

The third way: spermcast mating in sessile marine invertebrates

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Synopsis Marine invertebrates belonging to a broad range of taxa disperse aquatic spermatozoa to fertilize eggs that are retained rather than spawned. We outline the occurrence of this mechanism, which we refer to as spermcast mating, and identify tentative generalizations relating to it. Contrasts are drawn where appropriate with broadcast spawning of both eggs and sperm for external fertilization, and with copulation or pseudocopulation. Spermcast mating may involve the gradual accumulation of long-lived spermatozoa from dilute suspension, probably during suspension feeding, and the subsequent storage of spermatozoa by the recipient (acting female) prior to fertilization. This process may involve extensive contact between spermatozoa and recipient (maternal) tissue. Mating may be influenced by compatibility systems, and receipt of compatible allosperm may trigger female investment, giving apparent scope for sexual conflict over levels of maternal investment. External fertilization of cohesive egg masses remaining close to the acting female may appear somewhat intermediate between spermcast mating and broadcast spawning but, while it may be possible to envisage a continuum between the 2 modes, the end points are distinct, commonplace, and involve contrasting reproductive characteristics. Three variants of the typical pattern of spermcast mating are briefly discussed: the spawning of zygotes (rather than the more usual brooding of progeny), polyembryony, and the dispersal of spermatophores rather than individual spermatozoa.

Introduction

Some marine invertebrates mate by the release of eggs and sperm to meet in the water column, others by the direct delivery of sperm to maternal individuals via copulation or pseudocopulation. These mechanisms are widely recognized, but a third has been less well appreciated: many sessile (that is, attached) marine invertebrates are known, or can be inferred, to mate by releasing unpackaged spermatozoa to be dispersed to conspecifics where they fertilize eggs that have been retained by their originator. These include most sponges, many hydroids and some corals (Cnidaria), some polychaetes (Annelida), some bivalve Mollusca, most or all Entoprocta, some articulate Brachiopoda, all Ectoprocta, most or all pterobranchs (Hemichordata), the great majority of colonial, and a few unitary, ascidians (Chordata: Tunicata) (Pemberton and others 2003: electronic appendix). Many algae mate in an analogous way (tabulated in Pemberton and others 2003: electronic appendix). Some pelagic tunicates may have a similar basic mechanism (Miller 1994), albeit with the possibility of swarming during sperm release (Miller and Cosson 1997).

In the past, there was considerable doubt (reviewed by Ryland and Bishop 1993) that internal

cross-fertilization could be accomplished by the release, dispersal, and uptake of free spermatozoa, and some relatively recent articles, although otherwise very insightful, have apparently overlooked or discounted this possibility in discussing potential mating mechanisms of marine animals (for example, Hadfield and Hopper 1980; Strathmann 1990). This mating process stands in contrast to the release of both eggs and spermatozoa for external fertilization, generally referred to as broadcast or free spawning. Because the mechanism has tended to be overlooked in the absence of a name, and to promote precision of meaning, the introduction of a distinctive term for this mating process was advocated by Pemberton and others (2003: electronic appendix). We refer to it as spermcast mating. Other workers have not favored this usage; for instance, Johnson and Yund (2004) and Phillippi and others (2004) refer to species showing this pattern as egg-brooding free spawners.

The great majority of spermcasters brood their embryos following fertilization. Particularly in modular, colonial forms, larvae are then released at an advanced stage and have an abbreviated pelagic phase (Jackson 1985, 1986).

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Below, we suggest 6 tentative generalizations concerning spermcast mating in marine invertebrates, based on information in the literature. Particular comparison is possible between 3 species that are relatively well studied: the colonial ascidians *Botryllus schlosseri* (Pallas) and *Diplosoma listerianum* (Milne Edwards) (Deuterostomia: Chordata: Tunicata), and the bryozoan *Celleporella hyalina* (L.) (Protostomia: Ectoprocta). The colonies of all 3 species are hermaphroditic; whereas the 2 ascidians have only one type of module, that is, hermaphroditic zooids that also feed, *C. hyalina* has 3 distinct polymorphs: feeding, male, and female zooids. We then consider 3 variants of the basic pattern of spermcast mating.

Spermcast mating

Spermatozoa are relatively long lived in water

Half-lives of spermatozoa in dilute suspension have been reported as follows: c. 8 h at 16.5°C in *D. listerianum* (Bishop 1998); c. 1.2 h at 12°C, 16°C, and 18°C in *C. hyalina* (Manríquez and others 2001); and 16.1 h at 22°C and 26.3 h at 15°C in *B. schlosseri* (Johnson and Yund 2004). These values suggest that spermcast spermatozoa retain the ability to fertilize longer at comparable concentrations (relevant because of the respiratory dilution effect) than typical spermatozoa of broadcast spawners; comparisons are drawn by Manríquez and others (2001) and Johnson and Yund (2004). It has been suggested (Bishop 1998; Johnson and Yund 2004) that spermcast spermatozoa may be inactive during dispersal, conserving energy and thus promoting longevity, perhaps with activation upon reaching a conspecific individual. Temkin (1991, 1994) reported intermittent swimming or quiescence by spermatozeugmata of the cheilostome bryozoan *Membranipora membranacea* after spawning, with commencement of strong undulating movements upon being drawn into a conspecific lophophore; contact with the lophophore's intertentacular organ, through which spermatozeugmata enter the recipient zooid's coelom, apparently triggered the adoption of a distinct reverse waveform (Temkin and Bortolami 2004). In the internally fertilized salp *Thalia democratica*, Miller (1994) also noted alternating inactivity and swimming by spermatozoa for at least an hour after spawning, with continuous activity once drawn into the atrium of a female-stage salp.

Spermatozoa can be utilized from very dilute suspension

As an adjunct to extended sperm longevity, fertilization can be maintained from low concentrations relative to those necessary to avoid sperm limitation in broadcast

spawners. Appreciable rates of background fertilization in experimental mating arrays of both *B. schlosseri* and *C. hyalina* suggested that long-distance fertilizations could occur, implying utilization of dilute spermatozoa (Yund and McCartney 1994), and in *B. schlosseri* fertilization by spermatozoa originating more than 40 m away has been detected (Yund 1998). Pemberton and others (2003) estimated in the laboratory that maximum production of zygotes could occur down to sperm concentrations of 500 sperm.ml⁻¹ for *D. listerianum* and 50 sperm.ml⁻¹ for *C. hyalina*, and made explicit comparison with published *in vitro* values for the onset of sperm limitation in broadcast spawners (see tabulation in their electronic appendix); they also estimated from field trials that sperm supply at a minimum distance of 0.9 m from a wild population was several times that required to maintain female-role reproductive output. Phillippi and others (2004) reported the maintenance of high fertilization rates within low-density and/or small artificial mating arrays of *B. schlosseri*, and within low-density natural populations.

It thus appears that sperm limitation may be less of a threat for spermcasters than for broadcast spawners, particularly given a capacity of spermcasters to store allosperm once acquired (see below). With regard to spermcast fertilization efficiency, a distinction may be necessary between actively pumping species such as the 3 discussed here, which are suspected to extract spermatozoa efficiently from their incoming feeding/respiratory current, and passive feeders reliant on ambient flow, such as cnidarians (Yund 2000; Pemberton and others 2003; Phillippi and others 2004). Thus variable and generally low fertilization success is reported in the gorgonian coral *Briareum asbestinum*, which broods externally after internal fertilization (Brazeau and Lasker 1992). However, Miller (1994) describes the conditioning of ambient flow by polyps of hydroids of the genus *Tubularia* to create downstream eddies that potentially retain spermatozoa in the vicinity of the gonophoral openings, which release chemoattractant. Despite having nonflagellated male gametes (and no feeding current!) sperm limitation was not detectable in the spermcast-mating red alga *Gracilariaria gracilis* inhabiting intertidal rock pools (Engel and others 1999).

Spermatozoa can be stored by the recipient prior to fertilization

Allosperm can be stored in the female reproductive tract of zooids of *D. listerianum* for over 7 weeks prior to fertilization (Bishop and Ryland 1991; Burighel and Martinucci 1994a; Bishop 1998). In

C. hyalina, the passage of inseminated eggs into the ovicells to commence development may occur up to several weeks after uptake of allosperm by adult colonies (Manríquez 1999). Sperm precociously penetrate immature ovarian oocytes in a range of cheilostome bryozoans (Temkin 1996), including *C. hyalina* (Ostrovsky 1998), with the male pronucleus remaining separate in the cytoplasm until syngamy occurs near the time of passage of the egg from the coelom of the maternal zooid to the site of brooding. This pattern has the effect of sperm storage, that is, a significant delay between uptake of spermatozoa and the production of zygotes. In addition, juvenile (3-zooid) colonies of *C. hyalina* lacking sexual zooids can acquire spermatozoa that are subsequently used to fertilize eggs some weeks later following colony maturation (Hughes and others 2002a); it thus appears that spermatozoa are captured by autozooids of *C. hyalina* and can be stored in the absence of female zooids, although the site of storage is at present unknown.

Water-borne spermatozoa of the hydroid *Campanularia flexuosa* can enter the gonangia of female colonies while they contain immature oocytes, and can await oocyte maturation for at least 4 days before effecting fertilization (O’Rand 1972). The spermcasting polychaete *Spirorbis spirorbis* has a spermatheca adjacent to the mouth, at the base of the feeding tentacles, in which allosperm are stored in close association with spermathecal cells (Daly and Golding 1977; Picard 1980).

In contrast, *B. schlosseri* is not known to store allosperm. Developmental failure occurs if allosperm are not available for uptake during a critical phase following the opening of the siphons by each generation of zooids (Stewart-Savage and others 2001).

There may be extensive contact between allosperm and somatic tissue of the recipient

In order to access eggs, spermatozoa of the colonial ascidians *D. listerianum* and *B. schlosseri* must reach regions downstream of the filtering apparatus (branchial basket) of recipient zooids. Spermatozoa in *D. listerianum* must then pass up the narrow (and selective) fertilization duct (anatomically the oviduct, opening distally into the colonial exhalant space), be stored in the ovarian lumen, and must pass through the ovarian epithelium to reach the oocyte (Burighel and Martinucci 1994a, 1994b; Bishop 1996; Bishop and Sommerfeldt 1996); the spermatozoa undergo marked morphological changes in the female tract (Burighel and Martinucci 1994a). Spermatozoa of *B. schlosseri* must reach the ovulated egg in the water-filled atrial cavity of the recipient zooid and must interact

with the egg envelope (chorion and diploid inner follicle cells) surrounding the ovum (Mukai 1977). In *C. hyalina*, spermatozoa must enter the zooidal coelom, penetrate the ductless ovary, and pass into the cytoplasm of an immature oocyte (Temkin 1996; Ostrovsky 1998).

Internal fertilization in sponges involves the phagocytosis of the spermatozoon by a choanocyte of the recipient; the choanocyte then becomes modified into an amoeboid carrier cell that transfers the sperm head, as a spermicyst housed in an intracellular vacuole, to an oocyte (Gatenby 1920; Fell 1989; Maldonado and Bergquist 2002). Spermatozoa of the hydroid *C. flexuosa* adhere to the gonangial epithelium and move along it as they migrate from the entrance of the gonangium to the eggs (O’Rand and Miller 1974); interaction with the gonangial epithelium induces a capacitation-like change in the sperm (O’Rand 1972, 1974). In the polychaete *S. spirorbis*, the head of a stored spermatozoon becomes embedded in a cell of the spermatheca (storage organ), with extensive areas of specialized contact between the respective membranes of the 2 cells (Daly and Golding 1977; Picard 1980). An extreme case of interaction with maternal tissue during spermcast mating, albeit in a pelagic rather than a sessile tunicate, is provided by the salp *Pegea socia*; before reaching the egg, spermatozoa must pass through the atrial epithelium, then the solid cells that constitute the oviduct, and finally the follicular epithelium (Holland and Miller 1994).

This extensive contact during spermcast mating may be presumed to afford the opportunity for the acting female to impose safeguards against hybrid fertilization and polyspermy that, along with sperm limitation, are threats to the success of external fertilization following broadcast spawning (Franke and others 2002; Levitan 2002; Marshall 2002; Levitan and others 2004).

Compatibility systems are often involved

Selfing is substantially or completely prevented by incompatibility mechanisms in at least some populations of *Botryllus* spp. (Oka 1970; Scofield and others 1982; but see Sabbadin 1971), in UK populations of *D. listerianum* (Ryland and Bishop 1990; Bishop 1996) and in European populations of *C. hyalina* (Hoare and others 1999; Hughes and others 2002b). In *B. schlosseri* and *C. hyalina*, inbred crosses also experience partial fertilization success, reduced fecundity or reduced fitness (Oka 1970; Sabbadin 1971; Scofield and others 1982; Hoare and Hughes 2001). *D. listerianum* shows pronounced incompatibility between particular pairs of potential mates, commonly so in 2 out of 3 geographical populations investigated; this incompatibility

may be asymmetrical between members of a pair (Bishop and others 1996; Pemberton and others 2004; A. J. Pemberton and J. D. D. Bishop unpublished data). Contrary to the initial report (Bishop and others 1996), in a larger data set incompatibility between pairs of genotypes of *D. listerianum* drawn from a wild population did not correlate with relatedness estimated by DNA band-sharing following RAPD-PCR amplification (Pemberton and others 2004).

Thus, spermcast mating frequently involves self-incompatibility, which may extend to various levels of inter-individual incompatibility in cross pairings. In such cases paternity will not directly reflect the numerical representation of spermatozoa received from different sources.

Receipt of compatible allosperm may trigger female investment

Vitellogenic egg growth leading to ovulation is triggered by receipt of compatible allosperm in *D. listerianum* and *C. hyalina* (Bishop and others 2000). Furthermore, unrelated or half-sib spermatozoa trigger the budding of additional female zooids in *C. hyalina* (Hughes and others 2002a). Histological observations suggestive of the triggering of egg growth by allosperm in sponges have been reported (reviewed by Bishop and others 2000).

However, reliance on allosperm to trigger investment is clearly not universal in spermcast-mating species. Each generation of zooids in a *B. schlosseri* colony produces eggs before uptake of spermatozoa can occur. In the absence of allosperm, there is wastage of unfertilized eggs (Yund and McCartney 1994; Stewart-Savage and others 2001) or delayed selfing presumed to reflect the decay of a gametic incompatibility mechanism (Scofield and others 1982). When the unitary, hermaphroditic, spermcast polychaetes *Spirorbis spirorbis* and *Janua pagenstecheri* are reared from the early juvenile phase in reproductive isolation, broods of eggs are nevertheless produced (Neal 2004); the eggs fail to develop, indicating the absence of emergency selfing.

Variants of the pattern of spermcast mating

Spawning of zygotes

Some species are known to spawn the zygotes resulting from uptake of sperm and internal fertilization, rather than brooding the resulting embryonic stages; we would include such a pattern within spermcast mating. Examples include the gymnolaemate bryozoans *M. membranacea* (Temkin 1994), *Electra pilosa*, and *Alcyonidium* sp. (Temkin 1996), and many or most

“oviparous” sponges (Maldonado and Bergquist 2002). It is perhaps puzzling that, based on current information, this is not a commoner pattern. It may avoid the potential constraints on fecundity imposed by brooding while allowing the gathering of spermatozoa from dilute suspension, potentially into storage, and providing more female control over the process of fertilization than is possible with broadcast spawning.

Brooding necessitates fertilization at or in the female; in cross-fertilizing sessile animals this is likely to involve the indirect transmission of sperm, and thus spermcast mating (an exception being the pseudocopulation of thoracican barnacles). It is not known whether evolutionary pressure to brood has been responsible for the origin of sperm uptake, or if spermcast mating arose for different reasons before the adoption for brooding; if the latter, examples in which brooding does not take place may offer particular insights into the evolution of sperm uptake. Such investigations seem amenable to phylogenetic analysis.

Polyembryony

As far as is known, after spermcast mating all bryozoans of the order Cyclostomata (with the exception of the family Cinctiporidae: Boardman and others 1992) produce multiple clonal embryos from each zygote, resulting in the release of genetically identical larvae from enlarged brood chambers. This pattern is discussed by Hughes and others (2005). To some extent it parallels the cloning of pelagic larvae by a variety of mechanisms, known in broadcast-spawning echinoderms belonging to various classes (Bosch and others 1989; Balsler 1998; Eaves and Palmer 2003). However, larval cloning is a postembryonic process potentially undergone by numerous genetic individuals from a clutch of eggs, each arising from an independent fertilization, and therefore does not incur the restriction of genetic diversity amongst the primary sexual progeny involved in early embryonic cloning as seen in the Cyclostomata.

Dispersal of spermatophores

Amongst sessile or tubicolous invertebrates, mating by the dispersal of water-borne spermatophores (encapsulated packages of numerous spermatozoa) occurs in phoronids (Zimmer 1967), vermetid gastropods (Hadfield and Hopper 1980), and some polychaete annelids (pogonophorans: Webb 1963; Spionidae: Rice 1978; Onuphidae: Hsieh and Simon 1990). All else being equal, the availability of fewer “parcels” of gametes in the environment, compared with the commoner dispersal of individual spermatozoa, would be expected to increase the variance in reproductive success of both sexes in sessile species, including

enhanced risk of the complete failure of an individual to transmit or collect sperm. For female function, the storage of excess spermatozoa from spermatophores would be expected to counteract unpredictable sperm supply. Storage of allosperm in “spermatophore sting” species has been documented in vermetid gastropods (Hadfield and Hopper 1980) and in spionid and onuphid polychaetes (Rice 1978, 1987; Hsieh and Simon 1990), as well as in the motile bivalve mollusc *Mysella tumida* (Ó Foighil 1985). In the onuphid and the bivalve, reported levels of fertilization success are very high ($\geq 99\%$: Ó Foighil 1985; Hsieh and Simon 1990).

Spermatophore size and shape vary considerably both within and between species (for example, Zimmer 1967; Hadfield and Hopper 1980), but we are unaware of data describing differences in numbers of spermatozoa. Although data are few, sperm longevity has the potential to be high when spermatozoa are encapsulated, and thus enjoy a relatively sheltered environment and are retained at high density. Sperm heteromorphism within spermatophores is well developed in vermetids (Hadfield and Hopper 1980) and also in the motile bivalve mollusc *Mysella bidentata* (Ockelmann and Muus 1978; Jespersen and Lutzen 2001). The presence of apyrene spermatozoa might contribute to the longevity of eupyrene (fertilizing) spermatozoa. Hadfield and Hopper (1980) report viability of spermatozoa in released vermetid spermatophores lasting several hours to a few days. Such longevity does not clearly exceed that of freely dispersing spermatozoa of spermcasting species (see above), but would nevertheless be expected to have important effects on sperm-mediated gene flow.

The arrival of numerous spermatozoa packaged in a spermatophore seems to create scope for seminal materials or bulk characteristics of sperm to influence the pattern of mating. The situation thus becomes similar to transfer of an ejaculate during copulatory mating, with the important distinction that “precopulatory” screening of mates is absent. There is every reason to expect posttransfer (“postcopulatory”) sexual selection in such a mating system. The ejaculatory tube fluid and its inclusions in the spermatophores of vermetids, detailed by Hadfield and Hopper (1980), perhaps exemplifies the potential for delivery of accessory paternal fluids.

The motile relatives of vermetid gastropods within the superfamily Cerithioidea have aphyallic males and mate by the transfer of spermatophores (Hadfield and Hopper 1980; Glaubrecht and Strong 2004), so the production of spermatophores probably predates the adoption of a sessile existence in the evolutionary

history of vermetids, and may perhaps have aided this transition by providing a ready-made form of remote mating. The interception of spermatophores avoids the necessity to collect individual spermatozoa while feeding with an external mucous net, which would perhaps be problematic.

Some bryozoans, such as *M. membranacea* (Temkin 1994) and *E. pilosa* (Franzén 1956; Temkin 1996), release unencapsulated bundles (spermatozeugmata) of 32 or 64 spermatozoa. Temkin (1994) documented mating by the release and uptake of spermatozeugmata in *M. membranacea*, a process arguably intermediate between the dispersal of free spermatozoa and of spermatophores. Sperm heteromorphism exists within spermatozeugmata of the bivalve *Pseudopythina macrophthalmensis* (Jespersen and others 2001). In contrast to the situation for spermatophores (above) and spermatozeugmata, sperm heteromorphism is, to our knowledge, unknown in species dispersing individual spermatozoa, in agreement with expectations on theoretical grounds.

Compared with the collection of single spermatozoa in regular spermcast mating, the uptake of a spermatophore (or spermatozeugma) increases the probability of a high level of full sibship within progeny arrays (Strathmann 1990), equivalent to correlated mating in flowering plants (for example, Trapnell and Hamrick 2005). The transfer of packets (pollinia) of numerous pollen grains by members of the flowering plant families Asclepiadaceae (including the milkweeds) and Orchidaceae (orchids) suggests parallels to mating with spermatophores, albeit with the involvement of an insect pollinator (Strathmann 1990).

Final comments

Spermcast mating differs from other, more widely recognized, modes of animal mating. Hermaphroditic mating by copulation or through other forms of direct contact between individuals (reviewed by Michiels 1998) may involve elaborate mutual assessment of the potential mates, sometimes leading to assortative mating (Vreys and Michiels 1997); conflict over favored mating roles may be resolved by contest or by reciprocal gamete trading (Fischer 1984; Michiels 1998; Michiels and Newman 1998; Sella and Lorenzi 2000). Insemination is typically reciprocal in a broad range of species that pair up for mating (reviewed by Michiels 1998). In contrast, spermcast mating by sessile hermaphrodites cannot involve prior screening of potential mates, and direct contest or conditional reciprocity is not feasible (and incidental reciprocity may be unpredictable) since ambient water movements mediate gamete transfer.

During spermcast mating, the collection of spermatozoa from dilute suspension and their subsequent storage appears to lead inevitably to receipt of sperm from a variety of sources (and thus to sperm competition, and potentially cryptic female choice, if sperm supply exceeds the level necessary to ensure complete fertilization). The seeming inevitability of polyandry, referred to by Bishop and Pemberton (1997) as obligatory female promiscuity, and the absence of an ejaculate delivering seminal materials additionally distinguish spermcast mating from the established model species for mating in gonochorists and hermaphrodites. Rather than other animal processes, spermcast mating appears most comparable to wind pollination in land plants, since male genetic material is passively dispersed between sessile mates through an external fluid medium.

It is notable that the patterns of mating are more similar between the compound ascidian *D. listerianum* and the bryozoan *C. hyalina* than between the 2 ascidians *D. listerianum* and *B. schlosseri*. The former pair shares true internal fertilization and the storage of allosperm, which triggers female investment; these are presumably convergent adaptations.

The triggering of egg growth (and, in *C. hyalina*, of the budding of additional female zooids) appears to offer scope for sexual conflict between the male and female roles over the level of female investment elicited by allosperm during spermcast mating. In terms of lifetime reproductive success, optimal levels of maternal investment are likely to be higher for the source of successfully transmitted spermatozoa than for the recipient, particularly given the requirement in the recipient, if a modular hermaphrodite, to partition resources between female function, male function, and somatic growth (with addition of more modules potentially increasing future fecundity). In the case of broadcast spawning an entirely different form of sexual conflict has been postulated (Franke and others 2002; Levitan 2004), concerning male selection on spermatozoa for rapid, efficient fertilization in the face of competition between males, and female selection at high sperm density to reduce egg fertilizability and thus avoid wastage through polyspermy.

It is perhaps possible to envisage a continuum between spermcast mating and broadcast spawning, taking in the fertilization by dispersing spermatozoa of individual eggs retained externally by the acting female or of an externally spawned egg mass retained close to a sessile female in a viscous matrix (for example, Marshall 2002). However, the fertilization of an external egg mass must evidently involve the dilemma, inherent in broadcast spawning but (arguably) largely avoided in spermcast mating, between sperm

limitation and polyspermy (Franke and others 2002; Marshall 2002), with the attendant risk of hybrid fertilization if closely related species co-spawn (Levitan 2002; Levitan and others 2004). The utilization of very dilute sperm appears unlikely (but see Meidel and Yund 2001 for an example of gradual fertilization of external egg masses, potentially involving relatively dilute sperm), and it is not feasible that allosperm are stored and trigger egg provisioning as is the case in some spermcast systems.

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