

Mating behaviour in the terrestrial slug *Deroceras gorgonium*: is extreme morphology associated with extreme behaviour?

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Abstract—Mating in *Deroceras* consists of an investigation phase (precourtship), then a long courtship involving mutual stroking with the extruded sarcobelum, then sperm exchange (copulation). The penial gland, if present, everts over the partner's skin during copulation: this is hypothesised to apply a secretion manipulating the partner to use received sperm. *Deroceras gorgonium* has a particularly large penial gland divided into many finger-like branches. We studied *D. gorgonium* mating behaviour in the hope of further indications of the gland's function. Precourtship and courtship together last longer than in other *Deroceras* (ca. 6 h to >9 h); precourtship is highly variable, often with many bouts of different behaviours, including seemingly inactive phases. During most of the courtship partners remain apart waving their particularly long, pointed sarcobela; only at a later stage do the tips of these contact the partner. This waving alternates with circling for half a turn. For the first time in *Deroceras* we observed the sarcobelum transferring a secretion. The copulation is amongst the fastest: genital eversion and sperm exchange occur within 1 s, and slugs separate 18-25 s later. The penial gland is everted immediately after sperm exchange, but, surprisingly, is often spread underneath the partner rather than over its back and, if on top, is not always fully spread over the partner's body. We discuss these observations with respect to penial gland morphology and in the light of possible sexual conflicts. The long courtship and distant sarcobelum waving might reflect attempts to transfer, but not receive, secretion, and the circling might serve for size assessment.

Keywords: courtship; genital morphology; Pulmonata; sexual conflict; simultaneous hermaphrodite.

INTRODUCTION

Deroceras (Gastropoda: Pulmonata: Agriolimacidae) is the most speciose genus of terrestrial slugs. Its over 100 species are externally rather uniform yet differ considerably in penis morphology, not only in overall shape but also in the presence

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and form of various appending and internal structures. The function of most of these structures is unknown.

Also the mating behaviour varies considerably between species of *Deroceras* (reviewed by Reise, in press). The mating system in these simultaneous hermaphrodites is characterised by a long precopulatory phase and a usually (though not always) short copulation phase. Precopulatory behaviour comprises precourtship (with initial investigations and sometimes trail following) and courtship, during which each slug strokes its partner with the sarcobelum, a highly manoeuvrable penial structure which is extruded at the beginning of this phase. During copulation, ejaculates are exchanged mutually via everted and more or less entwined penes. External sperm exchange from penis to penis is considered unique to pulmonate gastropods, within which it has evolved at least four times independently (Ember-ton, 1994). Note that we apply the term 'copulation' exclusively to this phase of sperm exchange, not as a synonym for mating behaviour in general; and by a 'mating' we do not imply that mating behaviour proceeded to completion. Species differ considerably in the durations of individual mating phases, the occurrence and intensity of trail following during precourtship, the manner and intensity of stroking with the sarcobelum, the degree of aggressiveness, and, associated with the latter, how close partners get during early parts of courtship.

Mating behaviour is likely to be correlated with the penial structures involved, and Reise (in press) suggested that comparative studies of mating behaviour might suggest the functions of these structures. One approach is to focus on species with particularly enlarged or bizarrely shaped penis parts. Some examples are the elongated and helicoidally coiled penis of *Deroceras helicoidale* Rähle, 1998, huge flat sarcobela which can cover large areas of the partner's back during courtship, as in *Deroceras fatrense* Mácha, 1981, and the very large appending penial glands of *Deroceras gorgonium* Wiktor, Vardinoyannis and Mylonas, 1994, the focus of this paper.

The appending penial gland of *Deroceras* is a particularly interesting organ consisting of one or more finger-like appendages located more or less at the end of the penis (fig. 1). *Deroceras gorgonium* has by far the largest penial gland in the genus (Wiktor, 2000), consisting of a huge bundle of very many tangled branched processes (fig. 1). There are two main branches attached to a very robust basal stem. From these main branches, many side branches arise, all on the posterior side. These side branches are themselves branched at least once. The number of side branches, their robustness and branching vary within populations and we suspect that the complexity might correlate with maturity. The penial gland shows considerable interspecific diversity in *Deroceras*: it may be branched or unbranched, sometimes it is reduced to a small nodule, whereas others species have branches of similar length to *D. gorgonium*, but no other species has such a high level of branching and none is so massive (Wiktor, 2000).

At least in those *Deroceras* species in which mating has been observed carefully, the penial gland is everted during copulation and spread onto the partner's body.

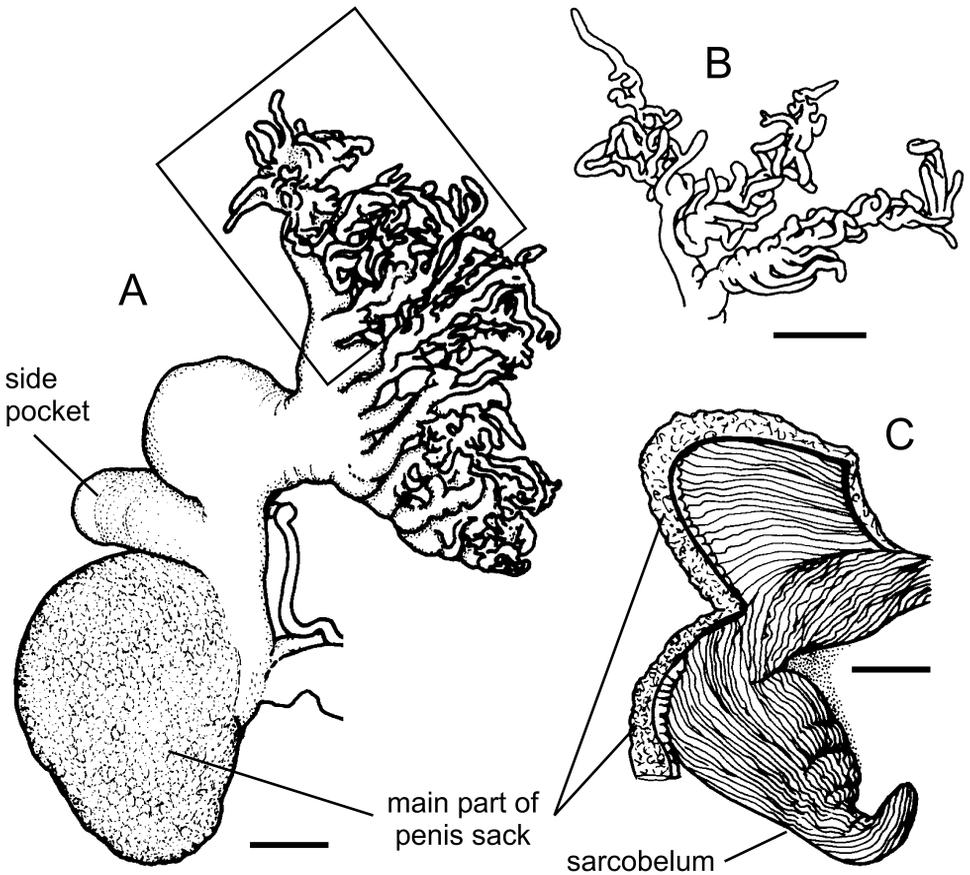


Figure 1. Morphology of distal male genitalia of *Deroceras gorgonium*. A: The whole penis; B: Component part of the penial gland (position shown boxed in A); C: The sarcobelum revealed by cutting open the main part of the penis sack: the cut reveals how thick the glandular part of the penis wall is where the sarcobelum inserts. (Scale bars = 1 mm.)

It has been shown in *Deroceras panormitanum* (Lessona and Pollonera, 1882) and *Deroceras reticulatum* (Müller, 1774) that, during early courtship, secretions are produced in the gland (or transported there from elsewhere) and transferred onto the partner's skin when the gland everts (Sirgel, 1973; Nicholas, 1984; Benke, 2006). There are also other glandular areas of the penis, and the sarcobelum also most probably transfers secretions onto the partner's body surface.

These diverse secretions might have several functions, including physiological stimulation and synchronisation, partner manipulation or labelling (Reise, in press). However, in *D. panormitanum* the penial gland is everted only after sperm exchange, which rules out stimulation and synchronisation as its function (but not for the role of the sarcobelum, for instance). One possibility is that the penial gland secretion remains on the outside of the recipient and acts as a label. The label might inform the donor that it has already mated with this individual (both partners might

want to avoid remating with each other), or advertise to other potential partners that this individual has recently mated, and so should be avoided as a mate (to avoid sperm competition or because it is sperm depleted), which would be to the benefit also of the partner that has just donated sperm and left the label (Andersson et al., 2004; Haase and Karlsson, 2004; Ivy et al., 2005). Another possibility is that the secretion could be absorbed and act as an all hormone manipulating the recipient (Reise and Hutchinson, 2001; Reise, in press), for instance not to mate again, to uptake more of the sperm or to digest less of it, or to increase egg laying using the donated sperm (e.g., Johnstone and Keller, 2000; Koene et al., 2005, 2006). For example, the helicid snail *Cornu aspersum* (Müller, 1774) uses the love dart to inject the partner with a secretion which has the effect of closing the opening of the sperm-digesting organ so that more sperm reaches the sperm storage organ, increasing paternity in situations of sperm competition (Koene and Chase, 1998; Landolfi et al., 2001; Rogers and Chase, 2001, 2002; Chase and Blanchard, 2006). Koene (2005) suggested that hermaphrodites are more vulnerable than gonochorists to manipulation by all hormones. However, known examples of all hormones are delivered by injection or in the semen, rather than by external application.

If such manipulations by the sperm donor involve a cost to the sperm receiver, that is there is a sexual conflict, then counteradaptations are to be expected (Rice, 2000; Parker, 2006). Sexual conflicts in simultaneous hermaphrodites are thought to be as strong a force driving the evolution of genital morphology and sexual behaviour as in some gonochorists (Leonard, 1990; Michiels, 1998; Arnqvist and Rowe, 2005). A comparative study of the morphology of love-dart shooting helicid snails (Koene and Schulenburg, 2005) has provided convincing evidence for an evolutionary arms race between male and female functions in these simultaneous hermaphrodites (see also Beese et al., 2006). A number of characteristics of reproduction in *Deroceras* provide the arena for sperm competition and partner conflict: individuals mate repeatedly, clutches can have mixed paternity, one insemination is sufficient for several clutches, some species are able to self-fertilise, and there is a sperm-digestion organ (Reise, in press). An arms race might have led to rapid speciation and to some of the bizarre morphologies and behaviours in this genus (Arnqvist et al., 2000; Gavrillets and Hayashi, 2005; Parker, 2006; Reise, in press).

Prior to this study *Deroceras gorgonium* had only been found at two localities, both on the Greek island of Crete, and, except for the anatomy, nothing further was known about its biology. The aim of this study was to describe its mating behaviour and to compare it with other *Deroceras* species, with particular emphasis on any unusual features that might relate to the form of the penial gland.

MATERIALS AND METHODS

Slugs

Adult and immature slugs were collected on northern Crete (Greece) at three localities between Sises and Aloides (Nomos Rethymnis) on 5 and 6 February 2005:

(1) 35°22'34.5"N, 24°52'43.1"E, altitude 436 m, exposed slope with rocks, low phrygana scrub and poor ground vegetation; (2) 35°22'45.0", 24°53'04.5", 377 m, ground vegetation similar to (1), but taller and denser macchia (mainly *Pistaccia lentiscus*), at its margin mixed in with phrygana scrub; (3) 35°22'05.3", 24°52'14.0", 357 m, at the bottom of a dry creek in an olive grove, rich ground vegetation of grass and *Oxalis pes-caprae*. All collecting sites lay within 2.5 km of the type locality, and at each *D. gorgonium* co-occurred with *Deroceras rethimmonensis* de Winter and Butot, 1986.

At the time of collection the temperature was around or below freezing, with patches of snow at localities (1) and (3), but some animals were active at all three sites. Some individuals were isolated immediately and others kept communally until they could be transferred into a constant-temperature chamber in Görlitz 1 week later.

Externally *D. gorgonium* looks like a typical *Deroceras*. Those adults killed in carbonised water soon after collection and preserved in 70% ethanol are 20-33 mm long. All specimens are stored in the collection of the State Museum of Natural History Görlitz.

Laboratory culture and matings

Slugs were kept in plastic containers with several layers of wet tissue paper and fallen beech leaves; single slugs were kept in Petri dishes (diameter 90 mm, height 15 mm), and groups in larger boxes (120 × 120 × 60 mm). The food consisted of lettuce, carrot, oats and cat-food pellets. Containers and food were changed twice a week. The containers were kept in temperature- and light-controlled chambers (15°C and 12 L:12 D).

We used the large boxes also for the mating trials; the bottom was lined with wet tissue paper, and a beech leaf placed centrally. We started the trials in the daytime, keeping the lighting low yet sufficient for observation; temperatures varied between 15 and 21°C. In each box we placed a pair of individuals isolated several days earlier, except that in one case three slugs were put together to improve the chance of a mating and then one slug was removed once all three had everted their sarcobela. Observations were made directly as well as with a video recorder, sometimes set on interval mode. Also one couple was killed rapidly with boiling water during copulation and then transferred into 70% ethanol.

Dissections at the time of collection established that, while some specimens were adult (developed penis and spermoviduct), other smaller specimens were still immature; we therefore reared small individuals for some weeks before attempting to mate them. Mating trials ran between 18 February and 31 March 2005, but none of the eight trials after 11 March led to mating behaviour, perhaps because the slugs were by then too mature. Other *Deroceras* species that we have kept in captivity ceased to mate at a later stage of their life history despite continuing to lay eggs.

When assigning individuals to potential partners, we initially tended to put individuals of similar size together (because small individuals were potentially

immature). They were immediately isolated again at the end of each mating trial. If there had been no copulation, we would continue to try for another day or two with the same partner. After repeated failures, or after a successful mating followed by an interval of a few days, we would swap partners so as to obtain data from many combinations despite the limited number of specimens available.

Altogether 14 wild slugs were used for 32 mating trials. At least four slugs laid fertile egg clutches. These were all laid after a mating, suggesting that at least these matings were successful (although we cannot rule out that the slugs had mated earlier or self-fertilised). All but one of the 14 slugs mated apparently successfully – and the exception was first tried probably too late in the season – but not all combinations showed mating behaviour. Also, some stopped mating behaviour during precourtship or courtship, or they were interrupted by us because we ran out of time to finish observing the mating. Owing to the long duration of mating, not all matings were fully observed during all phases. For these reasons, not all data are available from all couples, and sample sizes vary. When we give a sample size in the sections below, n refers to the number of trials, not of individuals.

At collecting site (2), one couple was found mating during daytime under a stone. We observed courtship for ca. 30 min until the couple separated; quite plausibly this was owing to the disturbance, but our laboratory observations later revealed that temporary separations were common during courtship in this species (see below).

RESULTS

As in other *Deroceras* species, four main phases of mating can be distinguished (reviewed by Reise, in press): i) precourtship phase: the partners encounter and investigate each other; ii) courtship phase: both partners have the sarcobelum protruded from the genital opening and they assume a position with their genital pores facing each other, forming a circle or yin-yang configuration (fig. 2); iii) copulation phase: the slugs evert their penes, entwine them and mutually transfer ejaculates; and iv) withdrawal phase: the penes are retracted together with the attached sperm masses. Behaviours during these phases are described in detail below.

Of 32 mating trials, 22 led to some mating behaviour and 12 of these to copulations. Eight of the ten couples showing no interest in mating were set up after the last successful mating, so the slugs were possibly too mature. Of the ten instances of mating behaviour which did not lead to copulation, one couple broke off mating during the precourtship phase (38 min after its start), eight couples were separated by us during precourtship (one) or courtship (seven), and we interrupted a further couple during pre-courtship when it was unclear whether they had stopped of their own accord. Amongst the interrupted couples were four couples for which precopulation (precourtship + courtship) had already lasted for more than 10 h (up to 12 h 29 min).

Many slugs mated repeatedly when given the chance: eight individuals copulated two or three times with different partners and another pair twice mated successfully with each other. Time intervals between successful matings were 7-13 days, but in most cases slugs were separated for at least 1 week after a successful mating so did not have the chance to remate earlier. Once slugs started to mate there were no obvious differences in behaviour depending on whether we had observed them mating earlier, but even if we had not it could be that they had already mated in the field or while being transported.

Precourtship

Precourtship is by far the most variable mating phase. Measured from the first signs of interest by either partner (investigation with tentacles, trail following, etc.), this phase took 32-412 min (mean 165 min, $n = 17$, excluding cases when we interrupted during precourtship or missed the exact transition to courtship). If we consider only those couples that successfully mated, the range was still 32-285 min (mean 139 min, $n = 11$). Also, the duration and patterning of the constituent behaviours was highly variable. These behaviours include unilateral or mutual investigations with tentacles (often face to face), trail following, circle formation (bodies curved to the right, head near the partner's tail), and phases of seemingly no sexual interest. They may also spend time stationary in a position typical of that when they wave their sarcobela at each other during courtship (see that section), but with only one or neither sarcobelum extruded (by definition, if both were extruded it would count as courtship: Reise, in press). Each of these behaviours could be shown once or repeatedly (alternating with other patterns) or not at all. Also the duration of single bouts of a certain behaviour varied considerably. Trail following and investigations with tentacles tended to occur earlier during precourtship, while circling and the waving position occurred only later. Most couples started precourtship with trail following, changed repeatedly between mutual investigation, trail following and inactivity, and took up a circle position before the start of courtship (i.e. extrusion of the second sarcobelum or both).

In 15 out of 17 couples adequately observed during this phase, precourtship consisted of repeated alternations between different behavioural patterns. Bouts of trail following (maximum ten in a precourtship) lasted for about 1 up to 40 min each. Similarly, there could be none, one, or several phases of inactivity, each lasting from a few to more than 30 min. Even the fastest precourtship (32 min) included two bouts of inactivity.

Trail following in mating *Deroceras* is characterised by one slug crawling closely behind the other. The front slug's tail is usually slightly flattened and most of the time lies between the second slug's tentacles (Reise, in press). In *D. gorgonium*, trail following almost always occurred at some stage during precourtship, but occurred far less than in *D. panormitanum* (Benke, 2006; Reise, in press), and there was no pronounced tail lashing as in that species. Only one couple showed no trail following

during precourtship (but one partner followed at some distance). In ten out of 15 couples, it was always the same individual following the other. In four (possibly only three) couples the partners swapped roles once, and in one couple four times, but in the latter case one partner was leader only for short periods. The roles during trail following were good predictors of who would extrude the sarcobelum first: in ten out of ten unambiguous cases this was the leading slug. This agrees with observations on *D. panormitanum* (Benke, 2006; Reise, in press) and indicates that trail following is not a case of one keener individual following a reluctant partner eager to escape. When roles had been swapped, the last role distribution decided the order of sarcobelum extrusion even if the other slug had been leading for most of the time.

With eight couples, the two sarcobela were extruded at roughly the same time (up to a few minutes apart), whereas in another ten couples the second sarcobelum was extruded 14-94 min later (mean 42 min). In six of these ten cases, the first sarcobelum to extrude was retracted once or repeatedly before the second slug extruded (mainly during the phases of inactivity, particularly if the partner appeared not to show much interest), and both sarcobela could also be retracted later, during courtship, but then for only shorter periods (see below).

Seven couples were in a circle position when the second sarcobelum was extruded, two couples were in the waving position, and two couples were sitting face-to-face touching each other with their tentacles (one of the latter changed to the circle position immediately afterwards).

In one case, three individuals (A, B, C) were involved in precourtship (they are not included in the durations given above). There were several short bouts of trail following where either A followed B or C followed A. After ca. 2 h, A and C extruded their sarcobela and investigated each other. B joined the couple after 20 min, extruded its sarcobelum 6 min later, and then all three slugs formed a circle with each individual following the tail of another. B was then removed.

Courtship

Because there could be an interval between extrusion of the first and second sarcobelum, and because both sarcobela were often extruded and retracted repeatedly, the separation between precourtship and courtship was much less clear than in other *Deroceras* species. If we follow Reise's (in press) definition of the start of courtship (extrusion of both sarcobela), courtship lasted 254-434 min (mean 327 min, $n = 10$); thus its duration was highly variable although the minimum time was longer than most precourtships in this species. This variability is not reduced if the first protrusion of the first sarcobelum is considered as the beginning of courtship. However, length of precourtship was negatively correlated with length of courtship, so that the variance in the total duration of precourtship and courtship (353-589 min, mean 475 min, $n = 10$) was no more than in the duration of courtship alone.

Retractions of one or both sarcobela, as well as phases of inactivity, could occur during almost all stages of courtship, but this happened in only three of the 12

successful couples. In two of these couples these 'fall backs' into behaviour typical of precourtship were brief (1-10 min), but in another couple both sarcobela were retracted for 43 min, and one of them for an additional 66 min.

Usually just after both sarcobela had been extruded (but sometimes shortly before sarcobelum extrusion, or up to 26 min after), the partners started the typical courtship behaviour that occupied the major part of this phase. It consisted of a frequent alternation between two constituent behaviours (fig. 2A-D). One of these was 'waving' (fig. 2A, C): the slugs stood diagonally to each other, each facing the partner's extruded sarcobelum, which was directed more or less straight out to the side and performed fore-and-back waving movements. Extruded sarcobela were much longer than when in their withdrawn position within the penis (fig. 1C), and the pointed tips were usually bent towards the partner. The tails were usually curved to the right, but could also be straight. The behaviour with which waving alternated was 'circling' (fig. 2B, D): the slugs circled half a turn around each other in a clockwise direction. From the waving position, they first crawled more or less straight, passing each other in an antiparallel direction. Once their heads became level with the partner's tail, they turned right and crawled back roughly into the position where the partner had been before, and then stopped and resumed waving. The typical frequency of circling was one half circle (taking about 1 min) every 2-4 min, but it could be less often.

During the course of courtship, the partners gradually got closer. For a long time circling would almost never involve touching with the sarcobela (fig. 2B), although occasionally the mouth or tentacles might touch the partner's tail. Later, they would pass each other more closely so that the stretched sarcobela stroked along the partner's side (fig. 2D), first with the tips only, then more intensely. Similarly, the partners' decreasing distance apart altered the waving position. In the first stage of courtship, the sarcobela waved in front of the partner's face without touching (fig. 2A); the rest of the body was more or less motionless, except occasionally in some couples when one slug suddenly stretched forward and protruded the mouth, snapping into the air towards the partner. This aggressive behaviour was usually rare, but occurred repeatedly over a 140 min period in one couple. Later during courtship, the slugs would be sitting slightly closer, their faces now roughly alongside so that the tips of their waving sarcobela often touched each other. After this intermediate stage, the slugs got even closer so that their sarcobela were roughly side by side (antiparallel). Now the tips of the waving sarcobela often touched the partner's face or the base of its sarcobelum (fig. 2C). The touching tended to alternate between partners; in typical cases, a touch occurred about every 1-26 s. If individuals were of very different sizes, the sarcobelum of the larger one tended to touch the partner's face while that of the smaller one touched the base of the partner's sarcobelum. Occasional snapping still occurred at this stage, but rarely, and could now result in successful bites into the base of the partner's sarcobelum or into a tentacle, usually followed by a temporary increase of distance between the partners.

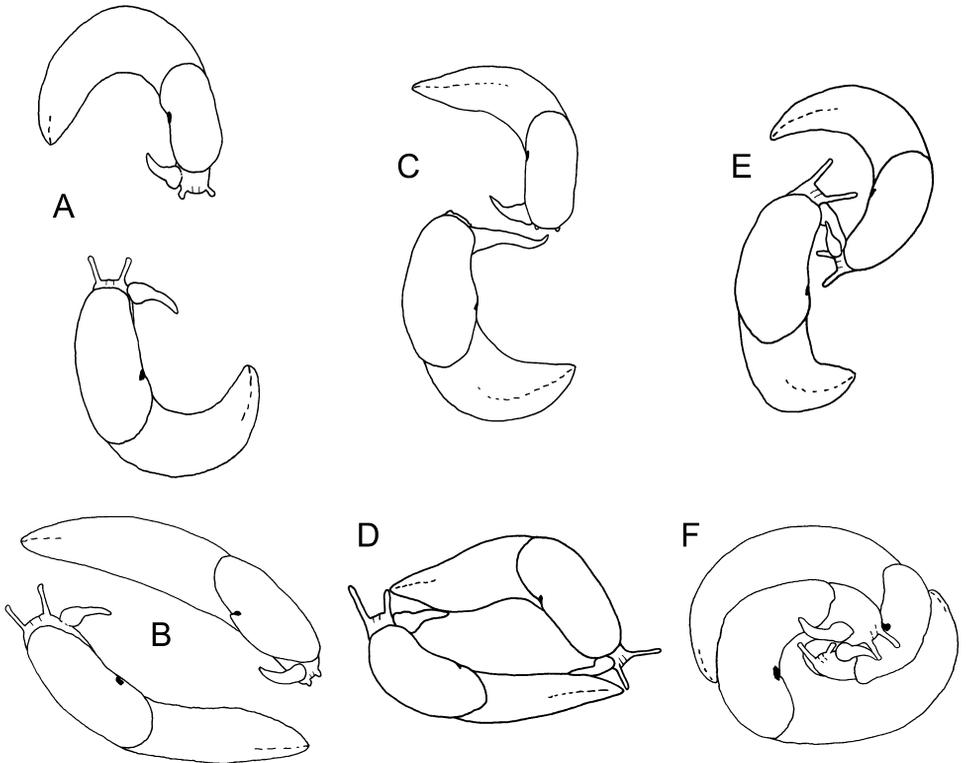


Figure 2. Courtship of *Deroceras gorgonium*, based on video stills. A, B: Distant waving and circling during early courtship; C, D: Closer waving and circling with body contact characteristic of later in courtship; E: Close contact between sarcobela; F: Close yin-yang.

In a few cases, we observed the transfer of a secretion via the sarcobelum: a droplet appeared on the side of one slug's sarcobelum (the side facing the partner), and moved slowly towards the tip from where it was transferred onto the base of the partner's sarcobelum during the next contact.

The major part of courtship, the alternations of waving and circling, finished when both slugs assumed a yin-yang position: they curved their bodies even more to the right, and pressed their heads against the partner's right side. In this way, the bodies themselves got into close contact for the first time, and also the genital openings were now close to each other. This last stage was rather short, 5-34 min before copulation. The sarcobela now touched the partner's body or sarcobelum for most of the time. Occasionally, the partners put the bases of their sarcobela together, probably placing their genital pores into direct contact.

Usually at this point tighter and looser yin-yang positions alternated, with the sarcobela gaining and losing contact repeatedly. However, the slugs maintained the yin-yang position and almost never reverted to earlier behavioural patterns: the exception was one couple in which the initial yin-yang lasted much longer than usual (73 min), they then reverted to waving and circling, but 54 min later did

copulate following another yin-yang of normal duration. Often, one partner was consistently the one to loosen the position by turning its head away, which could considerably delay copulation. Such a difference, one partner apparently striving for progress and the other reluctant, could also often be observed in the earlier, long stage of courtship, but this had no relationship with which slug had first extruded its sarcobelum. Immediately before copulation, the yin-yang became even tighter, the anterior bodies swelled and lay slightly on the left side, and the sarcobelum bases were pressed against each other (fig. 2F).

Copulation and withdrawal

The copulation phase was extremely short and started very suddenly. Both penes everted simultaneously and entwined within 1 s. The ejaculates, amorphous white packages probably transported into the penis during courtship, were sticking to the inner penis wall and were brought outside with the penis eversion. At maximum eversion of the penis sacks (after 1 s, fig. 3A), they were 'slapped' onto the partner's everted penis (probably onto a defined receiving area on it), and the donating penis parts slackened immediately after this. The longish shape of this sperm donating part implies that this is the side pocket of the penis (fig. 1A). Investigation of the pair killed at this stage showed two longish ejaculate packages each sticking tightly to the wall of a penis and stretching from the side pocket to the base of the sarcobelum. In those cases in which copulation was filmed or sufficiently well observed, both partners donated and received ejaculates simultaneously.

One to 3 s after the start of copulation, the penial glands began to evert and reached maximum eversion 0.5-2.1 s later (fig. 3B). The eversion process looked like a timelapse film of roots growing, and often the two main branches of the gland were

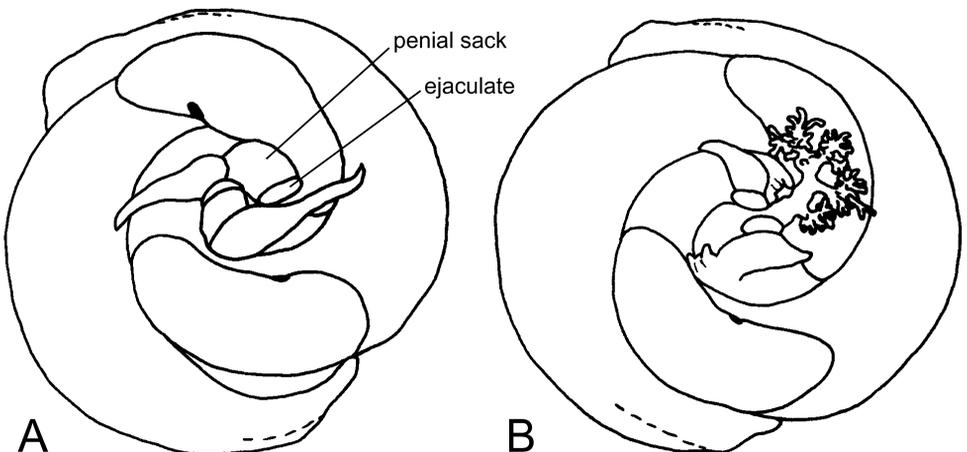


Figure 3. Copulation of *Deroceras gorgonium*, traced from video stills. A: Mutual ejaculate transfer onto the bases of the sarcobela (the labels indicate the penial sack and ejaculate of the right-hand slug); B: Eversion of appending penial gland (the gland of only the right-hand slug is visible).

distinguishable. However, in only three couples were both penial glands clearly visible and laid onto the partner's mantle or side. In five couples only one gland was laid on top of the partner, and in one couple no gland was visible. This might lead to the conclusion that, in contrast to other *Deroceras* species, often one partner does not evert the penial gland. However, examination of the pair killed during copulation showed that the apparently non-everted gland was actually spread under the partner; re-examination of the video recordings then established this also in two of the other four couples with apparently only one everted gland. However, we cannot exclude the possibility that four penial glands were not everted at all, as has been reported for two individuals of *D. panormitanum* (Benke, 2006). The eversion was rarely directly onto the partner's body surface as happens in *D. panormitanum*; instead the gland was initially stuck up into the air and then laid down onto the partner, but sometimes the gland retracted before the distal parts of the glands were lowered enough to make contact. Whether the penial gland lay under or over the partner seemed not to be related to body size or roles during trail following, and varied between matings of the same individual or even the same couple.

Immediately after maximum eversion, the gland was retracted, and full retraction into the penis (although most probably not yet into the original position) was reached 10-24 s later. The genitalia lost contact and the slugs separated 18-25 s after the start of copulation. The entire penes were retracted ca. 1-5 min after the start of copulation.

DISCUSSION

The general mating pattern of *D. gorgonium* was like that of other *Deroceras* species (Wiktor, 2000; Reise, in press). However, a number of features were peculiar to *D. gorgonium*: i) extremely long precopulatory phases; ii) highly variable duration and patterning of certain constituent behaviours during precourtship, including trail following typically occurring in several short bouts; iii) intermittent phases of seemingly no sexual interest (during precourtship often and sometimes for long periods, whereas during courtship occasionally and for short periods); iv) partners showing little activity and very little body contact during most of the courtship; and v) the penial gland often spread underneath the partner rather than on top.

Long precopulatory phases

Precourtship and courtship together lasted almost 6 to more than 9 h, much longer than in any other species studied. Some couples were interrupted by us after more than 12 h. Each of the two phases was longer than in most other species. While precourtship took a considerable part of the entire mating in *D. gorgonium*, it is usually much less pronounced in the other 12 species of *Deroceras* for which there are data. In some species, individuals investigate each other only briefly before everting their sarcobela, so that precourtship is hardly recognisable as a

phase. Species with trail following usually show a somewhat more pronounced precourtship phase; in *D. panormitanum*, the species with the most intense trail following, precourtship can last up to 28 min (Benke, 2006; Reise, in press). Previously the longest courtship, of sometimes over 240 min, had been observed in *D. turcicum* (Simroth, 1894) (Reise, in press). Other species have courtships of 20-210 min.

The long precourtship and courtship phases contrast strongly with the extremely fast copulation. Sperm exchange happens within 1 s, and partners separate after 18-25 s. Long courtship followed by a quick copulation does occur in some other *Deroceras* species, such as *D. rodnae* Grossu & Lupu, 1965 and *D. turcicum* (Reise, 1995, in press). However, quick copulation is not always correlated with long precopulation phases. For example in *D. praecox* Wiktor, 1966, copulation takes less than 30 s, yet precourtship is very brief and courtship can be as short as 20 min (Reise, 1995, in press).

Given that other congeners manage with a much shorter courtship, and given its variable duration within *D. gorgonium*, it seems unlikely that its long courtship has evolved to facilitate synchronisation or allow time for preparation of the sperm mass. One possibility is that a long courtship allows better assessment of a partner's qualities, upon which might depend decisions of whether to go ahead with copulation or how much sperm or eggs to allocate to this mating. Another possibility is that a long courtship increases the chance of further individuals getting involved, thus facilitating mate choice. That hypothesis predicts that the longest courtships occur when partner quality is more asymmetric, because only then will the better quality partner run little risk of being the individual left out of the copulation if another joins in the mating. Or the long courtship could be the outcome of an arms race in which each individual seeks to manipulate its partner yet avoid being manipulated.

Variable behavioural patterning

The long duration of precourtship may have to do with it consisting of many short bouts of different behaviours, mainly trail following, mutual investigations and inactivity. In other *Deroceras* species in which precourtship is pronounced, the sequence of behaviours is much more straightforward and predictable. Trail following is most pronounced in *D. panormitanum*, and there it is usually not interrupted by other intermittent behaviours, and roles are swapped only rarely (Benke, 2006; Reise, in press). In that species, at the end of trail following the leading slug turns back and extrudes its sarcobelum. The partners then usually form a circle and the second slug extrudes its sarcobelum. Exceptions to this regular pattern are uncommon and minor, such as trail following continuing a little longer after the sarcobela have been extruded. Trail following has also been observed in other *Deroceras* species, although less pronounced (Reise, in press). It remains to be investigated whether in these species it sometimes also consists of several bouts.

The precourtship behaviour of *D. gorgonium* suggests some uncertainty as to whether to proceed with mating or not. It may also involve partner assessment. However, even precourtships highly broken up into many phases, including inactivity, could proceed to copulation. It is probably misleading to envisage that the inactive phases signify a lack of sexual interest, because a sarcobelum was often extruded just after such a phase and inactivity (with both sarcobela retracted) was also observed 36 min before copulation.

We cannot exclude that this unusual patterning and duration of precourtship, and its variability, are an artefact caused by unnatural environmental conditions (e.g., light). However, both short and long precourtships often lead to successful matings, and even the shortest precourtship (32 min) consisted of several short bouts of different behaviours, including inactivity. Moreover, these unusual aspects of behaviour are not ones that have cropped up occasionally in our observations of other *Deroceras* species, some also from Crete, under the same laboratory conditions (Reise, in press, pers. obs.).

Little body contact during courtship

The most prominent behaviour during courtship in *Deroceras* is mutual stroking with extruded sarcobela and the entire phase consists of this behaviour in most of the species studied. *Deroceras panormitanum* is somewhat exceptional because of a high level of aggressiveness during early courtship, which is overcome slowly (Gerhardt, 1939; Barker, 1999; Benke, 2006; Reise, in press). During roughly the first half of courtship in *D. panormitanum*, partners maintain a distance and seem to try to touch the other one with the sarcobelum without being touched. There is also violent biting and tail lashing. However, even at this stage both sarcobela touch the partner often, and in the second half of courtship body contact and stroking resemble that in most other *Deroceras* species.

In *D. gorgonium* in contrast, although there is little aggressiveness (biting), the partners have little body contact during most of courtship. Moreover, they spend many hours sitting opposite each other and doing little else but waving their sarcobela alternated with circling half a turn. During the first few hours *D. gorgonium* seems not even to try seriously to touch the partner. What is the purpose of this? The exact positioning, the waving and the regular alternation of waving and circling are reminiscent of a ritualised combat during which duellists threaten each other by pointing their swords. However, as we observed one mating in the wild under a stone, and thus in the dark, it is questionable whether the waving is an optical signal.

Although large glandular areas at the base of the sarcobelum as well as the longitudinally ridged surface had already prompted the suggestion that the sarcobelum of *Deroceras* transfers secretions (Nicholas, 1984; Reise, in press), our observation of droplets transferred from the tip of the sarcobelum onto the partner is the first clear evidence of this. The long sarcobelum of *D. gorgonium* (fig. 2A-D) seems ideal for long-distance touching, which might be adaptive if slugs seek to touch but not

be touched. The very slow decrease of distance between the partners might reflect these two conflicting interests and their initial uncertainty as to how far the partner's sarcobelum can reach. In the sea slug *Siphopteron quadrispinosum* Golsiner, 1989, the first partner to inject a tranquiliser into the other is able to donate sperm without receiving it (Anthes and Michiels, 2007). Whilst the situation in *Deroceras* is certainly not so extreme, it is possible that the individual that lands more secretion onto the partner early in precopulation gains a disproportionate quantitative advantage by thereby suppressing reciprocal behaviour in the partner.

Secretions transferred onto the face or base of the sarcobelum (during waving) might be more effective than onto the body side (during circling), because they might penetrate the skin better or be less easily shed; also face and sarcobelum are near the distal genitalia, which might be the target of allohormones. This would explain why the slugs always returned to their standard waving position, from which the face or sarcobelum were reachable. However, this does not explain why waving was so often interrupted by circling. One possible explanation of the intermittent circling bouts is that the partners are assessing each other's size, similar to the proposed function of the 'sandwich' posture of the planarian flatworm *Dugesia gonocephala* Dugès, 1830 (Vreys et al., 1997). During circling, both slugs slowly pass each other antiparallel and turn once they have reached the end. At later stages of courtship they stroke along the partner's flank with the tip of their sarcobelum, which might be an even better way to estimate body size; it would be interesting to seek histological evidence for a sensory function of the sarcobelum, which has not been suggested before. Body size might indicate both genetic quality and quality as an egg producer, and thus be relevant to decide the amount of sperm to donate and whether to use received sperm for fertilisation.

Eversion of the penial gland under the partner's body

According to observations on other *Deroceras* species, appending penial glands, if present, are always everted during copulation and spread on the partner's upper body surface (Reise, in press). Surprisingly, the gland of *D. gorgonium* was not always spread on the partner's upper body surface, but instead underneath it in at least three copulations, and potentially in more than half of the copulations observed. This has never been reported from other *Deroceras* species.

In *Deroceras reticulatum* (Müller, 1774) and *D. panormitanum*, the penial gland transfers a secretion onto the partner's body surface (Nicholas, 1984; Benke, 2006). Benke (2006) observed that in *D. panormitanum* the slugs try to eat this secretion off their own body immediately after copulation. Unfortunately, we did not observe our slugs after withdrawal. An application of secretions underneath the partner makes the possible function of partner labelling (see Introduction) less probable, because it would seem harder to detect a label there. If we suppose instead that the secretion indeed manipulates the partner (Reise and Hutchinson, 2001), and that the partner tries to prevent this, gland eversion underneath the partner might have evolved as a counteradaptation to prevent removal. Secretions applied onto the sole

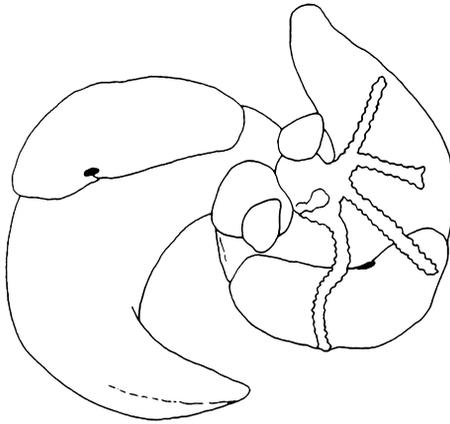


Figure 4. Penial gland eversion in *Deroceras panormitanum*, traced from a video still.

might penetrate the skin better than secretions on the back or mantle, and it might be more difficult for the receiver to eat the secretions on its sole than on its back. A flat shovel-like gland would seem better for application underneath, but numerous short fingers might also function well, and certainly have a higher surface-to-volume ratio.

The surface area and body region which can be covered by the appending gland, and probably the amount of secretion which can be transferred, depend on the size of the penial gland. The few fingers of the gland of *D. panormitanum* are comparatively long, and they stretch even more during eversion, extending over almost the entire body length (fig. 4). Considering its huge size in dissected specimens of *D. gorgonium*, we were surprised how little body surface the everted gland covered (fig. 3B). Moreover, often the gland seemed not to spread fully onto the partner's body surface, but large parts would stick up into the air. Such slugs probably did not succeed in applying all gland secretion onto the partner (in *D. panormitanum*, glands which were not fully spread on either the partner or the ground may still contain secretion: Benke, 2006). The multiple levels of branching in the gland of *D. gorgonium* may make it impossible for each branch to be directed in the appropriate direction to contact the partner's back. However, a gland everted under the partner will be squashed flatter so that more of it touches the partner's skin, and parts in contact with the ground could release secretion that will contact the partner as it crawls forward after the gland's rapid retraction. In this way the morphology of the gland would be associated with the habit of everting under the partner's body.

If there are these proposed advantages to everting the gland underneath the partner, why is the gland often everted onto the back? Perhaps each animal manoeuvres so as to avoid the partner's gland being everted over the most effective site for manipulation.

Eversion of the penis and all appending structures is probably entirely by haemolymph pressure, and there must be a limit to the volume which can be everted

by a slug of a given body size. The gland of *D. gorgonium* with its many short fingers and that of *D. panormitanum* with its few (3–6) long unbranched processes might represent two different evolutionary solutions to the same problem – transferring as much secretion as possible. Gland eversion and retraction in *D. gorgonium* are fast, possibly because the single fingers are rather short. The gland eversion in *D. panormitanum* is rather slow, so that the partner sometimes escapes before being treated with the secretion (Benke, 2006; Reise, in press).

We cannot exclude that in two out of five couples where only one or no gland was everted on top the glands we did not see were not everted at all. Benke (2006) observed two out of 25 copulations in *D. panormitanum* where one of the appending glands was not everted. In one of these cases, the uneverted gland was still filled with secretion after the end of copulation. To find out how often *D. gorgonium* everts underneath or not at all, one has to use high-resolution video or kill animals during copulation. It would be worthwhile to investigate the effect on fertilisation success of whether the gland was everted on top or underneath, analogous to the comparison of successful and unsuccessful love-dart shooters in *Cornu aspersum* (Landolfi et al., 2001; Rogers and Chase, 2001; Chase and Vaga, 2006). One might also test effects on sex allocation or remating interval.

CONCLUSIONS

By observing the mating behaviour of a species with an extremely large and elaborate appending penial gland, we hoped to learn about the function of this gland. Also, as the bizarre organ seemed best explained by an evolutionary arms race between male and female functions, we hoped for more indications of sexual conflict in the mating behaviour. We were somewhat disappointed to find that the gland eversion was much less spectacular than expected from the anatomy. On the other hand, the study revealed a number of unusual behavioural patterns which might indicate sexual conflict, but which are not all obviously related to the enormous size of the penial gland.

Further studies are necessary to understand the perplexing mating patterns in *D. gorgonium*. These include investigations of behaviour immediately after copulation, and of the reproductive consequences of where the penial gland was everted. It might be revealing to study the influence of absolute and relative body sizes on mating behaviour, and in this context to allow a third slug to be present in case there is a mate-choice component to precopulation. One should also check whether more natural environmental conditions alter the behaviour, for instance by studying mating in darkness using infrared light.

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