Natural History Miscellany

The Way of the Samurai Snail

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Sexual selection favors strategies that enhance the fertilization chances of sperm donors, and sexual conflict can ensue when these adaptations negatively affect sperm recipients (Arnqvist and Rowe 2005). Such conflict often causes counteradaptive coevolution that can result in bizarre mating behaviors in separate-sex (Arnqvist and Rowe 2005) and hermaphroditic species (Michiels and Newman 1998; Koene et al. 2005). For simultaneous hermaphrodites, dart shooting is perhaps the best known of these sexually selected behaviors, and a recent comparative study supports morphological counteradaptive coevolution (Koene and Schulenburg 2005; Schilthuizen 2005). However, everything that is known about dart-shooting behavior is based almost entirely on a single species, the garden snail *Cantareus aspersus* (formerly *Helix aspersa*). The inclusion of other dart-bearing species in evolutionary studies may enable more general, broader inferences. Therefore, here we provide the first description of the unique dart-shooting behavior performed by the Japanese snail *Euhadra subnimbosa*.

Dart shooting in *C. aspersus* consists of stabbing a 9-

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mm-long calcareous structure through the partner's skin, which results in transfer of mucus from the dart-sac-associated glands into the partner's blood (Adamo and Chase 1990). Previous research demonstrated that the dart increases fertilization success of the sperm that are transferred after shooting (Chase and Blanchard 2006), most likely by reducing sperm digestion (Koene and Chase 1998). This is evidently beneficial for the shooter. However, we wondered whether there are any behavioral adaptations that might improve the dart's effect. One strategy might be to reuse darts rather than dispose of them, as does *C. aspersus*.

In the best-studied family, the dart is bladed and shot once into the partner, where it stays behind in the skin (Helicidae: e.g., *C. aspersus*, *Helix pomatia*). However, some species seem to stab their partner repeatedly with the same dart (Bradybaenidae: *Bradybaena similaris stimpsoni*, Emura 1932; Helminthoglyptidae [syn. Xanthonychidae]: *Helminthoglypta* spp., Webb 1942; *Polymita muscarum*, B. Reyes-Tur and J. M. Koene, unpublished data). In order to understand how such behavior fits with what is known about dart evolution, we investigated dart shooting in *E. subnimbosa* (Bradybaenidae) and several congeners.

Once mating partners of the species *E. subnimbosa* have positioned themselves in the face-to-face mating position and inserted their penes simultaneously, both of them stab their dart repeatedly (2.52 stabs/s) and continue to do so for nearly 1 hr. The dart pierces so deep into the partner's right side that its tip comes out through the partner's foot. The average individual stabs its partner a staggering 3,311 times (fig. 1A, 1B; video available in the online edition of the *American Naturalist*), continuing even as the copulants withdraw so the darts no longer penetrate the skin (fig. 1C). At this point in the mating, it becomes clear that every sheathing of the dart passes it afresh through a mucus reservoir of the dart gland (see fig. 1C). The advantage, therefore, to the stabbing snail might be that the transfer of more mucus confers greater paternity, probably via a reduction of sperm digestion in the receiving snail. Dissections confirm that the dart is not lost after shooting
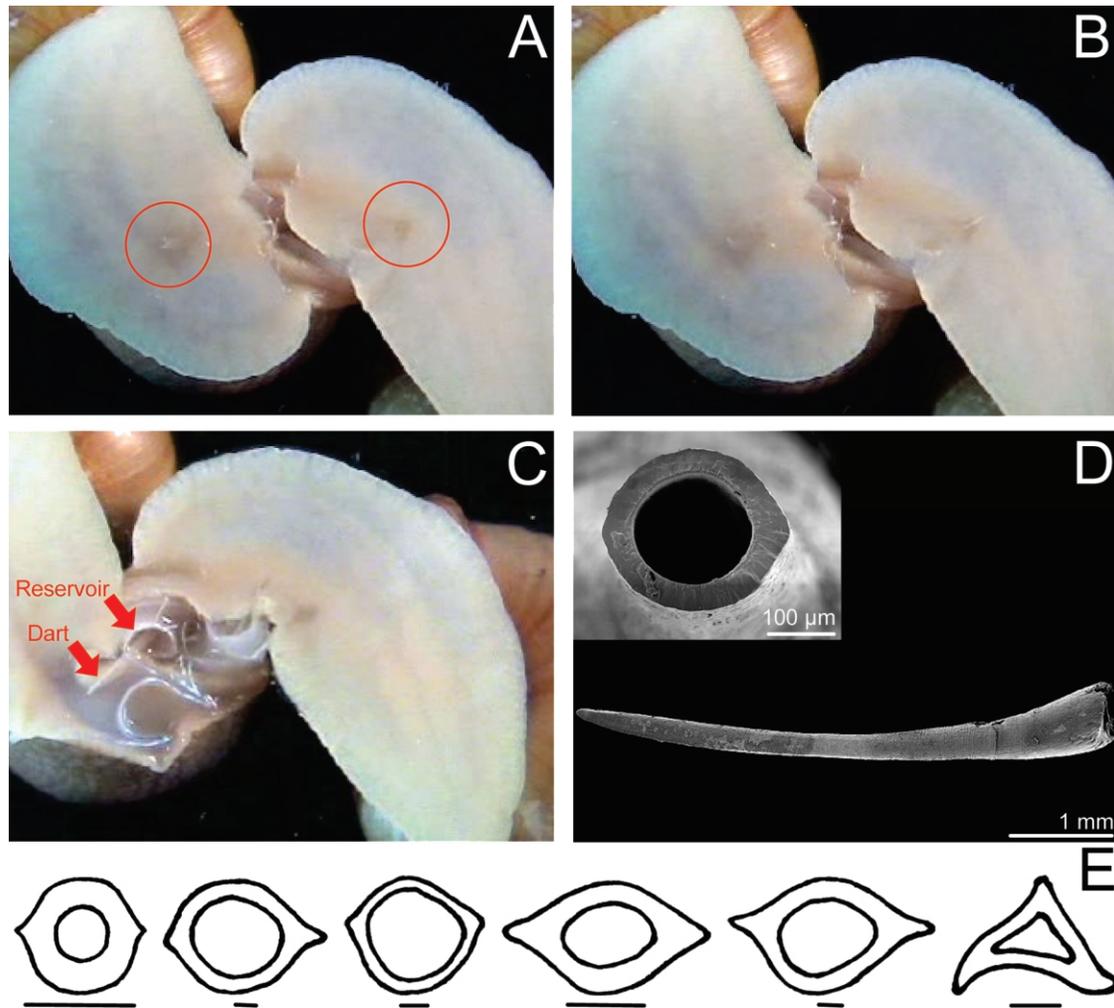
Figure 1: Dart-shooting behavior and love dart of the simultaneously hermaphroditic Japanese land snail *Euhadra subnimbosa*. Note that animals are seen from below through a glass plate because dart shooting is not visible from above. The shooting phase takes up 56.6 ± 10.7 min (*N* = 11 pairs) of the 153.0 ± 24.1 min that total copulation lasts. *A, B,* Early stage of dart shooting, when the dart is penetrating the partner's skin (lasting 21.9 ± 16.0 min, resulting in 3,311 ± 2,419 stabs). The frames are 200 ms apart and taken from a digital video sequence (see below). The repeated and forceful stabbing results in a hole that extends from the right side through to the bottom of the foot. The partner's dart can be seen coming out through the foot of each snail (*A, circles*). *C,* Later stage of dart shooting when the dart no longer penetrates the partner's skin (lasting 37.6 ± 10.7 min). The dart and mucus reservoir at the base of the dart sac, the latter permanently everted during the shooting phase, are clearly visible. *D,* Electron microscopic photographs showing a side view and cross section of the love dart, illustrating that it is half the size of *Cantareus aspersus*'s dart and has no prominent surface-enlarging blades. *E,* Line drawings of cross sections of the love darts of—from left to right—*Bradybaena similaris, Euhadra sandai, Euhadra peliomphala, Euhadra brandtii, Euhadra quaesita,* and *Euhadra latispira*. Black scale bars indicate 100 μm. A video, available in the online edition of the *American Naturalist*, shows a mating pair of the Japanese land snail *Euhadra subnimbosa* seen from below, sitting on a glass plate. The partners repeatedly stab each other, which results in a hole that extends from the right side through to the bottom of the foot. The tip of the partner's dart can be seen coming out through the foot of each snail at regular intervals. Note that the video plays in real time.

and that the spermatophore is transferred only after dart shooting (*N* = 7 pairs).

We also compared dart shooting of several closely related species (*B. similaris, Euhadra sandai, Euhadra peliomphala, Euhadra brandtii, Euhadra quaesita,* and *Euhadra latispira*). They all use their darts repeatedly on their partners before sperm transfer. Interestingly, the number of stabs is highest when darts have tiny blades (over 3,000 times in *E. subnimbosa,* fig. 1*D;* 900 in *B. similaris,* and 1,200 in *E. sandai,* fig. 1*E*) and is below 100 when prominent blades are present (e.g., *E. quaesita* and *E. latispira;* fig. 1*E*). Moreover, looking at what is known about dart-

shooting behavior in families within the superfamily Helicoidea partly reveals how the different shooting types evolved. Like Bradybaenidae, Helminthoglyptidae (syn. Xanthonychidae) also shoot repeatedly and have very simple, bladeless darts that are not lost (Webb 1942; B. Reyes-Tur and J. M. Koene, unpublished data). Interestingly, both families are ancestral to the Helicidae (e.g., Koene and Schulenburg 2005), denoting that repeated shooting may be more ancestral than the disposable dart shooting of the Helicidae. Evidently, the most parsimonious ancestral shooting type would then be a single stab with a reusable dart. Whether this is the case or not could be confirmed by investigating dart-shooting behavior of the phylogenetically older Hygromiidae, a family that has remained unexplored to date (Koene and Muratov 2004).

Previous work already demonstrated that elaborate darts and glands coevolved, most likely to optimize mucus transfer (Koene and Schulenburg 2005). Now, besides reporting a striking reproductive behavior, we show that the available behavioral data indicate that repeated stabbing with a simple, reusable dart is more ancestral. This finding highlights that sexual selection plays a crucial role in the evolution of courtship and mating behaviors of simultaneous hermaphrodites, as it does in species with separate sexes.

Acknowledgments

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