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The distinction between retractor and protractor muscles of the freshwater snail's male organ has no physiological basis

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SUMMARY

Many animals are equipped with organs that can be everted, a notable example being male copulatory organs. The ability to protrude or evert an organ generally requires protractor and retractor muscles. Male copulatory behaviour of the pond snail *Lymnaea stagnalis* (L.) involves eversion (protraction) and retraction of the relatively large penis-carrying organ. For this preputium, protractor and retractor muscle bands have been defined, which implies eversion and retraction through the activity of these muscle bands. However, no physiological data are available that confirm that the terms protractor and retractor are appropriate. To test whether eversion and retraction are possible without protractor and/or retractor muscle bands, lesion experiments were performed. The results show that with either one or several muscle bands lesioned, snails were still capable of everting their preputium and using it for copulation. However, the majority of animals that had six or more muscle bands lesioned were unable to retract its preputium. Hence, retractor muscle bands serve their designated function whereas protractor muscle bands do not. We therefore suggest that a different terminology is used in which all muscle bands are retractors and, based on their location, are either called distal or proximal retractors. The findings furthermore indicate that the preputium muscle bands are normally contracted, possibly in a catch state, retaining the organ inside without high-energy expenditure.

Key words: Basommatophora, catch, copulation, mating, Mollusca, Pulmonata, *Lymnaea stagnalis*.

INTRODUCTION

Protrusible and evertible organs are often equipped with protractor and retractor muscles to, respectively, externalise and internalise the organ (e.g. Neustadter et al., 2007; Paul et al., 2002). Most gastropod molluscs have a male copulatory organ that is everted during mating possibly involving the activity of protractor muscles. After sperm is transferred, the organ is retracted back into the body by retractor muscles. For freshwater snails both protractor and retractor muscles have been described (Holm, 1946) and both terms are used by taxonomists (e.g. Paraense and Pointier, 2003; Paraense, 2006), while for brackish water, marine and terrestrial molluscs only retractor muscles have been described (e.g. Berry et al., 1967; Blankenship et al., 1977; Gittenberger, 1979; Jaeger, 1963; Wabnitz, 1976). Is this a fundamental difference between these different species of simultaneous hermaphrodites or simply a misconception?

Before the penis-carrying organ – the preputium – is slowly everted in *L. stagnalis*, the individual performing the male role goes through a series of behavioural elements (Van Duivenboden and Ter Maat, 1988; De Boer et al., 1996; Koene and Ter Maat, 2005). The future sperm donor first mounts a mating partner (mounting), circles its shell (circling) and finally positions itself at the right edge of the shell (positioning). From this position the female gonopore can be reached and the fully everted preputium (i.e. full eversion) is used to find this opening (probing). Once the opening is found the penis is everted at the tip of the preputium and inserted into the vaginal duct of the sperm recipient (intromission). When sperm transfer is complete, the preputium is retracted.

When not copulating in the male role, the animal keeps its preputium inside. The organ is hollow and cylindrical in shape and is composed of longitudinal and circular muscles (Plesch et al., 1975). It is attached to the body wall by a number of muscle bands that have been labelled as retractors and protractors (Holm, 1946). For eversion, the organ is turned inside out *via* the male gonopore, located behind the right tentacle. The preputium carries the penis that is enveloped by the penis sheath. The penis itself is only everted when the tip of the preputium is pressed against the female gonopore. As a consequence, the penis cannot be observed during intromission because it is entered directly into the vaginal duct of the recipient. Sperm and seminal fluid is, respectively, transferred from the seminal vesicles and prostate gland *via* the vas deferens and penis into the vaginal duct (Van Duivenboden and Ter Maat, 1988; Loose and Koene, 2008).

The central control of male behaviour and preputium eversion has been extensively studied. Five neuronal clusters are known to innervate the preputium *via* the penial nerve (De Boer et al., 1996). These regions of the central nervous system produce a number of neuropeptides and neurotransmitters, some of which affect the preputium and/or its muscle bands (for a review, see Chase, 2002). In the preputium itself, immunocytochemical research has revealed the presence of APGWamide, SEEPLY, DEILSR and *Lymnaea* Inhibitory Peptide (LIP) in the longitudinal muscles of the outer sheath of the preputium, and DEILSR and serotonin (5HT) in the circular muscles of this outer sheath (De Lange et al., 1998). In the muscle bands, myomodulins, APGWamide, DEILSR and LIP have been found (De Lange et al., 1998). *In vitro*, APGWamide, *Lymnaea* Neuropeptide Y (LyNPY), GDPFLRF and SDPFLRF have a relaxing effect on the muscle bands (Croll et al., 1991; Li et al., 1992; Van Golen et al., 1995a) whereas 5HT, FMRFamide and FLRFamide have a contracting effect (Croll and Chiasson, 1989; Croll et al., 1991; Van Golen et al., 1995a). Some other substances

seem to modulate the magnitude of these contractions. LIP A, B and C reduce the amplitude of the contractions (Van Golen et al., 1995b; Smit et al., 2003), as do two of the myomodulins [SLSMLRL and GLQMLRL (Van Golen et al., 1996)]. The other two myomodulins increase the contraction's amplitude [SMSMLRL and PMSMLRL (Van Golen et al., 1996)].

In sum, the above indicates that many neuropeptides, and several neuronal clusters that produce them, are involved in the control of movement of the preputium. Moreover, this implies an extensive control and fine-tuned regulation of eversion and/or intromission of the male copulatory apparatus. One key neuropeptide for preputium eversion seems to be APGWamide from the anterior lobe of the right cerebral ganglion. De Boer et al. showed that electrical stimulation of the anterior lobe, as well as injection of its main neuropeptide APGWamide into the blood, induces preputium eversion (De Boer et al., 1997a; De Boer et al., 1997b). Moreover, the right anteromedial region of the cerebral ganglion has been demonstrated to play a key role in the control of male mating behaviour in gastropods in general (Koene et al., 2000).

Although much is known about the neuro-endocrine control of preputium eversion, the functional role of the different muscle bands that are attached to the male copulatory organ remains unknown. Knowledge about the use of these muscles is essential for proper interpretation of the functional significance of the reported involvement of the neurones and of the effects of their neuromodulators on these muscles. Holm's terminology (Holm, 1946) for these muscle bands of freshwater snails clearly suggests distinct functions for the proximal and distal bands (respectively, for eversion/protraction and retraction). We, therefore, set out to test the contribution of these different muscle bands to eversion as well as retraction of the preputium.

MATERIALS AND METHODS

Animals

Adult specimens of the great pond snail *L. stagnalis* with shell heights between 30 and 38 mm were used. These animals had been raised and kept in large breeding tanks under standard laboratory conditions with running fresh low-copper water of 20°C and a light:dark cycle of 12 h:12 h. Lettuce leaves were provided *ad libitum*.

Surgery

Before lesioning one or more protractor and/or retractor muscle bands, animals were anaesthetised by injecting approximately 2 ml of 50 mmol l^{-1} $MgCl_2$ into the foot of the animal. A skin incision of 3–4 mm was made in the head region on the dorsal side just right of the median. After identification of the muscles either one or several muscle bands were cut or the animal was used for the sham-operated group. Thus, sham-operated animals went through the whole surgical procedure but their muscle bands were left intact. Animals were allowed to recover over the following eight days (see below). At the end of the experiment, all animals were killed to verify whether the operations were performed correctly.

Behavioural observations

We made use of the previous finding that a period of sexual isolation increases male sexual drive in this species (Van Duivenboden and Ter Maat, 1988; De Boer et al., 1997a). Isolation was achieved by keeping the animals individually in perforated jars in the tank for eight consecutive days. Following isolation, male sexual behaviour was assessed as follows. All lesioned, sham-operated and control animals were paired with non-operated, non-isolated individuals. The animals were paired in transparent, unperforated jars, which were filled with water from the breeding tank and placed in a temperature-controlled room (20±1°C). Pairs were observed for at least three hours by a naïve observer (i.e. unaware of which particular treatment each snail had undergone). During the observation period the male behavioural elements that were scored were: mounting, circling, positioning, partial eversion of the preputium, full eversion of the preputium and intromission [according to De Boer et al. (De Boer et al., 1996)]. Whenever an animal was engaged in male copulatory activities, the observation was continued until these activities stopped. Following the experiment, female copulants were dissected and checked for the presence of semen in the vaginal duct to determine whether transfer of semen had taken place. This is easily detected by the white swollen appearance of the vaginal duct, which is thin and flat when no semen has been received (Loose and Koene, 2008).

RESULTS

Morphology and nomenclature

Fig. 1 shows a schematic drawing of the preputium and the attached muscle bands. Based on their morphology, we make a distinction between the proximal and distal muscle bands, which can be located either dorsally or ventrally. Thus, the protractor muscles defined by Holm (Holm, 1946) are those bands that are positioned proximally (Fig. 1: no. 4–6 and 8–10). These proximal muscle bands are on one side attached to the preputium near the male gonopore and on the other side to the dorsal and lateral body wall. The retractor muscles that Holm (Holm, 1946) defined have a distal position (Fig. 1: no. 1–3 and 7). These distal muscle bands are on one side attached to the preputium near the penis sheath and on the other side either to the dorsal or lateral body wall. The latter attachment to the body wall is more posterior than that of the proximal muscle bands.

The muscle bands are also arranged in two distinct lateral lines on the preputium, one on the dorsal side and one on the ventral side. The preputium muscle bands that are commonly found in *L. stagnalis* were numbered along these lines in order to distinguish between the different muscles. We found that the total number of muscles may vary slightly between individuals. Two variations were observed. Firstly, an additional small muscle was sometimes attached to the most proximal end of the preputium, either dorsally or ventrally. Secondly, the two most-distal muscle bands on the ventral side (Fig. 1, no. 1 and 2) were sometimes found to be partially fused (as indicated by the broken lines between no. 1 and 2 in Fig. 1). In these rare instances where the additional muscle band was present, either all of the muscles were cut or the animal was not used for the experiments. Likewise, if muscle bands no. 1 and 2 were partially fused, either none of the bands or both bands were lesioned.

The muscle bands of the preputium have different attachment sites on the body wall. All ventral muscle bands are attached to the columellar muscle. Among the dorsal muscle bands, muscle bands no. 9 and 10 are attached to the lateral body wall near the gonopore. Muscle bands no. 7 and 8 are attached to the lateral body wall. When the preputium was inside, muscle bands no. 1–3 make a 70–90 deg. angle with the preputium whereas all of the other muscle bands make 25–40 deg. angles.

Effect of lesions of the preputium muscle bands on male sexual activity

To test whether the muscle bands attached to the preputium are involved in protraction and retraction, 79 operations were performed in which single, several or all muscle bands were lesioned. Of the

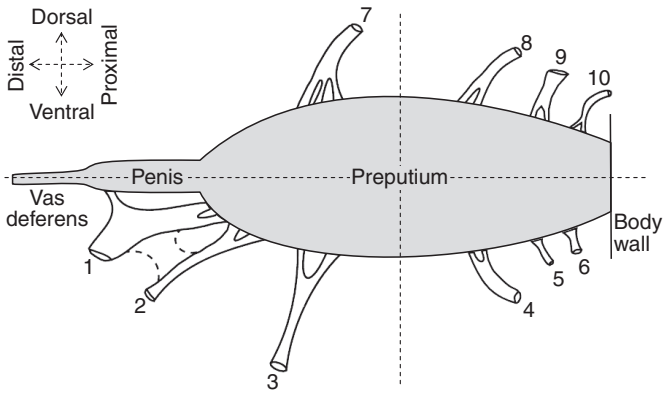

Fig. 1. Morphology of preputium muscle bands. Location of the muscle bands in *L. stagnalis* on the preputium. The muscle bands are drawn after cutting them near their attachment site on the body wall. Only part of the vas deferens is shown. The dorsal–ventral and proximal–distal coordinates are indicated with broken lines. For clarity, upon eversion the preputium is turned inside out towards the right in this drawing. The two most-distal muscle bands on the ventral side can sometimes be partially fused (as indicated by the broken lines between no. 1 and 2).

operated animals 32 died within one to four days following the operation, the remaining 47 animals survived. The animals that died following the operation all had more than three muscle bands lesioned (Table 1). The difference in death rates was significant (Pearson χ^2=23.77; d.f.=8; P=0.003); for this and subsequent comparisons animals were grouped based on the number of muscle bands that were cut (see Fig. 1). Fifteen of these animals kept their preputium everted for two to three days before they died (Table 1). None of these 15 animals retracted their preputium back into the body cavity. These findings indicate that lesioning of the muscle bands can cause spontaneous eversion of the preputium and impairs the ability to retract the preputium.

The 47 animals that did survive the operation were used to test their performance during male copulatory activity. In the behavioural experiments, 42 lesioned animals mounted the shell of the potential partner, 27 showed circling and 20 showed positioning. For the effects of the lesions we were primarily interested in the animals that showed eversion, intromission and retraction of the preputium. In some groups of animals that had received the same lesion, either no behavioural acts were observed or only acts without preputium movements. Table 2 lists the lesion groups in which at least one animal showed preputium eversion. The table reveals that animals with most, but not all, of the muscle bands lesioned were still able to evert the penis, probe for the female aperture and ejaculate (Pearson χ^2=4.31; d.f.=8; P=0.83). Even retraction was possible in all surviving animals except one. As indicated above, most animals with six or more bands lesioned everted spontaneously and died. After full eversion, these animals were simply unable to retract the preputium (Table 2). This inability to retract the preputium was significantly different (Pearson χ^2=22.68; d.f.=8; P=0.004). Sham-operated and control snails also showed male copulatory behaviour, including preputium eversion, intromission and retraction.

Because survival and retraction showed significant effects, as reported above, we split these data in two partitions. Assuming that there is a critical number of muscle bands at which these effects arise, we determined the most significant split of the treatments into two groups, using the χ^2 criterion. For survival we found this split to lie where animals had four and more muscle bands cut (χ^2=34.62; P<0.05); for retraction we found it to lie where animals had six and more muscle bands cut (χ^2=3.80; P<0.05). All of the findings are summarised in Table 3, which shows the survival as well as the ability to evert and subsequently retract the preputium. In sum, lesioning several preputium muscle bands impairs survival and the ability to retract the preputium (and thereby survival) but does not affect the ability to evert the organ.

DISCUSSION

In the present study, we found no evidence for the presence of a protractor function of the preputium muscle bands of the snail *L. stagnalis*. The experiments clearly show that lesioning the proximal muscle bands, which were previously labelled as protractors, had no effect on eversion but can impair retraction. Some of the animals with lesions of several distal and proximal muscle bands did not retract their preputium. This occurred both in animals that everted their preputium during copulation and in animals that everted during the recovery period. This indicates that both the proximal and distal muscles are involved in retraction rather than eversion (protraction).

In total 40.5% of the animals from which preputium muscle bands were lesioned did not survive the recovery period. A possible cause of this high mortality rate could be severe blood loss. In order to gain access to the muscle bands for lesioning it was necessary to make a rather large incision in the body wall. Such a wound in the body wall may have been too large to heal quickly enough to prevent excessive blood loss. Another reason for the high mortality might be infection. Especially in animals that were unable to retract the preputium, this organ may have become infected.

Our results indicate that preputium eversion during copulation thus (most likely) requires the relaxation of the preputium muscle bands. When the preputium is everted, these muscles are extended to about four times their resting length (P.A.C.M.deB., unpublished). This is in agreement with previous *in vitro* experiments that demonstrated that, for example, APGWamide causes relaxation of

Table 1. Effect of lesions of the muscle bands attached to the preputium on survival and spontaneous preputium eversion during the recovery period

Muscle band lesions					
Location	Side	No.	*N*	Died	Spontaneous eversion
Proximal	Both	4–6 and 8–10	8	4	4
Distal and proximal	Ventral	1–6	3	2	2
	Dorsal	7–10	9	9	
	Both	1–3 and 7–10	2	1	1
	Both	1–10	10	8	8

The different lesions are indicated based on the band's location, side and number (see Fig. 1). The animals had no access to copulation partners. All animals that everted their preputium permanently died within the next two to three days following eversion. The proximally located muscle bands were previously called protractors, the distally located ones called retractors.

Table 2. Effect of lesions of the muscle bands attached to the preputium on male copulatory activity involving preputium eversion and intromission

Muscle band lesions				Eversion			
Location	Side	No.	*N*	Partial	Full	Intromission	Retraction
Distal	Ventral	1	5	2	2	1	1
	Ventral	2	6	3	3	1	3
	Ventral	3	2	1	–	–	1
	Dorsal	7	3	2	2	2	2
Proximal	Dorsal	9	1	1	1	1	1
	Dorsal	8 and 9	2	1	1	1	1
	Both	4–6 and 8–10	8	4	4	3	4
Distal and proximal	Dorsal	7–10	5	2	2	2	2
	Both	3, 8 and 9	1	1	1	1	1
	Both	1–10 (all)	5	2	2	1	0
Sham-operated	–	–	4	3	3	2	3
Unoperated	–	–	4	1	1	1	1

The different lesions are indicated based on the band's location, side and number (see Fig. 1). Sham-operated and unoperated snails were included as controls. Lesions of the muscle bands primarily affect the ability to retract the preputium but not the ability to evert. The proximally located muscle bands were previously called protractors, the distally located ones called retractors.

the preputium muscle bands (Croll et al., 1991; Li et al., 1992). *In vivo*, the injection of this neuropeptide into the blood indeed induces eversion of the preputium (De Boer et al., 1997b). In addition, APGWamide is probably released during the electrical activity of the neurons of the right anterior lobe, because these neurons have been found to be electrically active only during preputium eversion (De Boer et al., 1997b). Besides APGWamide, a whole slew of other neuropeptides seems to be involved in the movement of the male copulatory apparatus (e.g. De Lange et al., 1998; Croll et al., 1991; Li et al., 1992; Van Golen et al., 1995a, Van Golen et al., 1995b; Smit et al., 2003). Based on our findings we suggest that many of these will be involved in the fine-tuned movements of the male copulatory apparatus after eversion, i.e. during probing, intromission and insemination.

Although the terms protractor and retractor given by Holm (Holm, 1946) seemed reasonable from a morphological point of view and are still in use (e.g. Paraense and Pointier, 2003; Paraense, 2006), our results indicate that these muscle bands are not used for eversion. Rather, all of the muscle bands that are attached to the preputium are involved in retraction of the preputium. This, therefore, calls for a new nomenclature in which all preputium muscle bands are called retractors. The terminology for basommatophoran pulmonates then comes into agreement with the nomenclature in brackish water, marine and terrestrial molluscs (Berry et al., 1967; Blankenship et al., 1977; Gittenberger, 1979; Wabnitz, 1976). We further propose, at least for *L. stagnalis*, to divide the preputium muscle bands into either a distal and proximal group, both on the dorsal and ventral side (Fig. 1). This results in distal ventral (no. 1, 2 and 3), proximal ventral (no. 4, 5 and 6), distal dorsal (no. 7) and proximal dorsal (no. 8, 9 and 10) retractor muscles. Holm noted that the large ventral, most-distal retractor (no. 1) has one branch that is attached to the penis sheath instead of the preputium and called this the penis retractor muscle (Holm, 1946). Whether this is a proper term remains to be tested.

The foregoing also indicates that the preputium muscle bands are contracted to retain the preputium inside the animal. The soft parts of the body are shaped by the hydrostatic skeleton where muscle activity maintains hydrostatic pressure (Chapman, 1958; Page, 2007). Clearly, eversion of the preputium involves haemolymph movement and, possibly, this is sufficient for eversion to occur. Given that the preputium is only everted during male copulation, this indicates that the preputium muscle bands are contracted most of the time. Continuous or tonic contraction of muscle bands is found in several molluscs and has been described as 'catch', in which three different states can be distinguished: relaxed state, active state and catch state (for reviews, see Watabe and Hartshorne, 1990; Castellani and Cohen, 1992). The essential property of a catch muscle is the maintenance of tension in the catch state without causing high-energy consumption (Johnson and Twarog, 1960; Watabe and Hartshorne, 1990). Given our findings, it seems probable that the preputium muscle bands of *L. stagnalis* have such catch properties. This is further supported by the presence of myomodulins in the male reproductive system of *L. stagnalis* (Li et al., 1994; Van Golen et al., 1996). The amino acid sequence of these peptides shows strong resemblance with the catch-relaxing peptide (CARP) and may form part of a myomodulin-CARP-related peptide family (e.g. Hernádi et al., 1995). In *L. stagnalis*, five different myomodulin peptides are encoded by a myomodulin gene (Kellett et al., 1996) and the different myomodulins modulate the contractions of the preputium retractor muscles (Li et al., 1994; Van Golen et al., 1996). Given their contractile and relaxing properties, these peptides may thus

Table 3. Summary of the effect of the number of muscle bands that were cut on survival and ability to evert and subsequently retract the preputium

No. of bands cut	*N*	Survival	Eversion	Retraction
0 (unoperated)	4	4	1	1
0 (sham-operated)	4	4	3	3
1	17	17	9	9
2	2	2	1	1
3	1	1	1	1
4	14	5*	2	2
6	19	13*	10	4*
7	2	1*	1	0*
10	15	7*	10	0*

The survival data include all animals that died either in the days following the operation or as a result of permanent preputium eversion (which was fatal after a maximum of four days). The ability to retract includes both spontaneous eversions and eversions during copulation. The asterisks (*) indicate the most significant split in two partitions between the different number of muscle bands cut ($P<0.05$); thus, indicating the critical number of muscle bands at which survival and retraction were affected.

very well be involved in the maintenance and release of the catch response in concert with APGWamide. Evidently, maintaining the muscle bands in catch state will keep the preputium inside the animal without requiring large energy expenditure.

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REFERENCES

Berry, A., Loong, S. C. and Thum, H. H. (1967). Genital systems of *Phytia*, *Cassidula* and *Auricula* (Ellobiidae, Pulmonata) from Malayan mangrove swamps. *Proc. Malacol. Soc. London* **37**, 325-337.

Blankenship, J. E., Rock, M. K. and Hill, J. (1977). Physiological properties of the penis retractor muscle of *Aplysia*. *J. Neurobiol.* **8**, 549-568.

Castellani, L. and Cohen, C. (1992). A calcineurin-like phosphatase is required for catch contraction. *FEBS Lett.* **309**, 321-326.

Chapman, G. (1958). The hydrostatic skeleton in the invertebrates. *Biol. Rev. Cambridge Phil. Soc.* **33**, 338-371.

Chase, R. (2002). *Behavior and its Neural Control in Gastropod Molluscs*. Oxford: Oxford University Press.

Croll, R. P. and Chiasson, B. J. (1989). Postembryonic development of serotoninlike immunoreactivity in the central nervous system if the snail, *Lymnaea stagnalis*. *J. Comp. Neurol.* **280**, 122-142.

Croll, R. P., Van Minnen, J., Kits, K. S. and Smid, A. B. (1991). APGWamide: molecular, histological and physiological examination of the novel neuropeptide involved with reproduction in the snail, *Lymnaea stagnalis*. In *Molluscan Neurobiology* (ed. K. S. Kits, H. H. Boer and J. Joose), pp. 248-254. Amsterdam: North-Holland.

De Boer, P. A. C. M., Jansen, R. F. and Ter Maat, A. (1996). Copulation in the hermaphrodite snail *Lymnaea stagnalis*: a review. *Invert. Reprod. Develop.* **30**, 167-176.

De Boer, P. A. C. M., Jansen, R. F., Koene, J. M. and Ter Maat, A. (1997a). Nervous control of male sexual drive in the hermaphroditic snail *Lymnaea stagnalis*. *J. Exp. Biol.* **200**, 941-951.

De Boer, P. A. C. M., Ter Maat, A., Pieneman, A. W., Croll, R. P., Kurokawa, M. and Jansen, R. F. (1997b). Functional role of peptidergic anterior lobe neurons in male sexual behavior of the snail *Lymnaea stagnalis*. *J. Neurophysiol.* **78**, 2823-2833.

De Lange, R. P. J., Joosse, J. and Van Minnen, J. (1998). Multi-messenger innervation of the male sexual system of *Lymnaea stagnalis*. *J. Comp. Neurol.* **390**, 564-577.

Gittenberger, E. (1979). *Elona* (Pulmonata, Elonidae, Fam. Non.). *Malacologia* **18**, 139-145.

Hernádi, L., Terano, Y., Muneoka, Y. and Kiss, T. (1995). Distribution of catch-relaxing peptide (CARP)-like immunoreactive neurons in the central and peripheral nervous system of *Helix pomatia*. *Cell Tissue Res.* **280**, 335-348.

Holm, L. W. (1946). Histological and functional studies on the genital tract of *Lymnaea stagnalis appressa* Say. *Trans. Amer. Micr. Soc.* **65**, 45-68.

Jaeger, C. P. (1963). Physiology of Mollusca 4. Action of serotonin on penis retractor muscle of *Strophocheilos oblongus*. *Comp. Biochem. Physiol.* **8**, 131-136.

Johnson, W. H. and Twarog, B. M. (1960). The basis for prolonged contractions in molluscan muscles. *J. Gen. Physiol.* **43**, 941-960.

Kellett, E., Perry, S. J., Santama, N., Worster, B. M., Benjamin, P. R. and Burke, J. F. (1996). Myomodulin gene of *Lymnaea*: structure, expression, and analysis of neuropeptides. *J. Neurosci.* **16**, 4949-4957.

Koene, J. M. and Ter Maat, A. (2005). Sex role alternation in the simultaneously hermaphroditic pond snail *Lymnaea stagnalis* is determined by the availability of seminal fluid. *Anim. Behav.* **69**, 845-850.

Koene, J. M., Jansen, R. F., Ter Maat, A. and Chase, R. (2000). A conserved location for the central nervous system control of mating behaviour in gastropod molluscs: evidence from a terrestrial snail. *J. Exp. Biol.* **203**, 1071-1080.

Li, K. W., Smit, A. B. and Geraerts, W. P. M. (1992). Structural and functional characterization of neuropeptides involved in the control of male mating behavior of *Lymnaea stagnalis*. *Peptides* **13**, 633-638.

Loose, M. J. and Koene, J. M. (2008). Sperm transfer is affected by mating history in the simultaneously hermaphroditic snail *Lymnaea stagnalis*. *Invert. Biol.* **127**, 162-167.

Neustadter, D. M., Herman, R. L., Drushel, R. F., Chestek, D. W. and Chiel, H. J. (2007). The kinematics of multifunctionality: comparisons of biting and swallowing in *Aplysia californica*. *J. Exp. Biol.* **210**, 238-260.

Page, L. R. (2007). Shrinking to fit: fluid jettison from a haemocoelic hydrostatic skeleton during defensive withdrawals of a gastropod larva. *Proc. R. Soc. B - Biol. Sci.* **274**, 2989-2994.

Paraense, W. L. (2006). The Afro-American Biomphalariae. *Mem. Inst. Oswaldo Cruz* **101**, 21-27.

Paraense, W. L. and Pointier, J. P. (2003). *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae): a study of topotypic specimens. *Mem. Inst. Oswaldo Cruz* **98**, 513-517.

Paul, J., Roces, F. and Holldobler, B. (2002). How do ants stick out their tongues? *J. Morphol.* **254**, 39-52.

Plesch, B, Janse, C. and Boer, H. H. (1975). Gross morphology and histology of the musculature of the freshwater pulmonate *Lymnaea stagnalis* (L.). *Neth. J. Zool.* **25**, 332-352.

Smit, A. B., Van Kesteren, R. E., Spijker, S., Van Minnen, J., Van Golen, F. A., Jiménez, C. R. and Li, K. W. (2003). Peptidergic modulation of male sexual behavior in *Lymnaea stagnalis*: structural and functional characterization of -FVamide neuropeptides. *J. Neurochem.* **87**, 1245-1254.

Van Duivenboden, Y. A. and Ter Maat, A. (1988). Mating behaviour of *Lymnaea stagnalis*. *Malacologia* **28**, 23-64.

Van Golen, F. A., Li, K. W., De Lange, R. P., Jespersen, S. and Geraerts, W. P. M. (1995a). Mutually exclusive neuronal expression of peptides encoded by the FMRFa gene underlies a differential control of copulation in *Lymnaea*. *J. Biol. Chem.* **270**, 28487-28493.

Van Golen, F. A., Li, K. W., De Lange, R. P., Van Kesteren, R. E., Van Der Schors, R. C. and Geraerts, W. P. M. (1995b). Co-localized neuropeptides conopressin and ALA-PRO-GLY-TRP-NH2 have antagonistic effects on the vas deferens of *Lymnaea*. *Neuroscience* **69**, 1275-1287.

Van Golen, F. A., Li, K. W., Chen, S., Jiménez, C. R. and Geraerts, W. P. M. (1996). Various isoforms of myomodulin identified from the male copulatory organ of *Lymnaea* show overlapping yet distinct modulatory effects on the penis muscle. *J. Neurochem.* **66**, 321-329.

Wabnitz, R. W. (1976). Mechanical and electromyographic study of penis retractor muscle (PRM) of *Helix pomatia*. *Com. Biochem. Physiol. A - Physiol.* **55**, 253-259.

Watabe, S. and Hartshorne, D. J. (1990). Paramyosin and the catch mechanism. *Com. Biochem. Physiol. B - Biochem. Mol. Biol.* **96**, 639-646.