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The influence of mating system on seminal vesicle variability among gobies (Teleostei, Gobiidae)

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Abstract

A variety of sexual selection mechanisms have been implicated to drive the variability of the male reproductive tract in internal fertilizers, while studies on external fertilizers have been largely limited to exploring the influence of sperm competition on testis size and sperm number. Males in the Gobiidae, a speciose teleost family of demersal spawners with external fertilization, are known to be characterized by accessory structures to the sperm duct called seminal vesicles. These seminal vesicles secrete a mucus-enriched seminal fluid. Seminal vesicle size and function have been demonstrated to be influenced by sperm competition at the intraspecific level. With the aim to test the factors influencing the development of these male organs at the interspecific level, an independent contrast analysis was performed on 12 species, differing in mating system type, sperm competition risk, and duration of egg deposition. The type of mating system appears to be the main factor significantly affecting development of seminal vesicles, with males of monogamous species completely lacking or having extremely reduced organs.

Key words: Male accessory structures – mating system – sexual selection – sperm competition – Teleostei – comparative analysis

Introduction

The role of sexual selection mechanisms in generating the extraordinary variability of the male reproductive tract has been extensively investigated in internal fertilizers. Indeed, the role of sperm competition, cryptic female choice, female quality, and mating opportunities in shaping male primary sexual traits has been documented in a wide range of internal fertilizers, at both the inter- and intraspecific levels (Birkhead and Møller 1998; Birkhead and Pizzari 2002; Wedell et al. 2002; Pizzari et al. 2003; Hosken and Stockley 2004). On the contrary, studies on the causes of diversity of the male reproductive tract in external fertilizers have been limited to and largely focused on the influence of sperm competition and environmental factors, such as turbulence or water current intensity, on male gonad size and sperm number (Levitan and Petersen 1995; Stockley et al. 1997; Levitan 1998; Petersen and Warner 1998; Yund 2000).

In addition to testis size, other aspects of male reproductive anatomy, physiology and behaviour may vary among externally fertilizing species in response to sexual selection. For example, male mating opportunities, expressed as monogamy versus polygyny or the degree of polygyny, as well as the intensity of sperm competition, might affect ejaculate characteristics, including not only sperm number and features (size, motility, longevity, etc.), but also seminal fluid quantity and quality. In this scenario, structures devoted to seminal fluid production or ejaculate release might also be affected by sexual selection pressures.

In teleost fish, variation in seminal fluid composition both within and between species are well known in the Gobiidae, a family of demersal spawners in which males defend or build a nest and perform parental care until hatching (Miller 1984; Mazzoldi 1999). Gobies inhabit different environments, show different types of mating systems, from monogamy to polygyny, and experience a range of risk and intensity of sperm competition (Miller 1984; Mazzoldi 1999, 2001; Pilastro et al. 2002). A major component of their seminal fluid is mucin, in particular sialoglycoproteins (Young and Fox 1937; Fishelson 1991; Lahnsteiner et al. 1992), whose viscosity strongly affects

ejaculate performance (Marconato et al. 1996). Several gobies lay sperm in the form of sperm trails, bands of mucins that, dissolving slowly, release sperm into the water for several hours (Marconato et al. 1996) and whose longevity has been demonstrated to depend on the trail's mucin content (Scaggiante et al. 1999; Mazzoldi et al. 2000; Rasotto and Mazzoldi 2002). Females can take a long time to lay eggs (up to several hours in large species: Mazzoldi 1999; Scaggiante et al. 1999) and males release sperm trails on the nest surface immediately before and during egg deposition (Marconato et al. 1996; Scaggiante et al. 1999). The intermittent gamete release that characterizes the laying of sperm trails enables parental males to spend part of the mating time patrolling nest entrances from incursions by predators or opportunistic males trying to sneak fertilizations (Mazzoldi 1999). In species with alternative male mating tactics, sneaker males produce sperm trails poorer in mucins, with higher sperm density, and with a shorter duration than those of parental males (Mazzoldi et al. 2000; Rasotto and Mazzoldi 2002). These characteristics confer different qualities to ejaculates, with parental male trails guaranteeing a low but steady supply of sperm for hours, while sneaker males release a high and quickly diffusing number of sperm that outnumber those of parental male for a short time (Scaggiante et al. 1999; Mazzoldi et al. 2000; Rasotto and Mazzoldi 2002).

Sperm trail mucins are produced in male accessory structures, defined as seminal vesicles or sperm duct glands, which are enlargements of the sperm duct. These organs (Eggert 1931; Miller 1984) are considered to be a synapomorphic trait of the whole suborder Gobioidae (Miller 1992) and show a conspicuous variability both among (Fishelson 1991) and within species (Mazzoldi 1999; Scaggiante et al. 1999; Rasotto and Mazzoldi 2002; Drilling and Grober 2005). Intraspecific analyses of the male reproductive apparatus and ejaculate characteristics have demonstrated that seminal vesicle development is positively related to sperm trail longevity (Scaggiante et al. 1999; Mazzoldi et al. 2000; Rasotto and Mazzoldi 2002). In species where alternative male mating tactics occur, differences in ejaculate performances among males are paralleled by differences in seminal vesicle size and function, with

the seminal vesicles of parental males well developed and filled with mucins, while those of sneakers are smaller, used for storing sperm rather than production of copious mucin (Mazzoldi 1999; Scaggiante et al. 1999; Rasotto and Mazzoldi 2002; Drilling and Grober 2005).

Goby seminal vesicles, because of their primary role in influencing ejaculate performances, their noteworthy inter- and intraspecific variability, and the widespread occurrence in the family, represent an excellent model to test the effects of different selection mechanisms in driving morphological diversity of the male reproductive system. This study was specifically aimed to analyse, at the interspecific level, the influence of three factors on the development of these organs: (1) The type of mating system, used as an indicator of mating opportunities. Prudence in sperm allocation may be particularly rewarding in polygynous species, in which males have to fertilize eggs, simultaneously or successively, from different females, than for monogamous ones. (2) The occurrence of alternative male mating tactics, and hence of sperm competition. When sperm competition occurs, producing sperm trails with higher longevity and slower sperm release could be beneficial if it increases a male's ability to behaviourally exclude other males, but it might decrease successful sperm competition with males releasing high numbers of short-lived sperm. (3) The duration of egg deposition. We expect higher allocation to mucins with longer duration of egg deposition.

Materials and Methods

Study species

Twelve goby species, belonging to eight different genera, were used for the analyses. Six species – *Pomatoschistus marmoratus* (Risso, 1810); *Pomatoschistus minutus* (Pallas, 1770); *Gobius niger* (L., 1758); *Knipowitschia panizae* (Verga, 1841); *Zosterisessor ophiocephalus* (Pallas, 1814); and *Padogobius bonelli* (Bonaparte, 1846) = *Padogobius marteni* (Günther, 1861) (see Kottelat 1997; Froese and Pauly 2004) – are temperate, Mediterranean and/or Atlantic (Whitehead et al. 1986), while the others six – *Amblygobius nocturnus* (Herre, 1945); *Amblygobius rainfordi* (Whitley, 1940); *Valenciennesa muralis* (Valenciennes, 1837); *Valenciennesa sexguttata* (Valenciennes, 1837); *Valenciennesa strigata* (Broussonet, 1782); and *Cryptocentrus lutheri* (Klausewitz, 1960) – are tropical, living in the Indian and Pacific Ocean (Froese and Pauly 2004). All species but *P. bonelli* are marine. Species have been selected on the basis of the available information on a full set of characteristics concerning the type of mating system, the occurrence of sperm competition, the duration of egg deposition, the investment in seminal vesicles, and their phylogenetic relationships. When a trait was unknown, data have been specifically collected (gonadal and seminal vesicle investment in six species), back calculated from a regression (egg deposition duration in eight species) or estimated or inferred from published accounts of behaviour or personal observations of specimens or behaviour in the field (degree of polygyny, and degree of sperm competition in one species).

Data

The reproductive characteristics under analysis have been calculated and coded as described in Table 1. For males, we only used data from parental individuals, and excluded potential sneaker individuals from the analysis.

For six species, *A. rainfordi*, *V. sexguttata*, *V. strigata*, *C. lutheri*, *P. bonelli*, and *K. panizae*, information on gonad and seminal vesicle investment were not available from the literature and fresh or museum preserved specimens were analysed. In particular, four species, *A. rainfordi* (three males, two females), *V. sexguttata* (three males, two females), *V. strigata* (two males, two females), and *C. lutheri* (two males, two females) have been analysed through museum samples,

Table 1. Description and coding of reproductive traits considered in the analyses and investment in seminal vesicles and gonads

Variable	Description and coding
TL	Mean total length (mm) of males
GSI	Gonosomatic index ($100 \times W_g/W_t$)
SVSI	Seminal vesicle somatic index ($100 \times W_{sv}/W_t$)
Polygyny	0, monogamy; 1, most nests with one clutch, but polygyny occurs; 2, > 1 egg clutch/nest common; 3, always > 1 egg clutch/nest
Sperm competition	0, absent; 1, present, low number of sneakers in the population; 2, present, sneakers usually found around nests during spawning with quick incursions; 3, present, usually > 1 sneaker male present in nest during spawning
Duration of egg deposition	Mean duration of female deposition

W_g , gonadal weight; W_{sv} , seminal vesicle weight; W_t , total weight.

while specimens of *P. bonelli* (16 males, four females) and *K. panizae* (17 males, four females) have been specifically collected during their reproductive season, brought to the laboratory and sacrificed with an excess of anaesthetic (MS222). For each specimen, total length (to the nearest millimetre) and, for fresh specimens, body weight (to the nearest milligram) were recorded. The entire reproductive apparatus was removed with the aid of a stereomicroscope. In wild caught fish, gonads and seminal vesicles were separately weighed (to the nearest mg), and gonosomatic (GSI) and seminal vesicle somatic (SVSI) indices were calculated, as reported in Table 1. For museum samples, preservation procedures affect weight measurements, consequently total, gonad and seminal vesicle weights were not measured, and GSI and SVSI were not calculated. However, we did note the presence or absence of seminal vesicles. In *A. rainfordi*, the only species where seminal vesicles were found in museum preserved specimens, seminal vesicles were observed but were smaller than the seminal vesicles observed in any other species where they were present. We assigned two different SVSI values for this species: either equal to the smallest value found in our species set (SVSI = 0.5) and or half that value (SVSI = 0.25).

Seminal vesicles were also examined histologically for the presence of mucopolysaccharides. Seminal vesicles were fixed in Dietrich's solution, embedded in paraplast, sectioned transversally and serially at 7 μ m, and mounted on slides. Sections were stained with Harris's haematoxylin–eosin and histochemical stains for protein and mucopolysaccharides were performed (see Scaggiante et al. 1999 for a complete methodology).

The duration of egg deposition was available for four species, *Z. ophiocephalus*, *G. niger*, *P. marmoratus*, and *K. panizae* (Table 2, reference column). These species showed a strong relationship between female size and duration of egg deposition ($r = 0.99$). As a result of this strong correlation, we used fish length as a proxy for duration of egg deposition in the comparative analysis.

The mating system for several of the study species was already known, with multiple polygynous species (*Z. ophiocephalus*, *G. niger*, *P. minutus*, *P. marmoratus*, *K. panizae*, and *P. bonelli*) as well as several species known to be monogamous (*V. strigata*, *V. sexguttata*, *V. muralis*, and *A. nocturnus*) (Table 2, reference column). A consistent feature of the monogamous gobies is their occurrence in pairs, often jointly digging one or more burrows in the sand (several species of *Valenciennesa*, *Amblygobius*, and *Signigobius*; Hudson 1977; Hoese and Larson 1994; Reavis 1997b; Takegaki and Nakazono 1999; Clark et al. 2000; Takegaki 2000; Mazzoldi 2001). As *C. lutheri* consistently occurs in pairs (Randall 1996; A.C. Gill, personal communication) we classified it as a monogamous species. In contrast, *A. rainfordi* does not seem to use a burrow and it is usually found in small groups (Myers 1989; A.C. Gill, personal communication). As a consequence, *A. rainfordi* has been considered a polygynous species but different possible values were used for its degree of polygyny and degree of sperm competition. In monogamous gobies, the occurrence of alternative male mating tactics and sperm competition has never been

Table 2. Reproductive traits of the analysed goby species

Species	TL (mm)	GSI	SVSI	Polygyny	Sperm competition	Egg dep. (min)	Reference
<i>Pomatoschistus marmoratus</i>	44.6	0.47	0.47	2	0	120	1,2
<i>Pomatoschistus minutus</i>	67.6	0.33	0.54	2	1	170 ²	3,4, 5, pres. pap.
<i>Knipowitschia panizzae</i>	36.7	0.74	0.60	1	0	90	6, pres. pap.
<i>Padogobius bonelli</i>	66.1	0.93	1.00	2	0	160 ²	7, pres. pap.
<i>Gobius niger</i>	113.3	0.66	0.65	3	2	300	8,9
<i>Zosterisessor ophiocephalus</i>	187.7	1.04	1.06	3	3	420	10,11
<i>Amblygobius nocturnus</i>	46.7	0.12	0	0	0	130 ²	12, pres. pap.
<i>Amblygobius rainfordi</i>	33.0		0.25–0.5	1–2 ^{1,2}	0–1 ^{1,2}	100 ²	Pres. pap.
<i>Valenciennea strigata</i>	110.6		0	0	0	300 ²	13,14, pres. pap.
<i>Valenciennea sexguttata</i>	92.2		0	0	0	240 ²	15, pres. pap.
<i>Valenciennea muralis</i>	63.4	0.09	0	0	0	160 ²	12, pres. pap.
<i>Cryptocentrus lutheri</i>	80.2		0	0 ²	0 ²	200 ²	Pres. pap.

Egg dep.: duration of egg deposition; pres. pap.: present paper.

¹Comparative analyses performed using different possible values; ²inferred data, see text for explanation

References: 1, Mazzoldi and Rasotto (2001); 2, Mazzoldi et al. (2002); 3, Lindström (1988); 4, Forsgren (1997); 5, Mazzoldi (1999); 6, Massironi et al. (2005); 7, Marconato et al. (1989); 8, Mazzoldi and Rasotto (2002); 9, Rasotto and Mazzoldi (2002); 10, Scaggiante et al. (1999); 11, Mazzoldi et al. (2000); 12, Mazzoldi (2001); 13, Reavis (1997a); 14, Reavis (1997b); 15, Hoese and Larson (1994).

reported (Reavis 1997b). Consequently, a null value of sperm competition has been assigned to monogamous species included in the analysis.

Data analyses

We controlled for phylogenetic non-independence in this comparative analysis by applying the independent contrasts method (Felsenstein 1985; Harvey and Pagel 1991), using the Computer programs for the statistical analysis of comparative data COMPARE, version 4.5 (Martins 2003). All regressions and correlations involving contrasts were forced through the origin, as recommended by Garland et al. (1992). Stepwise multiple regression analyses (tolerance = 0.0001; F to include = 1; F to remove = 0) was performed, with contrasts in SVSI as the dependent variable and contrasts in polygyny, sperm competition and total length (TL) as independent variables. Statistical analyses were performed using STATISTICA 5.0 for Windows.

Phylogenetic relationships

All species belong to the same subfamily: Gobiinae (Pezold 1993). While for Mediterranean species phylogenetic relationships and branch lengths, estimated from distances among species in mitochondrial DNA sequences, are available (Penzo et al. 1998), for Indo-Pacific species only relationships between genera are reported (Hoese and Larson 1994; Thacker 2003). Consequently, we set all branch lengths equal to 1 (Purvis et al. 1994). Within the genus *Valenciennea*, species relationships are not completely resolved, so we performed the analyses using all the three different possible trees. The phylogenetic relationships used in the comparative analyses are illustrated in Fig. 1.

Results

Data on seminal vesicle development and other reproductive traits considered in the analysis are reported in Table 2.

Histological analyses

All the analysed fishes were sexually mature. Seven of the 12 study species had seminal vesicles (Table 2). For the six species where no data existed on the presence and/or size of seminal vesicles, these structures were present in three species (*P. bonelli*, *K. panizzae* and *A. rainfordi*) and absent in the other three (*C. lutheri*, *V. strigata* and *V. sexguttata*). In addition to visual inspection at dissection, absence was verified by examining histological sections for any enlargement of the sperm duct. When they were present, seminal vesicles appeared

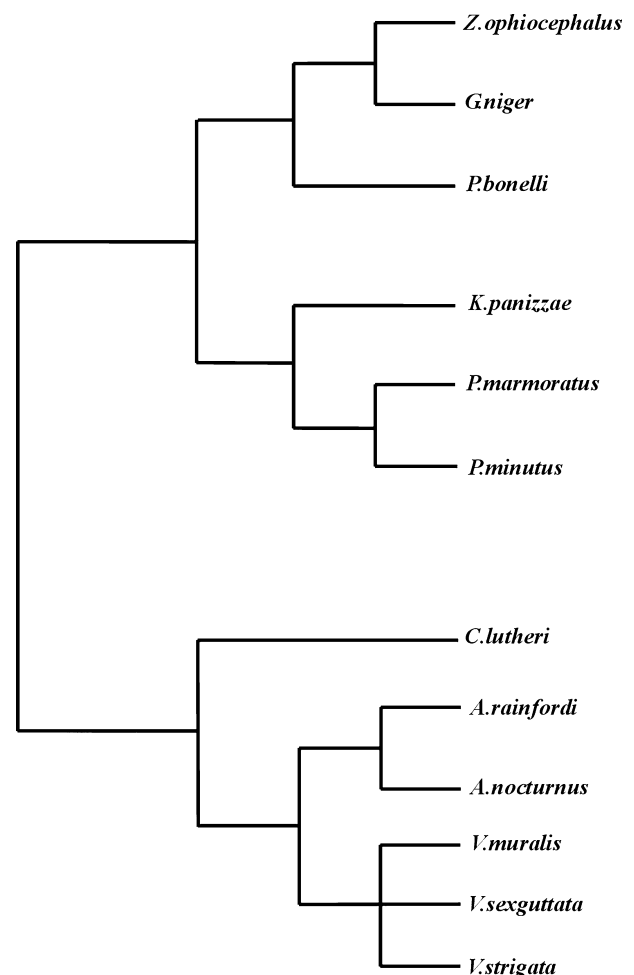


Fig. 1. Phylogenetic relationships among the studied goby species, used in the comparative analyses. Tree has been artificially reconstructed on the basis of the available literature information, consequently, only relationships among the considered species are reported

as multichambered, paired organs, opening into the terminal portion of the sperm duct. Chamber walls consisted of an inner single layer of epithelial cells, a basal lamina and an external

thin layer of connective tissue. Chamber lumina were filled with secretion, positively reacting to stains for sialoglycoprotein. Intraspecific differences in the morphological organization and/or function of seminal vesicles were not observed. The testes of all the analysed species were of the unrestricted lobular type (Grier 1993), showing a cystic spermatogenesis in *P. bonelli* and *K. panizzae*, and a semicyclic one (Lahnsteiner et al. 1990) in the species of the genera *Valenciennea*, *Cryptocentrus* and *Amblygobius*.

Comparative analysis

The type of mating system had a demonstrable effect on seminal vesicle size in the 12 goby species in this study. Indeed, polygyny was the only independent variable to have a statistically significant effect in the multiple regression analyses with contrasts in SVSI as dependent variable. Neither sperm competition nor TL had strong-enough effects to be included in the stepwise multiple regression analyses. This result occurred regardless of the value of SVSI attributed to *A. rainfordi* (SVSI = 0.5: $F_{1,10} = 7.03$, $p = 0.024$, $r = +0.64$, Fig. 2; SVSI = 0.25: $F_{1,10} = 5.29$, $p = 0.044$, $r = +0.59$). The different values of degree of polygyny or sperm competition attributed to *A. rainfordi*, or the different solutions for the polytomic node of *Valenciennea* species did not affect the multiple regression results.

Although the analysis showed a statistically significant result for the effect of polygyny on the development of seminal vesicles, several of the pairwise comparisons showed relatively high deviation from the regression (Table 3). The residuals of three contrasts appear particularly high in absolute values: the contrast between the node calculated from the two *Pomatoschistus* species and *K. panizzae*, with *K. panizzae* presenting a higher value than expected; the contrast between *G. niger* and *Z. ophiocephalus*, with *Z. ophiocephalus* presenting a higher value than expected; the contrast between the node calculated from *G. niger* and *Z. ophiocephalus* and *P. bonelli*, with *P. bonelli* presenting a higher value than expected. This high deviation suggests that factors in addition to the degree of polygyny may also affect the development of seminal vesicles in gobies.

Discussion

The development of seminal vesicles in parental males among goby species appears to be significantly correlated with the degree of polygyny. Seminal vesicles were absent or rudimentary in the monogamous species, while they were well developed in the polygynous species. The influence of mating system on seminal vesicle development provides support to the hypothesis that the mucins produced by these organs play an important role in sperm release, particularly in extending the duration and reducing the rate of sperm release. In monogamous species males usually maintain the pair bond for several consecutive spawning events, through which they fertilize just one egg batch at a time, and extra-pair spawning has never been observed (Reavis 1997b; Takegaki and Nakazono 1999; Takegaki 2000; Mazzoldi 2001). In addition, the nest entrance is plugged during spawning (Takegaki and Nakazono 1999), thus isolating the mating pair from outside, hence drastically reducing predation risk but also sperm dispersal and the potential of sperm competition. Consequently, males of these species might not need either a large sperm supply or mucus

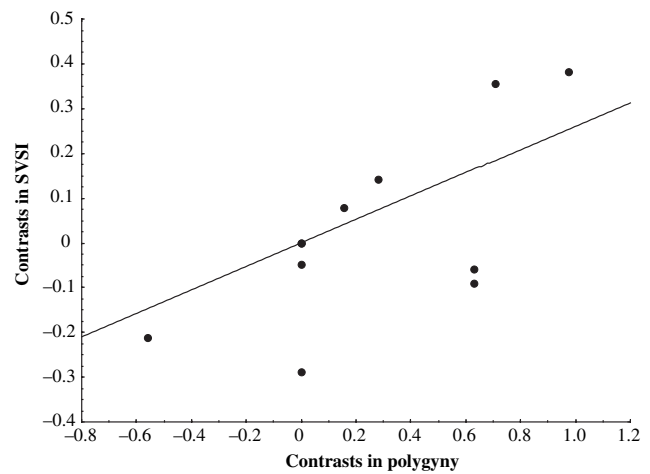


Fig. 2. Relationship between contrasts in polygyny and contrasts in SVSI

Table 3. Values of the residuals of the regression among contrasts in SVSI and polygyny

Contrasts	Residuals
1: <i>P. marmoratus</i> – <i>P. minutus</i>	–0.05
2: 1 ¹ – <i>K. panizzae</i>	–0.22
3: <i>G. niger</i> – <i>Z. ophiocephalus</i>	–0.29
4: 3 ¹ – <i>P. bonelli</i>	–0.25
5: 2 ¹ –4 ¹	–0.07
6: <i>A. rainfordi</i> – <i>A. nocturnus</i>	0.17
7: <i>V. muralis</i> – <i>V. strigata</i>	0
8: 7 ¹ – <i>V. sexguttata</i>	0
9: 6 ¹ –8 ¹	0.07
10: 9 ¹ – <i>C. lutheri</i>	0.04
11: 10 ¹ –5 ¹	0.12

¹Node relative to the contrast indicated by the number.

production to limit sperm release rate. It is interesting to note that, in addition to lacking seminal vesicles, males of the analysed monogamous species exhibit an asynchronous type of spermatogenesis, enabling them to mature, at any time, a smaller amount of sperm compared with the synchronous type, the most common among teleosts (Grier 1981; Lahnsteiner et al. 1990; Mattei et al. 1993; Manni and Rasotto 1997; Mazzoldi 2001). The interval between expected subsequent egg laying should guarantee males of monogamous species enough time to mature the needed amount of sperm. By contrast, in polygynous species, parental males fertilize eggs from several females, sometimes simultaneously (Mazzoldi et al. 2000; Mazzoldi and Rasotto 2002). Consequently, they may need a larger gamete supply; in addition an increase in sperm retention at spawning can be particularly rewarding to them.

This lack of seminal vesicles in several goby species also affects the general notion that the presence of seminal vesicles in males is ubiquitous within the Gobiidae (Miller 1984, 1992). As male accessory structures are considered a synapomorphic traits not only of this family but of the whole suborder Gobioidae (Miller 1992), our results suggest that, given the considered phylogeny, they might have been either lost twice or lost once and regained once in accordance with the mating system.

The development of seminal vesicles in relation to the type of mating system might be a consistent pattern in the

Table 4. Literature data on the presence of seminal vesicles and mating system of goby species

Species	Seminal vesicles	Mating system	References
<i>Gobius cobitis</i>	+	Polygynous	Gibson (1970), Fishelson (1991)
<i>Gobius paganellus</i>	+	Polygynous	Vivien (1938), Miller (1961)
<i>Pomatoschistus microps</i>	+	Polygynous	Miller (1984), Magnhagen (1992)
<i>Gobiusculus flavescens</i>	+	Polygynous	Fishelson (1991), Amundsen and Forsgren (2001)
<i>Coryphopterus nicholsi</i>	+	Polygynous	Cole (1982, 1983, 1990)
<i>Lythrypnus dalli</i>	+	Polygynous	St Mary (1993, 1994), Black, M. P., Moore, B. Canario, A. V. M., Ford, D., Reavis, Q. W. and Grober, M. S. (unpublished work)
<i>Gobiodon histrio</i>	+	Monogamous	Munday et al. (1998)
<i>Gobiodon quinquestriatus</i>	+	Monogamous	Cole (1990), Nakashima et al. (1996)
<i>Paragobiodon echinocephalus</i>	+	Monogamous	Cole (1990), Kuwamura et al. (1993)
<i>Trimma okinawe</i>	+	Polygynous	Sunobe and Nakazono (1990, 1993)
<i>Asterropteryx semipunctata</i>	+	Polygynous	Fishelson (1991), Privitera, L. A. and Smith, R. J. F. (unpublished work)
<i>Eviota prasina</i>	+	Polygynous	Cole (1990), Sunobe and Nakazono (1999)
<i>Bathygobius fuscus</i>	+	Polygynous	Fishelson (1991), Hishida (2002)

Gobiidae, as it also has been shown to be well developed in several other polygynous species (Table 4). The only exception to the correlation between mating system and seminal vesicle development is represented by species belonging to the closely related genera *Gobiodon* and *Paragobiodon*, in which seminal vesicle development appears to have evolved quite differently than in other gobies. *Gobiodon* and *Paragobiodon* differ from other gobies in two ways: first, one type of accessory structure occurs in both males and females. Secondly, males have a second accessory structure (Cole 1990; Cole and Hoese 2001). The presence of two pairs of accessory structures in males and one pair in *Gobiodon* females is peculiar and suggests that these organs could be involved in functions additional or alternative to fertilization dynamics (Cole and Hoese 2001). Considering that these species inhabit corals and eggs are released and cared for on their surfaces, the mucins produced by their accessory structures could be involved in protecting eggs and parents from toxic substances on coral surfaces. Mucus is well known to protect the skin against toxins or pathogens (Mittal et al. 1981; Shephard 1994; Ebran et al. 2000; Kosuga et al. 2000) but, recently, in a freshwater species with male parental care, it has also been demonstrated to play a major role in preserving egg batches from microbial infections (Knouft et al. 2003).

Accessory structures to the male reproductive apparatus have been described in several other teleost families, belonging to different orders (i.e. Siluriformes: Sneed and Clemens 1963; Rastogi 1969; Nayyar and Sundararaj 1970; Van den Hurk and Resink 1992; Osmeriformes: Pusey and Stewart 1989; Batrachoidiformes: Barni et al. 2001; Perciformes: Rasotto 1995; Richtarski and Patzner 2000). In some species these organs have been shown to be related to mucin production (Rasotto 1995; Richtarski and Patzner 2000; Barni et al. 2001; Mansour et al. 2004), and their intraspecific variability has been related to male mating tactics in some species (Bass 1992; Ruchon et al. 1995; Oliveira et al. 2001). It is noteworthy that these species are, like gobies, demersal spawners. Consequently, a role of the viscous seminal fluid in limiting sperm dispersal, as well as an influence of sexual selection on the development of these accessory organs, might be expected.

Contrary to expectation, neither variation in sperm competition nor duration of egg deposition appear to influence, at the interspecific level, the development of seminal vesicles.

Considering the small sample of analysed species we cannot exclude a data limitation effect. However, the observed pattern, in addition with the analysis of residuals, provides some hypotheses on other factors possibly affecting seminal vesicles development that can be tested when more data become available.

The most atypical result is the apparent lack of influence of sperm competition on possibly both seminal vesicle and testis size, as suggested by the strong covariance of GSI with SVSI ($r_s = +0.86$, $p = 0.014$, $n = 8$). If this pattern is not due to the limited data set it could indicate that the actual mucin content in sperm trail already guarantees parental males a release frequency (Mazzoldi et al. 2000; Mazzoldi and Rasotto 2002) consistent enough to successfully reduce cuckoldry. Indeed, in both *Z. ophioccephalus* and *G. niger*, territorial males, in the presence of sperm competitors, respond by allocating more energy to mate and nest guarding rather than adjusting ejaculate expenditure (Scaggiante et al. in press).

The analysis of the residuals of the multiple regression suggests that seminal vesicle size might be influenced by other factors, such as scaling, nest type, and water salinity. In the cases, where the residuals differed most from the general pattern, there were either differences between pairs in individual size (*Pomatoschistus* versus *K. panizae*), type of nest substrate (*Z. ophioccephalus* versus *G. niger*) or differences in habitat (freshwater *P. bonelli* versus all other species). The trends suggested by these single comparisons are interesting, but at present the causes for the differences observed in these comparisons are unclear. Given the high species diversity in this group of fishes, gobies may represent fruitful additional material for testing evolutionary hypotheses using comparative phylogenetic analysis.

Reference for museum samples: *C. lutheri*: BMNH 2000.4.19.1100-1103; *A. rainfordi*: ACG-PR 2, 4, 10; *V. sexguttata*: BMNH 1960.3.15.1411-1423; 1994.1.18.125-128; *V. strigata*: University of Washington Collection, Category number 7439.

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Riassunto

Influenza del sistema riproduttivo sulla variabilità delle vescicole seminali nei gobiidi.

Nelle specie a fecondazione interna è stato ampiamente dimostrato il ruolo di diversi meccanismi di selezione sessuale nel modellare l'estrema variabilità delle strutture genitali maschili. Nelle specie a fecondazione esterna le informazioni sono, invece, limitate principalmente all'influenza della competizione spermatica sulle dimensioni dei testicoli e sul numero di spermatozoi. Nei Gobiidae, famiglia di pesci teleostei caratterizzata da un elevato numero di specie e dalla produzione di uova demerse, i maschi presentano strutture accessorie allo spermidotto, chiamate vescicole seminali, che secernono un liquido seminale particolarmente ricco di mucine. A livello intraspecifico è stato dimostrato come le dimensioni e la funzione delle vescicole seminali sia influenzata dalla competizione spermatica. Con lo scopo di studiare quali fattori a livello interspecifico influenzino lo sviluppo di questi organi, è stata applicata l'analisi comparativa dei contrasti indipendenti a 12 specie di gobiidi che differiscono nel sistema riproduttivo, nel livello di competizione spermatica e nella durata della deposizione. L'unico fattore che influenza significativamente lo sviluppo delle vescicole seminali è risultato essere il sistema riproduttivo, infatti nelle specie monogame i maschi non hanno, o hanno estremamente ridotte, vescicole seminali.

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