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.
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...
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

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).

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 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, Tarin 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., Burrue 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.

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 × E interactions

**Sperm competition**

Sperm phenotype = male genotype

Diversification via G × G interactions

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**Unit of study**

- **Sperm fitness**
  - Overall E effect on sperm phenotype
  - Time
  - E effect over time (e.g., fitness episodes 1 to 4)

**Origin of sperm phenotypic variation**

Environmental: Different environments cause different sperm functions, different temporal variation (polyandry or monogamy):

**Evolution of sperm genotypes**

Divergent natural selection of different genotypes in different environments:

**Maintenance of sperm genotypic diversity**

- Only environmental effect
- G × E interaction effect on sperm phenotype
- G × E × time interaction effect on sperm phenotype

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**General observation**

Total sperm fitness of sperm genotype considered; temporal and environmental variation not relevant

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**G × G interaction**

Sperm competition nontransitive

Only genotypic effect

Sperm competition transitive (good genes)

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**G × E interaction**

Sperm competition

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**Female**

- Competitor sperm mutant favored under polyandry

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**Genotypic (mutations)**

E1 E2 E3

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**E1 E2**

- Overall E effect
- E × time effect
- E × time effect, with trade-offs

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**Maintenance of sperm genotypic diversity**

- Diversity maintained
- Diversity not maintained
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.

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 effects on sperm within ecology and evolution.
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.

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 phenotypic 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.
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 (Viljantari 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 ($G \times E$), 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 directly favor sperm traits (Figure 4). Sperm phenotypic plasticity opens a second route for selection: Male traits may be favored that directly favor sperm traits (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](image)

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.

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

<table>
<thead>
<tr>
<th>Type of evidence</th>
<th>Level of comparison</th>
<th>Group</th>
<th>Sperm function and environments examined</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G × E</td>
<td>Interspecific</td>
<td>Sea urchin</td>
<td>Fertilization ability, but not motility, of sperm decreased with increasing CO₂ water concentration in the water in only one species.</td>
<td>Sung et al. 2014</td>
</tr>
<tr>
<td>G × E</td>
<td>Interspecific</td>
<td>Sea urchin</td>
<td>Species differed in their sperm motility response to temperature.</td>
<td>Rahman et al. 2009</td>
</tr>
<tr>
<td>G × E</td>
<td>Interspecific</td>
<td>Bird</td>
<td>Species differed in their sperm motility response to cooling protocols.</td>
<td>Blanco et al. 2000</td>
</tr>
<tr>
<td>G × E</td>
<td>Interspecific</td>
<td>Cichlid fish</td>
<td>Consistent differences in sperm motility pattern existed between mouthbrooding and externally spawning species.</td>
<td>Reinhardt &amp; Otti 2012</td>
</tr>
<tr>
<td>G × E</td>
<td>Single nucleotide polymorphism</td>
<td>Ram</td>
<td>Temperature treatment increased DNA fragmentation in sperm only in bearers of one allele but not another.</td>
<td>Ramon et al. 2014</td>
</tr>
<tr>
<td>G × E</td>
<td>Karyotype</td>
<td>Mice</td>
<td>After irradiation, sperm defragmentation was larger in Y-bearing than in X-bearing sperm, leading to reduced egg-binding ability.</td>
<td>Kumar et al. 2013</td>
</tr>
<tr>
<td>G × E</td>
<td>Population</td>
<td>Cattle</td>
<td>Altitude and season produced changes in motility that differed between two breeds of cattle.</td>
<td>Barros et al. 2006, Chacur et al. 2013</td>
</tr>
<tr>
<td>G × E</td>
<td>Population</td>
<td>Chicken</td>
<td>Sperm of four chicken breeds differed in their susceptibility to different freezing methods.</td>
<td>Schramm 2008</td>
</tr>
<tr>
<td>G × E, phenotypic plasticity</td>
<td>Population</td>
<td>Guppies</td>
<td>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.</td>
<td>Breckels &amp; Neff 2014</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Humans</td>
<td>Exposure of males to certain environments caused altered morphology or chromatin integrity in only some male genotypes (review).</td>
<td>Axelsson et al. 2010</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Fruit fly</td>
<td>Larval-rearing density affected the sperm length of only some genotypes.</td>
<td>Morrow et al. 2008</td>
</tr>
<tr>
<td>G × E</td>
<td>Isogenic line</td>
<td>Fruit fly</td>
<td>Laboratory × genotype interaction effects accounted for 12–19% of the variation in paternity.</td>
<td>Clark et al. 1999</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Flour beetle</td>
<td>Different genotypes varied in their sperm defense ability (P1) in relation to nutritional manipulation.</td>
<td>Lewis et al. 2012</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Honeybee</td>
<td>Colonies showed an age × genotype interaction effect on sperm viability.</td>
<td>Stirrup et al. 2013</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Cod</td>
<td>Significant, but likely small, G × E interaction effect was found on sperm velocity.</td>
<td>Purchase et al. 2010</td>
</tr>
<tr>
<td>G × E</td>
<td>Genotype</td>
<td>Bedbug</td>
<td>Twice as many female genes were differentially expressed in response to G × E interaction effects as opposed to genotype effects.</td>
<td>Otti et al. 2015</td>
</tr>
</tbody>
</table>

(Continued)
Table 1  (Continued)

<table>
<thead>
<tr>
<th>Type of evidence</th>
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<th>Sperm function and environments examined</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local adaptation</td>
<td>Interspecific</td>
<td>Sea urchin</td>
<td>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.</td>
<td>Mita et al. 2002</td>
</tr>
<tr>
<td>Local adaptation</td>
<td>Interspecific</td>
<td>Fish</td>
<td>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.</td>
<td>Lindroth 1947</td>
</tr>
<tr>
<td>Local adaptation</td>
<td>Population</td>
<td>Fruit fly</td>
<td>Local adaptation in male fertility to temperature was found.</td>
<td>Rohmer et al. 2004</td>
</tr>
<tr>
<td>Local adaptation</td>
<td>Population</td>
<td>Cichlid fish</td>
<td>Populations from two different habitats differed in their activation threshold of sperm motility, and the threshold reflected the ionic concentrations in these habitats.</td>
<td>Morita et al. 2010</td>
</tr>
<tr>
<td>Local adaptation</td>
<td>Population</td>
<td>Stickleback fish</td>
<td>Sperm from males of a saltwater population were motile in saltwater, but sperm from freshwater or brackish water populations were not.</td>
<td>Elofsson et al. 2003</td>
</tr>
<tr>
<td>Local adaptation</td>
<td>Individual</td>
<td>Bees</td>
<td>Ejaculates contained much more live sperm when males were reared at natural temperatures in a hive versus when reared at lower or higher temperatures.</td>
<td>Bienkowska et al. 2011</td>
</tr>
<tr>
<td>Adaptive phenotypic plasticity?</td>
<td>Individual</td>
<td>Bedbug, cricket</td>
<td>Adaptive variation in sperm metabolic rates was found between male and female sperm stores.</td>
<td>Reinhardt &amp; Ribou 2013, Ribou &amp; Reinhardt 2012</td>
</tr>
<tr>
<td>Adaptive phenotypic plasticity</td>
<td>Individual</td>
<td>Tubeworm</td>
<td>Sperm kept under low salinity produced offspring that survived better under low salinity.</td>
<td>Ritchie &amp; Marshall 2013</td>
</tr>
</tbody>
</table>

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?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED


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, 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 thiolis. *Biol. Reprod.* 87:110


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


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


Gasparini C, Evans JP. 2014. Ovarian fluid mediates the temporal decline in sperm viability in a fish with sperm storage. PLOS ONE 8:e64431


Hayes TB. 2011. Arratine has been used safely for 50 years? Emerg. Top. Ectotoxicol. 3:301–24


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SUPPLEMENTAL MATERIAL

1. FUNCTIONAL COMPONENTS OF THE SPERM PHENOTYPE CONSIDERED

The main functions of the sperm cell are the transport of the genetic material, the fertilization of the egg and, as increasingly recognized, the provision of the zygote. Selection will favor male traits that increase any of these three functions but the various research fields differ in how these aspects are examined. Before examining how the sperm phenotype is influenced by environmental factors, we provide a brief overview of the most commonly measured components of the sperm phenotype.

We distinguish four phases, i) 'sperm manufacture' and consider ii) a post-ejaculation pre-fertilization period, iii) fertilization and iv) zygote and offspring development.

**Manufacture stage**
The manufacture of sperm requires four phases (White-Cooper et al. 2009): establishment of germline, proliferation of germ cells, production of spermatids by meiosis, and the differentiation of mature sperm. During manufacture, germ cell production may be disturbed such that fewer sperm are produced (this case is not considered here as it does not affect the function of manufactured sperm), increased sperm mortality, reduced DNA or membrane integrity and others. This has been most prominently known for endocrine disruptors, temperature shocks or radiation effects. During the transformation of spermatids to spermatozoa, when the nuclear material is packaged into the small nucleus of the sperm, histones are replaced by protamines (Oliva 2006, White-Cooper 2009). The ratio of the different protamines may be used as a proxy for altered sperm function because it can be related to offspring development (Oliva 2006) and hence represents an epigenetic signature. Other examples of possible epigenetic marks being imposed during the manufacture stage include variation in DNA methylation that was correlated with sperm motility (Pacheco et al. 2011), sperm DNA fragmentation (Tunc & Tremellen 2009) and embryo development (Hammoud et al. 2009).

**Post-ejaculation pre-fertilization period**
Mature sperm encounter more chemical and physical environments as they move from the manufacture site through the male and female reproductive tract or into the external environment. The chemistry and physics of the reproductive tract will be influenced by the male's and female's environment, lifestyle and parasitism (Fig. S1).

Many environment (E) effects act immediately but E effects may also act delayed. The most obvious example of a delayed action concerns sperm cells that result in altered offspring development because of environmental sperm damage or epigenetic alterations. Delayed effects may also simply occur because increased metabolic activity at one stage leads to lower availability of energy reserves at a later stage (trade-offs).

⇒ Figure S1

**Sperm morphology.**
The proportion of abnormal spermatozoa is used in hundreds of studies (WHO 2010). External morphological characteristics of sperm have received substantial attention
across many animal taxa, in particular sperm length and similar dimensions but "there is insufficient evidence at present to derive general biological principles regarding the evolution of sperm form" (Pitnick et al. 2009). Unusual sperm head dimensions have been used to infer improper morphological integrity, as have been the proportion of sperm with more than one tail, or with a liquid droplet exuding from the tail. 

*Intracellular morphological or ultrastructural studies* have received most attention but mainly concerned between-species comparisons (Jamieson 1987, reviewed by Pitnick et al. 2009) and so it is not clear whether sperm diversification is the result of direct selection on sperm traits, or a by-product of other processes.

**Sperm metabolism**

*Sperm chemistry.* The protein composition of sperm (Dorus & Karr 2009, Poland et al. 2011) provides insights into potential sperm function but can have limitations in predicting sperm function. For example, Poland et al. (2011) show differences in the abundance of a mitochondrial enzyme between ejaculated and stored sperm of honeybees but the physiological activity of the enzyme did not differ between the sperm types. Species appear to differ in the type of metabolism, some use primarily respiration (Warburg 1915 in Mann 1964, Werner & Simmons 2008, Koppers et al. 2008), others glycolysis (Bedford & Hoskins 1990, Werner & Simmons 2008) but both pathways seem possible in most, if not all, species (reviewed by Mann 1964, Bedford & Hoskins 1990, Baccetti 1998, Cummins 2009). Different types of metabolism may be linked to different sperm functions (Miki 2007) and so represent within-male variation. Not unrelated, the sperm metabolic rate can differ between males (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013) but also within males, measured when collected from the male or female (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013). The relationship between sperm metabolism and ROS production has also been considered (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013) and the nature of this relationship depends on environmental sources, particularly oxygen pressure (Balaban et al. 2005, Murphy 2009). ATP content of sperm has been used to characterize energy supplies (Burness et al. 2004). The tiny cytosol of sperm may require spermatozoa to use external metabolites (Mann 1964, Blum et al. 1962, Osanai et al. 1987), and the usage of external metabolites had been used to characterize sperm metabolism. *Mitochondrial function* has been used to describe sperm metabolism. Koppers et al. (2008) showed that 68% of sperm motility was determined by the sperm mitochondria. However, if the type of sperm metabolism is plastic, the study of mitochondrial function in sperm is not necessarily closely linked to fitness. Some results indicate that sperm function depends on the mitochondrial haplotype (Yee et al. 2013, but see Mossmann et al. 2009), or on its interaction with the nuclear genotype (Yee et al. 2013). ROS production also differs between mitochondrial haplotypes (Moreno-Loshuertos et al. 2006) and may have a direct impact on sperm function but also can be mediated by ubiquitin tagging of faulty mitochondria (Sutovsky 1999).

In summary, sperm metabolic traits have been widely measured but they are related to fitness in a straightforward way.

**Sperm motility**
Many aspects of sperm motility and velocity are measured in medical, veterinary and evolutionary research. The most common is the proportion of motile sperm (WHO 2010). There are many different ways of measuring sperm motility, sperm velocity, or sperm swimming speed (WHO 2010), which all mean slightly different things but are easily accessible by computer-assisted sperm analysis (e.g. Kime et al. 2001, WHO 2010). One rationale is that abnormal sperm may swim slower (Katz et al. 1982) and so may be less capable of reaching the egg or are outcompeted by faster, normal sperm (Froman & Feltmann 1998). Other rationales are that a male infertility factor may simultaneously affect sperm motility and fertilization ability (Sharpe 1994, Aitken & Baker 2004) and that faster sperm are assumed to reach the egg faster and so provide an advantage to the male under competition. For external fertilizers this rationale will hold true, at least partly, but the evidence is conflicting and studies have widely been plagued by methodological difficulties (Reinhardt & Otti 2012). For internal fertilizers it is less clear how sperm motility or swimming speed contributes to fertilization success (but see Froman et al. 2006 for a model in chicken). For humans, the WHO handbook (2010) recommends motility measurements but there seems no general consensus of how well motility predicts conception rate (Aitken 2006). Reasons for the hampered predictability include i) that the actual crawling movement of sperm of internal fertilizers along wall structures has little resemblance to swimming (Woolley 2003), ii) that male and female passive transport processes play important roles (e.g. Werner & Simmons 2008) and iii) the difficulty of identifying a time point at which it is useful to compare sperm motility (Kime et al. 2001, Rosengrave et al. 2009, Reinhardt & Otti 2012). Several studies measure flagellum beat frequency (Billard & Cosson 1992) or the intracellular Ca2+ influx or concentration, or pH, as these parameters can translate directly into sperm motility.

In summary, measurements of sperm motility are easy to obtain but there seems no general consensus of how good motility predicts fertilization ability under competitive or non-competitive (conception rate) situations. Sperm motility measurements frequently change during the life of a sperm cell, and in different environments.

Sperm mortality
For decades, now accelerated by the availability of a commercial kit, measuring the proportion of live sperm in a sperm population, or 'sperm viability' has been standard in medicine (reviewed Mann 1964, WHO 2011). This parameter became popular in ecology and evolution research (Holman 2009) though few of the difficulties outlined by Holman (2009) have been addressed since. For example, 'sperm viability' depends on total sperm number (Holman 2009). In addition, a snapshot value of say 50% dead sperm in a sperm population conveys little information about the performance of the 50% living sperm (Figure S2). Some of these criticisms may be circumvented by estimating sperm mortality from sperm viability measurements at repeated intervals (Otti et al. 2013). It is not clear whether a high proportion of dead sperm necessarily can be equated with low fitness (see 3.5. Sperm Viability Is Not a Good Fitness Indicator in the main text). Similar concerns apply to measuring the apoptotic activity in sperm. Sperm longevity is also measured, often expressed as the time when 90%, or 95% of sperm stop moving but this parameter is prone to be affected by trade-offs and may only be a useful fitness measure under some circumstances (e.g. Levitan 2000).
In summary, the proportion of dead (or live) sperm is only a useful measure if its change is assessed over time. Nevertheless, it is not clear whether more dead sperm equate with lower fitness.

**Fertilization stage**

**Fertilization ability.**

**DNA strand breakage or DNA fragmentation** assays (Simon & Carrell 2013) are used by hundreds of studies. There are three main issues of DNA damage in sperm. First, when it occurs during spermiogenesis it leads to apoptosis (Aitken & Baker 2014), i.e. the fitness loss for males consists in fewer functional sperm being produced. Second, DNA damage readily occurs in mature spermatozoa. Here it can cause fitness loss through reduced sperm mobility. Third, a substantial body of evidence has accumulated to show that DNA damage in sperm can disturb embryo development, result in miscarriage or increase offspring morbidity (Cooke et al. 2003, Borini et al. 2006, Aitken et al. 2009, Zini & Sigman 2009, Ribas-Maynou et al. 2012, Gawecka et al. 2013, Aitken et al. 2014). The latter notion is important because it shows that damaged spermatozoa not always get outcompeted by non-damaged spermatozoa, which is highly relevant to assisted reproduction technology in humans and other mammals but is also likely to have an evolutionary impact. That the proportion of sperm with strand breaks can be a strong predictor of fertility outcomes in a variety of organisms, including in humans and fish (Agarwal & Said 2003) suggests that any sperm functions occurring between the time of strand break measurements and fertilization are little influenced by DNA strand breaks. There are also differences in whether here are single-strand DNA fragmentation or double-strand breaks, the former impacting on the fertilization ability of sperm, the latter appear to influence the zygote (Ribas-Maynou et al. 2012). **Penetration assays.** Hamster-oocyte penetration assays (WHO 2010), gel-penetration essays (Froman et al. 2006), mucus penetration (Aitken 2006) or the ability of avian sperm to reach the perivitelline layer of the egg (Alexander et al. 1993). **Sperm zona binding,** the binding of sperm to the zona pellucida as well as other binding assays (Miller et al. 1998), **acrosome integrity** (WHO 2010) and **plasma membrane integrity** have also been used (WHO 2010).

Competitive fertilization ability, or **sperm competition** success is a standard measurement of evolutionary biology (Boorman & Parker 1976, Simmons 2001, Birkhead et al. 2009). It uses molecular or phenotypic markers to estimate the proportion of eggs fertilized, or offspring produced, by the sperm of two or more competing ejaculates. This character is somewhat difficult to directly take as a measure of sperm function because both of its components, **sperm offense** and **sperm defense** (Rice 2000) include effects of seminal fluids (which can be modulated independently from sperm - Wigby et al. 2009, Reinhardt et al. 2011) and which can have dramatic consequences for embryo development (Bromfield 2014). Sperm offense and defense also depend on the ejaculate characteristics of the competing male, as well as on characteristics of the female in which sperm competition is played out.

In summary, there is a variety of measures for fertilization ability, each with their advantages and disadvantages. For example, while DNA fragmentation has relatively strong correlations to offspring quality, the competitive fertilization ability can be investigated with large sample sizes and may provide a more sensitive measure than fertilization ability under non-competitive situations (Dziuk 1996).
Zygote and offspring development

Zygote and offspring development rate can be scored directly. However, there are also several surrogates. **Presence of essential RNAs.** In several mammals, small paternal RNA is present in the sperm nucleus. It is carried over from spermatogenesis (Miller 2000), transferred to the oocyte and incorporated into the zygote (Miller 2000, Ostermeier et al. 2004, Kravetz 2013). If missing, zygote development fails (Ostermeier et al 2004, Johnson et al 2011, Rousseaux & Khochkin 2011). There is also a range of epigenetic signatures that is related to offspring performance (Johnson et al. 2011).
2. CHARACTERIZATION AND QUANTIFICATION OF ENVIRONMENTAL VARIATION ON THE SPERM PHENOTYPE

Methods

Literature search. We searched the Web of Science using the following terms in the advanced search option:

TOPIC =(sperm NOT "sperm whale" AND (velocity OR "swimming speed" OR viability OR motility OR ATP OR metabol* OR morpholog* OR glycol* OR "oxidative phosphoryl* OR "membrane potential" OR "flagellum beat" OR "strand breakage OR fragmentat* OR apoptosis OR competition OR epigenetic* OR "fertilization potential" OR function OR fertility)) NOT research area = plant sciences NOT document type = (Meeting Abstract OR Meeting Summary OR Editorial Material). The final update on 22 Dec 2014 yielded 27514 articles and we restricted our search to articles published 2000-2015.

Separately for each environment, the abstracts were sorted alphabetically by the last name of the first author and divided into batches of 50. One early, one middle and one late batch of 50 were randomly selected and the abstracts examined in detail, sometimes using additional restrictive search terms (Table S1). Hence, for six environments, the abstracts of 900 articles were examined. Of these, 357 appeared suitable and the papers were included if they matched the following criteria:

1) At least one aspect of fitness-relevant sperm function considered (see FUNCTIONAL COMPONENTS OF THE SPERM PHENOTYPE). Effects on sperm counts, concentrations, ejaculate volume, fertility alone were not included as they do not represent aspects of the sperm phenotype

2) Not exclusively epigenetic effects (e.g. maternal or paternal exposure)

3) Original data, no reviews

4) Available through our institutional library, including open-access journals.

5) Containing at least semi-quantitative data (correlations, statements of the direction of the effect).

We calculated the mean ± 1 SD of relevant articles per 50-article batch per environment. We then used the lower standard deviation to predict a minimum number of papers being relevant among those published since 2000, and the upper standard deviation to predict a maximum number of relevant papers.

Table S1. Overview of articles resulting from a randomized selection of articles on environmental effects on sperm function.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Total number of articles found in database</th>
<th>Number of relevant articles, of 50 randomly selected ones (mean ± SD)</th>
<th>Projected number of relevant articles since 2000 min - max (mean)</th>
<th>Search terms used</th>
<th>Remarks, additional restrictive search terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1230</td>
<td>15.0 ± 4.0</td>
<td>271-467 (369)</td>
<td>temperature</td>
<td>includes season, cooling duration at</td>
</tr>
<tr>
<td>Source</td>
<td>Year</td>
<td>Mean ± SD</td>
<td>Min - Max (n)</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>-----------</td>
<td>---------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>2013</td>
<td>7.0 ± 1</td>
<td>241-322 (282)</td>
<td>oxidant stress OR antioxidant OR ROS OR reactive oxygen species OR oxygen radicals NOT contraceptive* OR &quot;maternal exposure&quot; OR &quot;paternal exposure&quot; OR irradiation OR &quot;phone*&quot; OR magnet* OR cryo*)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>722</td>
<td>3.0 ± 2</td>
<td>14-72 (43)</td>
<td>pH NOT TS=(ATPase OR NOX5 OR CatSper OR &quot;PH-20&quot; OR &quot;ion channel&quot; OR &quot;ion pump&quot; OR &quot;sodium channel&quot; OR &quot;calcium-dependent&quot; OR &quot;potassium channel&quot;)</td>
<td></td>
</tr>
<tr>
<td>Osmolarity</td>
<td>383</td>
<td>12.0 ± 3.46</td>
<td>65-118 (92)</td>
<td>salinity OR osmolarity OR osmolality includes seminal fluid or ovarian fluid dilution but not if merely two extenders were tested</td>
<td></td>
</tr>
<tr>
<td>Lifestyle</td>
<td>2369</td>
<td>17.0 ± 6.24</td>
<td>510-1101 (805)</td>
<td>diet OR food OR lifestyle OR exposure - NOT (&quot;sperm production&quot; OR &quot;intracytoplasmic sperm injection&quot; OR contraceptive* OR &quot;maternal exposure&quot; OR &quot;paternal exposure&quot; OR &quot;testicular injury&quot; OR irradiation OR &quot;mobile telephone&quot; OR &quot;cell phone&quot; OR magnet* OR cryo*) - does not includes maternal and paternal exposure and its effect on semen quality - does not include 'light exposure' or photoperiod or temperature</td>
<td></td>
</tr>
</tbody>
</table>
| Microbes | 1325 | 8.33 ± 0.57 | 205-236 (221) | microb* OR virus - does not include infection or
Summary of effects

Oxygen, reactive oxygen species (ROS) and oxidative stress. Sperm are well-known for their particularly high susceptibility to intracellular or extracellular oxygen radicals and the resulting oxidative stress. ROS have important cellular functions but when in imbalance cause oxidative stress to sperm, a topic that has been covered by numerous reviews (Zini & Al-Hathal 2011, Lavranos et al. 2012, Aitken et al. 2014). ROS are the proximate mechanisms for many, if not most, types of sperm damages by the environment, including male inflammation, diet, lifestyle, hormones or exposure to toxins, as well as sperm metabolism or bacterial attacks (Samanta & Chainy 1997, Fraczek et al. 2012, Aitken et al. 2014). The effects can be cumulative and generate continuous variation in sperm function but can also be rescued (Aitken et al. 2012).

Cellular episodes concerned: Variation in oxygen levels causes substantial variation in many aspects of sperm function, ranging from motility, longevity, mortality, metabolic changes as well as altered production of oxygen radicals (Menezo et al. 2010, Zini & Al-Hathal 2011, Aitken et al. 2014). ROS levels in stored sperm were also correlated to increasing sperm-related infertility (Reinhardt & Ribou 2013).

An interesting aspect is that ROS may damage the nuclear DNA but without affecting sperm motility and functionality. Selection may, therefore, not act before fertilization but the resulting offspring can be nonviable or have slower growth or higher morbidity (Ji et al. 1997, Aitken et al. 2009, Lee et al. 2009, Ribas-Maynou et al. 2012, Gawecka et al. 2013, Aitken et al. 2014). In two species of insects, females reduced the metabolic rate of sperm thereby reducing the production of oxygen radicals (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013).

Quantifying the impact of ROS is plagued by the fact that the most common method to measure ROS is based on chemoluminescence (Kashou et al. 2013), which has been shown to be unsuitable because the analytical reaction used to measure ROS itself produces ROS (Fridovich 1997).

Effect size: small for some functions, large to detrimental in others.

Onset: ROS impacts can be short or sustained, but they often accumulate (e.g. sperm ageing – Reinhardt 2007) and their effect is then also delayed. Some ROS damage can be repaired in the female (Menezo et al. 2010) but beyond a certain threshold, ROS effects seem irreversible. Many effects are long lasting within a male. For example, sperm
velocity in a songbird was altered six days after an immune challenge, an effect not seen with supplementation of antioxidants (Losdat et al. 2011). Similarly, a brief visit to high altitudes led to altered sperm morphology one month after the visit (Okumura et al. 2003). Effects can often be carried over to the offspring (see examples above).

**Temperature**
Sperm of many species survive substantial subzero temperatures for many years, with small to substantial damage (Leahy & Gadella 2011). High temperatures can kill sperm, affect sperm function per se but also via the viscosity the sperm cell experiences. The viscosity change caused by temperature changes contributed as much to the variation in sperm velocity as the temperature effect itself (Kupriyanova & Havenhand 2005). This notion deserves more widespread treatment for species where males and females move through a habitat or experience increased body temperature due to inflammation or infection. It is noteworthy that mammalian sperm possess thermotaxis, which is related to changes in motility pattern (Bahat & Eisenbach 2006).

The rich older literature on temperature effects on sperm motility is reviewed by Mann (1964) and Young (1961) with recent additions (Alavi & Cosson 2005, Werner & Simmons 2008).

A very detailed analysis by Purchase et al. (2010) shows that sperm motility varies with temperature, showing an intermediate peak, which disappeared, however, when sperm were examined after longer swimming periods. Temperature is an ideal candidate to affect the universal motility-longevity trade-off. Endothermic species can also possess marked temperature gradients in the male (Banks et al. 2005) and female reproductive tract (Hunter 2012). This suggests that successful sperm cells will need to be phenotypically plastic to respond to temperature. Studies finding no effect of temperature are rare but across a gradient for 18 to 26°C, Byrne et al. (2010) did not find the fertilization success of marine invertebrates affected. The effects of temperature acclimatization of males on sperm function have only begun to be addressed (Adriaenssens et al. 2012, Breckles & Neff 2014). It would be interesting to know whether any acclimatization effects are due to male or cellular plasticity.

Cellular episodes concerned: Many episodes are affected, including sperm activation rate and sperm longevity (Sharpe 2010, Prasad et al. 2011, Wang et al. 2011, Albright & Mason 2013, Stürup et al. 2013), fertilization rate (Fong et al. 1995), metabolism and motility (Richards 1963, Adriaenssens et al. 2012). Exposing males, sperm or sperm-storing females to low temperature extends sperm longevity (Mellanby 1939, Parker 1970, Hunter 2009). Stürup et al. (2013) report that heat treatment on male bees produced negative effects on sperm survival that were additive to heat treatment of sperm. Offspring effects were rarely examined. Sperm surviving a short pulse of high temperature produced normal offspring in guinea pigs (Young 1961). Temperature affected the protamination pattern of sperm (Evenson et al. 2000, Iguchi et al. 2006), which is an important epigenetic component of offspring health (Oliva 2006).

Effect size: Overall large, but moderate under temperatures representing natural ranges. Temperature usually produces an intermediate optimum.

Onset: Temperature action is typically sustained and immediate (activation, motility, metabolism) and most of its effects are reversible. When sustained it can have delayed effects, as seen in the case of sperm length variation with temperature in a fish
(Adriaenssens et al. 2012, Breckles & Neff 2014) and an insect (Blanckenhorn & Hellriegel 2002; Hellriegel & Blanckenhorn 2002). Temperature effects likely also cause delayed effects on sperm function via altered cellular energy depletion. Increased temperatures can increase DNA fragmentation in sperm (Table S1). Epigenetic effects have been little examined.

pH
The motility of sperm of virtually all species varies with pH (Alavi & Cosson 2005, Werner & Simmons 2008, Valdebenito et al. 2009, Lishko et al. 2012) because the ion channels have a pH-dependent Calcium ion influx that directly translates into motility (Lishko et al. 2012). The exhaustive early literature, reviewed by Mann (1964), shows that even small changes in pH can substantially alter sperm motility, metabolism and mortality, that these parameters usually peak at some intermediate value and that species differ in the pH at which sperm performance peaks. This is also supported by recent reviews on fish (Alavi & Cosson 2005, Valdebenito et al. 2009), insects (Werner & Simmons 2008) and mammals (Liu et al. 2012) as well as experimental studies (Ingermann et al. 2002, Ericson et al. 2010, Wang et al. 2011, Purchase and Moreau 2012, Albright and Mason 2013).

Cellular episodes concerned: activation, metabolism, motility, fertilization ability, survival. pH changes induce capacitation in mammals (Lishko et al. 2012) and probably in most other animals. The fertilization rate seems pH-independent in some species (Byrne et al. 2010) but not in others (Ericson et al. 2010, Albright & Mason 2013). Transgenerational effects have not been reported.

Effect size: large across the whole experimental spectrum, small to moderate across naturally occurring ranges.

Onset: Immediately, reversible, and due to energy expenditure partly cumulative. No delayed effects have been reported.

Osmolarity and salinity
The extensive early literature on osmotic effects on sperm functions has been reviewed by Mann (1964) and Young (1961), several more recent reviews cover osmolarity effects on sperm motility in fish (Alavi & Cosson 2006, Valdebenito et al. 2009) and insects (Werner and Simmons 2008). Recent reviews stress the role of ion channels (Lishko et al. 2012) and aquaporins, both of directly impact sperm motility (Chen & Duan 2011).

Cellular episodes concerned: Sperm volume (Chen & Duan 2011), activation rate (Mann 1964, Fong et al. 1995, Wang et al. 2011), motility (universal), fertilization ability (Fong et al. 1995) and sperm survival (Wang et al. 2011) are affected. A broadcast spawning marine worm showed reduced developmental success of offspring sired by sperm with decreased salinity (Ritchie & Marshall 2013). Most importantly, offspring performed better if they grew up in the same environment under which sperm lived.

Effect size: Under the range of natural osmolarities small to moderate effects.

Sperm motility typically varies quantitatively and peaks at intermediate osmolarities across several taxa (Shepherd 1974, Utsugi 1993, Tsui et al. 2012). The intermediate osmolarity peak for motility differs between species (Alavi & Cosson 2006, Werner & Simmons 2008, Valdebenito et al. 2009) and this peak seems to reflect the habitat conditions. For example, sperm motility was highest at seawater salinity in marine fish
(Yeganeh et al. 2008, Cosson et al. 2010). However, lower natural salinity increased sperm longevity in a fish (Cosson et al. 2010) and cuttlefish (Wang et al. 2011). Suboptimally high seminal fluid osmolarity (> 340 mOsm/L) had a negative effect on human sperm motility (Rossato et al. 2002).

Onset: Effects are immediate and usually sustained. They can be partially reversible (Alavi & Cosson 2006 for sperm motility) or permanent (via effects on sperm mitochondria and morphology - Utsumi 1993). Trade-offs with sperm longevity (Hughes & Davey, 1969) and energy depletion can cause osmolarity effects to be cumulative and delayed.

**Sexually transmitted and sperm-associated microbes**

Dozens of studies reveal that sperm function is affected by viruses (e.g. Foresta et al. 2010) and bacteria (Diemer et al. 2003, Eley et al. 2005, Fraczek et al. 2012) and this concerns humans (Diemer et al. 2003, Eley et al. 2005), livestock (Aurich & Spergser 2007) or insects (Otti et al. 2013). In addition to sexually transmitted diseases (Lockhart et al. 1996), microbes of urogenital or external origin (Diemer et al. 2003, Table S1) in case of injury other origin can affect sperm function. That the vulnerability of sperm to microbes has generally been recognized in animal breeding is obvious from the fact that even short-term storage media for sperm to be used in artificial insemination contain antibiotics (Aurich & Spergser 2007).

Cellular episodes concerned: Membrane properties, mitochondrial function, motility, and survival can be affected (e.g. Diemer et al. 2003, Fraczek et al. 2012, Otti et al. 2013). Sperm-egg interactions may also be affected (Karr et al. 2009) and offspring resulting from sperm attacked by microbes can have reduced survival ability (Sylla et al. 2005). Effect size: Reported reductions in sperm function range from no effect to almost total mortality. However, many studies are in vitro studies using concentration of unknown relevance to natural situations. Effects can be sustained (attachment to sperm) and cumulative (via ROS damage, see below and energy depletion) and visible immediately (reducing sperm survival) or delayed (accelerated energy expenditure of sperm, inflammation effects in males).

Onset: Immediately to probably delayed, depending on how precisely microbes affect sperm, viz competition between microbes and sperm for metabolites, microbe toxins or microbial alterations of the surrounding pH, mechanical attachment and hindrance (Foresta et al. 2010) as well as induction of apoptosis (Eley et al. 2005, Fraczek et al. 2012).

**Sperm density**

For a sperm cell, the density of the surrounding sperm cells is an important environment. Sperm density also varies greatly across reproductive episodes and different organs. At higher sperm densities, extracellular metabolites and oxygen availability are more limited, at least for the sperm in the densest areas (Mann 1964). This is especially marked in the so-called respiratory dilution effect, where endogenous respiration and osmotic stress temporarily reduce or slow down sperm function under high densities (Chia & Bickell 1983, Levitan 1995). Ex-vivo studies show that sperm live longer and retain fertilization ability for longer under high than under low sperm densities. This notion
applies to internal (Garner et al. 1997, Dowling et al. 2007) and external fertilizers (Levitan, 1995, Locatello et al. 2008).

Sperm swimming speed and sperm density were positively correlated in cuttlefish (Naud & Havenhand, 2006) or a bird (Mossman, 2008), negatively in Drosophila (Manier et al. 2013) whereas Rothschild & Swann (1951) did not find a change in sperm velocity in sea urchins when altering sperm concentrations. An alteration of the local sperm density is an effect, or side effect, of many ejaculate or non-ejaculate male or female traits that alter sperm function (Reinhardt 2007). It may be worth testing whether the respiratory dilution effect also explains why larger numbers of parasperm increase sperm viability in eusperm (Holman & Snook 2008).

Cellular episodes concerned: metabolism, motility, fertilization, survival.
Effects: small to very large. The effect of sperm density on fertilization ability is among the strongest environmental effects in external fertilizers.
Onset: Often sustained, but sudden changes between different reproductive episodes. Effects may be immediate and reversible for motility and metabolism. Delayed effects occur because of trade-offs or energy depletion.

**Male diet, lifestyle and exposure**
There is overwhelming evidence that a male lifestyle affects sperm functions. Lifestyle is a collective as well as a cumulative environmental effect, similar to age. The fact that exposure to pollutants, radiation, endocrine disruptors, metal ions, smoking, physical exercise, medical treatment, or infections of a male affects his sperm function is unquestionable, and this may occur without an effect on sperm numbers (e.g. Young 1961, Mann 1964, Fraga et al. 1996, Kumar 2004, Hayes 2011, Lewis & Ford 2012, Tavares et al. 2013, Aitken et al. 2014).

Rats that were fed low-protein diets had reduced sperm motility and showed increased sperm abnormalities (Vawda & Mandlwana 1990). In mice, a high-fat diet lowered the percentage of motile sperm, increased DNA damage and decreased sperm capacitation, oocyte binding and fertilization rates (Bakos et al. 2011). High cholesterol diets reduced capacitation ability in rabbit sperm (Diaz-Fontdevila & Bustos-Obregon 1993). A higher proportion of polyunsaturated fats altered sperm morphology and increased sperm velocity in fish (Alavi et al 2009).

Increased ascorbic acid (vitamin C) consumption reduced DNA damage in human sperm (Fraga et al 1991). In trout, ascorbic acid deficiency reduced sperm motility and therefore fertility (Ciereszko & Dabrowski 1995). Vitamin E, in interaction with beta-carotene, improved competitive fertilization ability the cricket Teleogryllus oceanicus (Almbro et al. 2011). In all cases, the protective effect against ROS is the suggested proximate mechanism of these vitamins. In healthy human males with a selenium-rich diet sperm motility was reduced (Hawkes & Turek 2001).

Cellular episodes concerned: All episodes seem to be affected, including motility, fertilization ability, the sperm protamine pattern (Oliva 2006) and transgenerational processes of unknown mechanism (Ng et al. 2010, Aitken et al. 2014)

Effect size: the entire spectrum, from absent to substantial. No, or no clear, effect of male diet restriction on sperm size was found in a moth (Gage & Cook 1994) and two fly species (Hellriegel & Blanckenhorn 2002, Amitin & Pitnick 2007). For specific
components, (including fat, selenium, vitamin C) it has been shown that an optimal level of consumption exists, i.e. the sperm function is reduced by under- or overprovision. Due to their impact via males, all lifestyle effects are delayed and not reversible, most are sustained. An important notion is that smoking-related DNA damage does not reduce sperm motility but carries information that increases offspring morbidity (Aitken et al. 2014).

Other environments

**Population density.** Morrow et al. (2008) found slightly larger sperm in males reared under high larval density compared to males reared under moderate density in *Drosophila melanogaster*. For the same species, Amitin & Pitnick (2007) report longer sperm when males were reared under low larval density. No effect of larval density on sperm length was found by Gay et al. (2009) in *Callosobruchus maculatus* beetles. Crean & Marshall (2008) as well as Crean et al. (2013) found sperm size, motility and longevity to be influenced by adult density in a marine broadcast spawner. Crean et al. (2013) could further demonstrate that the sperm character changes were adaptive.

**Elasticity of the medium.** Ho & Suarez (2001) found that hyperactivation pattern in mammalian sperm was highly dependent on viscoelasticity. They suggested that sperm may adjust their motility to elasticity. This points to a significance of elasticity to affect the motility-longevity trade-off (see also Kupriyanova & Havenhand 2005).

**Alien RNA/DNA.** Several proven or hypothetical possibilities exist for how small RNAs initiate RNA silencing pathways that protect the zygote from any sperm-delivered 'genome toxicity', including transposable elements (reviewed by Bourchis & Voinnet 2010). It is not clear whether this includes the extranuclear nucleic acids mentioned earlier but such genome protection may be important because posttranslational mechanisms to protect the genome integrity do not seem to exist in sperm (Bourchis & Voinnet 2010). The small RNA information can be further converted into chromatin-based information and so may allow epigenetic effects and both direct RNA action as well as the associated chromatin changes may contribute to postzygotic reproductive isolation (Bourchis & Voinnet 2010).

**UV radiation.** Lu & Wu (2005) and Nahon et al. (2009) report that UV radiation affects sperm mitochondrial function, motility and fertilization ability in a sea urchins, Rick et al. (2014) found reduced sperm velocity in stickleback males exposed to increased natural UV radiation.

**Unknown E effects.** Unknown E effects influencing the sperm phenotype can be seen when the repeatability of the sperm function is low within an ejaculate or within a male. While low within-ejaculate variation in sperm dimensions or function is reported by Morrow & Gage (2001), Gage et al. 2004, other studies show more variable parameters, i.e. low repeatability within a male (Birkhead and Fletcher 1995; Peters et al. 2004). Evidence that E effects influence the cellular phenotype of sperm also comes from studies showing that sperm of the same male or different males vary morphologically or metabolically between the male and the female sperm storage organ (Riemann & Thorson 1971, Renieri & Vegni Talluri 1974, Ribou & Reinhardt 2012, Reinhardt & Ribou 2013).


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, 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


Hayes TB. 2011. Atrazine has been used safely for 50 years? *Emerg. Topics Ecotoxicol.* 3:301-24


Mellanby K. 1939. Fertilization and egg production in the bed-bug, Cimex lectularius L. Parasitology, 31(02):193-9


Rothschild L, Swann MM. 1951. The fertilization reaction in the sea urchin. The probability of a successful sperm-egg collision. J. Exp. Biol. 28:403-16
Young EC. 1961. *Sex and internal secretions*, 3rd edn. Baltimore: Williams & Wilkins
Figure Legend

**Figure S1.** Environmental effects on the sperm phenotype. Starting at manufacture (spermatogenesis), sperm move along the time axis through different male environments (indicated by different shades of blue) and female environments (indicated by different shades of brown), passing significant events (empty arrows) in the sperm cell’s life. The immediate sperm environment (stippled circled line) can be influenced by the male and female environments (solid circled line). Effects on the sperm phenotype are either immediate (short, straight black arrows) or delayed (longer, curved black arrows). See also Fig. 3 for a more detailed scheme of immediate and delayed effects.