Review

Cite this article: Evans JP, Lymbery RA. 2020

Sexual selection after gamete release
in broadcast spawning invertebrates. *Phil.
Trans. R. Soc. B* **375**: 20200069.
<http://dx.doi.org/10.1098/rstb.2020.0069>

Accepted: 9 June 2020

One contribution of 22 to a theme issue 'Fifty
years of sperm competition'.

Subject Areas:

evolution

Keywords:

polyandry, cryptic female choice, sexual
cascade, broadcast spawner, marine,
ejaculate

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Sexual selection after gamete release in broadcast spawning invertebrates

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Broadcast spawning invertebrates offer highly tractable models for evaluating sperm competition, gamete-level mate choice and sexual conflict. By displaying the ancestral mating strategy of external fertilization, where sexual selection is constrained to act after gamete release, broadcast spawners also offer potential evolutionary insights into the cascade of events that led to sexual reproduction in more 'derived' groups (including humans). Moreover, the dynamic reproductive conditions faced by these animals mean that the strength and direction of sexual selection on both males and females can vary considerably. These attributes make broadcast spawning invertebrate systems uniquely suited to testing, extending, and sometimes challenging classic and contemporary ideas in sperm competition, many of which were first captured in Parker's seminal papers on the topic. Here, we provide a synthesis outlining progress in these fields, and highlight the burgeoning potential for broadcast spawners to provide both evolutionary and mechanistic understanding into gamete-level sexual selection more broadly across the animal kingdom.

This article is part of the theme issue 'Fifty years of sperm competition'.

1. Introduction

In the lowest classes the two sexes are not rarely united in the same individual, and therefore secondary sexual characters cannot be developed. In many cases in which the two sexes are separate, both are permanently attached to some support, and the one cannot search or struggle for the other. Moreover, it is almost certain that these animals have too imperfect senses and much too low mental powers to feel mutual rivalry, or to appreciate each other's beauty or other attractions

Charles Darwin (1871) *The Descent of Man*, ch. 4, [1, p. 321]

Although sperm competition was recognized as early as the fourth century by Aristotle (e.g. [2]), and indeed by his predecessors [3], it was not until 1970 that Geoff Parker [4] formally introduced the concept to evolutionary biologists. Parker [4, p. 527] originally defined sperm competition as the 'competition within a single female between the sperm from two or more males for the fertilization of the ova', although subsequent definitions were revised to accommodate mating systems where fertilization occurred outside the female's body [5]. Accordingly, sperm competition was envisaged as an extension of male-male competition, but in this case via contests that play out among ejaculates from different males after mating has occurred or after gametes have been released into the external environment.

The field of sperm competition has come a long way since Parker's [4] seminal and groundbreaking contributions. For example, Thornhill [6] and Eberhard [7] are justifiably credited as the pioneers of the field of 'cryptic female choice', defined as any female-mediated process that selectively biases fertilization toward a subset of competing ejaculates. However, it is important to acknowledge that Parker [4, p. 559] himself recognized the potential for post-copulatory female choice when concluding that the 'female cannot be regarded as an inert environment in and around which this form of adaptation evolves'.

Sperm competition and cryptic female choice, collectively termed 'post-copulatory sexual selection' [8], are studied across virtually every taxonomic group of animals and plants. Indeed, there are few sexually reproducing species

where polyandry (i.e. females mating with two or more males within a single reproductive episode) does not occur [9], and thus post-copulatory sexual selection is ubiquitous in nature [10]. However, the term *post-copulatory* sexual selection implies that these processes are confined to animals that ‘copulate’—that is, for the most part, internally fertilizing (mostly terrestrial) organisms. The term therefore excludes taxonomic groups with external fertilization, including numerous plants, fungi, amphibians, fishes and sessile or largely immobile aquatic invertebrates [11]. Regrettably, for much of its history, the field of post-copulatory sexual selection has predominantly adhered to this taxonomic bias. Yet, many of the groups that were largely discarded by Darwin [1] as the ‘lowest classes’ (see opening quote) exhibit the ancestral reproductive mode of broadcast spawning—the release of gametes from both sexes into the external environment for fertilization—which predisposes them to extreme levels of gamete competition [12]. Moreover, as Parker [13] notes, this ancestral state makes them highly relevant models for understanding evolutionary transitions towards sexual selection in derived taxa. Fortunately, thanks in large part to two landmark reviews by Levitan [14,15], research on gamete-level interactions in these historically neglected groups has now found its way into the mainstream literature on sexual selection [10,16]. The aim of the present review is to summarize current empirical and theoretical progress in this field, some of which extends the concepts outlined in previous reviews [14,15], and some that has revealed novel research directions. In doing so, we highlight how studies on broadcast spawners may be instrumental in improving our understanding of gamete-level sexual selection more broadly in nature.

By quoting Darwin’s [1] musings on the ‘lowest classes’ of animal at the start of this article we were being deliberately provocative. Our aim in this review is to explain why we think Darwin may have missed an opportunity by ignoring such organisms. Our review comprises four sections that together summarize what we have learned or can learn about sexual selection in these groups. Our focus is on marine invertebrates, but much of our discussion is relevant for other gonochoristic (separate sex) species exhibiting limited sexual dimorphism, sessile or largely sedentary lifestyles and external fertilization. First, we highlight their suitability for addressing, and sometimes challenging, conventional views of sexual selection and the unique insights we can gain from these groups in understanding evolutionary transitions in sexual reproduction. Second, we draw on recent studies that highlight the potential for mate choice at the level of gametes in these systems. Third, we explore how the continuum from sperm limitation to sperm abundance can have profound evolutionary consequences for patterns of sexual selection in these taxa. Fourth, we highlight recent studies that exploit many of these attributes to explore how sexual selection targets gamete traits. We conclude by considering a number of areas for future research.

2. Why study broadcast spawners?

Before highlighting the suitability of broadcast spawners as models for sexual selection, we must first refine our terminology for describing post-mating mechanisms of sexual selection in these systems. Two terms, in particular, are relevant in this context. First, throughout this article we will refer either to ‘sexual selection after gamete release’ or ‘gamete-level sexual

selection’ in place of the more common, but taxonomically restrictive, term ‘post-copulatory sexual selection’; external fertilizers do not *copulate*. Second, when referring to fertilization biases attributable to either sex (i.e. what would normally be referred to as either ‘cryptic female choice’ or ‘cryptic male choice’), we adopt Aguirre *et al.*’s [17] ‘gamete-level mate choice’, as this is relevant for organisms where mate choice can be constrained to act exclusively via gametes or their associated reproductive fluids or structures [16].

(a) Broadcast spawners are models for testing and extending sexual selection theory

The search for a unifying concept of sexual selection that closes the divide between disparate mating systems has been a long-standing problem for evolutionary biologists. Arnold [18] originally outlined the dilemma when seeking a definition of sexual selection that applies to both plants and animals, in essence trying to bridge the gap between botanical and zoological definitions of mate choice and mating competition (see also [19]). We suggest that such a divide exists *within* animal taxa when we compare (mostly terrestrial) animals with internal fertilization and (mostly aquatic) broadcast spawning organisms (which are arguably more similar to flowering plants than to copulating animals [11]). As we highlight here, theoretical models incorporating the distinctive dynamics of externally fertilizing systems have made considerable progress in bridging this divide (e.g. see review in [20]).

To briefly recap classic sexual selection theory, Bateman’s [21] famous experiments on *Drosophila melanogaster* revealed that the relationship between reproductive success and the number of mates differed between the sexes. Bateman concluded that this was owing to the fact that female fertility is limited by egg production, while males are seldom limited by sperm production and instead are limited by the availability of females. These insights led to three broad principles that underlie modern sexual selection theory. First, sex differences in gamete-production costs mean that males must compete for access to mates, and therefore males will exhibit greater variance in reproductive success than females. Second, males will exhibit higher variance in the number of mates than females. Third, because of the sex differences in gamete-production costs, and thus the variances in reproductive success and the number of mates, the relationship between reproductive success and the number of mates, the so-called ‘Bateman gradient’, will be largely linear for males and asymptotic for females (figure 1a).

Bateman’s ideas underwent radical development in the light of Trivers’s [23] insights on parental investment, and researchers continue to discuss and refine these concepts (e.g. [24–26]). However, the underlying principles from Bateman’s experiments remain cornerstones for modern sexual selection theory [27]. Nevertheless, many researchers continue to argue that these general principles do not apply universally across mating systems [22,28], particularly groups such as broadcast spawners and flowering plants, where patterns of selection on gametes can depend on locally variable conditions. Briefly, the predicted relationships between the number of mates and reproductive success may take any number of forms that differ from those envisaged by Bateman (figure 1b), such that linear and nonlinear relationships are present for both sexes [22]. When these ideas are applied to broadcast spawners, a number of extrinsic environmental factors known to influence gamete concentrations (for a

Figure 1. Bateman gradients; average reproductive success as a function of the number of mates. (a) ‘Traditional’ gradients from Bateman’s [21] experiments on *Drosophila*, showing (i) a linear relationship in males, and (ii) an asymptotic relationship in females. (b) In broadcast spawners (and other non-model organisms such as flowering plants), variations on traditional Bateman gradients can occur in both males and females, including (iii) diminishing returns and (iv) intermediate optimum functions. For example, when populations experience sperm limitation with low numbers of mates (i.e. gamete encounter rates) and both polyspermy and sperm competition with high numbers of mates, Bateman gradients may be linear (or diminishing returns) for males and intermediate optimum for females. Redrawn from Arnold [22].

theoretical perspective see [29]) can cause fundamental differences in Bateman gradients and ultimately alter the direction of sexual selection on gametes (i.e. from sperm competition to egg competition; see [28,30,31]).

In §4, we consider some of the evolutionary dynamics that may arise depending on where populations sit on the continuum between sperm limitation, where the availability of sperm limits female fertility and we might expect egg competition to occur, and sperm abundance, where sperm are saturating and sperm competition prevails. As we note there, both conditions can have profound evolutionary consequences, including selection for reproductive barriers driven via sexual conflict (e.g. [32–34]).

(b) Broadcast spawners are models for exploring evolutionary transitions in sexual selection: the sexual selection ‘cascade’

As we saw in §1, Parker [4] originally conceived sperm competition as a contest occurring within the female reproductive tract of internal fertilizers, and this has long been the dominant focus of research on post-copulatory sexual selection (e.g.

[9,35]). However, Parker [4] himself noted that gamete competition—and therefore sexual selection—almost certainly originated in external fertilizers, and that this was the catalyst for the series of evolutionary transitions that would eventually lead to internal fertilization. He later developed these ideas in more detail [13], arguing that the evolution of sexual reproduction in animals followed a logical series of steps from these beginnings in external fertilizers, events that he termed the ‘sexual cascade’ (see also [36]). There, Parker [13, p. 3] states that broadcast spawners ‘played a major role ... in the cascade of events in the evolution of sexual strategy that culminated in precopulatory sexual selection that Darwin so brilliantly perceived and described in 1871’.

External fertilizers were likely to be key players in two major transitions of the sexual cascade. First, the cascade began with an ancestral unicellular eukaryote, with undifferentiated gametes (i.e. isogamy) that were released externally. Theories for the transition to anisogamy, where gametes are differentiated by size into large eggs and small sperm, emphasize the critical selective forces of gamete limitation (e.g. [37]) and/or gamete competition (e.g. [38]). In both cases, it has been proposed that the production of fewer, large gametes (eggs) will be selectively favoured by increasing zygote survival (and possibly by providing larger targets for fertilization; [39]). Producing many smaller gametes (sperm) is beneficial either by increasing the probability of collision under gamete limitation, or by providing a competitive advantage over rivals under gamete competition [39]. Further discussion of this early transition in ancestral unicells is beyond the scope of this paper, and we point readers towards several theoretical papers and reviews on the topic (e.g. [39–42]).

Second, following the evolution of multicellularity and anisogamy (the former having arisen only once in metazoans [43]), the ancestral animal was probably a sessile or weakly mobile broadcast spawner. As we discuss in §4, extant marine invertebrates with these features can face sperm limitation, if spawning individuals are isolated, or sperm competition, if spawning in large aggregations [14]. Parker [13] suggests a likely series of events in the ancestral broadcast spawner, with sperm limitation and weak mobility favouring aggregation and synchronous spawning to increase the fertilization probability for both sexes. These spawning aggregations resulted in conditions of intense sperm competition and massive gamete expenditure, which are common features of extant broadcast spawners [12]. Sperm competition then favoured the targeting of females by males to gain a competitive advantage, i.e. the origin of pre-spawning sexual selection, ultimately leading to copulation, internal fertilization and familiar forms of mate choice and mating competition [13].

The key position of broadcast spawners early in the sexual cascade makes them uniquely suited for improving our understanding of sexual selection more broadly among animal systems. For example, we can gain novel insights into the factors that propelled major evolutionary transitions in sexual reproduction from empirical studies of extant species, comparing parameters such as spawning behaviour, mobility, gamete expenditure and the degree of sperm limitation and competition (e.g. [12]; see also §4). Furthermore, cellular-level communication systems among sperm and eggs were almost certainly the first mechanisms of reproductive competition and mate choice in ancestral broadcast spawning animals, and it has been argued that these mechanisms are most likely retained in some form in derived taxa [44]. Nevertheless, the

specific signalling molecules involved can be diverse [45] and, as we discuss in the next section, can most easily be observed in broadcast spawners.

3. Gamete-level mate choice

In a recent review, Kekäläinen & Evans [44] drew on three basic ideas to argue that gamete-level mate choice is likely to be of special importance in broadcast spawning taxa. First, as we note above (§2b), cellular-level processes of mate choice most likely arose soon after the evolution of syngamy (i.e. fusion of two separate gametes) and thus are probably present in extant groups displaying the ancestral reproductive strategy of broadcast spawning. Second, the mechanisms underlying sperm–egg interactions, which are well developed in many broadcast spawning taxa [44], provide scope for highly discriminating, even spermatozoa-specific, gamete selection (e.g. [46]). Third, gamete-level mate choice provides the only available mechanism of sexual selection in species where precopulatory sexual selection is not possible—i.e. groups that are sessile or weakly mobile and lack any obvious sexual dimorphism [16]. In this section, we briefly summarize recent progress in identifying putative processes of gamete-level mate choice in broadcast spawning taxa.

(a) Gamete-level mate choice for compatible gametes

Previous evidence for gamete-level mate choice in sea urchins [47,48] and ascidians [17] suggests that when egg clutches are given simultaneous access to ejaculates from multiple males (i.e. sperm ‘choice’ assays), they exhibit higher fertilization rates than the average success achieved when fertilizations are performed separately with individual ejaculates from the same males (i.e. ‘no-choice’ fertilization assays). Studies of the sea urchin *Heliocidaris erythrogramma* [47] and the ascidian *Ciona robusta* [17] suggest that the fertilization benefits arising from the sperm choice assays were driven by the disproportionately high success of the competing male whose sperm were most compatible with the focal female’s eggs. Indeed, in *H. erythrogramma* [47], the magnitude of the fertilization benefit under polyandry was strongly positively correlated with the differences in fertilization rates between the least and most ‘successful’ male–female pairing when gamete-level mate choice was experimentally prevented in no-choice assays (see also [48]). This latter finding suggests that under multiple-male spawning, fertilizations are biased in favour of sperm from the most compatible male (i.e. sexual selection rather than a ‘bet-hedging’ strategy, where fertilizations would be more equally shared by competing ejaculates [49,50]). Indeed, as predicted, the average fertilization rates of the male that did best under monandry were not significantly different from those achieved when ejaculates from both males competed for fertilization ([47], but see [48]). This result, along with the significant interacting effects of males and females on fertilization rates in *H. erythrogramma* and *C. robusta* [17,47], strongly implicated gametic incompatibilities as a selective force promoting gamete-level mate choice.

(b) Putative mechanisms of gamete-level mate choice

Given the importance of gamete compatibility more broadly in externally fertilizing animals [51], selection should favour the evolution of adaptations that increase the likelihood of fusion

between genetically compatible gametes. Accordingly, a series of recent studies on broadcast spawning marine invertebrates indicate that such mechanisms may be triggered through chemical and/or cellular interactions between eggs and sperm. For example, recent findings for the sea urchin *Lytechinus pictus* reveal that females exhibit significant individual variation in the production of egg chemoattractants [52], which act as chemical ‘signposts’ that direct sperm from conspecific males towards their source (i.e. the egg). Such intraspecific variation in egg chemoattractant production should provide scope for gamete-level mate choice in these taxa [16,44]. Indeed, a recent series of studies on the mussel *Mytilus galloprovincialis* confirms such a function; egg chemoattractants from particular egg clutches preferentially attract sperm from specific males [53], and these ‘preferences’ by sperm correspond with variation in sperm swimming behaviour and offspring survival [54]. Moreover, recent work employing mitochondrial sperm dyes reveals that such interactive sperm–egg chemoattractant effects are predictive of competitive fertilization success; sperm arising from males that are more compatible with egg chemoattractant donors have an advantage during competitive sperm chemotaxis assays over those from less compatible males [55].

Sexual selection can also target gamete reproductive proteins (GRPs) that operate downstream of chemical interactions between eggs and sperm (reviewed in [16]). Among the best-described GRP systems, those in echinoderms (sea urchins, sea stars, etc.) involve the sperm-binding surface protein Bindin and its corresponding egg binding receptor protein-1. Interestingly, in sea urchins GRPs can exhibit substantial variability among individuals of the same species, and this variation can influence fertilization rates under competitive and non-competitive fertilization scenarios [46,56–58]. The potential, therefore, exists for sexual selection to target GRP variants [16]. Several hypotheses have been proposed to explain how intraspecific variability in GRPs can be maintained, including the possibility that sexually antagonistic selection on reproductive proteins may favour rare GRP variants under certain conditions (e.g. [59–61]). We discuss these dynamics in detail in §4; briefly, under conditions of sperm limitation, high-affinity GRP variants will be favoured in both sexes. At high sperm concentrations, the risks of polyspermy (fertilization by more than one sperm leading to developmental failure; see below) and sperm competition can lead to evolutionary conflicts between the sexes [61,62]. If high sperm concentrations and polyspermy occur during non-competitive spawning conditions, there would be no conflict and alleles with lower affinities would be favoured in both sexes [61]. However, under the scenario of sperm overabundance during multi-male (i.e. competitive) spawning, there is likely to be an inherent conflict over the evolution of GRPs, whereby mutations that favour rare (low affinity) GRP genotypes in females (thereby reducing the likelihood of reproductive failure attributable to polyspermy) will be selectively excluded in males (where high sperm concentrations arising from multiple males select for high-affinity phenotypes that maximize competitive fertilization success) (see [61,62]).

4. Gamete competition and limitation in broadcast spawners

As we highlight above (§2a), patterns of sexual selection in broadcast spawners can differ radically from the

Darwin–Bateman paradigm of higher variance in reproductive success among males than females. In these species, local sperm densities (i.e. sperm concentrations within the ‘capture’ areas of ova; e.g. [62]) can vary considerably, even within the same spawning event, depending on several inter-related factors, such as density of spawning individuals, degree of clumping or dispersal of released gametes, and abiotic conditions such as current speeds [14]. Conditions of both sperm limitation and high sperm abundance can lead to substantial variance in reproductive success in both sexes. Under sperm limitation, fertilization rates for egg clutches can be much lower than 100% (in contrast to traditional assumptions for Bateman gradients), and both males and females are selected to increase fertilization rates. At high local sperm densities, where sperm outnumber eggs, there is the risk of polyspermy, and consequently embryonic failure [63], a situation that is more likely in external fertilizers compared to internal fertilizers as sperm cannot be ejected or destroyed by the female before reaching the egg (e.g. [64]). Under such conditions, females are likely to face selection to reduce fertilization rates. By contrast, however, high local sperm abundances are typically coincident with sperm competition among ejaculates from multiple males. High sperm abundance, in turn, is expected to lead to selection for males to increase fertilization rates and out-compete rivals, thus leading to sexual conflict over optimal fertilization rates (e.g. [31]).

Broadcast spawning species are typically characterized by massive gonad expenditure (gonado-somatic index or GSI; the proportion of adult body mass devoted to gonads) in both sexes [13]. Notably, male GSI is unusually high in broadcast spawners, typically equalling female GSI [12]. There has been considerable theoretical interest in understanding the relative importance of sperm limitation and sperm competition in driving selection on sperm investment, with both forces expected to increase expenditure on sperm [40]. However, recent theoretical findings suggest that sperm competition is often likely to dominate in this context [12,65]. Parker *et al.* [12] extended traditional game-theoretic models to incorporate sperm competition, sperm limitation and polyspermy, and found that the typical patterns of gamete expenditure in broadcast spawners (approximately equal GSI in males and females) were predicted when sperm competition was intense. Only a small proportion of broadcast spawning species show the pattern typical of most other taxa, which is lower male than female GSI; Parker *et al.*'s [12] models suggest these species are likely to experience either low sperm competition or polyspermy [12,34].

Beyond investment in gamete numbers, the continuum between sperm limitation and sperm overabundance can also alter individual gamete traits. In §5, we review the accumulating evidence from broadcast spawners that patterns of selection on ejaculates can vary with changes in sperm concentration. Interestingly, there is also evidence that differences in spawning densities can generate plasticity in both sperm and egg traits. For example, Crean & Marshall [66] showed that in the ascidian *Styela plicata*, males maintained at high densities produced larger, longer-lived and more motile sperm than those maintained at low densities. The authors concluded that these changes were adaptive by reducing the risk of polyspermy, based on the finding that sperm from high-density males had higher success in non-competitive fertilization trials when sperm concentrations were high. However, an

alternative but untested possibility is that these changes to ejaculates improve *competitive* fertilization success in the presence of rivals. Egg traits also exhibited plasticity under different spawning densities; females from low-density treatments produced larger eggs, while those maintained at high densities reduced their egg size [66]. Such plasticity in egg size may allow for an increase in sperm–egg collision rates under sperm limitation, while serving to reduce the probability of polyspermy at high sperm concentrations.

The substantial variance in female reproductive success in broadcast spawners raises the possibility that under some conditions sexual selection may act on females through egg competition, where eggs from different females compete for fertilization. As noted by Levitan [14], this is most likely in situations where sperm are dilute and eggs tend to remain clumped near spawning females, meaning that individual clutches can deplete the sperm available to others. In the only empirical test so far, to our knowledge, of egg competition in broadcast spawners, Marshall & Evans [30] simulated fertilizations under realistic current regimes in the tubeworm *Galeolaria caespitosa*, and found that the presence of rival egg clutches upstream significantly reduced the fertilization success of downstream clutches. Moreover, this effect was owing to both an overall depletion of sperm numbers, and by preferential use of more motile sperm by the competitor clutch [30]. Although studies of egg competition in any species are extremely rare [67], it is likely that the phenomenon is widespread among broadcast spawning taxa.

The occurrence of egg competition in broadcast spawning species raises the fascinating prospect that selection might act on female traits that increase competitive success, such as strategic egg release, egg size or through the release of sperm attractants (see §3b). Intriguingly, there might be a balance between promoting competitive success over rival clutches, and avoiding wasteful competition between sister eggs in the same clutch (termed ‘local egg competition’ in [32]). For example, Okamoto [68] found that at low, constant sperm densities, high local egg concentrations caused nonlinear reductions in within-clutch fertilization rates in the sea urchin *Strongylocentrotus purpuratus*, consistent with competitive sperm chemoattraction by individual eggs. Similarly, Marshall & Bolton [69] found in *G. caespitosa* that attachment of multiple sperm to individual eggs, prior to the establishment of permanent sperm blocks, can decrease fertilization rates of other eggs within a clutch. However, neither of these studies involved competition among eggs from *different* females; indeed, we are not aware of any studies that have tested how specific female/egg traits influence competitive success among different egg clutches.

In addition to selection on individual sperm and egg traits, the dynamics of sperm limitation and sperm abundance can influence gamete compatibility through gamete recognition loci, as discussed in §3b. A considerable body of theoretical work indicates that (i) sperm limitation will favour well-matched sperm ligand and egg receptor alleles, and (ii) conditions of polyspermy and sperm competition will generate sexual conflict over fertilization rates, favouring mutations that decrease affinity in females and those that increase affinity in males [59–61,70]. This can lead to maintenance of GRP polymorphism within populations, promote reproductive divergence between allopatric populations, and in theory could also generate sympatric speciation [70,71]. Levitan [62] has recently highlighted the potential for spatial and temporal variation in selection on GRPs within the individual spawning events of a population.

He noted that for sperm competition to play a role in GRP evolution, multiple sperm must arrive at an egg in the interval between initial sperm contact and fertilization. In spawning experiments with a natural population of the sea urchin *Strongylocentrotus franciscanus*, he found evidence for both competitive and non-competitive fertilization events at this scale, depending on the distribution, abundance, synchrony and sex ratio of spawning individuals. This suggests that even within a single population, there can be variation in whether low- or high-affinity GRP genotypes are favoured in males and females.

5. Selection on ejaculates

Since Parker's [3] original review of sperm competition in the insects, there has been considerable interest in determining how selection acts on traits that improve the fertilization success of competing ejaculates. Two broad classes of trait have emerged as selective targets of sperm competition (see reviews in [72,73]). First, in many taxa, males that ejaculate more sperm have greater competitive success, and thus a common evolutionary response to increased levels of sperm competition is higher sperm production (e.g. see review in [35]). Nevertheless, these ideas have rarely been applied empirically to broadcast spawning invertebrates, where as we note above (see §§2a and 4) the theoretical predictions for ejaculate expenditure can differ radically from 'traditional' mating systems [34]. Second, selection may favour variation in the relative fertilization efficiency of competing ejaculates, often referred to as 'ejaculate quality'. Traits that determine ejaculate quality, including the phenotypic attributes of individual sperm cells (e.g. sperm size, shape or motility) and non-sperm components (e.g. seminal fluid), have received considerably less empirical attention than sperm numbers in any system [35,74].

A recent turning point in this field has been the recognition that different sperm and ejaculate traits are unlikely to evolve independently [72]. This is because many sperm and ejaculate traits are phenotypically and/or genetically correlated (e.g. [75–77]), such that the selective responses of individual traits will contain both a direct component of selection on the trait itself, and an indirect component owing to selection on correlated traits. Furthermore, combinations of sperm and ejaculate traits are likely to interact to determine fertilization efficiency of the entire ejaculate, meaning that selection itself may be correlative (i.e. acting on combinations of traits, regardless of whether the traits themselves are phenotypically correlated) [78]. These two considerations call for multivariate selection analyses, which are built on the multiple regression approach of Lande & Arnold [79]. This approach can be used to separate the indirect and direct components of selection on individual traits, and characterize patterns of multivariate directional, stabilizing, disruptive and correlational selection acting on the ejaculate as a whole.

Multivariate selection studies are logistically challenging, not least because they require large sample sizes in which 'relative fitness' and multiple relevant phenotypic traits are measured for every individual. In the context of sperm competition, relative fitness would need to come from an estimate of competitive fertilization success for each individual that is relativized across the population. Fortunately, the experimental tractability of broadcast spawners makes them highly

amenable for such studies. In these systems, it is relatively straightforward to collect spawned gametes from numerous individuals and conduct biologically relevant fertilization assays in the laboratory. Indeed, several recent studies have examined natural selection on broadcast spawner ejaculates using non-competitive fertilization trials, and these have reported complex patterns of linear and nonlinear selection on combinations of sperm traits [80–84].

In addition to the non-competitive studies mentioned above, broadcast spawners have recently been used to estimate multivariate *sexual* selection on ejaculates under conditions of sperm competition [85]. Specifically, the blue mussel, *M. galloprovincialis* was used in replicated trials, each involving multiple rival ejaculates competing for fertilization of a mixed pool of eggs. The competitive fertilization success of each focal male's sperm was then tracked individually through the use of fluorescent dyes. This method generated estimates of sperm competitive ability that were averaged across multiple rival competitors and multiple female egg donors (i.e. competitive fitness relative to the population; e.g. [86]). By incorporating sperm competition, this study revealed significant stabilizing sexual selection on combinations of sperm motility and morphology traits [85]. These patterns were broadly similar to a previous study of natural selection on ejaculates in *M. galloprovincialis* (i.e. non-competitive fertilization assays; see [80]), although the specific traits involved differed between non-competitive and competitive environments. More broadly, the consistent evidence for multivariate nonlinear selection in other broadcast spawning taxa (see also [82,83]) has implications for our understanding of ejaculate evolution. Importantly, these broadcast spawner studies suggest that evolution is unlikely to proceed along simple lines of directional selection on individual sperm traits, whether through natural or sexual selection (although formal predictions of responses to selection will also require elucidation of genetic covariation in traits [87]). Indeed, selection along axes that describe combinations of multiple traits are likely to strengthen genetic and phenotypic correlations among traits, and is consistent with the growing view of ejaculates as functionally integrated phenotypes (e.g. [35,74]).

An intriguing finding from several of the non-competitive studies mentioned above is that patterns of selection can be context-dependent [82–84]. Specifically, studies of marine tubeworms in the genus *Galeolaria* have reported dynamic patterns of linear [82] and nonlinear [83] selection on sperm morphology that depend on sperm density. However, as we highlight in this review, conditions of high sperm density are likely to coincide with the occurrence of multi-male spawning, and consequently sperm competition. It would therefore be highly relevant to extend these studies by incorporating competitive fertilization into the experimental design (e.g. [85]), thus enabling us to compare patterns of multivariate sexual selection between low- and high-density treatments.

6. Conclusion and future directions

One of the primary purposes of this review has been to showcase the extraordinary experimental and theoretical value of broadcast spawning species as models for studying gamete-level sexual selection. As Parker [13] notes, many of the proposed evolutionary steps in the sexual cascade are represented among extant broadcast spawners, ranging

from weakly mobile species characterized by sperm limitation, to those with dense spawning aggregations and gamete competition, and even some forms of female targeting. As evident from the present review, our understanding of evolutionary processes that have led to more familiar forms of sexual selection has been greatly enhanced by studies of these groups.

At a practical level, the ability to extract mature gametes from both sexes in broadcast spawners means that we can conduct controlled and biologically realistic spawning experiments in which sperm–egg interactions can be manipulated and assayed directly without the need for invasive procedures. In some cases, the availability of sperm dyes [88,89] means that we can quantify competitive fertilization success directly in real time without recourse to paternity analyses, which may otherwise conflate fertilization success with embryonic survival [90, p. 2354]. As we highlight above, such techniques have enabled us to make progress in exploring the mechanistic processes underlying sperm–egg interactions (see also [91]), and to predict patterns of phenotypic selection when sperm compete for fertilization (see §5). We anticipate that such techniques might also be used to study some of the more neglected components of gamete-level sexual selection, such as egg competition (see below).

We also highlight how the reproductive dynamics of external fertilization make broadcast spawners and others with analogous mating systems ideally placed to experimentally test, extend and sometimes challenge, traditional models of sperm competition [32]. Indeed, a key message that emerges from our review is that the interplay between theoretical and empirical research is crucial for our understanding of sexual selection in broadcast spawners. For example, theoretical work has shown that when the effects of gamete concentration are incorporated into classic game-theoretic models (e.g. [33]), the evolutionary predictions for sperm release strategies become radically different than those generated for species with ‘conventional’ (i.e. Darwinian) mating systems [34]. Explicit tests of these predictions are currently lacking, and we see enormous scope for empirical progress in this field. Along similar lines, Parker *et al.* [12] have recently highlighted how an understanding of the balance between sperm limitation and sperm competition will greatly improve our ability to interpret sex-specific patterns of reproductive investment. However, we currently lack sufficient empirical data to test the emerging predictions from these models, and the need for detailed field experiments to quantify sperm–egg interactions and fertilization rates poses a significant challenge in this area.

A key prediction arising from the theoretical literature on external fertilizers is that fertilization success in both male and female broadcast spawners will depend critically on local sperm densities [20]. As we discuss throughout this

review, empirical studies have described how density-dependent selection and sexual conflict influence individual sperm and egg traits (e.g. [66]), as well as compatibility at gamete recognition loci [57,58,62]. However, an area of study that has been almost entirely overlooked is the potential for egg competition under conditions of sperm dilution and limitation. To our knowledge, only one experimental study has tested the effects of competition among egg clutches from different females [30]. There is a compelling need for studies that address this gap by (i) testing the potential for variance in female reproductive success to generate egg competition, (ii) determining female/egg traits that influence competitive outcomes, and (iii) comparing selection on female traits from within- versus between-clutch competition. Such studies would not only advance our understanding of gamete-level sexual selection in broadcast spawning species, but would be highly relevant for the growing number of other taxa for which variable reproductive success among females has been characterized [67].

Finally, broadcast spawners have emerged as putative models for characterizing patterns of multivariate selection on ejaculates, even providing the capacity to estimate multivariate sexual selection during competitive fertilizations [85]. The logical next step in these studies is to extend competitive fertilization trials across different contexts, allowing us to determine how patterns of sexual selection vary with environmental conditions. Given the evolutionary significance of local variations in gamete density in these systems (§4), we see considerable scope for testing how factors such as local sperm density influence patterns of sexual selection on ejaculates. Furthermore, given the rapid changes in external conditions caused by anthropogenic activities, it would be interesting to explore how other (abiotic) factors alter patterns of selection. Along these lines, Chirgwin *et al.* [84] recently reported that selection on sperm morphology during non-competitive trials in *G. caespitosa* was altered by ocean warming. Extending such trials to incorporate competitive fertilizations under a range of environmental changes, such as warming, acidification, and chemical pollution, would provide rare opportunities to characterize human impacts on biologically relevant patterns of sexual selection.

Data accessibility. This article has no additional data.

Authors' contributions. J.P.E. conceived the original concept for review. J.P.E. and R.A.L. co-wrote the paper.

Competing interests. We declare no competing interests.

Funding. J.P.E. is funded by the Australian Research Council (DP170103290).

Acknowledgement. We thank Nina Wedell and Leigh Simmons for the kind invitation to contribute this article, and Geoff Parker for his inspirational work in this field. We also thank two anonymous reviewers for comments on an earlier draft of this manuscript.

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