The penises stayed everted throughout the interaction, occasionally intertwining with each other, and sometimes separating and making seemingly random movements. At the beginning of one mating event, we observed the tip of the penis

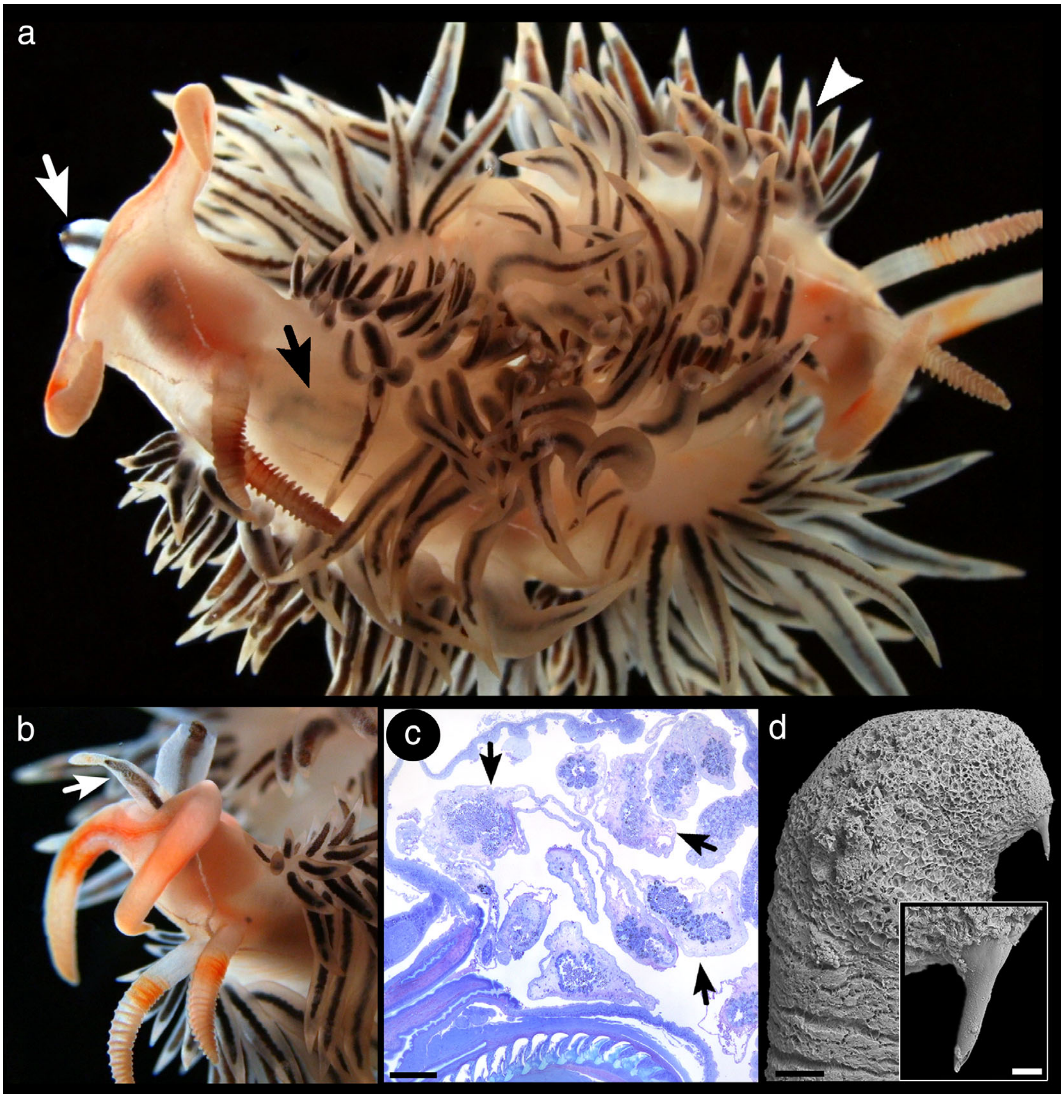

FIG. 1. Traumatic mating through cerata consumption in *Phidiana lynceus*. (a) Two individuals (approximately 15 mm long alive) during mating. The arrows point to cerata either being ingested (white arrow) or already within the stomach and visible through translucency (black arrow); the arrowhead points to intact cerata. (b) Ceras being ingested through the mouth (white arrow). (c) Histological section stained with toluidine blue, showing cerata within the stomach (black arrows). See also Appendix S1: Fig. S1 for a comparison between the histology of intact and ingested cerata. (d) Scanning electron micrograph of the penis distal region; the inset shows the stylet at the tip. Scale bars: (c) 500 μm, (d) 100 μm, (inset) 20 μm.

of one partner being thrust on the dorsum of the other for 16 s (Video S1: 00:00:29). Under scanning electron microscopy, the tip of the penis was shown to bear a stylet (Fig. 1d). It is unknown if the stylet could inject substances into the partner's body wall during mating (i.e., "traumatic secretion transfer"; Lange et al. 2013), since the penis has a glandular aspect (Fig. 1d) that deserves investigation. The interaction took 4 minutes and 10 s on average (minimum 3 minutes 20 s; maximum 4 minutes 54 s) and ended with sperm exchange, a process lasting a few seconds and observable due to the translucency of the penis. Sperm was deposited on the gonopore as a compact viscous mass (Video S1: 00:02:10).

A recent classification proposed three types of traumatic mating (Lange et al. 2013), including both intra- and extragenital damage. The type that would better represent the behavior of *P. lynceus* is extragenital "traumatic penetration" (Lange et al. 2013), a mechanism not associated with injection of secretions ("traumatic secretion transfer") or sperm ("traumatic insemination"). Studies aiming to test adaptive vs. collateral harm have mainly used species with intragenital traumatic mating as models and, in those cases, traumatic mating was associated with collateral harm (Hosken et al. 2003, Morrow et al. 2003, Hotzy and Arnqvist 2009, Hotzy et al. 2012). Choosing a model species to test the adaptive hypothesis is difficult, because most of the known cases of traumatic mating are intragenital and suspected to be associated with collateral harm (e.g., Lange et al. 2013). For example, the wound caused by the injection of substances or sperm into the partner tegument generally indicates collateral harm, because injected substances are known to manipulate the partner (Hotzy et al. 2012), or because sperm is injected nearer to the site of fertilization to counteract sperm competition (competition among male gametes from different partners for the fertilization of an individual's ova; Parker 1970) (Lange et al. 2013). The known cases of traumatic penetration of the genital tract also generally point to some degree of collateral harm (e.g., penile spines involved with an anchoring function or with displacement of previous sperm; Hotzy and Arnqvist 2009). The only type of traumatic mating for which it is difficult to envisage some obvious form of collateral harm is extragenital traumatic penetration. Being extragenital, this trauma would not be related to the direct manipulation of the female genital tract; and providing it does not involve injection of substances nor sperm, it would not be related to directly manipulating the partner's physiology nor be associated with avoiding sperm competition within the genital tract, respectively. However, this type of traumatic mating seems rare, particularly in hermaphrodites (Lange et al. 2013). The discovery of extragenital traumatic penetration in *P. lynceus*, therefore, opens doors for further experimentation to test adaptive harm.

Dissection of the specimens revealed that a mean of 13 cerata (minimum 6; maximum 26; $N = 7$ slugs) were ingested by each partner during their single mating event in the laboratory. In 7 out of the 10 individuals, cerata could be quantified, but not in the remaining three, in which the ingested cerata were in an advanced state of digestion. Considering that each individual had a mean of 152 cerata externally after the mating event (minimum 120; maximum 215; $N = 10$), ~8% of their cerata may be ingested per each mating on average. Although mating frequency is unknown for *P. lynceus*, all individuals readily mated once put together, as is usual for sea slugs (e.g., Karlsson and Haase 2002, Wyeth and Willows 2006). Therefore, cerata consumption during mating in *P. lynceus* should not be negligible.

Under the adaptive harm hypothesis, cerata consumption in the context of mating would enhance the fertilization success of the wound inflictor either by extending the remating interval of the partner, or reducing its survival perspectives, which would result in reallocation of resources toward reproduction and in the use of the available gametes (Michiels 1998, Johnstone and Keller 2000). Also, as usual for sea slugs, *P. lynceus* bears a sperm storage organ (Marcus 1957) and thus is potentially capable of storing sperm from many partners, which, consequently, would increase the pressure of sperm competition. Therefore, delaying remating would also benefit the sperm donor by reducing that pressure. Additionally, cerata consumption could presumptively provide the consumer with some benefits through the consumed cerata.

Cerata are blood-filled tubes (Appendix S1: Fig. S1) that contain branches of the digestive gland, playing important roles in gas exchange and digestive processes (Behrens and Hermosillo 2005). Eolid nudibranchs, which generally feed on cnidarians, can store intact nematocysts (organelles usually with stinging function) from their preys at the tip of their cerata (Appendix S1: Fig. S1), discharging them in their own defense (Goodheart and Bely 2016). Therefore, the costs of losing cerata during mating would not be restricted to the costs of the wounds, since the partner would also be partly deprived of important organs for gas exchange, digestion, and defense.

Interestingly, nudibranchs are known to use ceratal autotomy as a defensive mechanism (Goodheart and Bely 2016). Moreover, they can regenerate these structures; in *Phidiana crassicornis*, for example, complete ceratal regeneration takes ~24 d (Miller and Byrne 2000). If cerata regeneration is similar in *P. lynceus*, and considering the mean of 8% of cerata loss during each mating event, then a mating frequency higher than one copulation per 2 d would deprive the individual of complete cerata. These results thus suggest that the costs of traumatic mating should be quite substantial for *P. lynceus*.

Further studies should test if this behavior is indeed associated with adaptive harm. Would there be other benefits? Nuptial gifts, the donation of nutrients during mating, are generally predicted to be ineffective in simultaneous hermaphrodites, because, for example, of the potential risk of benefitting a future rival (Michiels 1998). Sexual cannibalism, too, seems not the case, since it generally implies the death of partner (Schneider 2014). Alternatively, this behavior may be sexually mutualistic, e.g., considering that the nudibranch cnidome (i.e., the set of nematocyst types of a given species) may vary according to their diet (Goodheart and Bely 2016), cerata consumption during mating may enable increasing the diversity of nematocysts of each partner.

Although the function of cerata consumption during mating remains uncertain, this finding clearly adds to the complexity of mating strategies in hermaphrodites. Traumatic mating is pervasive in hermaphrodites and ranges from traumatic insemination to traumatic secretion transfer (Lange et al. 2013). Although occasional "apophallation" (act of severing the partner's penis) was described for terrestrial slugs (Leonard et al. 2002), the behavior reported here for *P. lynceus* is unique regarding the nonlethal ingestion of extragenital body parts of the partner during mating in hermaphrodites.

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