The 'hybrid' character of the gametes and reproductive tracts of the African shrew, *Myosorex varius*, supports its classification in the Crocidosoricinae

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The Soricidae are generally considered to comprise two subfamilies – Crocidurinae and Soricinae – each of which has distinctive reproductive characteristics. Although *Myosorex varius* is classified as a crocidurine, the features of its reproductive system call that classification into question. Compared with three other shrew genera, *Myosorex* exhibited a number of specific features including a relatively prolonged time (about 22 h) for ovulation to be induced by hCG injection and the smallest diameter (75 µm) recorded for any mammal egg. Moreover, the relative testis mass and the number of epididymal spermatozoa were somewhat greater than in some other shrews studied recently. However, many reproductive features in *Myosorex* have a 'hybrid' character. The glans penis has spines similar to those evident in crocidurines but absent in soricines, yet the accessory sperm storage site, midway along the vas deferens, is similar to that in soricine shrews. The ultrastructure of *Myosorex* spermatozoa was primarily soricine, despite an unduly large acrosome, which reaches its apogee in the Crocidurinae. Whereas the Fallopian tube displays a crocidurine-type isthmus characterized by deep crypts throughout, the ampulla is richly endowed with ciliated crypts, which in soricines contain spermatozoa. The first polar body persists in the *Myosorex* ovum, as it does in the soricines *Cryptotis* and *Blarina*, but not in the crocidurine *Suncus* and *Crocidura*. However, the cumulus oophorus of *Myosorex* appears largely crocidurine by virtue of its persistent intercellular junctions, long term stability, and the absence of a matrix, lacking only the unique perizonal space that finally characterizes the cumulus of the crocidurines, *Suncus* and *Crocidura*. The 'hybrid' character of the reproductive system of *Myosorex* is more consistent with the proposal that the genus is a survivor of a primitive subfamily – the Crocidosoricinae – from which present day Soricinae and Crocidurinae have arisen.

Introduction

The evolution of therian mammals has seen the advent of several biologically novel features in the male and female reproductive tracts, in the design and behaviour of their gametes, and in the relationship of one to the other. Among advanced mammals, these features in the male include the scrotal condition, a variable development of different accessory glands, and the dependence of sperm maturation and, therefore, fertilizing ability on some essential factor(s) elaborated by the epididymis. Furthermore, storage of spermatozoa in the lower region of the epididymis is unusual in its acute dependence on testicular androgen and the lower temperature of the scrotum. Among the novel features seen in the female, spermatozoa at the site of fertilization in the ampulla of the oviduct need to

have undergone capacitation before they can fertilize, and, only in Eutheria, spermatozoa then face the cumulus oophorus, a mass of granulosa cells usually within a proteoglycan matrix, before negotiating the prominent zona pellucida around the tiny egg. The eutherian sperm head has an unusual flat, keratinoid nucleus and a stable posterior (equatorial) segment of acrosome, the plasma membrane over which provides a unique locus of fusion with the oolemma (see Bedford, 1991), which is perhaps related to the mode of its penetration through the unusually thick egg coat.

In the hope that particular comparative situations in more primitive mammals might provide useful insights into the significance of some of these developments, we have begun to study the *Insectivora*, specifically shrews. These have certain tooth and skull features indicating that they are mammals most closely related to the progenitors of modern Eutheria. After early histological studies of reproductive tracts in *Sorex*

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Fig. 1. Adult female *Myosorex varius*. Scale bar represents 1 cm.

(Brambell, 1935a, b) and in *Blarina* (Pearson, 1944), the structure of the gametes and their behaviour leading to fertilization have recently been described in three other genera (*Suncus*, *Crocidura* and *Cryptotis*). However, not only have such shrews been found to possess unusual features with regard to sperm structure, the disposition of spermatozoa in the oviduct, and the form and behaviour of the cumulus oophorus, but also the very few species studied display a considerable diversity of such features according to the subfamily, the Crocidurinae and the Soricinae (Bedford *et al.*, 1997a–d). Thus, so far, this line of enquiry has provided more questions than answers.

Since current opinion recognizes about 20 genera (Hutterer, 1985), an understanding of the events in more than the three genera recently examined might provide a clearer overall picture of conception in shrews. Therefore, we have investigated the gametes and reproductive tracts of *Myosorex varius*, a reputedly crocidurine African shrew. The genus *Myosorex* is believed to comprise 12 species (Hutterer, 1985), none of which has been examined in this regard. *Myosorex* is generally classified as crocidurine based on its lack of tooth pigmentation, on continuous articular facets on the mandibular condyle (Repenning, 1967), on anatomical structures related to habitat and life-style (Hutterer, 1985), and on the 'caravanning' behaviour of young (Baxter and Lloyd, 1980). Therefore, since it has been difficult on occasion to classify particular shrews (Martin, 1967; Mori *et al.*, 1991) it was anticipated that such an examination of *Myosorex* might also help to establish further archetypal crocidurine features useful for systematic studies.

Materials and Methods

Shrews were trapped in November and December in the vicinity of Grahamstown, and of the University of Fort Hare, Alice, in the Eastern Cape, South Africa. Observations were made on 10 males and 13 females. The conditions of their maintenance were in accordance with protocols approved by the Ethical Standards Committee of Rhodes University. They were housed individually at about 24°C in a 14 h light:10 h dark regimen, in aquaria with earth floors, with shredded paper and tubes for shelter, and with canned dog food and water *ad libitum*. Females were given an i.p. injection of 40 i.u. hCG (Profasi, Serono) to induce ovulation, and were then placed

Fig. 2. The extruded penis of *Myosorex varius* displays (a) a blunted form with bilateral skin swellings overlying a haematic sinus in the posterior glans region and (b) a dense complement of spines that extends to the external meatus. Scale bar represents (a) 1 mm and (b) 90 µm.

overnight with a male, identifiable as such only by the extrusion of the penis from the cloacal orifice.

Animals were killed with ether. The testis and attached epididymis and vas deferens were excised from the males, and selected regions were fixed for electron microscopy in 2.5% (w/v) phosphate-buffered glutaraldehyde followed by post-fixation in osmium tetroxide, dehydration in alcohols and propylene oxide, with final embedding in an araldite/TAAB mixture (Cross, 1989). The structure of spermatozoa was examined in thin sections of tissues from the caput and cauda regions of the epididymis using a transmission electron microscope (TEM). Spermatozoa released from successive regions of the tract into a culture medium of Dulbecco's modified Eagle's medium (DMEM) at 37°C were immediately assessed for motility on warm slides using phase contrast optics, and for the swelling response of the sperm nucleus when exposed to 1% (w/v) SDS. Total counts were made of the sperm populations in the caput plus the corpus, the cauda epididymidis and the vas deferens in two males using an improved Neubauer haemocytometer.

Oviducts and ovaries were excised from the females and dissected free of membranes 17–39 h after the hCG injection. Ovulation was assessed according to the presence of ripe follicles or of 'mushroom-like' young corpora lutea. Oviducts were mounted onto slides with the cover glass supported by wax spots. After slight flattening the oviducts were scanned,

Fig. 3. The special features seen in male reproductive tract of *Myosorex varius* include an unusual 'vas gland' (G) present in all shrews examined to date, the single duct segment (arrowhead) that connects the corpus to the cauda epididymidis, and the sperm store (S) located on the vas deferens, which seems the likely source of the ejaculated spermatozoa. The scale bar represents 1.2 mm.

using differential interference contrast (DIC) optics, for the character of the isthmus and ampulla, and for the presence and the state of cumulus–oocyte complexes (COCs). In some cases COCs were fixed *in situ* within a local segment of oviduct ampulla, as described for the male tract, and semi-thin and thin sections of the oviduct and the COCs were studied, respectively, in the light microscope and the TEM. Otherwise, COCs were extruded by stripping the ampulla off with fine forceps, and mounting the COCs again on wax-spot slides for further study.

Results

The nose–tail length of the adult *Myosorex varius* is about 10 cm in natural posture (Fig. 1). The mean bodyweight and mean testis masses were 11.65 g (range 8.2–15.2 g) and 85.9 mg (range 36.0–114.0 mg), respectively, for males with spermatozoa in the epididymis ($n = 7$). Three other males of bodyweight 7.7–9.6 g were not yet producing spermatozoa. The glans penis was characterized by bilaterally symmetrical skin folds that overlie a haematic sinus, and were covered by a rich array of epithelial spines oriented toward the base of the penis (Fig. 2). The active testis in mature males occupied an inguinal site with the cauda epididymidis preceding it within a cremaster sac. The testis was white, not pigmented green as it is in mature *Suncus*, *Crocidura* and *Cryptotis*. The form of the male tract was unusual in two respects. The epididymis tapered to an extreme degree in the lower corpus region, such that this was connected to the bulbous cauda by a single, almost straight duct segment (Fig. 3). The vas deferens was invested by a large multiloculated gland, as in all other shrews examined so far, but immediately before entering the gland the vas became highly corrugated to create an unusual sperm-filled swelling (Fig. 3). In

two males, between 5 and 7×10^6 spermatozoa were counted within each swelling, compared with 10.5 – 17.5×10^6 spermatozoa per cauda and 6 – 11×10^6 spermatozoa counted in each caput plus corpus region. However, in contrast to the cauda, which was consistently filled with spermatozoa, the accessory store in the vas deferens was essentially empty in three of the seven active males examined, suggesting a tendency for its evacuation.

The spermatozoon was characterized by a tail of $85 \mu\text{m}$ and by a head $9.0 \mu\text{m}$ in length, much of which was provided by a long rostral segment of acrosome (Fig. 4a). Spermatozoa, as seen in the TEM, were structurally similar in all regions of the epididymis, except that the plasmalemma tended to balloon, particularly over one face of the acrosome in cauda spermatozoa (Fig. 4b), whereas in caput spermatozoa the membrane was applied closely to the acrosome (Fig. 4c). Despite the unusually large acrosome, there was no 'rim' which reflects the unusual intimate relationship between outer acrosome membrane and plasma membrane seen in crocidurine spermatozoa (Mori *et al.*, 1991; Bedford *et al.*, 1994). However, in glancing sections, the periacrosomal surface of the plasmalemma was characterized by a regular 'bristly' periodicity not evident over other regions of the sperm surface. The interrupted nature of the perforatorium in some longitudinal sections (Fig. 4b, c) indicates that it has the barbed configuration seen in spermatozoa of other soricids and some bats (for discussion, see Bedford *et al.*, 1997d). The ultrastructure of the tail of the spermatozoa was notable for the asymmetrically prominent proximal dense fibres 5 and 6, as seen in other soricid spermatozoa, and outward displacement of fibres 3 and 8.

In regard to other aspects of epididymal maturation, the great majority of spermatozoa from the upper caput region showed no movement when released into DMEM, in contrast to an almost universal flagellation in those from the corpus

Fig. 4. (a) Phase contrast microscopy of the head of a spermatozoon of *Myosorex varius*. The rostral segment of the acrosome is exaggerated and extends well beyond the anterior border of the nucleus. Scale bar represents 6.25 μm . (b) Parasagittal section of a sperm head within the cauda epididymidis using transmission electron microscopy (TEM). Although somewhat asymmetrical in *M. varius*, ballooning of the periacrosomal region of the plasmalemma balloons is typical of mature spermatozoa of most mammals and of soricine shrews. The interrupted nature of the perforatorium (arrow) indicates that this has a barbed configuration in the coronal plane. Scale bar represents 0.5 μm . (c) Parasagittal section through sperm heads within the caput epididymidis of *M. varius* using TEM. The upper head shows evidence of an interrupted perforatorium. In contrast to cauda or vas spermatozoa, the periacrosomal plasmalemma of caput spermatozoa remains closely applied to the underlying acrosome. C: cytoplasmic droplet. Scale bar represents 0.5 μm .

region and vigorous motility of those from the cauda and the vas deferens. When exposed to 0.02%–0.05% SDS, most of the latter spermatozoa lost all trace of the prominent acrosome. However, whereas the nuclei of vas and cauda spermatozoa were unchanged even in 0.1% SDS, that concentration of SDS brought a major swelling of the nuclei of spermatozoa from the upper caput epididymidis.

None of the wild-caught females given hCG mated when left overnight with mature males. The general arrangement of the female reproductive tract and ovaries in *Myosorex* was similar to that in other shrews, typified by *Suncus* (Bedford *et al.*, 1997c). However, observation of the slightly flattened Fallopian tube (Fig. 5a) with DIC optics revealed not only deep crypts throughout the isthmus, as seen in the crocidurines, *Suncus* and *Crocidura*, but also a rich endowment of ciliated crypts throughout most of the ampulla, as seen in soricines such as *Cryptotis*, *Blarina* and three *Sorex* species (O. B. Mock

and J. M. Bedford, unpublished observations). Transverse sections of the lower ampulla (Fig. 5b) showed complex epithelial folds that create channels between the ciliated main lumen and the peripherally located ampullary crypts (Fig. 5c). As indicated by the occurrence of ovulation or development of a localized follicular vascularity before ovulation, the i.p. injection of hCG had stimulated ovarian follicles in all 13 females examined 17.0 h or more after the injection, including three females (Nos 12, 18, 20) in which corpora lutea had been counted, but the actual ovulation time had not been monitored. The mean number of follicles stimulated or ovulating was 3.3 per female (range 2.0–6.0). Ovulation did not occur for a relatively long period (about 22 h) after hCG (Table 1).

Eggs were surrounded by a dense ball of cumulus cells at ovulation (Fig. 6a), but were readily freed from the shell of the cumulus with 30 gauge needles. They were only 75 μm in diameter at the zona surface as measured directly from a

vernier scale. The COCs were closely surrounded by ampullary folds within the living oviducts and in semi-thin sections of the ampulla (Fig. 6b) as visualized *in situ* with DIC optics. The eggs studied always exhibited a first polar body even 18 h after ovulation (Fig. 6c). The cumulus oophorus had no intercellular matrix (Fig. 6d), and neighbouring cells were associated by way of desmosome-like complexes (Fig. 6e). The COCs gradually moved down the ampulla during the first hours after ovulation, but there was no change in the appearance of the cumulus oophorus around unfertilized eggs, with the exception of one of three COCs lying together in one ampulla (Fig. 6c) 18 h after ovulation in female No. 10.

Discussion

Any apparent trends or groupings in regard to reproductive characteristics of shrews must be viewed with caution owing to the very small number of the extant species that have been studied in this way. None the less, it is clear that various details of their reproductive tracts and the form and behaviour of their gametes differ quite obviously from one shrew to another, and 'diversity' already seems an appropriate first watchword for the reproductive system in shrews. So far, these differences have tended to distinguish the crocidurine subfamily, exemplified by *Suncus* and *Crocidura* (Bedford *et al.*, 1994, 1997a, c, d; T. Mori and J. M. Bedford, unpublished observations), from the soricine subfamily as represented by *Cryptotis* (Bedford *et al.*, 1997b), *Blarina* and, tentatively so far, three species of *Sorex* (O. B. Mock and J. M. Bedford, unpublished). Therefore, it was our expectation that *Myosorex*, as a putative member of the Crocidurinae, would help to substantiate as definitively 'crocidurine' some unusual reproductive characteristics manifested by *Suncus* and *Crocidura*, but not by indisputable soricines such as *Sorex*, *Blarina* and *Cryptotis*. These apparently crocidurine traits in *Suncus* and *Crocidura* include: (a) a giant acrosome whose plasmalemmal-outer acrosome membrane complex forms an unusual stable rim; (b) a Fallopian tube isthmus defined by deep crypts that house spermatozoa, and (c) an inert, long-lived cumulus oophorus that retreats from the zona pellucida to create a closed perizonal space that is the repository of cumulus-involved spermatozoa, motile or immotile, but

Fig. 5. (a) A Fallopian tube from *Myosorex varius* at its resting length after removal of mesosalpinx and membranes of the ovarian bursa as seen using differential interference contrast optics. Arrowheads mark the isthmo-ampullary junction. The isthmus is defined throughout by deep, essentially cilia-free, epithelial crypts, not evident at this magnification. The somewhat wider ampulla is distinguished along all but the uppermost region by a rich complement of peripherally located ciliated crypts. The arrow designates the level of the transverse section shown in (b). Scale bar represents 0.4 mm. (b) A transverse section of the ampulla of a Fallopian tube from *M. varius*, stained with Toluidine blue, illustrates the way the avenues between the epithelial folds that come from a central flat duct lumen eventually communicate with richly ciliated crypts situated at the periphery. Scale bar represents 660 μ m. (c) A peripheral segment of a transverse section, such as that in (b) illustrating in more detail the typical character of the ciliated crypts that are distributed throughout most of the ampulla. Scale bar represents 210 μ m.

Table 1. The ovulation response of *Myosorex varius* to hCG given i.p.

Female no.	Bodyweight (g)	Time after hCG (h)	Ovulation	Ovarian response
3	6.35	17.0	—	Right ovary – two follicles vascularized
9	8.20	20.5	—	Left ovary – three ripe follicles: eggs at Met II
16	12.80	20.8	—	Left ovary – four follicles vascularized Right ovary – one follicle vascularized
6 (P)	11.15	21.0	—	Left ovary – two follicles vascularized
4 (P)	9.85	22.0	—	Right ovary – three follicles vascularized
13	12.50	22.0	+	Left ovary – one ovulation Right ovary – one ovulation; both COCs in upper ampulla
22	12.00	22.5	+	Left ovary – two ovulations; bursal COCs Right ovary – one ovulation; COC in mid-ampulla
23	10.85	25.0	+	Left ovary – one ovulation; COC in upper ampulla Right ovary – two ovulations; two COCs in upper ampulla
11	8.15	27.5	+	Right ovary – two ovulations; two COCs in upper and lower ampulla
10	9.23	39.0	+	Left ovary – three ovulations; COC in lower ampulla Right ovary – one ovulation; COC in lower ampulla

(P): late pregnancy. COC: cumulus–oocyte complex.

all lacking acrosomes (Bedford *et al.*, 1994, 1997a, c, d). In addition, the glans penis in both *Suncus* and *Crociodura* is covered with spines.

In fact, the character of its reproductive system brings into question the designation of *Myosorex* as a crocidurine. *Myosorex* displayed one or two features specific to this genus, such as the relatively long interval, of about 22 h, from hCG treatment to ovulation, compared with 13–15 h in other shrews studied. Moreover, with a zona diameter of only 75 µm, the egg of *Myosorex* seems to displace that of the field vole (Austin, 1957) as the smallest mammalian egg recorded. However, *Myosorex* also exhibited a complex mix of other reproductive characters that, on the basis of previous observations in other shrews, are part soricine and part crocidurine. These characteristics are summarized in Table 2.

The field of spines over the glans penis in *Myosorex* is quite common among small mammals. However, while it mimics that in the crocidurines *Suncus murinus* and *Crociodura russula* and *C. dsinezumi* (Bedford *et al.*, 1997c; T. Mori and J. M. Bedford, unpublished observations), such spines are absent from soricines such as *Blarina* (Pearson, 1944), *Sorex cinereus*, *S. palustris*, *S. longirostris* and *Cryptotis parva* (O. B. Mock and J. M. Bedford, unpublished observations). The spermatozoon of *Myosorex* evidently undergoes a maturation in the epididymis similar to that in other mammals, at least in regard to its motility and stabilization of the sperm chromatin by covalent (—S—S—) bonds (see Bedford, 1991). Moreover, the changing disposition of the plasmalemma from a tight to a ballooned configuration between the caput and cauda regions is reminiscent of that which occurs in fixed spermatozoa from some other mammals, and provides the first indication in a shrew that the sperm membrane is modified during passage through the epididymis. The rather consistent one-sided ballooning of the mature periacrosomal plasmalemma hints at dorsoventral asymmetry, a character shown with surface markers for the spermatozoa of *Suncus murinus* (Cooper and Bedford, 1976).

However, while the spermatozoon evidently displays a barbed perforatorium (as indicated by the interrupted perforatorium in some sagittal sections), and a more distinct rostral elongation of the acrosome than in soricine shrews, its head is not crocidurine in type. The acrosome of *Myosorex*, though relatively large, is far smaller than the giant acrosome of *Crociodura* and *Suncus*, and it does not resist the concentrations of SDS that theirs do. In addition, the loose disposition of the mature periacrosomal plasmalemma and its 'bristly' surface are more like those of the soricines examined so far (Mori *et al.*, 1991; Bedford *et al.*, 1997b).

In *Myosorex*, an interesting feature of the male reproductive tract is the local aggregation of spermatozoa in the unusually convoluted distal segment of the vas deferens, which is also seen in various soricines and other shrews (O. B. Mock and J. M. Bedford, unpublished observations). This arrangement is rare in mammals, but has been noted as a feature in the Australian hopping mouse (Breed, 1986; Peirce and Breed, 1989). As suggested for that species, it appears that this expanded distal segment of the vas in *Myosorex* and soricine shrews acts as an accessory sperm store, and could be the immediate source of spermatozoa in the ejaculate, with gradual replacement of spermatozoa into this region from the cauda epididymidis in the period after ejaculation.

The Fallopian tube of *Myosorex* has a crocidurine-type isthmus (Bedford *et al.*, 1994, 1997a, c), defined throughout by deep crypts that are absent in the several soricines studied so far. However, its ampulla is endowed with many ciliated crypts similar to those seen in soricines (*Cryptotis parva*: Bedford *et al.*, 1997b; *Blarina brevicauda*, *Sorex palustris*, *S. longirostris*, *S. cinereus*: O. B. Mock and J. M. Bedford, unpublished), but which are absent in *Suncus* and relatively sparse in *Crociodura*. The persistence of the first polar body within the ovum in *Myosorex* is a trait not seen so far in the two crocidurines (where it soon disintegrates) but seen in two soricines (*Cryptotis parva*: Bedford *et al.*, 1997b; *Blarina brevicauda*: O. B. Mock

Fig. 6. (a) Two cumulus–oocyte complexes (COCs) from *Myosorex varius* extruded from the upper ampulla 22 h after hCG treatment (that is, at about the time of ovulation) visualized using differential interference contrast optics. The cumulus is ovulated as a matrix-free (non-expanded) dense mass of cells in contrast to that of most mammals. Scale bar represents 50 μm . (b) Transverse section of a COC, stained with Toluidine blue, *in situ* in the mid-ampulla, taken about 25 h after hCG treatment. The intact cumulus is enveloped by epithelial folds. Scale bar represents 45 μm . (c) Section through an unfertilized tubal egg in the ampulla, fixed 39 h after hCG (about 18 h after ovulation) and stained with Toluidine blue. Typically, and in contrast to the crocidurines *Suncus* and *Crocidura*, a prominent first polar body remains intact, as it does for many hours after ovulation in the soricines *Blarina* and *Cryptotis*. The cumulus is almost dispersed. A cumulus remained around each of two neighbouring unfertilized oocytes located in the same region of this tube. Scale bar represents 9.5 μm . (d) Segment of the cumulus oophorus of *M. varius* fixed 25 h after hCG (about 3 h after ovulation) visualized using transmission electron microscopy (TEM). Each cell associates with its neighbours by way of several junctional complexes. Although intercellular junctional complexes within the cumulus tend to disappear before ovulation in most mammals, these persisted for many hours after ovulation in *M. varius*, as they do in the crocidurines, *Suncus* and *Crocidura*. The black granules and larger black deposits in a few cells are believed to represent glycogen. ZP: zona pellucida. Scale bar represents 2.5 μm . (e) TEM showing the apposed borders of two cumulus cells in *M. varius* illustrating one of the desmosome-like complexes that probably maintain their relationship. Scale bar represents 0.45 μm .

Table 2. Subfamily affinities of some reproductive characteristics seen in *Myosorex varius*

Character in <i>Myosorex</i>	Soricine type	Crocidurine type
Vas deferens sperm store	<i>Cryptotis parva</i> <i>Blarina brevicauda</i> <i>Sorex palustris</i> <i>Sorex cinereus</i>	—
Penile spines	—	<i>Suncus murinus</i> <i>Crocidura russula</i> <i>Crocidura dsinezumi</i>
Sperm head structure	<i>Cryptotis parva</i> <i>Blarina brevicauda</i> <i>Sorex palustris</i> <i>Sorex cinereus</i>	—
Crypts in oviduct isthmus	—	<i>Suncus murinus</i> <i>Crocidura russula</i> <i>Crocidura dsinezumi</i>
Many crypts in oviduct ampulla	<i>Cryptotis parva</i> <i>Blarina brevicauda</i> <i>Sorex palustris</i>	—
Stable inert cumulus oophorus	—	<i>Suncus murinus</i> <i>Crocidura russula</i>
Persistent first polar body	<i>Cryptotis parva</i> <i>Blarina brevicauda</i>	—

and J. M. Bedford, unpublished observations). Yet the dense cumulus oophorus of *Myosorex* is almost crocidurine in its long-term inertness, in its intercellular junctions, and in the absence of postovulatory matrix secretion (compare with *Cryptotis*: Bedford *et al.*, 1997b), lacking only the significant perizonal space that develops as a unique characteristic of the cumulus in the true crocidurines studied (Bedford *et al.*, 1997a, d). How the cumulus behaves in *Myosorex* after fertilization awaits further study.

Although previous analyses of relationships have invoked penile morphology (Smith and Madkour, 1980), and sperm morphology (Bedford, 1974; Jamieson *et al.*, 1995), reproductive traits have not proved to be particularly useful as a basis for systematic or cladistic analysis (Luckett, 1980; Voss and Linzey, 1981). In the present case, however, reproductive traits do appear to be a useful basis. While the mix of reproductive traits in *Myosorex* is not consistent with it being classified simply as a crocidurine, they do support Reumer's (1987) proposal that *Myosorex* is a member of an older subfamily, the Crocidosoricinae, that gave rise to the present day Crocidurinae and Soricinae. The mix of reproductive characteristics is also consistent with the conclusion from recent alloenzyme analyses that *Myosorex* occupies an intermediate and primitive position in relation to soricines and crocidurines (Maddalena and Bronner, 1992). Finally, it accords with earlier reservations about the place of *Myosorex*, voiced on the basis of certain dentition and skull features (Meester, 1953; Heim de Balzac and Lamotte, 1956), and, perhaps, with recent observation of metabolic and behavioural traits that differ in certain respects from those of true crocidurines (Brown *et al.*, 1997).

'Difference' is another watchword that characterizes the gametes and reproductive tracts of the Soricidae and in this regard, the primitive status of *Myosorex* is of particular interest because the unusual reproductive features of shrews may eventually provide insights into some of the general questions raised in the Introduction about mammalian conception. Shrews exhibit novel features with regard to sperm morphology and to the configuration of male accessory glands. Shrews produce relatively few spermatozoa, and with the exception of cows, which have crypts at the uterotubal junction (Hunter, 1995), appear, so far, to be the only example in a eutherian in which the oviduct has differentiated crypts that house spermatozoa. It remains to be determined whether these crypts serve to store spermatozoa or, more likely, to limit the number at the site of fertilization in shrews. Furthermore, in keeping with the very small antrum of the mature ovarian follicle and narrow ampulla of the Fallopian tube, the soricid cumulus differs from that of most mammals in presenting as a dense ball that has no matrix. The indications are that the cumulus may be essential for fertilization in shrews, perhaps by virtue of a role in induction of the acrosome reaction (Bedford *et al.*, 1997a, b, d).

Such information about fertilization and cumulus behaviour has been obtained in other shrews after mating of adapted or captive-bred individuals. In the present series, the strategy of placing recently caught males and females together did not lead to mating. However, the use of captive-bred animals may in the future prove successful in the case of *Myosorex*, whose unusual features justify further efforts in that regard. *M. varius* presents a distinctly greater testis mass and sperm production,

and the most elaborate system of sperm crypts in the Fallopian tube seen thus far in any mammal. Finally, its sperm morphology suggests that, as with *Cryptotis* (Bedford *et al.*, 1997b) and other soricines (O. B. Mock and J. M. Bedford, unpublished), it may be possible to capacitate spermatozoa and fertilize eggs *in vitro*. Should this be the case, the fact that the cumulus can be dissected easily from newly ovulated eggs will facilitate investigation of the apparently essential role that this vestment has in fertilization in shrews.

Note added in proof. In subsequent studies, a female mated less than 24 h previously displayed moving spermatozoa in small groups within peripheral ciliated crypts located in the lower ampulla. However, the many active spermatozoa in the isthmus were essentially confined to the central lumen rather than to the crypts, which harbour isthmic spermatozoa in the crocidurines, *Suncus* and *Crociodura*. In addition, *in vitro* fertilization, whilst being possible with spermatozoa from *Cryptotis* and other soricines, could not be achieved in *Myosorex*. Under the same conditions (DMEM + 10% fetal calf serum at 37°C in 5% CO₂) active *Myosorex* spermatozoa from the cauda epididymis merely bound to the cumulus of, but did not penetrate, freshly ovulated *Myosorex* oocytes – a refractoriness observed previously in the crocidurine *Suncus murinus* (Bedford *et al.*, 1997d).

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