

The Cloaca and Spermatheca of the Female Smooth Newt, *Triturus vulgaris* L. (Amphibia: Urodela: Salamandridae)

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Abstract

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The anatomy and histology of the cloaca and spermatheca of 13 female smooth newts (*Triturus vulgaris*) were studied by light microscopy. The cloaca consists of a short, anterior tube (which receives the oviducts), which opens into a larger, more posterior chamber. The spermatheca, which is the only gland in the cloaca, consists of a mass of exocrine, acinar tubules which empty individually and dorsolaterally into the posterior part of the cloacal tube and all but the most posterior part of the cloacal chamber. Stored sperm are most abundant in the spermathecal tubules during the peak of the breeding season (in May and June); during their period of storage, the sperm do not appear to make intimate contact with the epithelial cells lining the spermathecal tubules. Long-term storage of viable sperm from one breeding season to the next is probably absent in the smooth newt. Sperm storage by and multiple insemination of females both occur in this species and are necessary preconditions for competition between the sperm of different males for the fertilization of eggs. However, sperm competition has not been demonstrated in the smooth newt.

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Introduction

In the majority of oviparous urodele amphibians fertilization is internal and sperm transfer is accomplished indirectly by means of a spermatophore. The latter is usually deposited on the substrate by the male during courtship and may then be picked up in the cloaca of the female. Within the female's body sperm are liberated from the spermatophore and are then stored in a specialized gland, the spermatheca, until fertilization occurs, just prior to oviposition. By dissociating the transfer of sperm and the deposition of fertilized eggs in both space and time, the female presumably is able to decrease the mortality risk to her developing embryos (Salthe and Mecham 1974, Nussbaum 1985). When coupled with multiple mating, sperm storage may also provide the female with a mechanism which enables her selectively to use the sperm of a

certain male(s) to fertilize her eggs (Eberhard 1985).

Kingsbury (1895) provided the first detailed descriptions of the anatomy and histology of spermathecal glands in female urodeles. Since the publication of this important paper a number of workers have investigated aspects of the structure, biochemistry, physiology and function of sperm storage organs in a wide range of urodele taxa (see reviews by Boisseau and Joly 1975, Halliday and Verrell 1984). The most recent interest in spermathecal glands has focused on their importance in sperm competition (e.g. Houck and Schwenk 1984) and their utility in the reconstruction of phylogenetic relationships (e.g. Sever in press).

The smooth newt (*Triturus vulgaris*) is the most widespread and abundant of the European urodeles in most parts of its range. In southern England adult smooth newts leave their terres-

trial hibernaculae in the months of February and March and migrate to their breeding ponds (Verrell in press). Breeding activity, observed as courtship, intermale competition and oviposition, is most intense in May and June (Verrell and Halliday 1985, Verrell *et al.* 1986). In the laboratory a female newt which has been inseminated with a single spermatophore will lay fertilized eggs for as long as 50 days; after this period, a few unfertilized eggs may be laid before the female remates and resumes laying fertilized eggs (Halliday and Verrell 1984, unpublished data). This behavioural evidence for sperm storage over at least part of the duration of the breeding season is supported by two morphological studies. Wahlert (1953) found that sperm are stored in a mass of cloacal tubules in females of several species in the genus *Triturus*. A more detailed description of the spermathecal region of *T. vulgaris* is that of Sever (in press), who noted that the spermatheca consists of numerous short tubules located in the dorsal and lateral walls of the cloacal chamber.

In this paper we provide a detailed description of the anatomy and histology of the cloaca and spermatheca of the female smooth newt, extending the previous work of Wahlert (1953) and Sever (in press). We also discuss several aspects of the functional significance of sperm storage organs in urodeles.

Materials and Methods

All of the material used in this study was taken from a larger sample of female *T. vulgaris* obtained during an investigation of the annual reproductive cycle of this species (see Verrell *et al.* 1986). Thirteen females were selected for detailed histological examination of the cloaca and spermatheca (Table 1), such that all stages in the annual cycle are represented. These newts were collected in 1983 from three similar sites in southern England: Linford (Milton Keynes), Walton (Milton Keynes) and Soulbury (Leighton Buzzard). All of these sites are within a 20 km radius of the campus of The Open University in Milton Keynes, Buckinghamshire.

Within 48 h of collection, the newts were killed by immersion in 1% aqueous solution of MS 222 (Sandoz) and snout-vent length (SVL) was measured to the nearest 0.5 mm. The newts were then incised through the ventral body wall and placed in individual bottles containing 10% unbuffered formalin solution, for fixation and preservation.

The cloacal region of each newt was dissected at varying intervals after fixation. Each specimen was embedded in paraffin wax by standard methods and sections were cut at a thickness of 8–10 μm . These sections were stained with either Harris' haematoxylin and eosin, for general histology, or periodic acid/Schiff's reagent (PAS), for general carbohydrates (see Humason 1979).

Observations

The cloaca of female *T. vulgaris* is very simple in its general anatomy. The most anterior portion, which receives the distal openings of the oviducts and is known as the cloacal tube, comprises approximately 15–20% of the total length of the cloaca. The mucosa of the cloacal tube and the more posterior cloacal chamber (into which the tube opens) is not particularly rugose. However, a conspicuous mid-dorsal fold appears at the anterior end of the cloacal tube and extends throughout the length of the cloaca. A pair of thick, lateral folds also occurs in the walls of the cloacal chamber, just dorsal to the region where the stratified epithelium evaginates into the cloacal orifice. The skin surrounding the latter contains many granular and mucous glands, the latter frequently occurring as demilunes on the former. Stratified epithelium is also found at the most posterior end of the cloacal chamber. Elsewhere, the cloacal epithelium is of the simple-columnar type, with a slight PAS-positive staining reaction present in the apical cytoplasm of the epithelial cells. The whole cloacal region is surrounded by a dense sheath of connective tissue, known as the superficial cloacal sheath. No cilia are evident in any part of the cloaca (Fig. 1A).

The spermatheca is the only gland opening into the cloaca in the female smooth newt (Sever in press). The tubules which comprise the gland are simple, exocrine structures that pass laterally from the dorsal half of the posterior cloacal tube and from all but the most posterior part of the cloacal chamber (Fig. 1A). It is difficult to count the total number of tubules with any real confidence, but we estimate the number to lie between 40 and 60. The epithelium which lines the spermathecal tubules exhibits a 'bunched' appearance in the absence of stored sperm (Fig. 1C); the epithelial cells are cuboidal-columnar in type and show a slight PAS-positive staining reaction. In tubules containing sperm the epithelial cells are of the squamous type and do not stain with PAS (Fig. 1B). The distal ends of the tubules are acinar and this is most conspicuous in glands which do not contain sperm. Each spermathecal tubule is surrounded by several concentric layers which appear to consist of smooth muscle fibres; at present, however, we cannot dismiss the possibility that these layers represent a myoepithelial basket or even collagen fibres. For this reason, we refer to these layers as a fibrous sheath (see Fig. 1D). This sheath is most easily seen in glands which do not contain sperm, due to the 'bunched' nature of the epithelium lining the tubules. Melanocytes are associated with the outermost part of the fibrous sheath and these cells give the cloacal lining in

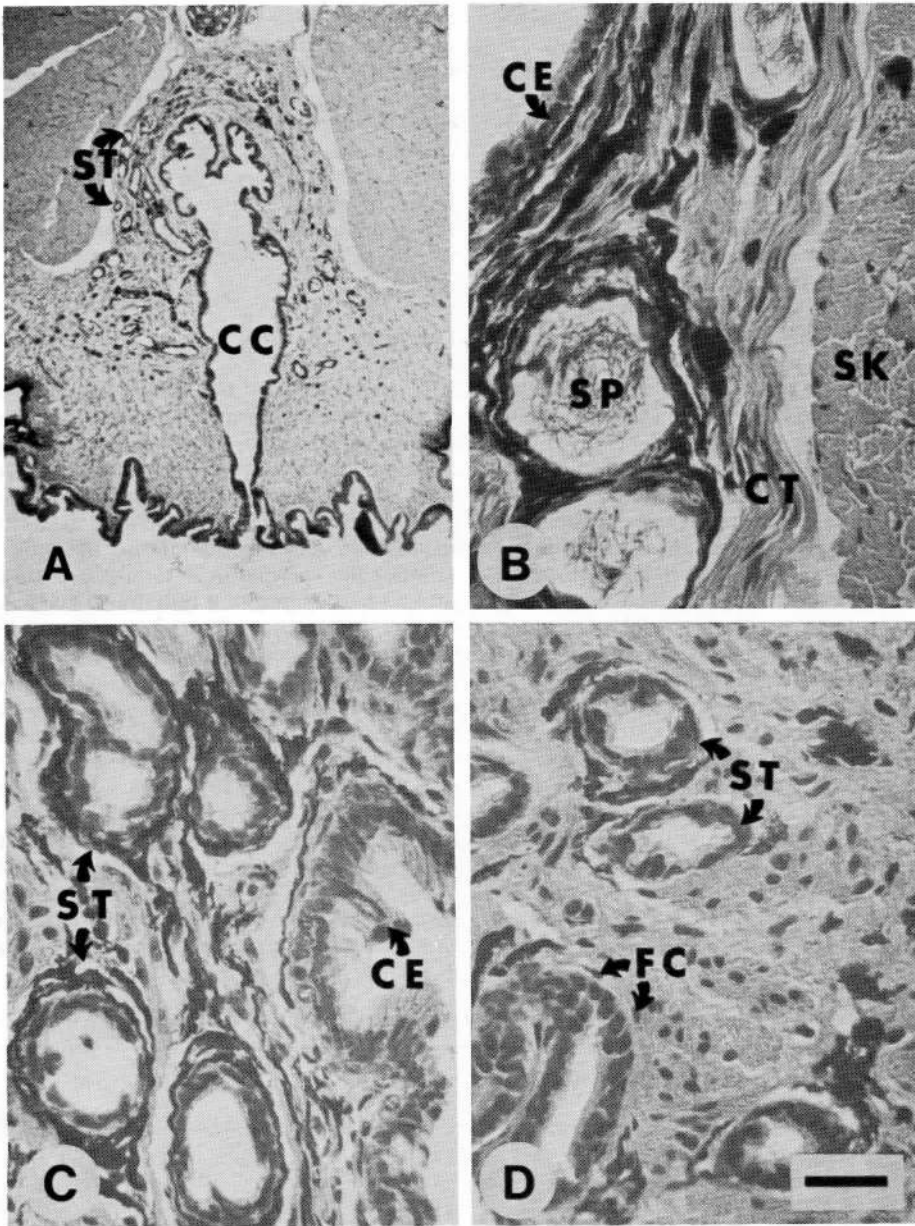


Fig. 1. Transverse sections through the spermatheca of *Triturus vulgaris*. (A) Anterior cloacal chamber of an individual 47.5 mm SVL collected 13 May in water. The spermathecal tubules of this specimen contain sperm. (B) Spermathecal tubules containing sperm of an individual 45.0 mm SVL collected 24 June in water. (C) Spermathecal tubules lacking sperm of an individual 40.0 mm SVL collected 6 March in water. (D) Spermathecal tubules lacking sperm of an individual 45.5 mm SVL collected 28 November moving away from water. All sections were stained with haematoxylin and eosin. Scale in lower right corner 45 μm for (A) and 5 μm for (B), (C) and (D). Abbreviations: CC cloacal chamber, CE epithelial lining of cloacal mucosa, CT dense connective tissue of superficial cloacal sheath, FC nuclei of cells in the fibrous sheath surrounding a spermathecal tubule, SK skeletal muscle, SP spermatozoa, ST spermathecal tubule.

the vicinity of the spermatheca a black appearance on gross examination.

When present in the spermathecal tubules, sperm do not appear to adopt any regular orientation, but seem to be aligned randomly (Fig. 1B). Occasionally a sperm head is seen in close apposition to a squamous epithelial cell, but the irregularity with which such contacts occur suggests that they are chance events. The reaction of stored sperm to staining with PAS is quite variable. In most of the specimens we examined in which sperm are present, both PAS-positive and PAS-negative (greenish in colour) sperm can be seen, apparently mixed in a random manner. Sperm in freshly deposited spermatophores stain positively with PAS (Verrell unpublished data).

Data on the 13 female smooth newts whose cloacae we examined are summarized in Table 1. Evidence for sperm storage from one breeding season to the next is sparse. An aquatic female collected in water after the breeding season (in August) and two females collected emigrating away from water in early winter did not contain sperm in their spermathecae. Of four females collected as they were immigrating towards water in the spring, two did not contain sperm, one contained unidentifiable debris (which may have included sperm) and another contained a small quantity of stored sperm; all of the latter were PAS-negative. Stored sperm were most abundant in females collected in May and June, the months when breeding activity is most intense in natural populations (Verrell and Halliday 1985). In females which have ovulated (i.e. have ova in their oviducts) the oviducts are hypertrophied

and the epithelial cells lining them are flattened and show a strong, apical PAS-positive staining reaction. The jelly coats surrounding the oviducal ova also exhibit a strong PAS-positive reaction. No females collected outside of the water contain ova in their oviducts, an observation in accord with previous work on the timing of ovulation in natural populations of smooth newts (Verrell and Halliday 1985, Verrell *et al.* 1986).

Discussion

The spermatheca of the female smooth newt is of the typical salamandrid type in its anatomy, consisting of a mass of blind tubules which open individually into the roof of the posterior cloacal tube and the cloacal chamber (Fig. 1). This configuration is similar to that found in the ambystomatids and dicamptodontids; in the latter two families the spermathecal tubules are clustered around and open into a mid-dorsal evagination. It also differs from the situation found in the plethodontids, in which all spermathecal tubules pass into a common duct (an autapomorphic character) which then opens into the cloacal chamber (Sever in press). The spermatheca is the only gland present in the cloaca of the female smooth newt, although female salamandrids of other species are known to possess ventral and/or dorsal glands as well (in *T. vulgaris* these two glands only occur in males; see Heidenhain 1890). Sever (in press) suggests that the ventral gland is a symplesiomorphic character in female urodeles and that the absence of such glands in

Table 1. Summary of data obtained for the 13 newts whose cloacae were examined

Date	Stage of cycle	SVL	Sperm*	Oviducal eggs†
6 March	Immigrating	40.0	—	—
7 March	Immigrating	45.0	++	—
9 March	Immigrating	48.0	+	—
23 March	Immigrating	41.5	—	—
21 April	In water	42.0	++	—
13 May	In water	47.5	+++	+
23 May	In water	41.0	+++	+
1 June	In water	45.5	+++	+
24 June	In water	45.0	+++	+
25 July	In water	43.0	++	—
19 August	In water	48.5	—	—
10 October	Emigrating	39.0	—	—
28 November	Emigrating	45.5	—	—

*—absent, + debris, ++ few, +++ many.

†—absent, + present.

female salamandrids (as well as in amphiumids and plethodontids) is due to secondary loss.

We obtained no evidence of regular, intimate contact between stored sperm and the spermathecal epithelial cells in our sample of newts. This is in contrast to the observation of Wahlert (1953) that the heads of *Triturus* sperm lie close to or even embedded within the epithelial cells. Such penetration has also been reported for the newt *Cynops pyrrhogaster* (Tsutsui 1931) and the salamanders *Plethodon glutinosus* (Trauth 1984) and *Salamandra salamandra* (Boisseau and Joly 1975). In other urodele species the sperm either lie free within the spermathecal tubules (e.g. *Hemidactylium scutatum*, Dieckmann 1927), or lie with their heads in contact with, but not buried within, the epithelial cells (e.g. *Desmognathus ochrophaeus*, Houck and Schwenk 1984, Sever and Houck 1985; *D. fuscus*, Marynick 1971; *Notophthalmus viridescens*, Benson 1968, Dent 1970; *Eurycea quadridigitata*, Pool and Hoage 1973). The functional significance, if any, of contacts between sperm and epithelial cells is uncertain; sperm which make such contacts may be inviable and about to be resorbed (Pool and Hoage 1973) or they may receive some form of sustenance from the epithelial cells, perhaps increasing their longevity (Sever and Houck 1985).

Ultrastructural and histochemical studies have revealed that the spermatheca produces acidic and neutral polysaccharides which either attract sperm as they leave the spermatophore and/or sustain the sperm during their storage period (Boisseau and Joly 1975, Halliday and Verrell 1984). The presence of contractile cells in the region surrounding the spermathecal tubules appears to be a common feature in urodeles; these cells may function to squeeze sperm into the cloacal tube and cloacal chamber as the ova pass through just prior to oviposition (Boisseau and Joly 1975).

Our data suggest that long-term sperm storage from one breeding season to the next is either rare or absent in *T. vulgaris*. Only one female appears to have stored sperm over this period, however, the staining reaction of these sperm with PAS suggests that they were non-functional, perhaps even dead (see also Sever and Houck 1985). Additional evidence for the absence of such long-term storage arises from the observation made by one of us (Verrell) that female newts collected on land in early spring and then kept in aquaria in the laboratory do not lay eggs until they have been inseminated. Data on the longevity and viability of stored sperm are available for only a few urodele species (reviewed by Halliday and Verrell 1984); in general, sperm

are not stored beyond the end of the breeding season. One exception is the salamander *Desmognathus ochrophaeus*, in which sperm can be stored from one season to the next, apparently in a viable state as judged by microscopic appearance (Houck and Schwenk 1984).

The storage of viable sperm, at least for part of one breeding season, is a necessary condition for sperm competition, defined as competition between the sperm of two or more males for the fertilization of a female's ova (see Parker 1970, Halliday and Verrell 1984). A second necessary condition is that the female is inseminated by more than one male. In *T. vulgaris* a female may be multiply inseminated in one of two ways. First, competitive interactions between males during an ongoing courtship encounter may result in the female being inseminated by both the male that has courted her and the male that interferes with the courtship (Verrell 1984). Secondly, some females will remate in the interval between a first mating and the onset of oviposition; this does not seem to occur frequently in the laboratory, because the majority of females become unresponsive to male courtship once they have been inseminated (Halliday and Verrell 1984). It thus seems likely that sperm competition occurs in *T. vulgaris*. Multiple paternity of egg clutches has been demonstrated in natural populations of two urodele species. In the newt *T. alpestris* 16 out of 17 females were found to be carrying clutches fertilized by more than one male (Rafinsky 1981). In the salamander *D. ochrophaeus* the frequency of multiple insemination has been estimated as at least 7% in one study (Tilley and Hausman 1976) and as at least 25% in another (Labanick 1983). A preliminary laboratory study of sperm competition in this salamander indicates that the number of ova fertilized by a male depends on the number of times his mate has been inseminated by other males (Houck *et al.* 1985); no clear advantage accrues to either the first or last male to mate, as occurs in many insects (Parker 1970). Sperm competition in the smooth newt is a topic which awaits empirical investigation.

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