An Ecology of Sperm: Sperm Diversification by Natural Selection

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Abstract

Using basic ecological concepts, we introduce sperm ecology as a framework to study sperm cells. First, we describe environmental effects on sperm and conclude that evolutionary and ecological research should not neglect the overwhelming evidence presented here (both in external and internal fertilizers and in terrestrial and aquatic habitats) that sperm function is altered by many environments, including the male environment. Second, we determine that the evidence for sperm phenotypic plasticity is overwhelming. Third, we find that genotype-by-environment interaction effects on sperm function exist, but their general adaptive significance (e.g., local adaptation) awaits further research. It remains unresolved whether sperm diversification occurs by natural selection acting on sperm function or by selection on male and female microenvironments that enable optimal plastic performance of sperm (sperm niches). Environmental effects reduce fitness predictability under sperm competition, predict species distributions under global change, explain adaptive behavior, and highlight the role of natural selection in behavioral ecology and reproductive medicine.

1. A FRAMEWORK OF SPERM ECOLOGY

In almost all species, only a tiny fraction of ejaculated sperm reaches an egg and interacts with it for fertilization. The function of these few sperm is central to the study of evolution because only those functioning sperm deliver genetic information to the next generation. Sperm function is also central to other biological areas. For example, reduced sperm function is one of the most important known causes of human infertility in the Western world (Hirsh 2003, Pizzol et al. 2014) and is central to assisted reproduction technologies. For other species on the planet, sperm function is the target of animal breeders to improve the reproductive capacity of livestock (Billard & Cosson 1992, Froman et al. 2006) and the target of geneticists to optimize conservation programs (Roldan & Gomendio 2009). It is under substantial scrutiny in ecotoxicology as a trait affected by environmental pollution (Hayes 2011, Tavares et al. 2013) and is used in a range of toxicity bioassays (Hoornstra et al. 2004, Rajkovic et al. 2006). A striking feature of sperm cells is their enormous evolutionary diversification, particularly in morphology (reviewed in Pitnick et al. 2009), which is currently attributed to sexual selection: Diversification in sperm form and function arises because the sperm of genetically different males compete, and the outcome of the competition varies within different female genotypes, thus leading to selection for competitive sperm (Birkhead et al. 2009, Manier et al. 2013).

Here we propose a framework for sperm evolution and diversification that incorporates the environmental and genetic components of sperm function. We start by briefly reviewing various ways in which the large number of environments affects many different sperm functions and their relative strengths. We then apply several simple ecological concepts to sperm biology to provide a more comprehensive view of its role in ecology, evolution, and medicine.

1.1. The Sperm Phenotype

Variation in sperm form and function—the cellular phenotype—comes from three sources and their interactions: the male nuclear genotype, the male mitochondria, and the environment (**Figure 1**). This definition extends previous ones that consider genetic variation in sperm form and function between males (Pizzari & Parker 2009), herein called the genotype effect. Research into sperm biology has been organized roughly into these three main sources of variation (**Figure 1**). In this review, we do not cover variation in the sperm phenotype that might arise from variation in the genetic makeup of sperm within an ejaculate and any possible resulting differences in haploid gene expression (Parker & Begon 1993). Although this issue is very interesting, we focus on environmental effects.

1.2. Male Nuclear Genetic Effects on Sperm Phenotype

By suggesting that "sperm phenotypes are predominantly determined by testicular gene expression and hence the diploid genome of the male," Pitnick et al. (2009, p. 75) implied that environmental sources are not important in explaining the sperm phenotype and sperm diversification. This summary reflects four decades of intense research on sperm competition (Bernasconi et al. 2004; Birkhead et al. 1998, 2009; Parker 1970). This view is also implicit in the literature on animal breeding as well as in procedures within medicine that seek correlations between sperm function and genotype (so-called sperm function tests) (Aitken 2006, World Health Organization 2010). Although sperm competition is a successful research field, a need to extend this view is apparent from the fact that male genotype effects explain only a small to moderate proportion of variation in sperm function (Dowling et al. 2010, Simmons et al. 2014). For example, crosses of six male and female *Drosophila melanogaster* genotypes were carried out under highly controlled laboratory conditions (Clark et al. 1999), but genotype explained only 6–11% of the variation in paternity.

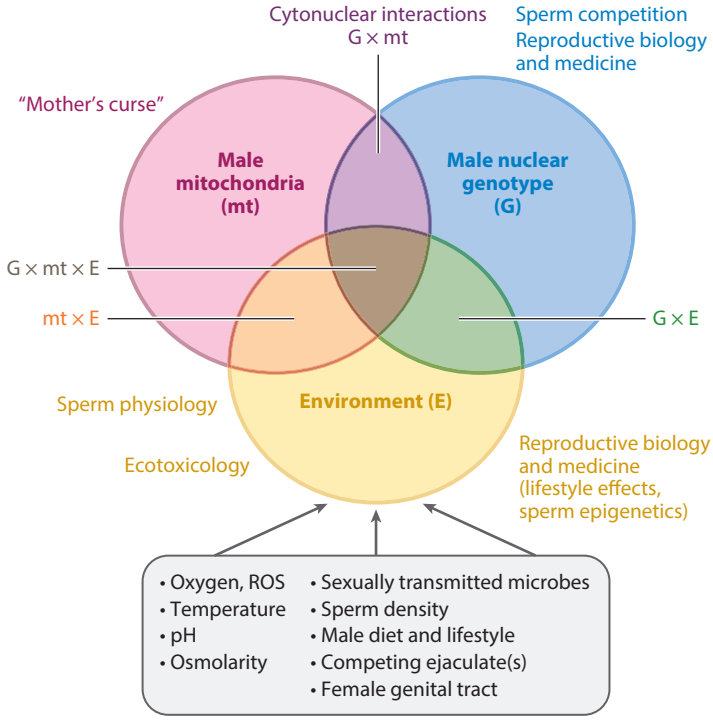


Figure 1

Three major influences on phenotypic sperm function and their corresponding research fields. The male nuclear genotypic contribution to sperm function is studied mainly in the context of sexual selection and male reproductive medicine (andrology) (*blue*). The male mitochondrial contribution to sperm function, and its interaction effects with the male nuclear genotype, is studied within evolutionary genetics in the context of "mother's curse" and cytonuclear interactions (*magenta*). External environmental effects are studied in the context of sperm physiology, ecotoxicology, and reproductive medicine. Genotypically fixed aspects of competing ejaculates and female reproductive tracts are also important in studies of sperm competition. Sperm ecology considers environmental contributions to the sperm phenotype (*yellow*) and how they can lead to genotypic variation. Examples of major sperm environments are listed in the gray-shaded box (*bottom*). Abbreviation: ROS, reactive oxygen species.

Similarly, despite intense research efforts in reproductive medicine, approximately 10–15% of infertility cases are currently attributed to genetic factors in males (Pizzol et al. 2014).

1.3. Male Mitochondrial Effects on Sperm Phenotype

Mitochondria affect many aspects of the sperm phenotype (Aitken et al. 2009, Dowling et al. 2007, Froman & Kirby 2005, Innocenti et al. 2011, Yee et al. 2013, Zini & Al-Hathal 2011). In particular, mitochondrial genetic variation can have a substantial impact (but see Friberg & Dowling 2008): Variation in mitochondrial production of reactive oxygen species (ROS) explained 68% of the variation in sperm motility in humans (Koppers et al. 2008). Few studies, however, have experimentally manipulated the mitochondrial haplotype (but see Friberg & Dowling 2008, Yee et al. 2013) and, therefore, separate mitochondrial and mitochondrial-genotype (mt × G) effects on sperm function. Fruit fly sperm carrying mitochondrial haplotypes combined with a foreign

Reactive oxygen species (ROS): a by-product of normal metabolism; these chemically reactive molecules can cause damage to membranes and DNA and also act as cellular signals

nuclear background have had, on average, a 30% lower sperm competitive ability than they do when expressed with their coevolved background (Yee et al. 2013).

Sperm trait: a sperm character explained by male nuclear and mitochondrial genotype

Genotype-by-environment (G × E) interaction: different genotypes respond differently to various environmental conditions

Two aspects of mitochondrial effects on the sperm phenotype relate to sperm diversification. First, the exclusive maternal inheritance of mitochondria (in almost all species) reduces the possibility that sperm functions can evolve via sperm competition if mitochondria govern these sperm functions. Selective advantages may occur through local mitochondrial adaptations in females (Rand 2001). However, mutations with a negative effect on sperm function can accumulate if these mutations have only small, or positive, effects on females (Innocenti et al. 2011, Yee et al. 2013), a process known as "mother's curse." Second, molecular signaling from mitochondria to the nucleus, such as variable ROS production, can differ between environments (Murphy 2009, Wallace et al. 2011). This provides an opportunity for mitochondrial-environmental (mt × E) interactions and perhaps for local adaptations of mitochondria (Dowling 2014, Wolff et al. 2014).

1.4. Environmental Effects on Sperm Phenotype

In the eighteenth century, Spallanzani observed that snow-chilled sperm recover their motility in warmer temperatures (Mann 1964). Mann (1964) added that the nineteenth century "abounds in studies on the effect of changes in the medium on sperm motility and survival" (p. 54). Despite this history, and in contrast to recent extensive research on genotype and mitochondrial effects, current evolutionary and ecological research has largely ignored environmental effects on the sperm phenotype and sperm diversification (but see Blanckenhorn & Hellriegel 2002; for ecotype effects on sperm cells in plants, see Delph et al. 1997). Instead, several currently unconnected research fields deal with environmental effects on the sperm phenotype, i.e., effects that go beyond a mere variation in sperm number: (*a*) the substantial medical literature of lifestyle effects on sperm function (Aitken et al. 2014, Fraga et al. 1996, Yauk et al. 2008); (*b*) the literature on fertilization biology in marine systems (Adriaenssens et al. 2012; Jensen et al. 2014; Levitan 1995, 2000; Schlegel et al. 2014); (*c*) ecotoxicological research on the effects of environmental pollutants and endocrine disruptors on sperm function across a wide range of taxa (Hayes 2011, Lewis & Ford 2012, Tavares et al. 2013); (*d*) applied research on storage, transport, and long-term cryostorage of sperm (Leahy & Gadella 2011, Mann 1964); and (*e*) sperm aging, which encompasses the successive or collective accumulation of damage across all the environments through which a sperm cell has passed (e.g., Pizzari et al. 2008, Reinhardt 2007, Siva-Jothy 2000, Tarín et al. 2000). Environmental effects on the sperm phenotype can also be deduced from the fact that intramale variation (Pitnick et al. 2009) and intraejaculate variation for sperm traits are abundantly reported. Finally, sperm epigenetics (offspring variation based on environmental alterations of sperm cells), just like sperm aging, describes collective and cumulative environmental effects, often without specifying the underlying molecular mechanism. This is an emerging field, but most effects concern epigenetic alteration at the spermatid stage (Dada et al. 2012, Jenkins & Carrell 2012, Johnson et al. 2011) rather than of mature sperm (but see Marshall 2015).

1.5. Introducing the Research Field of Sperm Ecology

By applying basic individual-level approaches, which have been successful in developing whole-organism ecology, sperm ecology aims to characterize interactions between sperm cells and their environment and to examine the consequences of this interaction. This aim requires a consideration of the nuclear genotypic, mitochondrial, and environmental components of the sperm phenotype and their interrelations, such as genotype-by-environment (G × E) and mitochondria-by-environment (mt × E) interaction effects (**Figure 1**). By using the concept of the sperm phenotype, sperm ecology extends existing research areas by combining the focus on additive genetic

effects in research on sperm competition (Simmons & Moore 2009) with the environmental effects that ecotoxicology, reproductive medicine (i.e., lifestyle effects on sperm function), and other fields outlined in Section 1 have identified. In addition, specifying the environments that sperm cells encounter in different female genotypes may provide a useful route to characterize the outcome of reproductive interactions (see, e.g., Aranha et al. 2008, Rosengrave et al. 2009, Yeung et al. 2006). The fact that sperm function in individual males is not always highly repeatable (Birkhead & Fletcher 1995, Garcia-Tomas et al. 2006, Peters et al. 2004; but see Gage et al. 2004) suggests a role for environmental effects in explaining fitness variation in nature.

Environmental effects on sperm may be apparent as a temporal variation in sperm function. In contrast to the concept of sperm competition, for which the evolutionary outcome is important (i.e., only the end points of competition), sperm ecology takes a longitudinal, cellular-lifetime approach (**Figure 2**, **Supplemental Figure 1**; for all **Supplemental Material**, follow the corresponding link from the Annual Reviews home page at **http://www.annualreviews.org**), yielding several advantages. First, sperm may be in competition for a variable amount of time; hence, temporal variation will help to predict the end points of sperm competition in these cases while simultaneously considering the universal cellular trade-off between energy expenditure and life span (**Figure 2**) (for various examples of cellular trade-offs in sperm, see Burness et al. 2004, Gage et al. 2004, Hughes & Davey 1969, Levitan 2000, Reinhardt & Otti 2012, Ribou & Reinhardt 2012).

Second, the longitudinal approach incorporates delayed environmental impacts on sperm (see below) including the view that differences in offspring phenotype or quality arise because fertilizing sperm have different exposure histories or durations (e.g., Burruel et al. 2013, Ghaleno et al. 2014, Immler et al. 2014, Lane et al. 2014, Marshall 2015). Third, a lifetime view allows one to consider sperm physiological changes as phenotypic plasticity at the cellular level. Despite Spallanzani's early observations, and despite the central position of phenotypic plasticity in whole-organism biology, the issue of sperm phenotypic plasticity has not been addressed by sperm competition and has been addressed only rarely in other areas of evolutionary and ecological research (Crean et al. 2013, Jensen et al. 2014, Poland et al. 2011, Purchase et al. 2010). We further argue that male and female reproductive traits evolve to accelerate sperm function via sperm phenotypic plasticity. This process is similar to niche construction in whole-organism ecology, and we refer to such created sperm environments as sperm niches.

Finally, by testing for adaptive G × E or mt × E interactions, sperm ecology aims to describe whether, and how, natural selection favors specific sperm phenotypes in specific environments. This approach may also include sexual selection if male and female genotypes are regarded as specific environments for sperm. Therefore, sperm ecology contributes to explaining sperm diversification. Importantly, sperm ecology does not necessarily require competition between genotypes to produce evolutionary changes (**Figure 2**) and, as such, is a parsimonious concept. In summary, by integrating sperm biology with four basic ecological concepts, e.g., environmental variation, phenotypic plasticity, niche construction, and G × E interactions (local adaptation), sperm ecology may contribute to explaining phenotypic adaptations as well as sperm diversification in three important ways: (*a*) characterizing and quantifying the effects of environmental variation on sperm function, (*b*) assessing the role of natural selection in sperm diversification, and (*c*) suggesting a pathway for the evolution of male and female reproductive traits.

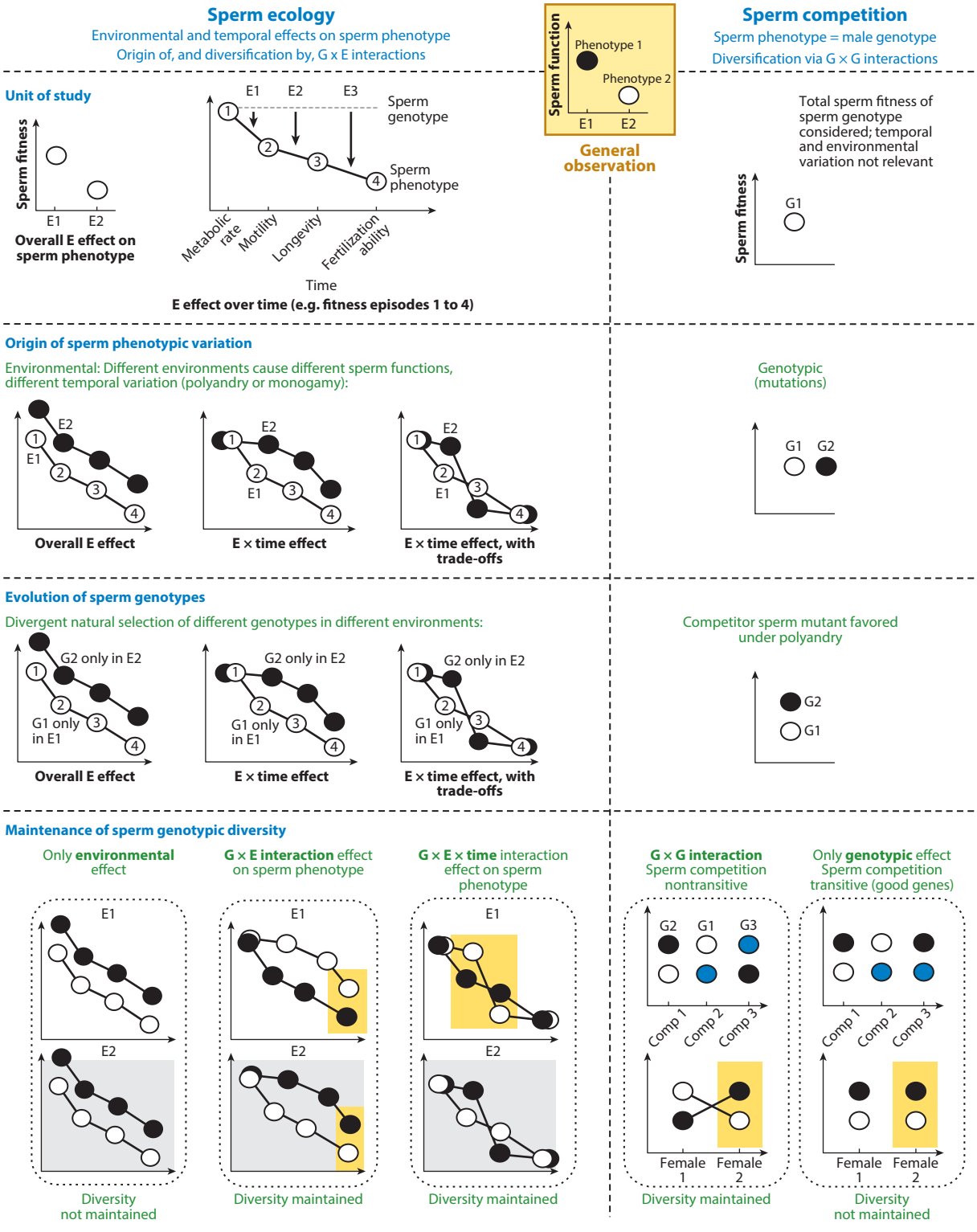
Phenotypic plasticity: the ability of a genotype to vary phenotypically under different environmental conditions

Local adaptation: a form of genotype-by-environment interaction where the fitness of a genotype is highest in the environment in which that genotype evolved

Supplemental Material

2. CHARACTERIZING AND QUANTIFYING THE EFFECTS OF ENVIRONMENTAL VARIATION ON THE SPERM PHENOTYPE

Environmental variation can act on sperm in several ways. First, an external environment (such as temperature for ectothermic animals; water pressure, UV radiation, and salinity for


Sperm ecology
Environmental and temporal effects on sperm phenotype
Origin of, and diversification by, G x E interactions
Sperm function
Phenotype 1
Phenotype 2
E1
E2
General observation
Sperm competition
Sperm phenotype = male genotype
Diversification via G × G interactions
Unit of study
Sperm fitness
E1
E2
Overall E effect on sperm phenotype
E1
E2
E3
Sperm genotype
Sperm phenotype
Metabolic rate
Motility
Longevity
Fertilization ability
Time
E effect over time (e.g. fitness episodes 1 to 4)
Total sperm fitness of sperm genotype considered; temporal and environmental variation not relevant
Sperm fitness
G1
Origin of sperm phenotypic variation
Environmental: Different environments cause different sperm functions, different temporal variation (polyandry or monogamy):
Overall E effect
E × time effect
E × time effect, with trade-offs
Genotypic (mutations)
G1
G2
Evolution of sperm genotypes
Divergent natural selection of different genotypes in different environments:
G2 only in E2
G1 only in E1
Overall E effect
E × time effect
E × time effect, with trade-offs
Competitor sperm mutant favored under polyandry
G2
G1
Maintenance of sperm genotypic diversity
Only environmental effect
G × E interaction effect on sperm phenotype
G × E × time interaction effect on sperm phenotype
G × G interaction
Sperm competition nontransitive
Only genotypic effect
Sperm competition transitive (good genes)
Diversity not maintained
Diversity maintained
Diversity maintained
Diversity maintained
Diversity not maintained
G2
G1
G3
Comp 1
Comp 2
Comp 3
Female 1
Female 2

broadcast-spawning organisms; or in vitro treatments for laboratory cases) directly impacts sperm cells. Second, environments can act on males and females and generate microenvironments for sperm cells that are different from the external environment (**Supplemental Figure 1**). For example, smoking results in increased systemic levels of ROS, including in reproductive compartments; food items may affect the pH in seminal fluid; or increased temperature may alter ion or enzyme concentrations. Third, longitudinal variation in sperm function may vary across different environments, such as reduced metabolism under hypoxia.

Supplemental Material

We use two literature search methods to describe environmental effects on sperm: (*a*) a random selection of relevant articles and (*b*) a directed search by specific environments on specific sperm functions (see **Supplemental Table 2**). Our literature search on environmental effects on sperm yielded 27,514 articles, or 8,042 if restricted to articles published in 2000–2014. Of the latter, 900 articles were randomly chosen, of which 178 articles (19.8%) (**Supplemental Table 1**) matched our criteria (**Supplemental Table 2**). These articles provide an estimate of the significance of environmental effects on sperm within ecology and evolution.

2.1. Many Environmental Factors Affect Sperm Function

Phenotypic sperm function is affected by various environments, including temperature, pH, osmolarity and concentration of specific ions, oxygen concentration, oxygen radicals and antioxidants, diet (male, maternal, and paternal as well as its amount and composition), larval or adult population density, photoperiod, UV radiation, sexually transmitted microbes, viruses, exposure to airborne or food-borne chemicals (male, maternal, and paternal), external nucleic acids, and sperm density (**Supplemental Table 2**). The data may be biased, as results that show no environmental effect on sperm were published less frequently (but for a discussion of the substantial number of studies reporting the absence of environmental effects, see Section 2.2). Yet, it seems most environments tested show some effect on sperm.

2.2. The Literature on Environmental Effects on Sperm Phenotype Is Large and Largely Neglected by Ecology and Evolution Research

After applying our search criteria (**Supplemental Table 2**), accounting for the fact that different environments were studied to a varying extent (**Supplemental Table 1**) and excluding 597 articles that appeared under more than one environment (e.g., two environments examined by one paper), we suggest that between the years 2000 and 2014 an estimated 1,293–2,180 (mean = 1,736) articles explored environmental effects on sperm (**Supplemental Table 1**). The 178 articles we studied in detail examined a total of 458 environment–sperm function combinations (2.57 per study). Of these, 356 combinations (78%) reported in 163 studies (91.6%) showed at least one environmental effect on at least one function, whereas 64 studies (35.9%) showed no effect on at least one function. Projecting to the other articles, we find the abundance of environmental

Figure 2

Origin, evolution, and maintenance of genetic variation in sperm function as envisaged by the concept of sperm ecology presented here and compared with the concept of sperm competition. Sperm ecology considers temporal variation in sperm function, i.e., over various fitness episodes, noting how different environments increase deviation in sperm phenotype from the sperm genotype. This allows for the consideration of delayed environmental effects on sperm (for details, see **Figure 3**) and cellular trade-offs. Natural selection favors certain sperm phenotypes in some environments, without requiring a sperm competitive advantage. G × E interactions increase sperm function diversity (for details, see **Figure 4**). Abbreviations: Comp, competition; E, environment; G, male nuclear genotype.

effects is remarkable: Of the projected 1,736 (1,293–2,180) relevant articles, 1,590 articles may have explored 4,086 environment–sperm function combinations, with 3,187 environment–sperm function combinations potentially showing environmental effects on sperm function.

Supplemental Material

Although the assertion that environment shapes the sperm phenotype (as proposed in **Figure 1**) may not seem surprising, it is noteworthy that only very few of these articles originate from evolution and ecology research. Broadly defining "ecology and evolution journals" (**Supplemental Table 2**), only 8 (4.5%) articles from our random search concerned ecology and evolution. A similar number resulted from our directed search: only 40 (5.4%) out of 7,445 articles, including 18 that examined consequences of predicted global change on sperm function, were found.

2.3. Magnitude and Shape of Environmental Effects on Sperm Phenotype

Our summary (**Supplemental Table 2**) revealed that some environments such as brief high altitude visits (Okumura et al. 2003), brief pollution events, and brief temperature elevations (Paul et al. 2008) affect the sperm phenotype after even a short transient impact, whereas others were found after a sustained period of action. Some environmental effects became apparent immediately, whereas others, including those found in offspring, appeared much longer after the environmental impact. Many environmental effects, such as DNA damage, membrane damage, and sperm mortality, are irreversible and hence permanent at the cellular level or in males. However, examples also show that other environmental effects are reversible at those levels (Aitken et al. 2012, Bencic et al. 2000, Le Comber et al. 2004, Okumura et al. 2003, Villegas et al. 2003).

Among the fitness-related aspects of sperm function, none appeared so canalized as to be consistently inert to environmental effects. Across species, sperm morphology, metabolism, motility, longevity, fertilization ability, epigenetic signatures on the nuclear genome, and offspring health were all affected. Within species, these characters were not equally affected, and sometimes the effects were not positively correlated with each other (**Supplemental Table 2**). The magnitude of effects was so variable as to prevent any generalization. Compared with controls, environmental effects on sperm populations or sperm cells ranged from no, or minute, effects to substantial reductions in sperm function, including complete failures. Even natural variation in environmental conditions (such as temperatures >37°C or changes in pH or osmolarity) generated substantial variation in sperm function (**Supplemental Table 2**).

2.4. Phenotypic Plasticity in Sperm Function

Almost all studies incorporated in our literature search assessed environmental effects against control sperm from the same male, the same genotype, or the same population. In other words, almost all 397 environment–sperm function combinations represent phenotypic plasticity at either the genotype or male population level. Some studies even demonstrated plasticity at the level of the individual sperm cell or the ejaculate, for example, by showing that in vitro effects were reversible (Le Comber et al. 2004, Otti et al. 2013) or that human sperm repeatedly bind and unbind to the epithelium (Pacey et al. 1995) or move in and out of a hyperactivated state (Mortimer & Swann 1995). It may be noteworthy that even sperm morphology can be plastically (but not necessarily reversibly) affected by the environment. For example, compared with ejaculated sperm, the female sperm storage organ of several insect species shows substantial membrane alteration (Renieri & Vegni Talluri 1974, Riemann & Thorson 1971; for a review of phenotypic plastic, environmental effects on sperm size, also see Marshall 2015).

Physiological responses in sperm, equivalent to cellular phenotypic plasticity, are not unexpected, given the diverse chemistry of male and female genital tracts that sperm cells have to

master. However, explicitly spelling out the existence of sperm phenoptypic plasticity may help to formalize predictions of when such plasticity would be adaptive. Adaptive plasticity depends on how often a given environment is encountered, the duration of the encounter, and the reliability of information (for a general framework, see Pfennig et al. 2010). Interestingly, these predictor variables of sperm phenotypic plasticity can now be linked to the substantial body of cell biology studies that examine the considerable ability of individual sperm cells to respond to chemical, surface, or other conditions encountered in situ (Alvarez et al. 2012, 2014; Babcock et al. 2014; Bahat & Eisenbach 2006; Friedrich & Jülicher 2007).

2.5. The Relative Size of Genotypic, Mitochondrial, and Environmental Effects on Sperm Phenotype

Surprisingly few studies have estimated the relative size of genotype and environmental effects simultaneously in the same system (and possibly none have separated genotype, mitochondrial, and environmental effects). One study used female gene expression after sperm receipt as a parameter of sperm fitness and varied sperm age (environmental effect) within three different sperm genotypes (populations) (Otti et al. 2015). Approximately 16 times as many female genes were differentially expressed in response to environmental effects (79 genes) as opposed to genotype effects (5 genes). When this comparison was restricted to genes with substantial differences, 5 times as many genes were still expressed in response to environmental than to genotype effects (Otti et al. 2015). However, even though quantifying the number of differentially expressed genes in females may be useful, this number does not necessarily translate directly into differences in sperm fitness. The statistical table in the *Drosophila melanogaster* study by Clark et al. (1999) suggests less than 1% of the variation in paternity was explained by environmental (laboratory) effects, compared with 6–11% explained by male genotype effects. Both studies have the limitation that they include seminal fluid effects. More closely related to sperm function are the careful analyses by Purchase & Moreau (2012) and Purchase et al. (2010) on sperm swimming speed in fish across a pH and temperature gradient. These studies showed that genotype explained 3 times as much variance as did pH, whereas temperature explained 1.3 times as much variance as did genotype.

We conclude that environmental effects on sperm function are ubiquitous, take many forms, and may be as large as genotype effects, or even larger. Given our randomized literature search, it is not tenable to assume that male genotype effects almost exclusively shape the sperm phenotype. Environmental effects can be direct, indirect, or phenotypically plastic. They can be caused by sustained action or brief impact and are permanent or reversible. Evolutionary and ecological research should not ignore environmental effects when examining variation in reproductive success.

3. CONSEQUENCES OF ENVIRONMENTAL EFFECTS ON SPERM PHENOTYPE

3.1. Reduced Significance and Hampered Predictability of Sperm Competition

The observations that sperm functions vary between environments and that sperm can accumulate damage and information during their passage through an environment have important consequences. First and foremost, in many species the sperm genotype can occupy a very large phenotypic space (**Figure 3**). This fact severely hampers our ability to predict sperm function on the basis of genotype alone, in both the absence and the presence of sperm competition. Whenever sperm of two males compete, their sperm phenotypes have "stored" an environmental component, and this history may be decisive in their competition, even if both compete in the same environment.

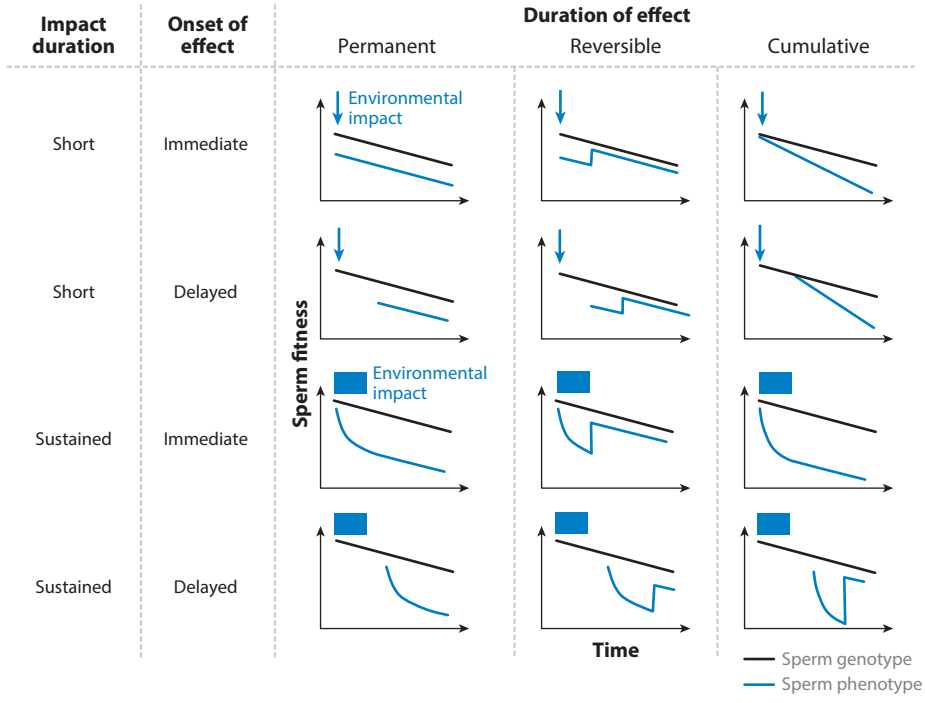


Figure 3

Summary of environmental impacts on the sperm phenotype (*blue lines*) generating deviations from the sperm genotype (*black lines*). The sperm genotype contains an a priori aging component, hence a decline over time. Impacts can directly affect the sperm cell and act immediately or in a delayed fashion, or they can indirectly affect the male, in which case they are inherently delayed. The impact can be a short burst or sustained, leading to permanent, reversible, or cumulative effects. In sum, the sperm phenotype can occupy almost any value under the sperm genotypic curve.

Supplemental Material

Importantly, this history will not give consistent differences between two competing males unless the sperm function follows a linear decline over time (cf. Reinhardt 2007, figure 1). Therefore, the loaded raffle in sperm competition (Parker 2009) cannot be expressed by a loading coefficient that is independent of time and environment. In nature, there will hardly ever be two males with identical lifestyles, habitat utilization, or age at the time of mating (when their sperm compete); hence, they will not have sperm with an identical environmental component (**Supplemental Figure 1**). We therefore predict that such carryover environmental effects on sperm competition or fertility are a universal feature in the animal kingdom. However, its testing is severely hampered by the paucity of studies addressing the impact of environmental effects on postejaculatory reproductive success (Almbro et al. 2011, Breckels & Neff 2014, Gasparini & Evans 2014, Mehlis & Bakker 2014, Vasudeva et al. 2014).

3.2. Sperm Function May Determine Species Range

In certain cases, sperm velocity was not affected by environmental variation, such as increased temperature and decreased water pH in several marine invertebrates (Byrne et al. 2010), the Atlantic cod (Frommel et al. 2010), or the oyster (Havenhand & Schlegel 2009). But in other

cases, males within a population were affected differently by environmental variation such that consistent variation between populations did not emerge. Examples of the latter include responses of sperm motility to ocean acidification in a polychaete (Schlegel et al. 2014) and a sea urchin (Schlegel et al. 2012). These examples show that sperm phenotypic plasticity (in males or at the population level) can buffer environmental variation and enable population persistence.

However, there are also dozens of studies showing that some sperm functions have optima at certain intermediate states that are close to current environmental conditions. In these cases, environmental effects on sperm function may limit a population's range or its ability to cope with altered environmental conditions. This has been suggested for some species under predicted global climate change: For example, reduced sperm velocity is expected under higher UV radiation doses [stickleback (Rick et al. 2014), sea urchins (Lu & Wu 2005, Nahon et al. 2009)], increased CO_2 concentration in the water [sea star (Uthicke et al. 2013), mussels (Vihtakari et al. 2013), oyster (Barros et al. 2013), coral and sea cucumber (Morita et al. 2010)], and increased water temperature [guppy (Breckels & Neff 2013)]. Sperm longevity may be reduced under increased water temperatures [sea urchin (Binet & Doyle 2013)] or may undergo altered trade-offs with sperm velocity [sea urchin (Caldwell et al. 2011), stickleback (Mehlis & Bakker 2014)]. Similar effects may occur under decreased water pH [sea urchin (Caldwell et al. 2011)]. Acclimatization by males to higher temperatures, possibly representing indirect environmental effects, may not shift thermal critical limits of sperm velocity (Adriaenssens et al. 2012).

3.3. Intraejaculate Heterogeneity

As males pass through different environments but continue to produce sperm, ejaculates become heterogeneous in terms of sperm age (Reinhardt & Siva-Jothy 2005), epigenetic marks (Aoki et al. 2006), and other characters (Dorado et al. 2013, Satake et al. 2006). Immler et al. (2014) found that sperm from the same ejaculate produce different offspring phenotypes when sperm are exposed to different treatments. An important consequence is that different environments may cause different patterns of ejaculate heterogeneity and so contribute to phenotypic and genetic divergence of populations that live in different habitats.

3.4. The Brynhild Effect

Some sexual selection models have suggested that females benefit from creating barriers to sperm that only the best sperm can pass to fertilize eggs (Birkhead et al. 1993). The observation that sperm cells experience, or even accumulate, environmental damage is not entirely consistent with those models. Instead, sperm ecology predicts the adaptive evolution of filter mechanisms against damaged sperm regardless of their genotype (see also Siva-Jothy 2000, Reinhardt 2007). Additionally, stronger female barriers that represent harsh environments for sperm are predicted to cause greater damage to sperm. Termed the Brynhild effect, this situation is similar to that of the eponymous female character in the Nordic epic saga *Nibelungenlied* (Song of the Nibelungs) who resided inside a ring of fire. Noble men aiming to get across to marry Brynhild either died or were injured. The man who finally succeeded needed magical powers to cross the fire.

3.5. Sperm Viability Is Not a Good Fitness Indicator

Some environments may be so stressful that sperm apoptosis is initiated, for example, during attacks by retroviruses that inject foreign RNA or DNA into sperm DNA. Aitken & Baker (2013) pointed out that apoptosis may then be selectively advantageous: "Selective deletion of damaged germ cells

is clearly a critical component of the mechanisms used to safeguard the genome of a given species" (p. 265). Though expressed in a group selectionist way, this remark illustrates how sperm viability or apoptotic activity per ejaculate is not necessarily a sign of low male quality but may be adaptive for a male if apoptosis prevents damaged sperm that would result in lower-fitness offspring from outcompeting his own genetically undamaged sperm (Aitken & Koppers 2011, Aitken et al. 2013). Aitken & Baker (2013) also highlighted another benefit of apoptosis: "By engaging in regulated cell death exhibiting many features of apoptosis, moribund spermatozoa ensure they can be efficiently removed from the male or female reproductive tract without provoking a damaging inflammatory response" (p. 266).

As a consequence, the widely used proportion of dead sperm per ejaculate (or proportion of live sperm/sperm viability) may be an indicator of the environmental history of a male or a positive indicator of the ability of a genotype to respond to its environment (G × E), rather than exclusively reflecting a negative genotype.

3.6. Variance Effects in Numerical Sperm Competition

Sperm competition predicts a numerical advantage for males delivering more sperm. Because of the environmental component of the sperm phenotype, sperm ecology also specifies this prediction of male advantage. Continuous, and constant, sperm production automatically results in ejaculates with more sperm that also contain more recently produced sperm, i.e., sperm that have been exposed to an environment for shorter periods (Reinhardt 2007). The general numerical advantage seen in sperm competition may therefore be due to the fact that in larger ejaculates more sperm are present in the fresh cohort.

3.7. Mean Ejaculate Traits May Be Noninformative

In most species, only a few sperm reach an egg. As selection acts to maximize sperm functions, mean ejaculate values of sperm motility or longevity may be less informative in predicting paternity than are some maximum values (Holt & van Look 2004, Mossman et al. 2010, Reinhardt & Otti 2012). We suggest that current medical diagnostics of infertility (World Health Organ. 2011) may benefit from considering this notion.

3.8. Trade-Offs in Sperm Function Can Hamper Comparability

Sperm function can decline within seconds of activation (see examples in Levitan 1995, Purchase et al. 2010, Reinhardt & Otti 2012). Trade-offs in sperm function associated with such rapid decline can severely hamper the comparison of individuals and lead to false conclusions (as illustrated in Reinhardt & Otti 2012, figure 2).

3.9. Adaptive Habitat Choice

Given the environmental effects on sperm function and that altered sperm function translates into reproductive success, we predict that males and females are under selection to choose specific environmental conditions that positively affect sperm function.

3.10. Selection for Sperm Niches

Alternatively to adaptive habitat choice, environment-dependent sperm function can select for male and female traits that create environments in which sperm function is improved. These

so-called sperm niches may, for example, allow an organism to colonize new habitats. This has been suggested by Elofsson et al. (2003), who argue that ovarian fluid chemistry allowed sticklebacks to overcome the osmotic constraints on sperm imposed by a new freshwater habitat. The most obvious sperm niche is seminal fluid, an evolutionarily diverse character (Avila et al. 2011, Poiani 2006). Seminal fluid fulfills niche functions by buffering the pH for sperm, reducing oxidative stress to sperm, supporting motility, extending longevity, and improving offspring development across a variety of taxa (e.g., Aitken & Clarkson 1988, Bromfield et al. 2014, Heise et al. 2010, Kang et al. 2008, Rickard et al. 2014, Scaggiante et al. 1999, Shaliutina-Kolesova et al. 2014). Specific examples include seminal antioxidants (Avila et al. 2011, Poiani 2006) or antimicrobial properties (Otti et al. 2009, 2013; Poiani 2006). Other niches may transiently reduce sperm motility and save cellular energy resources by containing sperm in bundles, via spermatophores, or through additional sperm types (Reinhardt 2007).

Many female traits also serve as niches by plastically improving sperm function. Reinhardt (2007), Holt & Lloyd (2010), and Heifetz & Rivlin (2010) review female traits that reduce sperm metabolism and sperm oxidative stress, thereby extending sperm longevity during prefertilization storage. These traits include hypothermia at the sites of sperm storage, reduction of sperm motility by binding sperm to epithelia, as well as packing sperm tightly or organizing them in bundles. Sperm niche functions are also found in immunological and antioxidant protection, interference with sperm metabolism, reduction in the number or size of mitochondria, or delays of sperm activation. Recent work on insects suggests that the process by which sperm metabolism is reduced is adaptive to females (i.e., it delays infertility) (Reinhardt & Ribou 2013, Ribou & Reinhardt 2012). Both Reinhardt & Ribou (2013) and Ribou & Reinhardt (2012) supported the idea that this effect is specifically directed toward sperm phenotypes, and not sperm genotypes, because the sperm genotype could not be predicted on the basis of sperm metabolism.

3.11. Selection May Be Directed Against Environmental, not Genotypic, Components of the Sperm Phenotype

If environmental effects on sperm are often damaging, then male and female traits that discriminate against sperm on the basis of the sperm phenotype unrelated to the sperm genotype are predicted to evolve. In males, such traits include those that specifically disfavor aged sperm phenotypes, for example, repeated mating with the same female (though functional for sexual selection, mating with different females also gives rise to this trait), sperm transfer to other males, sperm discard without copulation, continuous sperm production, and the many ways of bringing reproductive events forward in time, i.e., closer to sperm production (reviewed in Reinhardt 2007). In females, repeated mating to the same male reduces representation of aged or environmentally damaged sperm phenotypes in the fertilization set. Sperm dumping by females also has this effect if it is related to sperm storage time. If sperm stratify in males by age or quality cohorts, the behavior of mate copying by females would automatically increase the representation of higher-quality sperm (reviewed in Reinhardt 2007).

However, though these traits automatically alter ejaculate variability in terms of environmental effects, relatively few empirical tests exist. For example, in a cricket species, nonused sperm were expelled by males while younger sperm were more successful in reaching the female sperm storage organ (Reinhardt & Siva-Jothy 2005). In bedbugs, Otti et al. (2013) demonstrated that antibiotic activity in the seminal fluid transferred during one mating was sufficient to reduce sperm mortality caused by simultaneously transferred bacteria. Finally, because experimental scrotum insulation results in reduced sperm motility or DNA fragmentation (Banks et al. 2005, Brito et al. 2003), we may conclude that the scrotum evolved to reduce environmental effects on sperm.

In summary, environmental effects on the sperm phenotype yield substantial conceptual and methodological consequences for ecological and evolutionary research. Even though some predicted consequences remain untested, they substantially alter our understanding of variation in fitness and reproductive success. We suggest that the consideration of environmental effects on fitness is a worthwhile scientific enterprise.

4. NATURAL SELECTION AND DIVERSIFICATION IN SPERM PHENOTYPE

4.1. A Model for Phenotypic Sperm Evolution

Sperm phenotypic diversification is possible along two principal paths: via sperm environments enabling sperm phenotypic plasticity and via sperm traits. Both ways can lead to adaptive population genetic changes and contribute to evolutionary change. Selection can operate on increased male reproductive success by directly favoring sperm traits (**Figure 4**). Sperm phenotypic plasticity opens a second route for selection: Male traits may be favored that create sperm niches in which phenotypically plastic sperm function better than they would in the absence of such niches (**Figure 4**), making niches adaptive paternal effects. An obvious example of the evolution of a sperm niche is seminal fluid. Sperm cells experiencing a newly evolved sperm niche may then allow further sperm diversification by providing an arena in which novel sperm traits evolve (**Figure 4**) [cf. genetic assimilation (West-Eberhard 2003)].

Alternatively, sperm niches may reduce the opportunity for sperm evolution because sperm experience a stable environment without selection pressure. An imaginary example is a sperm phenotype that has benefitted through a male mutation that led to increased sugar content in the seminal fluid (**Figure 4**). This sperm phenotype may now benefit from a male mutation that

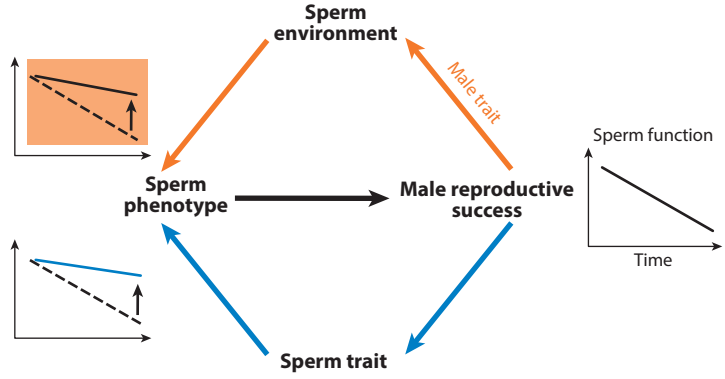


Figure 4

Model of sperm phenotypic variation leading to genotypic variation. Increased male reproductive success (with or without competition) may be generated if male traits are favored that directly code for sperm traits (*blue arrows*). Alternatively, male traits are favored that indirectly affect sperm function by creating environments that improve sperm function, but without altering sperm traits (*orange arrows*). Under this simple model, sperm environments can either shield sperm from external influences or generate niches that allow an increase in phenotypic space. These new sperm environments may then canalize specific phenotypes (*blue arrows*) and allow for selection of new genotypes or further extension of the sperm niche. The most obvious candidate for this model is the evolution of seminal fluid, but it also accommodates male-female coevolution.

generates, say, thermal stability in the sperm environment (**Figure 4**) and so optimizes energy expenditure (thus, no change in sperm traits). Alternatively, this sperm phenotype may benefit from a mutation that increases the permeability of the sperm membrane for sugars (**Figure 4**). These kinds of successive changes can explain environment-mediated sperm diversification if the male environment changes (e.g., generally increased sugar availability to the male), but they do not require such changes (e.g., mutations that increase male sugar uptake or allocation to seminal fluid).

Although this verbal model is exceedingly simple, we propose it may, for example, explain the evolution of species-specific ion channels or surface proteins in sperm in response to altered internal conditions (Lishko et al. 2012) as well as the evolution of seminal fluid complexity (Avila et al. 2011, Poiani 2006). Alternatively, it may provide a plausible mechanism for examples of ecological speciation in which divergence in diet specialization translated into postmating reproductive isolation (Nosil 2012). This model may also help to explain how males may adaptively alter the fertilization ability of their sperm such that sperm, or ejaculates, function best under their paternal environments (Marshall 2015). And it can incorporate coevolutionary interactions such that male traits that induce females to create sperm niches evolve (such as male seminal substances that manipulate the sperm storage ability of females; see Avila et al. 2011). Whereas genotype effects encompass the sperm-activating ability by seminal fluids of other males, even of another species (den Boer et al. 2010, Morrell et al. 2014, Usinger 1966), our model can generate coevolution-like dynamics between sperm and males, where males evolve niches to harness their own sperm.

4.2. Genotype-by-Environment Interaction Effects on Sperm Phenotype

In general, G × E interaction effects on the sperm phenotype have rarely been addressed. For example, of the 178 articles in our literature research that contained relevant data, 6 articles (3%) presented the data separately for two, or more, breeds or separate populations (broadly equivalent to genotypes). This suggests that very few of the thousands of articles reporting effects on sperm cells caused by diet, parental smoking habits, caffeine consumption, pH or temperature, salinity, antioxidants or their addition or blocking, vaginal lubricants, traditional medicinal herbs, etc., examined the generality of the effect beyond one genotype. None of these studies reported a formal G × E analysis.

We have extended our literature search in a second step and assessed the title and abstracts of all 7,445 articles without search terms (manually) for evidence of G × E interactions and of local adaptation. This resulted in a number of additional articles. Whereas no significant G × E interaction effect was reported for the sperm motility decline in two trout populations over decreasing pH (Purchase & Moreau 2012) or in two very different environments for sperm motility of Atlantic cod (Beirao et al. 2014), most other studies seem to indicate the presence of G × E interaction effects (**Table 1**). The common existence of species-specific maxima of sperm motility at different temperatures, osmolarities, or pH values that correlate to habitat conditions (**Supplemental Table 2)** (Alavi & Cosson 2005) suggests that local adaptation in sperm is common.

Supplemental Material

5. CONCLUSIONS

G × E interaction effects are likely relatively common. However, their adaptive significance and magnitude await further quantification, especially in the light of widespread sperm phenotypic plasticity. This is a major task for sperm ecology.

The coevolution-like dynamics between sperm and internal environments of males or females have evolutionary and medical consequences that can be summarized in one sentence: Do not take sperm out of context. The lack of a coevolved sperm niche for sperm tested in medical sperm

Table 1 Examples of studies examining genotype-by-environment (G × E) interaction effects or adaptive phenotypic plasticity on the sperm phenotype

Type of evidence	Level of comparison	Group	Sperm function and environments examined	Reference(s)
G × E	Interspecific	Sea urchin	Fertilization ability, but not motility, of sperm decreased with increasing CO_2 water concentration in the water in only one species.	Sung et al. 2014
G × E	Interspecific	Sea urchin	Species differed in their sperm motility response to temperature.	Rahman et al. 2009
G × E	Interspecific	Bird	Species differed in their sperm motility response to cooling protocols.	Blanco et al. 2000
G × E	Interspecific	Cichlid fish	Consistent differences in sperm motility pattern existed between mouthbrooding and externally spawning species.	Reinhardt & Otti 2012
G × E	Single nucleotide polymorphism	Ram	Temperature treatment increased DNA fragmentation in sperm only in bearers of one allele but not another.	Ramon et al. 2014
G × E	Karyotype	Mice	After irradiation, sperm defragmentation was larger in Y-bearing than in X-bearing sperm, leading to reduced egg-binding ability.	Kumar et al. 2013
G × E	Population	Cattle	Altitude and season produced changes in motility that differed between two breeds of cattle.	Barros et al. 2006, Chacur et al. 2013
G × E	Population	Chicken	Sperm of four chicken breeds differed in their susceptibility to different freezing methods.	Schramm 2008
G × E, phenotypic plasticity	Population	Guppies	Populations responded to experimental evolution under altered temperatures with an increase in sperm length, but not sperm motility. Populations also displayed phenotypic plasticity in sperm length and sperm motility.	Breckels & Neff 2014
G × E	Genotype	Humans	Exposure of males to certain environments caused altered morphology or chromatin integrity in only some male genotypes (review).	Axelsson et al. 2010
G × E	Genotype	Fruit fly	Larval-rearing density affected the sperm length of only some genotypes.	Morrow et al. 2008
G × E	Isogenic line	Fruit fly	Laboratory × genotype interaction effects accounted for 12–19% of the variation in paternity.	Clark et al. 1999
G × E	Genotype	Flour beetle	Different genotypes varied in their sperm defense ability (P1) in relation to nutritional manipulation.	Lewis et al. 2012
G × E	Genotype	Honeybee	Colonies showed an age × genotype interaction effect on sperm viability.	Stürup et al. 2013
G × E	Genotype	Cod	Significant, but likely small, G × E interaction effect was found on sperm velocity.	Purchase et al. 2010
G × E	Genotype	Bedbug	Twice as many female genes were differentially expressed in response to G × E interaction effects as opposed to genotype effects.	Otti et al. 2015

(*Continued*)

Table 1 (*Continued*)

Type of evidence	Level of comparison	Group	Sperm function and environments examined	Reference(s)
Local adaptation	Interspecific	Sea urchin	Sperm motility, metabolism, and temperature-dependent motility differed across four closely related species such that sperm function was highest under conditions resembling the native habitat.	Mita et al. 2002
Local adaptation	Interspecific	Fish	Sperm motility, metabolism, and temperature-dependent motility differed across four closely related species such that sperm function was highest under conditions resembling the native habitat.	Lindroth 1947
Local adaptation	Population	Fruit fly	Local adaptation in male fertility to temperature was found.	Rohmer et al. 2004
Local adaptation	Population	Cichlid fish	Populations from two different habitats differed in their activation threshold of sperm motility, and the threshold reflected the ionic concentrations in these habitats.	Morita et al. 2010
Local adaptation	Population	Stickleback fish	Sperm from males of a saltwater population were motile in saltwater, but sperm from freshwater or brackish water populations were not.	Elofsson et al. 2003
Local adaptation	Individual	Bees	Ejaculates contained much more live sperm when males were reared at natural temperatures in a hive versus when reared at lower or higher temperatures.	Bienkowska et al. 2011
Adaptive phenotypic plasticity?	Individual	Bedbug, cricket	Adaptive variation in sperm metabolic rates was found between male and female sperm stores.	Reinhardt & Ribou 2013, Ribou & Reinhardt 2012
Adaptive phenotypic plasticity	Individual	Sea squirt	Fertilization ability of sperm varied adaptively with population density.	Crean & Marshall 2008, Crean et al. 2013
Adaptive phenotypic plasticity	Individual	Tubeworm	Sperm kept under low salinity produced offspring that survived better under low salinity.	Ritchie & Marshall 2013

function tests may provide an explanation for the poor predictive power of sperm function tests for conception and paternity (Aitken 2006; but see Birkhead et al. 1999, Froman & Feltmann 1998). Also consistent with this assertion, the predictive power of sperm function tests would likely be further improved if sperm function were measured after sperm have had contact with the female reproductive tract (Glazener et al. 2000; also Holman & Snook 2008).

SUMMARY POINTS

1. Sperm cells have been suggested to be the morphologically most diverse cell type to have evolved via sperm competition. Here we add that sperm cells show substantial phenotypic diversity caused by environmental effects.
2. Direct and indirect environments can act immediately or in a delayed way and shape sperm function in a decisive manner.

3. Male and female environments (indirect sperm environmental effect) can differ between or—as a result of ecological specialization—within populations. In this way, natural selection may substantially contribute to the evolution of sperm diversity via local adaptation of sperm.
4. Regardless of whether postejaculatory sexual selection, i.e., sperm competition or female sperm choice, augments the explanation of sperm diversification, sexual selection will benefit from the consideration of environmental influences on sperm.
5. Applying ecological concepts may help formalize descriptions of sperm biology. Such application may also help to identify the (currently largely lacking) mechanistic basis of competition between sperm.

FUTURE ISSUES

1. What is the relative significance of sperm phenotype evolution via sperm niches (environmental effects) and via sperm traits (genotype effects)?
2. How frequently do reproductive traits evolve that act on sperm phenotypic variation that is not related to sperm genotypic characters?
3. To what extent is the outcome of sperm competition between two males repeatable across environments?
4. Are large environmental effects on the sperm phenotype the reason why sperm competition ability has low heritability (Simmons & Moore 2009), and are they the reason why genotype explains only a low proportion of reproductive success (Pischedda & Rice 2012)?
5. What are the extent and mechanisms of sperm epigenetic alterations and their effect on offspring characters?
6. Is there support for Marshall's (2015) idea that external fertilizers are more likely to show adaptive sperm phenotypic plasticity than are internal fertilizers?

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LITERATURE CITED

Adriaenssens B, van Damme R, Seebacher F, Wilson RS. 2012. Sex cells in changing environments: Can organisms adjust the physiological function of gametes to different temperatures? *Glob. Change Biol.* 18:1797–803

Aitken RJ. 2006. Sperm function tests and fertility. *Int. J. Androl.* 29:60–75

Aitken RJ, Baker MA. 2013. Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. *Int. J. Dev. Biol.* 57:265–72

Aitken RJ, Bronson R, Smith TB, De Iuliis GN. 2013. The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. *Mol. Hum. Reprod.* 19:475–85

Aitken RJ, Clarkson JS. 1988. Significance of reactive oxygen species and anti-oxidants in defining the efficacy of sperm preparation techniques. *J. Androl.* 9:367–76

Aitken RJ, De Iuliis GN, McLachlan RI. 2009. Biological and clinical significance of DNA damage in the male germ line. *Int. J. Androl.* 32:46–56

Aitken RJ, Gibb Z, Mitchell LA, Lambourne SR, Connaughton HS, De Iuliis GN. 2012. Sperm motility is lost in vitro as a consequence of mitochondrial free radical production and the generation of electrophilic aldehydes but can be significantly rescued by the presence of nucleophilic thiols. *Biol. Reprod.* 87:110

Aitken RJ, Koppers AJ. 2011. Apoptosis and DNA damage in human spermatozoa. *Asian J. Androl.* 13:36–42

Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. 2014. Oxidative stress and male reproductive health. *Asian J. Androl.* 16:31–38

Alavi SMH, Cosson J. 2005. Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biol. Int.* 29:101–10

Almbro M, Dowling DK, Simmons LW. 2011. Effects of vitamin E and beta-carotene on sperm competitiveness. *Ecol. Lett.* 14:891–95

Alvarez L, Dai L, Friedrich BM, Kashikar ND, Gregor I, et al. 2012. The rate of change in Ca^{2+} concentration controls sperm chemotaxis. *J. Cell Biol.* 196:653–63

Alvarez L, Friedrich BM, Gompper G, Kaupp UB. 2014. The computational sperm cell. *Trends Cell Biol.* 24:198–207

Aoki VW, Emery BR, Liu L, Carrell DT. 2006. Protamine levels vary between individual sperm cells of infertile human males and correlate with viability and DNA integrity. *J. Androl.* 27:890–98

Aranha I, Bhagya M, Yajurvedi HN. 2008. Concentration of cations in different parts of male reproductive system and their influence on in vitro sperm motility in lizard, *Mabuya carinata* Schneider. *Ind. J. Exp. Biol.* 46:720–24

Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF. 2011. Insect seminal fluid proteins: identification and function. *Annu. Rev. Entomol.* 56:21–40

Axelsson J, Bonde JP, Giwercman YL, Rylander L, Giwercman A. 2010. Gene-environment interaction and male reproductive function. *Asian J. Androl.* 12:298–307

Babcock DF, Wandernoth PM, Wennemuth G. 2014. Episodic rolling and transient attachments create diversity in sperm swimming behavior. *BMC Biol.* 12:67

Bahat A, Eisenbach M. 2006. Sperm thermotaxis. *Mol. Cell. Endocrinol.* 252:115–19

Banks S, King SA, Irvine DS, Saunders PTK. 2005. Impact of a mild scrotal heat stress on DNA integrity in murine spermatozoa. *Reproduction* 129:505–14

Barros CM, Pegorer MF, Vasconcelos JLM, Eberhardt BG, Monteiro FM. 2006. Importance of sperm genotype (*indicus* versus *taurus*) for fertility and embryonic development at elevated temperatures. *Theriogenology* 65:210–18

Barros P, Sobral P, Range P, Chicharo L, Matias D. 2013. Effects of sea-water acidification on fertilization and larval development of the oyster *Crassostrea gigas*. *J. Exp. Mar. Biol. Ecol.* 440:200–6

Beirao J, Purchase CF, Wringe BF, Fleming IA. 2014. Sperm plasticity to seawater temperatures in Atlantic cod *Gadus morhua* is affected more by population origin than individual environmental exposure. *Mar. Ecol. Prog. Ser.* 495:263–74

Bencic DC, Cloud JG, Ingermann RL. 2000. Carbon dioxide reversibly inhibits sperm motility and fertilizing ability in steelhead (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* 23:275–81

Bernasconi G, Ashman T-L, Birkhead TR, Bishop JDD, Grossniklaus U, et al. 2004. Evolutionary ecology of the prezygotic stage. *Science* 303:971–75

Bienkowska M, Panasiuk B, Wegrzynowicz P, Gerula D. 2011. The effect of different thermal conditions on drone semen quality and number of spermatozoa entering the spermatheca of queen bee. *J. Apic. Res.* 55:161–68

Billard R, Cosson MP. 1992. Some problems related to the assessment of sperm motility in freshwater fish. *J. Exp. Zool.* 261:122–31

Binet MT, Doyle CJ. 2013. Effect of near-future seawater temperature rises on sea urchin sperm longevity. *Mar. Freshw. Res.* 64:1–9

Birkhead TR, Fletcher F. 1995. Male phenotype and ejaculate quality in the zebra finch *Taeniopygia guttata*. *Proc. R. Soc. Lond. B* 262:329–34

Birkhead TR, Hosken DJ, Pitnick S, eds. 2009. *Sperm Biology: An Evolutionary Perspective*. New York: Academic

Birkhead TR, Martinez JG, Burke T, Froman DP. 1999. Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. R. Soc. Lond. B* 266:1759–64

Birkhead TR, Møller AP, Sutherland WJ. 1993. Why do females make it so difficult for males to fertilize their eggs? *J. Theor. Biol.* 161:51–60

Blanckenhorn WU, Hellriegel B. 2002. Against Bergmann's rule: fly sperm size increases with temperature. *Ecol. Lett.* 5:7–10

Blanco JM, Gee G, Wildt DE, Donoghue AM. 2000. Species variation in osmotic, cryoprotectant, and cooling rate tolerance in poultry, eagle, and peregrine falcon spermatozoa. *Biol. Reprod.* 63:1164–71

Breckels RD, Neff BD. 2013. The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *J. Exp. Biol.* 216:2658–64

Breckels RD, Neff BD. 2014. Rapid evolution of sperm length in response to increased temperature in an ectothermic fish. *Evol. Ecol.* 28:521–33

Brito LFC, Silva AEDF, Rodrigues LH, Vieira FV, Deragon LAG, Kastelic JP. 2003. Effects of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* AI bulls in Brazil. *Anim. Reprod. Sci.* 70:181–90

Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ, Robertson SA. 2014. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *PNAS* 111:2200–5

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R. 2004. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* 56:65–70

Burruel V, Klooster KL, Chitwood J, Ross PJ, Meyers SA. 2013. Oxidative damage to rhesus macaque spermatozoa results in mitotic arrest and transcript abundance changes in early embryos. *Biol. Reprod.* 89:72

Byrne M, Soars NA, Ho MA, Wong E, McElroy D, et al. 2010. Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Mar. Biol.* 157:2061–69

Caldwell GS, Fitzer S, Gillespie CS, Pickavance G, Turnbull E, Bentley MG. 2011. Ocean acidification takes sperm back in time. *Invert. Reprod. Dev.* 55:217–21

Chacur MGM, Mizusaki KT, Gabriel LRA, Oba E, Ramos AA. 2013. Seasonal effects on semen and testosterone in Zebu and Taurine bulls. *Acta Sci. Vet.* 41:1110

Clark AG, Begun DJ, Prout T. 1999. Female × male interactions in *Drosophila* sperm competition. *Science* 283:217–20

Crean AJ, Dwyer JM, Marshall DJ. 2013. Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology* 94:2575–82

Crean AJ, Marshall DJ. 2008. Gamete plasticity in a broadcast spawning marine invertebrate. *PNAS* 105:13508–13

Dada R, Kumar M, Jesudasan R, Fernández JL, Agrawal A. 2012. Epigenetics and its role in male infertility. *J. Assist. Reprod. Genet.* 29:213–23

Delph LF, Johannson MH, Stephenson AG. 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* 78:1632–39

den Boer SPA, Baer B, Boomsma JJ. 2010. Seminal fluid mediates ejaculate competition in social insects. *Science* 327:1506–9

Dorado J, Acha D, Galvez MJ, Ortiz I, Carrasco JJ, et al. 2013. Sperm motility patterns in Andalusian donkey (*Equus asinus*) semen: effects of body weight, age, and semen quality. *Theriogenology* 79:1100–9
Dowling DK. 2014. Evolutionary perspectives on the links between mitochondrial genotype and disease phenotype. *Biochim. Biophys. Acta* 1840:1393–403
Dowling DK, Nowostawski AL, Arnqvist G. 2007. Effects of cytoplasmic genes on sperm viability and sperm morphology in a seed beetle: implications for sperm competition theory? *J. Evol. Biol.* 20:358–68
Dowling DK, Nystrand M, Simmons LW. 2010. Maternal effects, but no good or compatible genes for sperm competitiveness in Australian crickets. *Evolution* 64:1257–66
Elofsson H, Van Look K, Borg B, Mayer I. 2003. Influence of salinity and ovarian fluid on sperm motility in the fifteen-spined stickleback. *J. Fish Biol.* 63:1429–38
Fraga GG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames B. 1996. Smoking and low antioxidants levels increase oxidative damage to sperm DNA. *Mutat. Res.* 351:199–203
Friberg U, Dowling DK. 2008. No evidence of mitochondrial genetic variation for sperm competition within a population of *Drosophila melanogaster*. *J. Evol. Biol.* 21:1798–804
Friedrich BM, Jülicher F. 2007. Chemotaxis of sperm cells. *PNAS* 104:13256–61
Froman DP, Feltmann AJ. 1998. Sperm mobility: a quantitative trait of the domestic fowl (*Gallus domesticus*). *Biol. Reprod.* 58:379–84
Froman DP, Kirby JD. 2005. Sperm mobility: phenotype in roosters determined by mitochondrial function. *Biol. Reprod.* 72:562–67
Froman DP, Wardell JC, Feltmann AJ. 2006. Sperm mobility: deduction of a model explaining phenotypic variation in roosters (*Gallus domesticus*). *Biol. Reprod.* 74:487–91
Frommel AY, Stiebens V, Clemmesen C, Havenhand J. 2010. Effect of ocean acidification on marine fish sperm (Baltic cod: *Gadus morhua*). *Biogeosciences* 7:3915–19
Gage MJG, MacFarlane CP, Yeates S, Ward RG, Searle JB, Parker GA. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: Relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* 14:44–47
Garcia-Tomas M, Sanchez J, Rafel O, Ramon J, Piles M. 2006. Variability, repeatability and phenotypic relationships of several characteristics of production and semen quality in rabbit. *Anim. Reprod. Sci.* 93:88–100
Gasparini C, Evans JP. 2014. Ovarian fluid mediates the temporal decline in sperm viability in a fish with sperm storage. *PLOS ONE* 8:e64431
Ghaleno LR, Valojerdi MR, Hassani F, Chehrazi M, Janzamin E. 2014. High level of intracellular sperm oxidative stress negatively influences embryo pronuclear formation after intracytoplasmic sperm injection treatment. *Andrologia* 46:1118–27
Glazener CM, Ford WC, Hull MG. 2000. The prognostic power of the post-coital test for natural conception depends on duration of infertility. *Hum. Reprod.* 15:1953–57
Havenhand JN, Schlegel P. 2009. Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics in the oyster *Crassostrea gigas*. *Biogeosciences* 6:3009–15
Hayes TB. 2011. Atrazine has been used safely for 50 years? *Emerg. Top. Ecotoxicol.* 3:301–24
Heifetz Y, Rivlin PK. 2010. Beyond the mouse model: using *Drosophila* as a model for sperm interaction with the female reproductive tract. *Theriogenology* 73:723–39
Heise A, Kaehn W, Volkmann DH, Thompson PN, Gerber D. 2010. Influence of seminal plasma on fertility of fresh and frozen-thawed stallion epididymal spermatozoa. *Anim. Reprod. Sci.* 118:48–53
Hirsh A. 2003. Male subfertility. *Br. Med. J.* 327:669–72
Holman L, Snook RR. 2008. A sterile sperm caste protects brother fertile sperm from female-mediated death in *Drosophila pseudoobscura*. *Curr. Biol.* 18:292–96
Holt WV, Lloyd RE. 2010. Sperm storage in the vertebrate female reproductive tract: How does it work so well? *Theriogenology* 73:713–22
Holt WV, van Look KJW. 2004. Concepts in sperm heterogeneity, sperm selection and sperm competition as biological foundations for laboratory tests of semen quality. *Reproduction* 127:527–35
Hoornstra D, Andersson MA, Johansson T, Pirhonen T, Hatakka M, Salkinoja-Salonen MS. 2004. Mitochondrial toxicity detected in a health product with a boar spermatozoan bioassay. *Altern. Lab. Anim.* 32:407–16

Hughes M, Davey KG. 1969. The activity of spermatozoa of *Periplaneta*. *J. Insect Physiol.* 15:1607–16
Immler S, Hotzy C, Alavioon G, Petersson E, Arnqvist G. 2014. Sperm variation within a single ejaculate affects offspring development in Atlantic salmon. *Biol. Lett.* 10:20131040
Innocenti P, Morrow EH, Dowling DK. 2011. Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. *Science* 332:845–48
Jenkins TG, Carrell DT. 2012. The sperm epigenome and potential implications for the developing embryo. *Reproduction* 143:727–34
Jensen N, Allen RM, Marshall DJ. 2014. Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. *Funct. Ecol.* 28:724–33
Johnson GD, Lalancette C, Linnemann AK, Leduc F, Boissonneault G, Krawetz SA. 2011. The sperm nucleus: chromatin, RNA, and the nuclear matrix. *Reproduction* 141:21–36
Kang JH, Hakimov H, Ruiz A, Friendship RM, Buhr M, Golovan SP. 2008. The negative effects of exogenous DNA binding on porcine spermatozoa are caused by removal of seminal fluid. *Theriogenology* 70:1288–96
Koppers AJ, De Iuliis GN, Finnie JM, McLaughlin EA, Aitken RJ. 2008. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. *J. Clin. Endocrinol. Metab.* 93:3199–207
Kumar D, Upadhya D, Uppangala S, Salian SR, Kalthur G, Adiga SK. 2013. Nuclear DNA fragmentation negatively affects zona binding competence of Y bearing mouse spermatozoa. *J. Assist. Reprod. Genet.* 30:1611–15
Lane M, McPherson NO, Fullston T, Spillane M, Sandeman L, et al. 2014. Oxidative stress in mouse sperm impairs embryo development, fetal growth and alters adiposity and glucose regulation in female offspring. *PLOS ONE* 9:e100832
Le Comber SC, Faulkes CG, van Look KJW, Holt WV, Smith C. 2004. Recovery of sperm activity after osmotic shock in the three-spined stickleback: implications for pre-oviposition ejaculation. *Behaviour* 141:1555–69
Leahy T, Gadella BM. 2011. Sperm surface changes and physiological consequences induced by sperm handling and storage. *Reproduction* 142:759–78
Levitan DR. 1995. The ecology of fertilization in free-spawning invertebrates. In *Ecology of Marine Invertebrate Larvae*, ed. LR McEdward, pp. 56–123. Boca Raton, FL: CRC
Levitan DR. 2000. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc. R. Soc. Lond. B* 267:531–34
Lewis C, Ford AT. 2012. Infertility in male aquatic invertebrates: a review. *Aquat. Toxicol.* 120:79–89
Lewis SM, Tigreros N, Fedina NT, Ming QL. 2012. Genetic and nutritional effects on male traits and reproductive performance in *Tribolium* flour beetles. *J. Evol. Biol.* 25:438–51
Lishko PV, Kirichok Y, Ren DJ, Navarro B, Chung JJ, Clapham DE. 2012. The control of male fertility by spermatozoan ion channels. *Annu. Rev. Physiol.* 74:453–75
Lindroth A. 1947. Time of activity of freshwater fish spermatozoa in relation to temperature. *Zoologiska Bidrag från Uppsala* 25:165–68
Lu XY, Wu RSS. 2005. Ultraviolet damages sperm mitochondrial function and membrane integrity in the sea urchin *Anthocidaris crassispina*. *Ecotoxicol. Environ. Saf.* 61:53–59
Manier MK, Lüpold S, Belote JM, Starmer WT, Berben KS, et al. 2013. Postcopulatory sexual selection generates speciation phenotypes in *Drosophila*. *Curr. Biol.* 23:1853–62
Mann T. 1964. *The Biochemistry of Semen and the Male Reproductive Tract*. London: Methuen
Marshall DJ. 2015. Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. *J. Exp. Biol.* 218:107–13
Mehlis M, Bakker TCM. 2014. The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evol. Ecol.* 28:655–67
Mita M, Uehara T, Nakamura M. 2002. Comparative studies on the energy metabolism in spermatozoa of four closely related species of sea urchins (genus *Echinometra*) in Okinawa. *Zool. Sci.* 19:419–27
Morita M, Awata S, Takahashi T, Takemura A, Kohda M. 2010. Sperm motility adaptation to ion-differing aquatic environments in the Tanganyikan cichlid, *Astatotilapia burtoni*. *J. Exp. Zool.* 313A:169–77
Morrell JM, Georgakas A, Lundeheim N, Nash D, Davies Morel MC, Johannisson A. 2014. Effect of heterologous and homologous seminal plasma on stallion sperm quality. *Theriogenology* 82:176–83

Morrow EH, Leijon A, Meerupati A. 2008. Hemiclonal analysis reveals significant genetic, environmental and genotype × environment effects on sperm size in *Drosophila melanogaster*. *J. Evol. Biol.* 21:1692–702
Mortimer ST, Swann M. 1995. Variable kinematics of capacitating human spermatozoa. *Hum. Reprod.* 10:3178–82
Mossman JA, Slate J, Birkhead TR. 2010. Mitochondrial haplotype does not affect sperm velocity in the zebra finch *Taeniopygia guttata*. *J. Evol. Biol.* 23:422–32
Murphy MP. 2009. How mitochondria produce reactive oxygen species. *Biochem. J.* 417:1–13
Nahon S, Porras VAC, Pruski AM, Charles F. 2009. Sensitivity to UV radiation in early life stages of the Mediterranean sea urchin *Sphaerechinus granularis* (Lamarck). *Sci. Total Environ.* 407:1892–900
Nosil P. 2012. *Ecological Speciation*. New York: Oxford Univ. Press
Okumura A, Fuse H, Kawauchi Y, Mizuno I, Akashi T. 2003. Changes in male reproductive function after high altitude mountaineering. *High. Alt. Med. Biol.* 4:349–53
Otti O, Johnston PR, Horsburgh GC, Galindo J, Reinhardt K. 2015. Female transcriptomic response to male genetic and nongenetic ejaculate variation. *Behav. Ecol.* 26:681–88
Otti O, McTighe AP, Reinhardt K. 2013. In vitro antimicrobial sperm protection by an ejaculate-like substance. *Funct. Ecol.* 27:219–26
Otti O, Naylor R, Siva-Jothy MT, Reinhardt K. 2009. Bacteriolytic activity in the ejaculate of an insect. *Am. Nat.* 174:292–95
Pacey AA, Hill C, Scudamore IW, Warren MA, Barratt CL, Cooke ID. 1995. Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpix. *Hum. Reprod.* 10:2603–9
Parker GA. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* 45:525–67
Parker GA. 2009. The sexual cascade and the rise of pre-ejaculatory (Darwinian) sexual selection, sex roles, and sexual conflict. In *The Genetics and Biology of Sexual Conflict*, ed. WR Rice, S Gavrilets, pp. 1–22. Cold Spring Harbor, NY: Cold Spring Harb. Lab. Press
Parker GA, Begon ME. 1993. Sperm competition games: sperm size and number under gametic control. *Proc. R. Soc. Lond. B* 253:255–62
Paul C, Murray AA, Spears N, Saunders PTK. 2008. A single, mild, transient scrotal heat stress causes DNA damage, subfertility and impairs formation of blastocysts in mice. *Reproduction* 136:73–84
Peters A, Denk AG, Delhey K, Kempenaers B. 2004. Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *J. Evol. Biol.* 17:1111–20
Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* 25:459–67
Pischedda A, Rice WR. 2012. Partitioning sexual selection into its mating success and fertilization success components. *PNAS* 109:2049–53
Pitnick S, Hosken DJ, Birkhead TR. 2009. Sperm morphological diversity. See Birkhead et al. 2009, pp. 69–149
Pizzari T, Dean R, Pacey A, Moore H, Bonsall MB. 2008. The evolutionary ecology of pre- and post-meiotic sperm senescence. *Trends Ecol. Evol.* 23:131–40
Pizzari T, Parker GA. 2009. Sperm competition and sperm phenotype. See Birkhead et al. 2009, pp. 207–45
Pizzol D, Ferlin A, Garolla A, Lenzi A, Bertoldo A, Foresta C. 2014. Genetic and molecular diagnostics of male infertility in the clinical practice. *Front. Biosci.* 19:291–303
Poiani A. 2006. Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* 60:289–310
Poland V, Eubel H, King M, Solheim C, Harvey Millar A, Baer B. 2011. Stored sperm differs from ejaculated sperm by proteome alterations associated with energy metabolism in the honeybee *Apis mellifera*. *Mol. Ecol.* 20:2643–54
Purchase CF, Butts IAE, Alonso-Fernández A, Trippel EA. 2010. Thermal reaction norms in sperm performance of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 67:498–510
Purchase CF, Moreau DTR. 2012. Stressful environments induce novel phenotypic variation: hierarchical reaction norms for sperm performance of a pervasive invader. *Ecol. Evol.* 2:2562–71
Rahman MS, Tsuchiya M, Uehara T. 2009. Effects of temperature on gamete longevity and fertilization success in two sea urchin species, *Echinometra mathaei* and *Tripneustes gratilla*. *Zool. Sci.* 26:1–8
Rajkovic J, Uyttendaele A, Deley M, Van Soom W, Rijsselaere A, et al. 2006. Dynamics of boar semen motility inhibition as a semi-quantitative measurement of *Bacillus cereus* emetic toxin (Cereulide). *J. Microbiol. Methods* 65:525–34

Ramon M, Salces-Ortiz J, Gonzalez C, Perez-Guzman MD, Garde JJ, et al. 2014. Influence of the temperature and the genotype of the *HSP90AA1* gene over sperm chromatin stability in Manchega rams. *PLOS ONE* 9:e86107

Rand DM. 2001. The units of selection on mitochondrial DNA. *Annu. Rev. Ecol. Evol. Syst.* 32:415–48

Reinhardt K. 2007. Evolutionary consequences of sperm cell aging. *Q. Rev. Biol.* 82:375–93

Reinhardt K, Otti O. 2012. Comparing sperm swimming speed. *Evol. Ecol. Res.* 14:1039–56

Reinhardt K, Ribou A-C. 2013. Females become infertile as the stored sperm's oxygen radicals increase. *Sci. Rep.* 3:2888

Reinhardt K, Siva-Jothy MT. 2005. An advantage for young sperm in the house cricket *Acheta domesticus*. *Am. Nat.* 165:718–23

Renieri T, Vegni Talluri M. 1974. Sperm modification in the female ducts of a grasshopper. *Monit. Zool. Ital.* 8:1–9

Ribou A-C, Reinhardt K. 2012. Reduced metabolic rate and oxygen radicals production in stored insect sperm. *Proc. R. Soc. Lond. B* 279:2196–203

Rick IP, Mehlis M, Esser E, Bakker TCM. 2014. The influence of ambient ultraviolet light on sperm quality and sexual ornamentation in three-spined sticklebacks (*Gasterosteus aculeatus*). *Oecologia* 174:393–402

Rickard JP, Pini T, Soleilhavoup C, Cognie J, Bathgate R, et al. 2014. Seminal plasma aids the survival and cervical transit of epididymal ram spermatozoa. *Reproduction* 148:468–78

Riemann JG, Thorson BJ. 1971. Sperm maturation in the male and female genital tracts of *Anagasta kühniella* (Lepidoptera: Pyralidae). *Int. J. Insect Morphol. Embryol.* 1:1–19

Ritchie H, Marshall DJ. 2013. Fertilisation is not a new beginning: sperm environment affects offspring development success. *J. Exp. Biol.* 216:3104–9

Rohmer C, David JR, Moreteau B, Joly D. 2004. Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. *J. Exp. Biol.* 207:2735–43

Roldan ERS, Gomendio M. 2009. Sperm and conservation. See Birkhead et al. 2009, pp. 539–64

Rosengrave P, Taylor H, Montgomerie R, Metcalf V, McBride K, Gemmell NJ. 2009. Chemical composition of seminal and ovarian fluids of chinook salmon (*Oncorhynchus tshawytscha*) and their effects on sperm motility traits. *Comp. Biochem. Physiol. A* 152:123–29

Satake N, Elliott RMA, Watson PF, Holt WV. 2006. Sperm selection and competition in pigs may be mediated by the differential motility activation and suppression of sperm subpopulations within the oviduct. *J. Exp. Biol.* 209:1560–72

Scaggiante M, Mazzoldi C, Petersen CW, Rasotto MB. 1999. Sperm competition and mode of fertilization in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). *J. Exp. Zool.* 283:81–90

Schlegel P, Havenhand JN, Gillings MR, Williamson JE. 2012. Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. *PLOS ONE* 7:e53118

Schlegel P, Havenhand JN, Obadia N, Williamson JE. 2014. Sperm swimming in the polychaete *Galeolaria caespitosa* shows substantial inter-individual variability in response to future ocean acidification. *Mar. Pollut. Bull.* 78:213–17

Schramm GP. 2008. Studies on genotype specific modified methods for cryopreservation of cock semen. *Züchtungskunde* 80:137–45

Shaliutina-Kolesova A, Gazo I, Cosson J, Linhart O. 2014. Protection of common carp (*Cyprinus carpio* L.) spermatozoa motility under oxidative stress by antioxidants and seminal plasma. *Fish Physiol. Biochem.* 40:1771–81

Simmons LW, Lovegrove M, Almbro M. 2014. Female effects, but no intrinsic male effects on paternity outcome in crickets. *J. Evol. Biol.* 27:1644–49

Simmons LW, Moore AJ. 2009. Evolutionary quantitative genetics of sperm. See Birkhead et al. 2009, pp. 405–34

Siva-Jothy MT. 2000. The young sperm gambit. *Ecol. Lett.* 3:172–74

Stürup M, Baer-Imhoof B, Nash DR, Boomsma JJ, Baer B. 2013. When every sperm counts: factors affecting male fertility in the honeybee *Apis mellifera*. *Behav. Ecol.* 24:1192–98

Sung CG, Kim TW, Park YG, Kang SG, Inaba K, et al. 2014. Species and gamete-specific fertilization success of two sea urchins under near future levels of pCO_2. *J. Mar. Syst.* 137:67–73

Tarín JJ, Pérez-Albalá S, Cano A. 2000. Consequences on offspring of abnormal function in ageing gametes. *Hum. Reprod. Update* 6:532–49

Tavares RS, Mansell S, Barratt CL, Wilson SM, Publicover SJ, Ramalho-Santos J. 2013. *p,p′*-DDE activates CatSper and compromises human sperm function at environmentally relevant concentrations. *Hum. Reprod.* 28:3167–77

Usinger RL. 1966. *Monograph of the Cimicidae*. Philadelphia: Entomol. Soc. Am.

Uthicke S, Pecorino D, Albright R, Negri AP, Cantin N, et al. 2013. Impacts of ocean acidification on early life-history stages and settlement of the coral-eating sea star *Acanthaster planci*. *PLOS ONE* 8:e82938

Vasudeva R, Deeming DC, Eady PE. 2014. Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition in *Callosobruchus maculatus*. *J. Evol Biol.* 27:1811–18

Vihtakari M, Hendriks IE, Holding J, Renaud PE, Duarte CM, Havenhand JN. 2013. Effects of ocean acidification and warming on sperm activity and early life stages of the Mediterranean mussel (*Mytilus galloprovincialis*). *Water* 5:1890–915

Villegas J, Kehr K, Soto L, Henkel R, Miska W, Sanchez R. 2003. Reactive oxygen species induce reversible capacitation in human spermatozoa. *Andrologia* 35:227–32

Wallace DC, Fan W, Procaccio P. 2011. Mitochondrial energetics and therapeutics. *Annu. Rev. Pathol. Mech. Dis.* 5:297–348

West-Eberhard MJ. 2003. *Developmental Plasticity and Evolution*. Oxford, UK: Oxford Univ. Press

Wolff JN, Ladoukakis ED, Enriquez JA, Dowling DK. 2014. Mitonuclear interactions: evolutionary consequences over multiple biological scales. *Philos. Trans. R. Soc. B* 369:20130443

World Health Organ. 2010. *WHO Laboratory Manual for the Examination and Processing of Human Sperm*. Geneva: WHO. 5th ed.

Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, et al. 2008. Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *PNAS* 105:605–10

Yee WKW, Sutton KL, Dowling DK. 2013. In vivo male fertility is affected by naturally occurring mitochondrial haplotypes. *Curr. Biol.* 23:R55–56

Yeung CH, Barfield JP, Cooper TG. 2006. Physiological volume regulation by spermatozoa. *Mol. Cell. Endocrinol.* 250:98–110

Zini A, Al-Hathal N. 2011. Antioxidant therapy in male infertility: fact or fiction? *Asian J. Androl.* 13:374–81