

REVIEW ARTICLE

The love-darts of land snails: integrating physiology, morphology and behaviour

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ABSTRACT

Several land-snail species of the helicoid and limacoid superfamilies possess one or more love-darts, which seem to have evolved as a result of conflict over the fate of donated sperm and/or as a way to select the most fit sperm donor. A love-dart is a calcareous stylet used during mating encounters to pierce the partner's body wall. When used, it carries accessory gland mucous products that influence the partner's physiology. Most of the knowledge on the effects of the glands' mucus derives from a single well-studied species, *Cornu aspersum*, in which the mucus increases the male reproductive success of the dart user. However, detailed descriptions on the use of the dart are limited to just a few other species. Hence, here we compare physiological, morphological and behavioural aspects concerning love-darts in several dart-bearing species. Patterns in the use of the dart are identified according to family and we discuss the coevolution of the morphology of the dart and anatomical traits of the reproductive system. The reported physiological effects caused by the dart's mucus suggest a common function of the dart in increasing male reproductive success. Nevertheless, caution is needed when generalizing the use and effects of the love-dart, which are predominantly based on one model organism.

INTRODUCTION

Sexual selection drives the evolution of traits that favour an organism in the competition for reproductive success (Darwin, 1871). The two sexes face this competition differently, since their reproductive contributions differ. In general, females invest more resources, leading them to be the more selective sex (Trivers, 1972). On the contrary, males produce relatively cheaper gametes and their reproductive success depends on how many eggs they are able to fertilize; for this reason they compete with each other for access to females or their eggs (Bateman, 1948). To succeed better in reaching their divergent reproductive goals, males and females have evolved sexually-selected traits involved in pre- and post-copulatory processes. Males have, for example, evolved antlers to challenge other males prior to copulation and fast-swimming sperm to promote high paternity during sperm competition within the female's genital tract after copulation (e.g. deer: Kodric-Brown & Brown, 1984; cichlids: Fitzpatrick *et al.*, 2009, respectively). In contrast, females select the fittest males before mating and often, if promiscuous, develop ways to control sperm usage after copulation (called cryptic female choice; reviewed by Eberhard, 1996). For instance, many species have evolved multiple sperm-storage organs that potentially enable them to favour the paternity of eggs by certain males

(e.g. Hellriegel & Ward, 1998). Females can also remove received sperm immediately after mating by either ejecting it from their reproductive tracts or by digesting it in specialized organs (e.g. feral fowls: Pizzari & Birkhead, 2000; *Succinea putris*: Dillen, Jordaens & Backeljau, 2009, respectively).

While these traits help the sexes to serve their respective reproductive interests, their sexual optima do not always coincide. As a result, the two sexes are often in conflict over the optimal outcome of a reproductive encounter (Arnqvist & Rowe, 2002). Such sexual conflict can lead to the evolution of traits that are beneficial for one sex but harmful for the other. Commonly cited examples are grasping devices of males used to force females into mating and mating plugs that are placed inside the female's genital tract to prevent a subsequent copulation with a rival male (e.g. water strider: Arnqvist, 1989; bumble-bee: Sauter *et al.*, 2001, respectively). From this perspective, antagonistic coevolution between the sexes over the manipulative traits of males and the mate-selection traits of females is expected. An interesting example of such antagonistic coevolution is found in seed beetles in which males have spines on their penes that damage the female tract (Crudgington & Siva-Jothy, 2000; Hotzy & Arnqvist, 2009). A comparative analysis by Rönn, Katvala & Arnqvist (2007) revealed that females belonging to species in which males have more elaborate penial spines had a thicker

connective tissue layer in the vagina, which has been proposed as a counter-adaptation to protect them from injury.

In the context of sexual selection, simultaneous hermaphrodites have been relatively neglected because it was long supposed that sexual selection could not act on them. This was due to either their limitation in expressing sex-specific traits, since these organisms are male and female at the same time, or their low sensory capability that would be limiting in detecting such traits in the partner (Darwin, 1871; Morgan, 1994; Greeff & Michiels, 1999). However, recent research clearly indicates that strong sexual selection does occur in these organisms (Baur, 1998; Michiels, 1998; Chase, 2007; Schärer & Janicke, 2009; Anthes *et al.*, 2010; Nakadera & Koene 2013). There is no inherent reason for sexual selection not to be able to affect traits of organisms that express both sexual functions simultaneously. By extension, sexual conflict can occur whenever two genetically different individuals decide to reproduce sexually, even when the two sexes are joined in one individual (Arnqvist & Rowe, 2005). Such conflicts about the outcome of a mating interaction commonly revolve around which sex role to perform and whether the donated gametes are used for fertilization (Michiels, 1998). One way to deal with the preference for a specific reproductive role is to transfer sperm reciprocally during any mating encounter, i.e. performing both sex roles with the same partner simultaneously or in sequence (Michiels, 1998). While this solves a precopulatory conflict, it enhances the potential for postcopulatory conflict over the use of donated sperm (Arnqvist & Rowe, 2005). Such postcopulatory conflicts occur whenever sperm from different mating partners are stored prior to the fertilization of eggs, allowing for sperm competition and cryptic female choice (Parker, 1970; Charnov, 1979; Eberhard, 1996).

This scenario also applies to the evolution of love-darts in land snails (Arnqvist & Rowe, 2005; Koene & Schulenburg, 2005). A love-dart is a sharp calcareous stylet carried in a muscular eversible dart sac (Tompa, 1984) and used during mating encounters to pierce the body wall of the partner. The location of the dart in the reproductive system is shown in Figure 1. While it is now generally accepted that love-darts have evolved as a result of conflict over the fate of donated sperm and/or as a way to select the most fit sperm donor, the discussion about their possible function has been long and controversial (reviewed by Chase, 2007). Decades ago, the love-dart was thought to be used to persuade the partner to mate (Adamo & Chase, 1988). However, the dart is utilized only when the partner is already stimulated and willing to mate, so additional motivation seems redundant (Chase & Vaga, 2006). Another hypothesis suggests that darts could be involved in recognizing conspecifics (Diver, 1940). However, species recognition in land snails happens earlier than dart usage, via chemical detection of the mucus trails left by other snails (Chase *et al.*, 1978) and body contact during courtship (Chase *et al.*, 2010). Another idea, that the love-dart could serve as a nuptial gift of calcium to the partner, was also rejected because the quantity of calcium contained in one dart is insignificant (equivalent to provisioning a single egg, while many more are laid within one clutch; Koene & Chase 1998b). Finally, the suggestion that the love-dart is used to show eagerness to transfer sperm was not supported by empirical evidence, revealing that snails delivered a spermatophore irrespective of whether they were hit by or used a dart (Rogers & Chase, 2001; Chase & Vaga, 2006). The one explanation that has withstood scrutiny of testing is the hypothesis that love-darts are used to enhance fertilization success of the donor by increasing the chance of fertilizing eggs. Experimental evidence clearly shows that mucous gland products, transferred on the dart into the partner's haemolymph, increase sperm storage and consequently the paternity of the dart user (Landolfi, Green & Chase, 2001; Rogers & Chase, 2001, 2002; Chase & Blanchard, 2006).

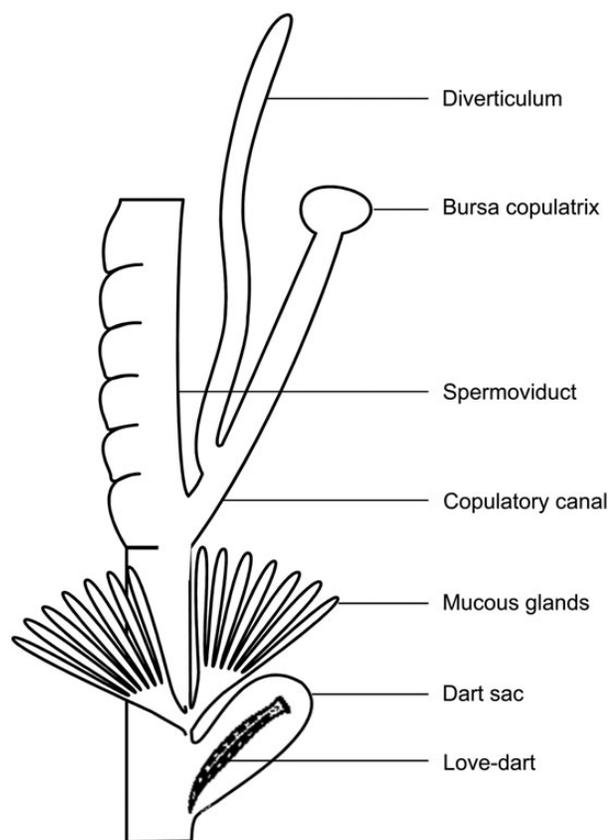


Figure 1. Portion of the reproductive system of *Cornu aspersum* showing the dart sac that hosts the love-dart; the mucous glands that produce mucus coating the dart's surface; the copulatory canal leading to the bursa copulatrix; the bursa copulatrix, which is responsible for digesting the sperm and the remains of the spermatophore; the diverticulum that receives the spermatophore of the partner; and the spermoviduct, through which the sperm of the partner travel to reach the spermatheca (sperm storage organ).

It is crucial to note that all the above findings have been obtained for only one model species, the brown garden snail *Cornu aspersum* (Helicidae). Extending these findings to other dart-bearing species has greatly facilitated an assessment of whether the above function of the love-dart is conserved across species and families.

As a result of repeated evolutionary events, rather than one single origin, several species possess one or more love-darts (Koene & Schulenburg, 2005). Some species may have gained it and subsequently lost it again in the course of evolution (e.g. *Cochlicella*, with an empty rudimentary dart sac, belongs within a taxon with darts; Davison *et al.*, 2005). The possession of a dart is associated with face-to-face mating behaviour (Davison *et al.*, 2005; Jordaens, Dillen & Backeljau, 2009) and low-spired shell shape (Davison *et al.*, 2005), but not all species with these characteristics have darts. Dart-bearing land-snail species are found in two superfamilies, Helicoidea (families: Bradybaenidae, Helicidae, Helminthoglyptidae/Xanthonychidae, Hygromiidae) and Limacoidea (families: Ariophantidae, Dyakiidae) (Davison *et al.*, 2005; Davison & Mordan, 2007; Koene *et al.*, 2013). Darts in these superfamilies appear to be ontogenetically homologous, arising from the same part of the reproductive system (Tompa, 1984). Detailed descriptions of how the dart is used during reproduction are available for only a few species within the Helicoidea. More work is clearly needed to test the generality of the conclusions reached from these studies.

To assist this, we bring together in this review information on several aspects related to the function of love-darts. First, we explain the effects of the mucus transferred along with the dart on the physiology of the recipient. Second, we focus on the dart morphology and its consequences on the anatomy of the reproductive system. Third, we describe the behavioural differences in the use of the love-dart among families. Finally, we synthesize the knowledge about these different subjects by suggesting useful avenues of research and by identifying the important gaps in our understanding of love-darts.

PHYSIOLOGY

The love-dart is used as a vehicle to inject mucus into the partner's haemolymph during mating encounters. This mucus coats the surface of the dart and is released by the mucous glands associated with the dart sac when the dart is employed. The effect of the mucus has been extensively investigated in *Cornu aspersum*. As shown by Koene & Chase (1998a), the mucus causes two major changes when added to an *in vitro* preparation of part of the female reproductive system of *C. aspersum*. First, it induces peristaltic contractions of the blind-ended tubular organ that receives the spermatophore, the diverticulum, which may facilitate the uptake of the spermatophore. Second, contractions of the copulatory canal are initiated, narrowing the tract leading to the sperm-digesting organ, called the bursa copulatrix. As a consequence, the entrance of the bursa copulatrix becomes less accessible. Normally, about 99.98% of the sperm received is digested (Rogers & Chase, 2001), but as an effect of the injected mucus, more sperm avoid digestion and can be stored, resulting in the dart user more than doubling its paternity of the partner's eggs (Chase & Blanchard, 2006). The dart may, therefore, benefit the male function, but this may impose a cost on the female function by preventing control over her fertilization processes.

The analysis of the bioactive substances in the mucus responsible for the physiological changes started with Chung (1986b), who identified a polypeptide that causes genital eversion in *C. aspersum*. Since then, no further research has been published in this field, but recently Stewart *et al.* (unpubl.) have identified a peptide called love-dart allohormone (LDA) as the substance inducing contractions of the copulatory canal. The gene coding for LDA shows homology to buccalin, a neuromodulator conserved in freshwater and marine molluscs. LDA is expressed by both *C. aspersum* and *Theba pisana* (both Helicidae).

Despite detailed knowledge of responses to the dart's mucus in *C. aspersum*, little is known for other dart-bearing species. Hence, it is important to extend our understanding of the effects of the mucus on physiology, especially since recent studies on the Bradybaenidae suggest other possible effects, also enhancing male reproductive success. For example, in *Euhadra quaesita* (Bradybaenidae), snails that are stabbed during mating will mate again only after a longer period of time (*c.* 10 d longer) than those not stabbed (i.e. those that had mated with virgin snails that had not yet formed a dart) (Kimura, Shibuya & Chiba, 2013). Suppressing a partner's willingness to mate again is also a common response to male accessory-gland substances in insects (e.g. *Drosophila melanogaster*: Scott, 1986; *Togo hemipterus*: Himuro & Fujisaki, 2008). For dart-shooting land snails, this effect represents a novel reaction to the mucus, benefiting the dart user in terms of minimizing future sperm competition. Moreover, Kimura *et al.* (2013) also suggest that in *E. quaesita* the mucus may also induce oviposition, ensuring higher paternity gain before the partner mates again. However, this effect on egg laying was only found in smaller snails that were artificially injected with mucous-gland extract once a week over a month. The dose of mucus injected into these smaller snails may have been unnaturally high. This experimental result needs treating

with caution and further investigation is required, since by using a behavioural approach Koene & Chase (1998b) found that receiving a dart during a single mating encounter does not affect oviposition in *C. aspersum*. In other species, results have been obtained similar to those for *C. aspersum*. For example, Kimura, Chiba & Koene (2014) found in an *in vivo* experiment on *E. peliomphala* that mucus closes off the tract leading to the bursa copulatrix. They demonstrated this by injecting snails with mucus extract near the genital pore and then injecting colorant through the genital opening, resulting in dye entering the oviduct but not the bursa copulatrix. This effect is the same as in *C. aspersum*, implying that similar physiological responses to the mucus are found across families of dart-bearing species.

MORPHOLOGY

Shape of the love-dart

Among dart-bearing species, dart shape is highly variable and species-specific. The most common form appears to be a sharp, conical, calcareous stylet that carries mucous products on its surface. The main characteristics possessed by darts, and the observed variation between species in these traits, are shown in Figure 2. First, the number of darts varies from one to eight. While many species possess only one dart, species of *Humboldtiana*, for example, have four dart sacs arranged around the vaginal duct, located under a ring of four mucous glands (Thompson & Brewer, 2000). Each sac can contain two darts (Webb, 1980; Schileyko, 1989). Second, love-darts differ in curvature, from straight, as in *Trichia hispida*, to curved, as in *Hygromia cinctella*, to curved and twisted, as in *Leptaxis erubescens*. Third, the dart can be bladeless as in *Polymita picta*, or bladed as in *Cepaea hortensis* (Koene & Schulenburg, 2005). When blades are present, they vary in number and length, as well as in presence of additional perpendicular blades, enlarging the surface area and possibly the amount of mucus that can be transferred. In general, families other than the Helicidae typically carry simpler darts with a smooth surface. In addition, multiple darts are found only in these families, each hosted in separate sacs (but see *Nesiiohelix bipiramidalis* for an exception, Azuma 1995).

While all the darts discussed so far carry the mucus on their outside, there is at least one alternative mechanism known to deliver mucus into the haemolymph of a mating partner. The dart of *Everettia corrugata corrugata* (Dyakiidae) has perforations on the sides and numerous channels inside (Koene *et al.*, 2013). Based on details of the connection of the dart gland with these channels, this dart is most likely used as a syringe that injects the mucus hypodermically via the perforations. This suggestion of a distinctive method of mucus delivery could be tested if the use of the dart during mating could be observed. It should be noted that *Everettia* belongs to the Limacoidea, a superfamily phylogenetically distant from the Helicoidea to which other studies refer (Wade, Mordan & Clarke, 2001).

Anatomy of the reproductive system relative to the shape of the love-dart

The anatomy of the reproductive organs in a wide range of animals shows evidence of modification as a consequence of sexual selection (Eberhard, 1996). For example, an elongated oviduct may counter male manipulation, such as where the male has traits that facilitate the transport of his sperm to outpace that of rivals. A longer oviduct might also select sperm that travel a long distance successfully or faster. These features and others reflect the conflict of interest between mating partners. As shown above, the morphology of the love-dart is species-specific. If the love-dart evolved as a result of sperm competition, to favour reproductive success of a particular male, or if it

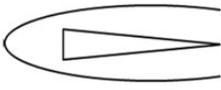
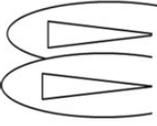
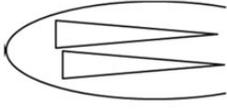
Number of darts	One (in one dart sac)  <i>Helix lucorum</i> (Helicidae)	Multiple (each in one dart sac)  <i>Trichia hispida</i> (Hygromiidae)	Multiple (in one dart sac)  <i>Nesiohelix solida</i> (Bradybaenidae)
Degree of curvature	Straight  <i>Chilostoma glaciale</i> (Helicidae)	Curved  <i>Hygromia cincitella</i> (Hygromiidae)	Curved and twisted  <i>Leptaxis erubescens</i> (Hygromiidae)
Surface enlargement	Bladeless  <i>Fruticicola fruticum</i> (Bradybaenidae)	Bladed  <i>Helix lucorum</i> (Helicidae)	Blades on blades  <i>Cepaea hortensis</i> (Helicidae)

Figure 2. The main morphological characteristics of the love-dart. The first row shows that one or multiple darts can be present, in the latter case they can be contained in separate dart sacs or in one sac (for this category the shapes of the darts have been simplified); the second row shows that darts can be straight, curved or even curved and twisted; the last row indicates that the surface of the dart can be smooth, enlarged with blades or even more by additional perpendicular blades on those blades (cross sections of the dart are shown). Examples of species carrying the love-dart, and their corresponding family in parentheses, are given. Note that, in principle, all row combinations are possible. Drawings from Koene & Schulenburg (2005).

evolved as a trait on which females base their paternity choices, counter-adaptations between male and female traits are expected (Arnqvist & Rowe, 2005).

Morphological coevolution does occur between the shape of the dart and the female reproductive tract, as revealed by a comparative analysis by Koene & Schulenburg (2005). This demonstrated that when the surface of the dart becomes relatively greater, due to an increased number of blades or their greater length, the number of darts possessed is reduced and the mucous glands increase in size, number and type of branching. Where these dart traits arise, the female function counter-adapts by evolving an additional spermatophore-receiving structure, the diverticulum, and the more the surface area of the dart is increased, the longer the diverticulum becomes (Fig. 3). A diverticulum is also present in other species, such as the Clausiliidae, which lack darts (e.g. Schilthuizen & Lombaerts, 1995). This family has high-spined shells and mate by shell mounting, probably making the evolution of the dart unlikely since it would be mechanically difficult to use (Davison *et al.*, 2005; Jordaens *et al.*, 2009). Moreover, morphological studies indicate that the diverticula of the Clausiliidae and Helicidae may be homologous structures with different functions (Visser, 1977; Gómez, 2001; Szybiak & Gabała, 2013).

In dart-bearing species, the evolution of the diverticulum as a counter-adaptation is advantageous, because it probably decreases the chance of sperm being stored. This is the result of the longer travel distance to the sperm storage organ and/or because sperm cannot exit the spermatophore safely (i.e. without being digested). It is known that sperm reach the storage organ more successfully if the spermatophore tail is protruding from the diverticulum into the vaginal duct, allowing some sperm a safe way out through the tail, while the spermatophore is digested (Lind, 1973). Hence, it might be beneficial for the donor to evolve a spermatophore with an extended tail. Accordingly, a long spermatophore tail relative to the diverticulum of the mating partner enhances male paternity in *C. aspersum* (Garefalaki *et al.*, 2010). Moreover, a longer tail might be beneficial in delaying the transport of the spermatophore into the bursa copulatrix. Since transportation is achieved through peristalsis (Lind, 1973), more energy and time would be required to move the spermatophore. With this delay, more sperm is likely to escape and get stored, as found in the *E. peliomphala* (Kimura & Chiba, 2013). Other aspects of spermatophore morphology may also be important. For example, the spines present on the tail of the spermatophore may impede and delay its passage up the bursa copulatrix when orientated opposite to

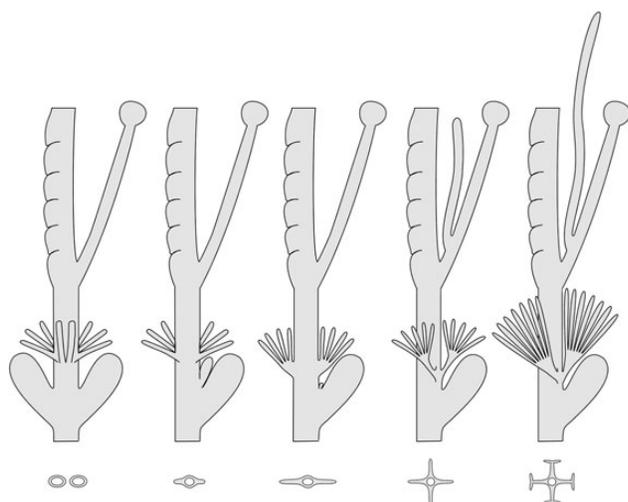


Figure 3. Schematic representation of the evolution of part of the reproductive system of land snails with love-darts. At the bottom, cross sections of a dart are shown. The simplified evolutionary sequence illustrates the association between dart traits and female reproductive system traits reported by Koene & Schulenburg (2005). From left to right, as the dart's surface is enlarged (due to the presence of blades and perpendicular blades on these blades), the number of darts is reduced and the mucous glands increase in size, number and complexity of branching. Meanwhile, female counter-adaptation is shown by the appearance and elongation of a diverticulum. It should be noted that this representation is simplified and not one linear sequence in the evolution of these structures.

the uptake direction (e.g. *Xerocrassa*: Sauer & Hausdorf, 2009; *E. peliomphala*: Kimura & Chiba, 2013; *P. picta*: Reyes-Tur et al., 2015).

Counter-adaptations of this sort are hard to demonstrate in single cases, but they may be revealed by comparative studies (Arnqvist & Rowe, 2002; Koene & Schulenburg, 2005; Beese, Beier & Baur, 2006). Although the love-dart seems to have evolved several times, morphological traits associated with dart structure have been achieved repeatedly by similar evolutionary pathways (Koene & Schulenburg, 2005).

BEHAVIOUR

Use of the love-dart

Although the term 'dart shooting' may suggest otherwise, the dart never gets airborne but rather is stabbed by a forceful eversion of the muscular dart sac. Nor does the dart contain sperm, a popular misconception. The dart shooting process tends to vary by family (Table 1). In Helicidae, the love-dart is used once per mating, before spermatophore transfer occurs. The dart is rapidly pushed out of the genital pore by muscular actions of the dart sac and when it hits the partner's body wall it detaches and remains temporarily lodged or, in rare cases, becomes internalized (Koene & Chase, 1998b). The dart is sometimes shot so weakly that it remains inside the dart sac of the shooter or it can even miss its target completely (8.3% of cases in *Cornu aspersum*; Koene & Chase, 1998b). After use, a new dart is formed within 5–7 d by the dart sac (*C. aspersum*: Tompa, 1981; *Helix pomatia*: Jeppesen, 1976). In this family the love-dart is generally used simultaneously by the two mating partners during courtship and is a standard component of the mating sequence. Exceptions to this rule are *C. aspersum*, *H. lucorum* and *H. pomatia*, in which both partners do shoot a dart, but with a delay between the two shootings (Lind, 1976; Giusti & Lepri, 1980; Adamo &

Chase, 1988; Koene & Chase, 1998b). Another exception is found in *Arianta arbustorum*, which uses its dart in only 23–50% of matings (Baminger, Locher & Baur, 2000); this species does not always shoot a dart even when one is present in the dart sac. In contrast, *C. aspersum* performs shooting behaviour when the dart sac is surgically removed, empty (in virgins) or while a new dart is growing (Chung, 1986a; Adamo & Chase, 1990).

One major difference between the Helicidae and the other families investigated (Hygromiidae, Bradybaenidae and Helminthoglyptidae/Xanthonychidae) is that in the latter the love-dart is normally not lost by the shooter, but instead remains attached to the dart sac. As a result, the dart can be used repeatedly to pierce the partner's body wall during courtship and in some species this behaviour extends throughout copulation (i.e. spermatophore transfer). The number of times a dart is used seems to be associated with the length of the blades, at least in the Bradybaenidae. As shown in closely related bradybaenids of the genus *Euhadra*, darts with short blades are stabbed from 900 to more than 3,000 times per mating encounter, while darts that possess larger blades are stabbed <100 times (Koene & Chiba, 2006). While most species use a general stabbing motion, some families employ the dart differently. For example, the genus *Polymita* (Helminthoglyptidae/Xanthonychidae) has a dart apparatus that can extend to different degrees and be used with great agility. This allows *P. muscarum* to rub the dart in circular movements over the partner's skin (Reyes-Tur & Koene, 2007) and *P. picta* to use the love-dart in three different ways: to rub, wipe and stab the partner's body wall (Reyes-Tur et al., 2015).

In conclusion, the love-dart can be utilized in different ways, yet following a pattern according to the family. Despite this variability, the location hit by the dart seems to be less flexible and is often constrained by mating position, as discussed in the following section.

Locations hit by the love-dart

Due to the face-to-face mating position, in which the genital openings are opposed, the love-dart hits mainly the right flank of the partner's body in all families investigated (and the left flank in sinistral species). In the Helicidae, it still remains unclear if snails target a particular part of this area. This so because, as observed by Chung (1987) in *C. aspersum*, the movements of the partners influence the exact place where the dart is received in the body. Hence, the orientation of the mate towards the shooting partner might be crucial for a successful hit, explaining why dart shooting is not consistent in consecutive matings in *C. aspersum* (Koene & Schulenburg, 2005). Notwithstanding, it appears that snails may attempt to stab the area next to the genital pore since it is the point most hit, but darts are also known to penetrate the foot or the head of the partner. In contrast, in families where multiple stabbing occurs, partners remain motionless, resulting in repeated stabs in the same place. One exception is the genus *Polymita*, where the agile and extensible dart apparatus enables various parts of the partner's body to be hit (Reyes-Tur & Koene, 2007; Reyes-Tur et al., 2015). In contrast, species of the bradybaenid genus *Euhadra* always stab the partner in the same area behind the genital pore. For example, the dart of *E. subnimbosa* is, on average, used for 3,311 stabs (2.52 stabs/s) per mating. The stabbing continues for nearly an hour, creating a hole in the lateral body wall that extends to the partner's foot (Koene & Chiba, 2006) and snails can be seriously wounded as a result.

Injury can occur even in helicids, which discharge a single dart. In Figure 4, a dart shot by the partner has passed through the head of this *C. aspersum*, emerging near the base of the superior tentacle with only the base still lodged in the snail. As a result, the stabbed snail could not fully extend its right tentacle for two weeks.

Table 1. Use of the love-dart in three different land-snail families belonging to the Helicoidea.

Family	Species	Love-dart type	Timing of use	No. of stabs	Place of stabbing	Use of love-dart	Mucus on love-dart	Reference
Helicidae	<i>Eobania vermiculata</i>	Disposable	Before copulation	Once	Right side 87.5% Head 12.5%	Simultaneous	Yes	Giusti & Lepri (1980)
	<i>Helix lucorum</i>	Disposable	Before copulation	Once	Right side 68.8% Head 6.2% Foot 25%	Delay	Yes	Giusti & Lepri (1980)
	<i>Cornu aspersum</i>	Disposable	Before copulation	Once	Genital pore 73.1% Foot 23.1% Head 3.8%	Delay	Yes	Giusti & Lepri (1980) Koene & Chase (1998b) Adamo & Chase (1988) Chung (1987)
	<i>Helix pomatia</i>	Disposable	Before copulation	Once	Genital pore Head Foot	Delay	Yes	Lind (1973) Lind (1976)
	<i>Theba pisana</i>	Disposable	Before copulation	Once	Neck 64.7% Head 17.6% Genital pore 17.6%	Simultaneous	Yes	Giusti & Andreini (1988)
	<i>Helix aperta</i>	Disposable	Before copulation	Once	Foot 30.4% Genital pore 26.2%	Simultaneous	Yes	Giusti & Andreini (1988)
	<i>Arianta arbustorum</i>	Disposable	Before copulation	Once				Baminger et al. (2000)
Bradybaenidae	<i>Cepaea nemoralis</i>	Disposable						Wolda (1967)
	<i>Euhadra subnimbosa</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Koene & Chiba (2006)
	<i>Bradybaena similaris</i>	Reusable	Before copulation	Repeatedly	Right/left side	Simultaneous	Yes	Koene & Chiba (2006)
	<i>Bradybaena pellucida</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Kimura & Chiba (2015)
	<i>Euhadra sandai</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Koene & Chiba (2006)
	<i>Euhadra peliomphala</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Koene & Chiba (2006) Kimura et al. (2014) Kimura & Chiba (2013)
	<i>Euhadra brandtii</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Koene & Chiba (2006)
<i>Euhadra quaesita</i>	Reusable	Before copulation	Repeatedly	Left side	Simultaneous	Yes	Koene & Chiba (2006) Kimura et al. (2013)	
Helminthoglyptidae	<i>Euhadra latispira</i>	Reusable	Before copulation	Repeatedly	Right side	Simultaneous	Yes	Koene & Chiba (2006)
	<i>Helminthoglypta californiensis</i>	Reusable	Before and during copulation	Repeatedly			Yes	Webb (1942)
	<i>Helminthoglypta dupetithouarsi</i>	Reusable	Before and during copulation	Repeatedly			Yes	Webb (1942)
	<i>Helminthoglypta umbilicata</i>	Reusable	Before and during copulation	Repeatedly			Yes	Webb (1942)
	<i>Cepolis maynardi</i>	Reusable		Repeatedly	Head Foot			Webb (1952)
	<i>Helminthoglypta traski fieldi</i>	Reusable	Rare					Webb (1952)
	<i>Polymita muscarum</i>	Reusable	Before, during and after copulation	Repeatedly	Foot Lip Tentacles Mantle edge Penial and vaginal lobes		Unclear	Reyes-Tur & Koene (2007)
	<i>Polymita picta</i>	Reusable	Before, during and after copulation	Repeatedly	Anterior region (head and everted genitalia) 61–71%		Yes	Reyes-Tur et al. (2015)

'Love-dart type' indicates whether the dart is shot and lost (disposable), or if it is reused. The 'Timing of use' during mating is either before, during or after spermatophore transfer (referred to as copulation). 'No. of stabs' indicates whether the partner is stabbed once or repeatedly, and 'Place of stabbing' indicates where the dart hits the mate (genital pore refers to the adjacent area) and percentages are given when available. 'Use of love-dart' refers to the timing when the partners employ the dart, either simultaneously or with a delay. 'Mucus on love-dart' specifies whether the dart was observed to deliver mucus on its surface or, in case of repeated stabbing, the mucous glands were observed to pulsate. References are provided and empty spaces occur when no information is available.

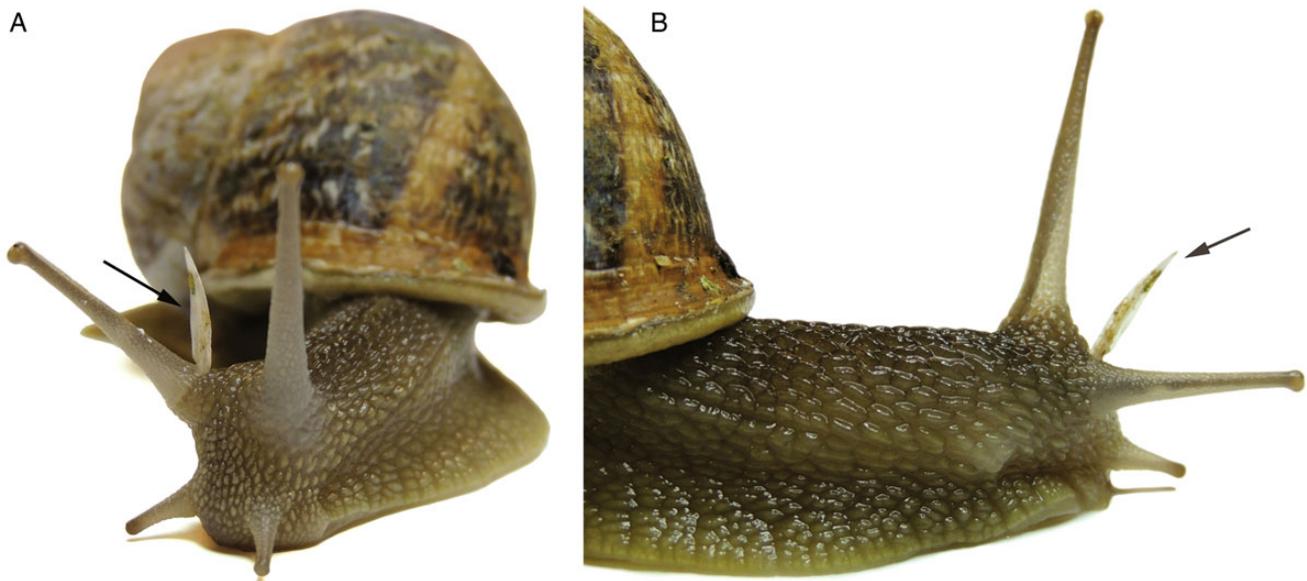


Figure 4. **A.** An individual of *Cornu aspersum* hit in the head by a love-dart (indicated with an arrow). The dart was so powerfully stabbed that it came out of the other side of the head, next to the base of the right eyestalk. The tip of the dart is clearly visible. **B.** The same individual seen from the side to illustrate that the love-dart stabbed through the head prevented the right eye stalk of the snail from fully extending so that it is shorter than the left one. Shell length of this animal was 34 mm.

Despite these injuries, hitting and being hit by the dart seems to be a standard component of mating. In the Helicidae, *C. aspersum* shows no indication of avoidance behaviour, since the outcome of dart shooting appears to be unpredictable and independent of the shooting performance of the partner (Chase & Vaga, 2006). Moreover, snails have a quick and minimal reaction when receiving the dart because the withdrawal reflex is inhibited during courtship by the central nervous system (Balaban & Chase, 1990). A minimal reaction to receiving the dart might also be the case for the repeatedly dart-stabbing families, where avoidance seems not to be an option. The partners are hit continuously, which is inevitable if they are both motivated to continue mating and in some cases mutual penis intromission has already occurred, preventing significant movement (Koene & Chiba, 2006).

We still do not exactly know how much damage is caused by receiving a dart, although severe injury is relatively rare (Chase & Vaga, 2006). Dorello (1925) suggested that the mucus on the dart may carry some coagulant to prevent leakage of haemolymph after dart shooting (because this is never observed, although this is also the case after injection with a sedative or other substances). Gastropods have a high regenerative capacity, hence receiving a dart might be less costly than in other taxa, even if vital organs are hit (Moffett, 1995). The only evidence of costs caused by receiving the dart is found in *Bradybaena pellucida* (Kimura & Chiba, 2015), in which the repeated dart stabbing decreases egg laying and reduces the life span by 16 d. However, this species has only one reproductive season in which it mates up to 15 times (in the laboratory). Other land snails usually live longer (e.g. *Arianta arbustorum* lives for 3–4 years as an adult; Baur & Raboud, 1988), so the damage might be spread over a longer period of time and fewer mating occasions per reproductive season. Experimental data quantifying the costs are needed for more dart-bearing species.

Transfer of mucus

As outlined earlier, stabbing with the dart facilitates the entry of hormone-bearing mucus into the body of the partner. In almost

all dart-bearing snails studied, transfer of mucus on the surface of the dart has been observed. In Helicidae, the digitiform glands release the mucus when the dart is pushed out. In contrast, in the repeatedly dart-stabbing families, the mucus secreted from the glands is partly loaded each time the dart retreats during multiple piercing (Koene & Chiba, 2006). This multiple stabbing behaviour could be explained if the physiological changes caused by the mucus are also dose-dependent as in *C. aspersum* (Koene & Chase, 1998a). Since these families carry simpler darts with a smaller surface area, repeated stabbing may be necessary to deliver enough mucus and to ensure that the manipulation via bioactive substances remains effective, as suggested by species that stab their mating partner throughout copulation. On the contrary, in the Helicidae the dart is used only once during courtship and its degree of penetration into the partner's body wall varies from being superficial (<1 mm deep) to its entire length (c. 9 mm in *C. aspersum*) (Landolfa, 2002). Hence, helicid snails might attempt to hit the partner forcefully and as a result the dart remains lodged for longer and more mucus can be introduced into the mate. There appear to be two strategies involved in mucus transfer: stabbing a simple dart repeatedly or using just once a dart that is more fully coated with mucus.

CONCLUSION

The comparative approach taken here indicates that the common feature in dart shooting across families is the enhancement of male reproductive success by transferring mucus from the shooter to the recipient, inhibiting the destruction of received sperm. The precise mechanisms may be family-specific or conserved across families (e.g. Kimura *et al.*, 2013; Kimura *et al.*, 2014, respectively). *In vivo* and *in vitro* investigations in this direction have only just begun, but are already showing promising results by highlighting differences and commonalities with the well-studied species *Cornu aspersum*. In this respect, particular families of interest to be investigated are the Helminthoglyptidae and Hygromiidae and the superfamily Limacoidea, for which no information is available.

The relationship between the shape and size of the dart, its manner of use and other traits of reproductive anatomy show some consistent differences among families. While the patterns are all consistent with expectations based on sexual conflict (Koene & Schulenburg, 2005), the evolution of such coadaptations cannot yet be traced. The relationships between dart-bearing families are still unclear and thus the ancestral conditions cannot be determined. While there is an ontogenetic homology in the tissues forming the dart and its accompanying structures, darts as such might have evolved more than once as a strengthening of noninjurious, external hormonal secretions. More work on phylogeny is needed.

More behavioural observations on the use of the dart are also necessary, especially for those species with multiple love-darts (e.g. the helicoid *Humboldtiana*). The quantification of the costs of receiving a dart has only just begun (Kimura & Chiba, 2015) and needs to be done for different species; it would be desirable to measure any immune or stress response following dart receipt (e.g. resulting from any bacterial infection due to the wounding by the dart). In our opinion the most promising and fruitful direction of investigation among all the aspects reviewed here is the physiological response of the receiver induced by the mucus delivered with the dart. As suggested by recent results, the dart seems to cause physiological changes that favour male reproductive success in more species than just *C. aspersum*. A broader study in this direction should assess the function of the dart in multiple species. Not only complete mucus extracts, but also the recently discovered LDA peptide (responsible for one of the physiological changes in both *C. aspersum* and *Theba pisana*), could be used experimentally. This opens up a new area of study that can investigate the expression and similarity of LDA in different dart-bearing species. This will enable a comparison across families at the all hormone level and is expected to shed light on the evolution of such substances. Such analysis can be further expanded when the peptides and proteins in the mucus responsible for different responses are identified, such as for muscular contractions, mating inhibition and increased sperm storage or paternity. To broaden our understanding of love-darts, a comparative approach across superfamilies will provide more reliable general conclusions than can at present be drawn based mainly on studies of *C. aspersum*.

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