© 2022. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2022) 225, jeb238782. doi:10.1242/jeb.238782

RESEARCH ARTICLE

Sexual inactivation induced by the mucus that covers land snail love darts: sexual selection and evolution of allohormones in hermaphrodites

Kaito Shibuya[1,‡], Satoshi Chiba[1] and Kazuki Kimura[1,2,‡,*]

ABSTRACT

The transfer of male accessory gland secretions is a well-investigated reproductive strategy for winning in sexual selection. An example of such a strategy is the conspicuous mating behaviour of simultaneously hermaphroditic land snails, the so-called shooting of love darts, whereby a snail drives a love dart(s) into the body of its mating partner. In the land snail *Euhadra quaesita*, it has been shown that a specific mucus which coats the love dart is transferred into the partner's haemolymph and that it suppresses subsequent matings in the darted individual. However, how the mucus of the love dart suppresses rematings remains unclear. In the present study, we tested the hypothesis that by injection of the dart mucus, love-dart shooters manipulate the physiology of a dart recipient and make the individual sexually inactive. In an experiment in which snails were provided with opportunities to encounter a potential mating partner, we found that the latency period to achieve sexual arousal was longer in snails injected with the dart mucus than in snails of the control treatments. This finding indicates that the dart mucus delays sexual arousal in injected snails. This delay in arousal is a novel example of the effects of the mucus in simultaneously hermaphroditic land snails. The remating suppression effect of the dart mucus is likely to occur through sexual inactivation.

KEY WORDS: Accessory gland secretions, Accessory gland proteins, Mate manipulation, Remating suppression, Simultaneous hermaphrodites

INTRODUCTION

If a female copulates with more than one male and stores their sperm, sexual selection is likely to continue after copulation via sperm competition and cryptic female choice. This post-copulatory sexual selection favours the males that show high efficiency in fertilising eggs in competition after mating (reviewed in Eberhard, 2009). The transfer of male accessory gland secretions (AGSs) is a well-investigated example of a reproductive strategy for achieving such high efficiency. When proteins in AGSs play a principal role in such a strategy, researchers use the term 'accessory gland proteins' (ACPs). There is ample evidence that males transfer AGSs into females during mating in many species (reviewed in Poiani, 2006; Avila et al., 2011). AGSs have a variety of effects on the physiology and behaviour of females after mating, such as promoting sperm uptake, increasing egg production and egg laying, and modulating feeding (Poiani, 2006; Avila et al., 2011). Suppression of female remating is also an example of the effects of AGSs and has been reported in many species, including fruit flies (Chen et al., 1988), ground beetles (Takami et al., 2008) and rats (Forsberg et al., 1990).

The present study focused on the transfer of AGSs and suppression of remating in simultaneously hermaphroditic land snails. Several species of such land snails show a conspicuous mating behaviour, the so-called shooting of love darts, which has post-copulatory effects on the physiology of sperm recipients (Schilthuizen, 2005; Chase, 2007; Lodi and Koene, 2016). Dart shooting is a behaviour in which a snail pushes its love dart(s) through its mating partner's body wall using a muscular dart sac. Because they intromit their penises simultaneously and reciprocally in a single mating event, both mating individuals perform dart-shooting behaviour. The timing of dart shooting varies among species and may occur in the courtship, copulation and/or post-copulation phases (Chase, 2007; Kimura and Chiba, 2013a; Reyes-Tur et al., 2015). The love darts are hard and sharp and made of a crystalline form of calcium carbonate called aragonite (Shimizu et al., 2019). Several studies addressing the functional aspects of dart shooting have been performed on species within the families Helicidae and Camaenidae (including Bradybaeninae), particularly on *Cornu aspersum*. During dart penetration in *C. aspersum*, a specific mucus is transferred into the recipient's haemolymph from glands associated with the sac containing the dart (Adamo and Chase, 1990). The dart's mucus causes temporary conformational changes in the female reproductive system by closing off the route to the bursa copulatrix, a gametolytic organ (in *C. aspersum*: Koene and Chase, 1998; in *Euhadra peliomphala*: Kimura et al., 2014). Subsequent studies have found that either successful dart shooting or experimental injection of the dart mucus alone increases sperm storage and paternity of the sperm donor in *C. aspersum* (Landolfa et al., 2001; Rogers and Chase, 2001; Chase and Blanchard, 2006).

Furthermore, a recent study reported that dart shooting decreases the likelihood of remating by darted individuals in *Euhadra quaesita*, and the suppression is induced by the chemical stimuli of the dart mucus, not by the physical stimuli of dart shooting (Kimura et al., 2013). Subsequently, Kimura et al. (2016) tested the hypothesis that the observed remating suppression resulted from physical damage in snails due to the chemical stimuli and revealed that the dart mucus has no short-term influence on the physical vigour of snails. Therefore, how the dart mucus suppresses remating remains unclear. To fully understand how and why this conspicuous mating behaviour in simultaneously hermaphroditic land snails has evolved, it is important to know the mechanisms underlying the effects of the mucus.

[1]Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, Kawauchi 41, Aoba-ku, Sendai 980-0862, Japan. [2]Department of Biology, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 41566, South Korea.
‡These authors contributed equally to this work

*Author for correspondence (k.kimura.000@gmail.com)

K.S., 0000-0002-0108-2285; K.K., 0000-0003-1091-2313

Received 6 October 2020; Accepted 24 January 2022

In the present study, we conducted a laboratory experiment to test the hypothesis that remating suppression is mediated through interruption of sexual activation and/or reduction of sexual attractiveness in snails receiving the dart mucus.

MATERIALS AND METHODS

Study species

We used the simultaneously hermaphroditic land snail *Euhadra quaesita* (Deshayes 1850) (Camaenidae Bradybaeninae). This species is widely distributed in the eastern region of Japan (Kimura and Chiba, 2010). When individuals of this species reach sexual maturity, they stop their shell growth and form a reflected lip at the shell aperture (adult shell diameter: 32–46 mm). Like many land snail species in Bradybaeninae, *E. quaesita* has a love dart, which is encased in a dart sac, and associated mucous glands, which produce the secretions covering the love dart (Nagasawa, 1991; Kimura et al., 2013). The mating process of *E. quaesita* consists of courtship behaviour and copulation. When two sexually mature individuals meet, they reach sexual arousal and tissues between the upper tentacles are expanded and protruded (Fig. 1). The protruded tissues are the so-called head wart (Taki, 1930, 1935) and are considered to release a sex pheromone (Takeda and Tsuruoka, 1979). The sexually aroused snails exhibit the protruded head wart before and during courtship behaviour in camaenid species, including the study species (e.g. Taki, 1930, 1935; Nagasawa, 1991). After the encounter, the two snails show courtship behaviour that consists of licking the anterior part of the potential mate's body and rubbing their anterior body against the potential mate. The duration of the behaviour is approximately 5–30 min. As in *E. peliomphala* (Kimura and Chiba, 2013a), courtship in this species does not include the 'circling phase' behaviour that is typical of certain land snails and slugs (Adamo and Chase, 1988; Reise, 1995). Copulation in this species lasts 100–150 min (Kimura and Chiba, 2013b). Before the spermatophore exchange, the two mating partners stab each other repeatedly with their love dart. At this time, they also reciprocally insert their penises. Although several land snail species omit dart shooting (e.g. Baminger et al., 2000), the use of the love dart during the mating process is most likely obligatory in non-virgin snails of *E. quaesita* (Kimura et al., 2013) as in *C. aspersum* (Chase and Vaga, 2006). However, virgin adults of *E. quaesita* have no love dart; these snails make a dart only after the first mating. As in *C. aspersum*, *E. quaesita* is believed to transfer secretions from the dart mucus glands into the mating partner with its love dart.

Adult snails were collected in 2012, 2013 and 2021 on Hachijo Island, Japan, and kept individually in plastic pots (450 ml) at 22°C and approximately 65% relative humidity. About 50 of the snails collected were used in our previous study (Kimura et al., 2016), and the remaining were used in the present study (i.e. the individuals used in the two studies were from the same population). All snails were maintained under the same photoperiod, though the photoperiod was not controlled. The snails were fed cucumbers coated with protein/calcium powder *ad libitum*, and the pots were cleaned every 2 weeks. We categorised the collected adult individuals as non-virgin snails because a field investigation revealed that adults already had stored allosperm in their bodies (Kimura et al., 2013). To minimise any influence of matings and oviposition behaviour preceding capture on the general activity of adult snails, laboratory experiments were performed at least 5 weeks after collection.

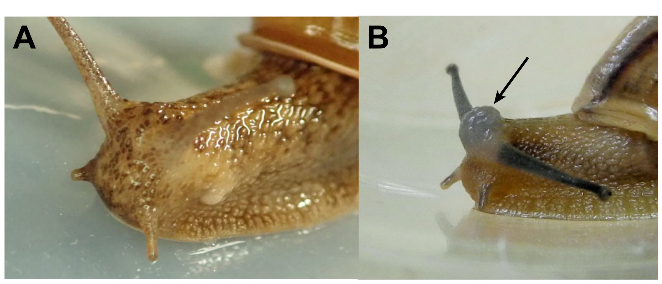

Fig. 1. The land snail *Euhadra quaesita*. (A) The head of a sexually non-aroused snail. (B) The protruded head wart in a sexually aroused snail.

Effect of dart mucus on sexual activation and attractiveness

An extract of dart mucus glands (242 mg in total) dissected out of four adult snails was made in a hand-held homogeniser with 3.0 ml of saline solution (for *C. aspersum*: Kerkut and Meech, 1966). Intact adult snails were anaesthetised with an injection of approximately 1.0 ml of 50 mmol l^{-1} $MgCl_2$ via the skin under the back of the shell with a 26-gauge syringe. Subsequently, the supernatant of the extracts of the dart glands (0.1 ml) was hypodermically injected after centrifugation (14,000 rpm, 30 s). By comparison with a saline solution without the dart mucus, it was calculated that a volume of 0.1 ml of the supernatant contained 4.3 mg of dart gland extract. It has already been shown that this supernatant contains substances inducing remating suppression in our study species (Kimura et al., 2013). This volume of the injected extract was equivalent to those used our previous study revealing the effect of remating suppression (Kimura et al., 2013), although examining the effect of the concentration of the dart mucus is one area for future research. The injection of the extract solution was made through the skin near the genital opening with a 26-gauge syringe because the love dart pierces this area in *E. quaesita*. To examine the effect of this dart mucus injection on the sexual arousal and attractiveness of individuals, each injected adult snail (n=20) was kept in a plastic pot (900 ml) with an intact adult snail and given the opportunity to have physical contact with the partner snail for 5 h (11:00 h to 16:00 h) every 2 days, from the day after the injection to 19 days after the injection, because the remating suppressive effect of the dart mucus lasts approximately 2 weeks (Kimura et al., 2013). When the snails were transferred from rearing pots to experimental pots, the insides of the experimental pots were moistened with a sufficient amount of water in order not to reduce the locomotor behaviour of snails. The state (sexually aroused or not) of the injected snails and partners was observed once for 30 min during the experiment. In this study, snails were considered to achieve sexual arousal when their head wart protruded by more than 1.0 mm (Fig. 1). After the observations, the rearing pots were cleaned, and then the snails were moved back into them. As control treatments, an identical procedure was followed except that 0.1 ml of the supernatant of extracts of a piece of snail foot, 0.1 ml of saline solution (without any extracts) or no solution was injected into the adult snails. A foot piece (160 mg) was cut from an adult snail and homogenised with 3.0 ml of saline solution to make foot extract. By comparison with the weight of saline solution, it was calculated that a volume of 0.1 ml of the supernatant of foot extract contained 4.2 mg of substances from the foot piece (i.e. equivalent to the consistency of the dart mucus solution). Snails were assigned to each of the four treatment groups in such a way that the mean of snail body size did not differ significantly among treatments. While the snails collected in 2012 and 2013 were used in dart mucus, saline solution and no-injection treatments, the individuals collected in 2021 were used in the foot extract injection treatment.

Journal of Experimental Biology (2022) 225, jeb238782. doi:10.1242/jeb.238782

Statistical analyses

Generalised linear models (GLMs) with a gamma distribution and log link function were used to evaluate the effect of the dart mucus on sexual activation in the injected snails. The latency period to achieve sexual arousal in the injected snail (i.e. latency to show a protruded head wart) was treated as the dependent variable. The type of treatment (dart mucus injection, foot extract injection, saline solution injection or no injection), the volume of the focal snail (shell diameter^2×shell height) and the partner snail volume were treated as fixed effects. The effect of the dart mucus on sexual attractiveness in the injected snail was also assessed using a similar GLM analysis. This analysis used latency to achieve sexual arousal in the partner snail as the dependent variable with the same set of fixed effects. Snail volume was natural log transformed before the GLM analyses. The significance of the fixed effects was assessed with likelihood ratio tests using Chi-square approximation. The models were simplified by stepwise deletion of non-significant (P>0.05) fixed effects starting with the effect showing the highest P-value (Zuur et al., 2009). All analyses were conducted in R 3.5.2 (http://www.R-project.org/) using the package lme4.

RESULTS

Effect of dart mucus on sexual activation and attractiveness

While all focal snails achieved sexual arousal by the fifth day of the experiment in the no-injection, saline and foot extract treatments (1.1 ±0.45, 1.2±0.62 and 3.2±1.44 days, respectively, means±s.d.), the mean (±s.d.) latency for sexual arousal was 13.1±4.7 days in the snails injected with the dart mucus (Fig. 2). The stepwise deletion of fixed effects selected a model including only an effect of treatment type (Table 1).

In all four treatments, all partner snails achieved sexual arousal by the third day of the experiment (1.3±0.73, 1.4±0.82, 1.2±0.62 and 1.3±0.73 days for no-injection, saline, foot extract and dart mucus treatments, respectively). The stepwise deletion of fixed effects selected a null model (Table 2).

DISCUSSION

In the artificial injection experiment, we found that the partner snails that were in contact with the snail injected with the dart mucus achieved sexual arousal in as short a time as the partner snails did in the foot extract, saline and no-injection treatments (Table 2). This result suggests that there was no effect of the dart mucus on the sexual attractiveness of sexually mature individuals and that the focal snails equally received mating attempts by the sexually aroused partner snails in the four treatments. However, we found that the mucus-injected snails had a longer latency to show a protruded head wart than the focal snails in the three control treatments (Fig. 2, Table 1). The dart mucus-injected snails did not achieve sexual arousal and did not attempt to mate with the partner snail even when receiving mating attempts by the partners for approximately 2 weeks after the injection. They appeared to actively avoid the partners exhibiting courtship behaviour (i.e. licking and rubbing behaviour). A similar tendency was also observed in the foot extract treatment. However, the dart mucus was found to be over 4 times as effective at delaying sexual arousal as the foot extract although the dart mucus and foot extract solutions used in our experiment were of equivalent consistency (Fig. 2). In the GLM analyses, we also found that the estimated coefficient for the effect of dart mucus was over twice as large as the coefficient for foot extract treatment and that the 95% confidence intervals for the two treatments had no overlap (Table 1). The snails used in the foot extract treatment were collected from the wild in a different year from that for the snails used in the other treatments. The effect of this difference in time of collection cannot be determined. However, all snails were maintained under laboratory conditions for at least 5 weeks after collection to minimise any influence of experience in the wild and, thus, the difference found between dart mucus and foot extract treatments is most likely attributable to difference in the substances injected. These findings suggest that the gland of the dart mucus produces distinctive biochemical substances that influence sexual activation, not sexual attractiveness, in receiving individuals.

Although dart shooting itself (consisting of the effects of the chemical stimuli in the dart mucus and the physical stimulus of being stabbed with the love dart) decreases the dart receiver's longevity (Kimura and Chiba, 2015), the chemical stimuli alone do not affect the receiver's physical condition for 2 weeks (Kimura et al., 2016). Therefore, it is unlikely that the mucus-injected snails suffered from a weakened physical condition and did not achieve sexual arousal. Also, although feeding and reproductive behaviour

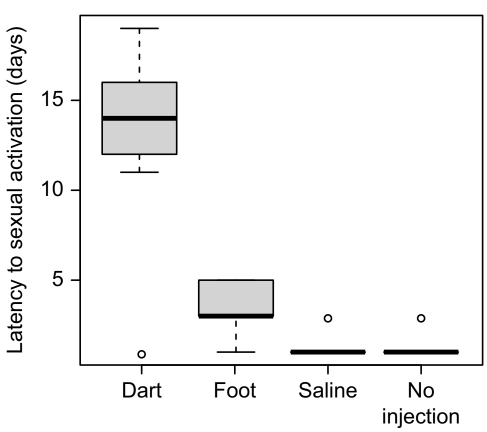

Fig. 2. Latency period to achieve sexual arousal in the four treatments. Snails were injected with dart mucus (N=20), foot extract (N=20) or saline (N=20), or were not injected (N=20). Boxes show the 25th, 50th and 75th percentiles, whiskers include data within 1.5 times the interquartile range and circles indicate outliers.

Table 1. Results of stepwise deletion of effects from initial GLM for the effect of the dart mucus on sexual activation

Variable	Intercept or coefficient (95% CI)	log-Likelihood ratio	d.f.	P
Intercept	0.095			
Treatment type	Saline: 0.18 (−0.01 to 0.37) Foot extract: 1.16 (0.97 to 1.35) Dart mucus: 2.57 (2.38 to 2.76)	450.06	3	**<0.01**
Focal snail volume		2.17	1	0.14
Partner snail volume		0.71	1	0.40

GLM, generalised linear model; CI, confidence interval. Bold indicates a significant effect.

Table 2. Results of stepwise deletion of effects from initial GLM for the effect of the dart mucus on sexual attractiveness

Variable	Intercept or coefficient	log-Likelihood ratio	d.f.	P
Intercept	0.26			
Treatment type		0.67	3	0.88
Focal snail volume		0.79	1	0.37
Partner snail volume		1.09	1	0.30

are correlated in land snails (Adamo and Chase, 1991), the dart mucus does not influence feeding in *E. quaesita* (Kimura et al., 2016). Therefore, it is also unlikely that the delay in achieving sexual arousal is produced by raising the priority of feeding in the mucus-injected snails. Our findings suggest that the dart mucus directly influences reproductive physiology in receiving individuals and makes them sexually inactive. It has been proposed that manipulating the reproductive physiology of mates by the transfer of biochemical substances can easily evolve through the sensory trap process in simultaneously hermaphroditic animals (Koene, 2005).

Unlike organisms with separate sexes, simultaneous hermaphrodites, by their very nature, produce and possess biochemical substances affecting both male and female functions to regulate their own functions. Such biochemicals can be diverted as AGSs to manipulate a mating partner's male and/or female functions. This evolutionary process may be the case for the effect of sexual inactivation found in the present study. To better understand the evolution of the love dart and dart mucus, it is essential to elucidate the substance(s) and the mechanism underlying the sexual inactivation.

Kimura et al. (2013) reported that the chemical stimuli of dart shooting decrease the likelihood of remating by a darted individual for about 2 weeks in our study species *E. quaesita*. In the present study, we followed the procedure of Kimura et al. (2013). Thus, the experimental conditions (e.g. snail volume, the dose of the dart mucus injected) were equivalent between the present study and the previous one. Although the amount of dart mucus transferred via actual dart shooting is still unclear in *E. quaesita*, the duration of sexual inactivation found in the present experiment was equivalent to the duration of remating suppression in Kimura et al. (2013). Therefore, it is plausible that the remating suppression found in the darted individuals occurs through the sexual inactivation effect of the dart mucus, although the relationship between the dose of the dart mucus and duration of sexual inactivation needs to be examined. A similar physiological effect of AGSs has been well studied in insects (Avila et al., 2011). In *Drosophila melanogaster*, transfer of ACPs causes a decrease in female receptivity to mating attempts by males (Chapman and Davies, 2004). Likewise, in the simultaneously hermaphroditic snail *Lymnaea stagnalis*, an avoidance behaviour towards courtship by a potential partner is induced by the transfer of ACPs (Daupagne and Koene, 2020). As *L. stagnalis* and *E. quaesita* are both simultaneously hermaphroditic snails (pulmonate snails), comparison of mechanisms underlying the avoidance behaviour and sexual inactivation [e.g. the identity of the substance(s) that induces the focal phenomenon] would shed some light on the evolutionary history of reproductive strategies in simultaneous hermaphrodites. In addition, understanding common and species-specific processes in the underlying mechanisms could contribute to the extermination of invasive alien pulmonate snails, such as the agricultural pest snail *Lissachatina fulica* (Vijayan et al., 2020) and the predatory and aggressive snail *Macrochlamys* sp. (Kimura, 2015; Pholyotha et al., 2018), without influencing native snails.

In conclusion, the mucus covering the land snail love dart affects reproductive physiology in the injected snails and prevents them from achieving sexual arousal. This effect of the dart mucus explains the mechanism underlying the function of dart shooting reported in Kimura et al. (2013).

Acknowledgements
We express our sincere gratitude to Yuichi Kameda, Yuta Morii, Taka Suzuki and Jotaro Urabe for helpful comments on this study and Takahiro Hirano for collecting material. We are also grateful to Joris M. Koene and an anonymous referee for improving the earlier manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.S., K.K.; Investigation: K.S., K.K.; Data curation: K.S., K.K.; Writing - original draft: K.S., K.K.; Writing - review & editing: S.C., K.K.; Supervision: S.C.; Funding acquisition: S.C.

Funding
This research was supported by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (no. 15H03743 to S.C.).

References
Adamo, S. A. and Chase, R. (1988). Courtship and copulation in the terrestrial snail *Helix aspersa*. *Can. J. Zool.* **66**, 1446-1453. doi:10.1139/z88-211

Adamo, S. A. and Chase, R. (1990). The "love dart" of the snail *Helix aspersa* injects A pheromone that decreases courtship duration. *J. Exp. Zool.* **255**, 80-87. doi:10.1002/jez.1402550111

Adamo, S. A. and Chase, R. (1991). The interactions of courtship, feeding, and locomotion in the behavioral hierarchy of the snail *Helix aspersa*. *Behav. Neural Biol.* **55**, 1-18. doi:10.1016/0163-1047(91)80123-V

Avila, F. W., Sirot, L. K., LaFlamme, B. A., Rubinstein, C. D. and Wolfner, M. F. (2011). Insect seminal fluid proteins: Identification and function. *Annu. Rev. Entomol.* **56**, 21-40. doi:10.1146/annurev-ento-120709-144823

Baminger, H., Locher, R. and Baur, B. (2000). Incidence of dart shooting, sperm delivery, and sperm storage in natural populations of the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Can. J. Zool.* **78**, 1767-1774. doi:10.1139/z00-113

Chapman, T. and Davies, S. J. (2004). Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides* **25**, 1477-1490. doi:10.1016/j.peptides.2003.10.023

Chase, R. (2007). The function of dart shooting in helicid snails. *Amer. Malacol. Bull.* **23**, 183-189. doi:10.4003/0740-2783-23.1.183

Chase, R. and Blanchard, K. C. (2006). The snail's love-dart delivers mucus to increase paternity. *Pro. Roy. Soc. B* **273**, 1471-1475. doi:10.1098/rspb.2006.3474

Chase, R. and Vaga, K. (2006). Independence, not conflict, characterizes dart-shooting and sperm exchange in a hermaphroditic snail. *Behav. Ecol. Sociobiol.* **59**, 732-739. doi:10.1007/s00265-005-0103-y

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M. and Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell* **54**, 291-298. doi:10.1016/0092-8674(88)90192-4

Daupagne, L. and Koene, J. M. (2020). Disentangling female postmating responses induced by semen transfer components in a simultaneous hermaphrodite. *Anim. Behav.* **166**, 147-152. doi:10.1016/j.anbehav.2020.06.009

Eberhard, W. G. (2009). Postcopulatory sexual selection: Darwin's omission and its consequences. *Proc. Natl. Acad. Sci. U.S.A* **106**, 10025-10032. doi:10.1073/pnas.0901217106

Forsberg, G., Bednar, I., Eneroth, P. and Södersten, P. (1990). β-Endorphin acts on the reproductive tract of female rats to suppress sexual receptivity. *Neurosci. Lett.* **115**, 92-96. doi:10.1016/0304-3940(90)90523-C

Kerkut, G. A. and Meech, R. W. (1966). The internal chloride concentration of H and D cells in the snail brain. *Comp. Biochem. Physiol.* **19**, 819-832. doi:10.1016/0010-406X(66)90438-5

Kimura, K. (2015). Interference effect of the alien land snail *Macrochlamys* sp. on the native land snail *Bekkochlamys perfragilis*. *Res. Bull. Environ. Edu. Center, Miyagi Univ. Edu.* **17**, 59-61.

Kimura, K. and Chiba, S. (2010). Interspecific interference competition alters habitat use patterns in two species of land snails. *Evol. Ecol.* **24**, 815-825. doi:10.1007/s10682-009-9339-8

Kimura, K. and Chiba, S. (2013a). Delayed spermatophore removal in the land snail *Euhadra peliomphala*. *Biol. J. Linn. Soc.* **108**, 806-811. doi:10.1111/bij.12008

Kimura, K. and Chiba, S. (2013b). Strategic ejaculation in simultaneously hermaphroditic land snails: more sperm into virgin mates. *BMC Evol. Biol.* **13**, 264. doi:10.1186/1471-2148-13-264

Kimura, K. and Chiba, S. (2015). The direct cost of traumatic secretion transfer in hermaphroditic land snails: individuals stabbed with a love dart decrease lifetime fecundity. *Pro. Roy. Soc. B* **282**, 20143063. doi:10.1098/rspb.2014.3063

Kimura, K., Shibuya, K. and Chiba, S. (2013). The mucus of a land snail love-dart suppresses subsequent matings in darted individuals. *Anim. Behav.* **85**, 631-635. doi:10.1016/j.anbehav.2012.12.026

Kimura, K., Chiba, S. and Koene, J. M. (2014). Common effect of the mucus transferred during mating in two dart-shooting snail species from different families. *J. Exp. Biol.* **217**, 1150-1153. doi:10.1242/jeb.095935

Kimura, K., Shibuya, K. and Chiba, S. (2016). Effect of injection of love-dart mucus on physical vigour in land snails: can remating suppression be explained by physical damage? *Ethol. Ecol. Evol.* **28**, 284-294. doi:10.1080/03949370.2015.1037359

Koene, J. M. (2005). Allohormones and sensory traps: a fundamental difference between hermaphrodites and gonochorists? *Invertebr. Reprod. Dev.* **48**, 101-107. doi:10.1080/07924259.2005.9652176

Koene, J. M. and Chase, R. (1998). Changes in the reproductive system of the snail *Helix aspersa* caused by mucus from the love dart. *J. Exp. Biol.* **201**, 2313-2319. doi:10.1242/jeb.201.15.2313

Landolfa, M. A., Green, D. M. and Chase, R. (2001). Dart shooting influences paternal reproductive success in the snail *Helix aspersa* (Pulmonata, Stylommatophora). *Behav. Ecol.* **12**, 773-777. doi:10.1093/beheco/12.6.773

Lodi, M. and Koene, J. M. (2016). The love-darts of land snails: integrating physiology, morphology and behaviour. *J. Molluscan Stud.* **82**, 1-10. doi:10.1093/mollus/eyv046

Nagasawa, T. (1991). Notes on the copulation and spawning of *Euhadra quaesita*. *Chiribotan* **22**, 65-68.

Pholyotha, A., Sutcharit, C. and Panha, S. (2018). The land snail genus Macrochlamys Gray, 1847 from Thailand, with descriptions of five new species (Pulmonata: Ariophantidae). *Raffles Bull. Zool.* **66**, 763-781.

Poiani, A. (2006). Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* **60**, 289-310. doi:10.1007/s00265-006-0178-0

Reise, H. (1995). Mating behaviour of Deroceras rodnae Grossu and Lupu, 1965 and *D. praecox* Wiktor, 1966 (Pulmonata: Agriolimacidae). *J. Molluscan Stud.* **61**, 325-330. doi:10.1093/mollus/61.3.325

Reyes-Tur, B., Allen, J. A., Cuellar-Araujo, N., Hernández, N., Lodi, M., Méndez-Hernández, A. A. and Koene, J. M. (2015). Mating behaviour, dart shape and spermatophore morphology of the Cuban tree snail *Polymita picta* (Born, 1780). *J. Molluscan Stud.* **81**, 187-195. doi:10.1093/mollus/eyu089

Rogers, D. W. and Chase, R. (2001). Dart receipt promotes sperm storage in the garden snail *Helix aspersa*. *Behav. Ecol. Sociobiol.* **50**, 122-127. doi:10.1007/s002650100345

Schilthuizen, M. (2005). The darting game in snails and slugs. *Trends Ecol. Evol.* **20**, 581-584. doi:10.1016/j.tree.2005.08.014

Shimizu, K., Kimura, K., Isowa, Y., Oshima, K., Ishikawa, M., Kagi, H., Kito, K., Hattori, M., Chiba, S. and Endo, K. (2019). Insights into the evolution of shells and love darts of land snails revealed from their matrix proteins. *Genome Biol. Evol.* **11**, 380-397. doi:10.1093/gbe/evy242

Takami, Y., Sasabe, M., Nagata, N. and Sota, T. (2008). Dual function of seminal substances for mate guarding in a ground beetle. *Behav. Ecol.* **19**, 1173-1178. doi:10.1093/beheco/arn090

Takeda, N. and Tsuruoka, H. (1979). A sex pheromone secreting gland in the terrestrial snail, *Euhadra peliomphala*. *J. Exp. Zool.* **207**, 17-25. doi:10.1002/jez.1402070103

Taki, I. (1930). Notes on a swelling on the head in land snails. *Zool. Mag.* **42**, 325-326.

Taki, I. (1935). Notes on a warty growth on the head of some land snails. *J. Sci., Hiroshima Univ., Ser. B, Div.* **13**, 159-183.

Vijayan, K., Suganthasakthivel, R., Sajeev, T. V., Soorae, P. S., Naggs, F. and Wade, C. M. (2020). Genetic variation in the Giant African Snail *Lissachatina fulica* (Bowdich, 1822) in its invasive ranges of Asia and West Africa. *Biol. J. Linn. Soc.* **131**, 973-985. doi:10.1093/biolinnean/blaa171

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. S. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York: Springer.