An Ecology of Sperm: Sperm Diversification by Natural Selection.

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Abstract

Using basic ecological concepts we introduce sperm ecology, a framework to study sperm cells. We first 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 conclude 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 on male and female micro-environments 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.

A FRAMEWORK OF SPERM ECOLOGY

In almost all species, only a tiny fraction of the ejaculated sperm reaches the egg and interacts with it for fertilization. The function of these few sperm, obviously vital for a male, is central to the study of evolution because only those functioning sperm deliver the 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 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 of geneticists to optimize conservation programs (Roldan & Gomedio 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 a 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 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 and so leads to selection for competitive sperm (Birkhead et al. 2009, Manier et al. 2013a).

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

The sperm phenotype

Variation in sperm form and function - the cellular phenotype – comes from three sources and their interactions: the male nuclear genotype (G), the male mitochondria (mt), and the environment (E) (**Figure 1**). This definition extends previous ones that considered genetic variation in sperm form and function between males (here the G effect) (Pizzari & Parker 2009). Research into sperm biology has organized itself into roughly the 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 make-up of sperm within an ejaculate and any possible resulting differences in haploid gene expression (Parker & Begon 1993). Although this issue is very interesting, we wish to focus on E (environmental) effects.

Male nuclear genetic effects on the 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) imply that environmental sources are not important in explaining the sperm phenotype and sperm diversification. This summary reflects four decades of intense sperm competition research (Parker 1970, Birkhead & Møller 1998, Bernasconi et al. 2004, Birkhead et al. 2009). This view is also implicit in procedures that seek correlations between sperm function and a male's genotype in medicine (so called sperm function tests – Aitken 2006, WHO 2011), and in the animal breeders' literature. Although sperm competition is a successful research field, a need to extend this view is apparent from the fact that male G effects explain only a small to moderate proportion of variation in sperm function (Dowling et al. 2010, Simmons 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 only 6-11% of the variation in paternity was explained by male genotype. 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).

Male mitochondrial effects on the sperm phenotype

Mitochondria affect many aspects of the sperm phenotype (Aitken et al 2009, Dowling 2007, Froman & Kirby 2005, Innocenti et al 2011, Yee et al. 2013, Zini & Al-Hathal 2011). The contribution by mt genetic variation can be substantial (but see Friberg & Dowling 2008): 68% of the variation in sperm motility in humans was explained by variation in mitochondrial production of reactive oxygen species (ROS) (Koppers et al. 2008). Few studies, however, experimentally manipulated the mt haplotype (but see Friberg & Dowling 2008, Yee et al. 2013) and, therefore, separate mt and mt x G effects. Fruit fly sperm carrying mt haplotypes combined with a foreign nuclear background had, on average, a 30% lower sperm competitive ability than when expressed with their co-evolved background (Yee et al. 2013).

Two aspects of mt 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 these sperm functions are governed by mitochondria. 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 signalling from mitochondria to the nucleus can differ between environments, such as a variable ROS production (Murphy 2009, Wallace et al 2011). This provides an opportunity for mt x E interactions and perhaps local adaptations of mitochondria (Wolff et al. 2014, Dowling 2014).

Environmental effects on the sperm phenotype

In the 18th century, Spallanzani observed that snow-chilled sperm recover their motility in warmer temperature, as cited by Mann (1964), who continues to say that the 19th century "abounds in

studies on the effect of changes in the medium on sperm motility and survival". Despite this history, and in contrast to the recent extensive research on G and mt effects, current evolutionary and ecological research has largely neglected ignored E effects on the sperm phenotype and to sperm diversification (but see Delph et al. 1997 for ecotype effects on sperm cells in plants). E effects on the sperm phenotype, i.e. effects that go beyond a mere reduction in sperm numbers, are dealt with by several, currently unconnected research fields: i) the substantial medical literature of 'lifestyle effects' on sperm function (Fraga et al. 1996, Yauk et al. 2008, Aitken et al. 2014), ii) the literature on fertilization biology in marine systems (Levitan 1995, 2000, Adriaenssens et al. 2012, Jensen et al. 2014, Schlegel et al. 2014), iii) ecotoxicological research on the effects of environmental pollutants and endocrine disruptors on sperm function across a wide range of taxa (Lewis & Ford 2012, Hayes 2011, Tavares et al. 2013), iv) applied research on storage, transport and long-term cryo-storage of sperm (Mann 1964, Leahy and Gadella 2011) and v) sperm aging, which encompasses the successive or collective accumulation of damage across all the environments a sperm cell has passed through (e.g. Tárin 2000, Siva-Jothy 2000, Reinhardt 2007, Pizzari et al. 2008). E influences on the sperm phenotype can also be deduced from the fact that intra-male variation (Pitnick et al. 2009) and intra-ejaculate variation for sperm traits are abundantly reported. Finally, sperm epigenetics (offspring variation based on environmental alterations of sperm cells), like sperm aging, describes collective and cumulative E effects, often without specifying the underlying molecular mechanism. This is an emerging field but most effects concern epigenetic alteration at the spermatid stage (Johnson et al. 2011, Dada et al. 2012, Jenkins & Carrell 2012) rather than mature sperm (but see Marshall 2015).

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 G, mt and E component of the sperm phenotype and their interrelations, such as G x E and mt x E interaction effects (**Figure 1**). By using the concept of the sperm phenotype, sperm ecology extends the existing research areas by combining the focus on additive genetic effects studied by sperm competition (Simmons & Moore 2009), with the E effects identified by ecotoxicology, reproductive medicine (i.e. lifestyle effects on sperm function), and other fields outlined in the Introduction. 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. Yeung et al. 2006, Aranha et al. 2008, Rosengrave et al. 2009). The fact that sperm function in individual males is not always highly repeatable (Birkhead and Fletcher 1995; Peters et al. 2004, Garcia-Tomas et al. 2006, but see Gage et al. 2004) suggests a role for E 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 the competition), sperm ecology takes a longitudinal, cellular lifetime approach (**Figure 2**). This approach has several advantages. First, sperm may be in competition for a variable amount of time and hence, the temporal variation will help to predict the end points of sperm competition and to consider the universal cellular trade-off between energy expenditure and lifespan (**Figure 2**) (Hughes & Davey 1969, Reinhardt & Otti 2012, Gage et al. 2004, Burness et al. 2004, Levitan 2000, Ribou & Reinhardt 2012 for various examples of cellular trade-offs in sperm).

Second, the longitudinal approach incorporates delayed environmental impacts on sperm (see below) including the view that differences in offspring phenotype or quality arise because the fertilizing sperm had different exposure histories or durations (e.g. Burruel et al. 2013, Ghaleno et al. 2014, Lane et al. 2014, Immler et al. 2014, Marshall 2015).

Third, a lifetime view allows one to consider sperm physiological changes as phenotypic plasticity at the cellular level. Despite Spallanzi's early observations, and despite the central position of phenotypic plasticity in whole-organism biology, the issue of phenotypic plasticity of sperm has not been addressed by sperm competition and only rarely in other areas of evolutionary and ecological research (Purchase et al. 2010, Poland et al. 2011, Crean et al. 2013, Jensen et al. 2014). We will further argue that male and female reproductive traits will 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, sperm ecology aims to describe whether, and how, natural selection favors specific sperm phenotypes in specific environments by testing for adaptive G x E, or mt x E interactions. This may also include sexual selection if male and female G effects are characterized as specific environments for sperm. Therefore, sperm ecology contributes to explaining sperm diversification. Importantly, sperm ecology does not necessarily require competition between genotypes in order to produce evolutionary changes and as such, is a parsimonious concept (**Figure 2**).

In summary, by incorporating four basic ecological concepts to sperm biology, viz environmental variation, phenotypic plasticity, niche construction and genotype-environment-interactions (local adaptation), sperm ecology may contribute to explaining phenotypic adaptations as well as sperm diversification in three important ways: i) characterization and quantification of environmental variation on sperm function, ii) assessing the role of natural selection in sperm diversification, and iii) suggesting a pathway for the evolution of male and female reproductive traits.

CHARACTERIZATION AND QUANTIFICATION OF ENVIRONMENTAL VARIATION ON THE SPERM PHENOTYPE

Environmental variation can act on sperm in several ways. First, there is an external environment that directly impacts on sperm cells, such as temperature in ectothermic animals, or water pressure, UV radiation, and salinity in the case of broadcast-spawning organisms, or *in-vitro* laboratory treatments. Second, environments can act on males and females and generate sperm environments that are different from the external environment. For example, smoking results in systemic increased levels of ROS, including in reproductive compartments; food items may affect the pH in the seminal fluid or increased temperature may alter ion or enzyme concentrations. Third, the longitudinal variation in sperm function might differ across different environments, such as reduced metabolism under hypoxia.

We use two literature search methods to describe E effects on sperm, i) a random selection of relevant articles and ii) a directed search for E effects by specific environments on specific sperm functions (Supplementary Material). Our literature search on E effects on sperm yielded 27,514 articles, or 8,042 if restricted to articles published 2000-2014. Of the latter, 900 articles were randomly chosen, of which 178 articles (19.8%) (Table S1) matched our criteria (Supplementary methods). These articles provide an estimate of the significance of E effects on sperm in ecology and evolution.

Many environmental factors affect sperm function

A large variety of environments affects phenotypic sperm function including temperature, pH, osmolarity and concentration of specific ions, oxygen concentration, oxygen radicals and antioxidants, diet (male, maternal, and paternal diet, and 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, or sperm density (Supplementary Material). There may be a bias in the data in that results that show no E effect on sperm were published less frequently (but see the next paragraph that shows a substantial

number of studies reporting the absence of E effects) but it seems that most environments tested show some effect on sperm.

The literature on E effects on the sperm phenotype is large and largely neglected by ecology and evolution research

After applying our search criteria (Supplementary Material), accounting for the fact that different environments were studied to a different extent (Supplementary Material: Table S1) 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 1293-2180 (mean: 1736) articles looked at E effects on sperm (Supplementary Material: Table S1).

Thereby, 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 E effect on at least one function; 64 studies (35.9%) showed no effect on at least one function. Projecting to the other articles, the abundance of E effects is remarkable: of the projected 1736 (1293-2180) relevant articles, one may predict that 1590 articles have looked at 4086 environment-sperm function combinations, of which potentially 3187 environment-sperm function combinations could show E effects on sperm function.

While it may not seem surprising that the environment shapes the sperm phenotype as proposed in **Figure 1**, it is noteworthy that only very few of these articles originated from evolution and ecology research. Broadly defining 'ecology and evolution journals' (Supplementary Material: Table S2), only 8 (4.5%) of the articles of our random search concerned ecology and evolution. A similar number resulted from our directed search: 40 (5.4%) out of 7445 articles, including 18 that examined consequences of predicted global change on sperm function.

Magnitude and shape of environmental effects on the sperm phenotype

Our summary (Supplementary Material: Table S2) revealed that some environments affect the sperm phenotype after even a short transient impact such as brief high altitude visits (Okumura et al. 2003), brief pollution events or brief temperature elevations (Paul et al. 2008), whereas others were found after a sustained period of action. Some effects became apparent immediately, others appeared much longer after the environment impact, including in offspring. Many effects, such as DNA damage, membrane damage or, of course, sperm mortality, are irreversible and hence permanent at the level of the cell, or the male. Examples also exist where effects are reversible at either the cellular level or at the level of the male (Bencic et al. 2000, Okumura et al. 2003, Villegas et al. 2003, Le Comber et al. 2004, Aitken et al. 2012).

Among the fitness-related aspects of sperm function none appeared as canalized as to be consistently inert to E effects. Across species, sperm morphology, metabolism, motility, longevity, fertilization ability as well as epigenetic signatures on the nuclear genome or effects on offspring health were all affected. Within species, these characters were not equally affected, and sometimes the effects were not even positively correlated with each other (Supplementary Material: Table S2). The magnitude of effects was so variable as to prevent any generalization. Compared to controls, sperm populations or sperm cells, E effects 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, pH or osmolaric changes) generated substantial variation in sperm function (Supplementary Material: Table S2).

Phenotypic plasticity in sperm function

Almost all studies incorporated in our literature search compared the E effects against control sperm from the same male, the same genotype or the same population. In other words, almost all of the 397 environment-sperm function combinations represent phenotypic plasticity at either the level of the male, genotype or population. 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), that human sperm repeatedly bind and unbind to epithelium (Pacey *et al.*, 1995) or move in and out of the hyperactivated state (Mortimer and Swann, 1995). It is perhaps noteworthy that even sperm morphology can be plastically (but not necessarily reversibly) affected by the environment. For example, several insect species show substantial membrane alteration in the female sperm storage organ, compared to ejaculated sperm (Riemann & Thorson 1971, Renieri & Vegni Talluri 1974). Effects on sperm size are reviewed by Marshall (2015).

Physiological responses in sperm, or cellular phenotypic plasticity, are not unexpected given the diverse chemistry of the male and female genital tracts that sperm cells have to master. However, explicitly spelling out the existence of sperm phenoptypic plasticity may help to formalize predictions of when it will be adaptive. Adaptive plasticity will depend on how often a given environment will be encountered, the duration of the encounter, and the reliability of information (see Pfennig et al. 2010 for a general framework). Interestingly, these predictor variables of sperm phenotypic plasticity are linked to the substantial body of cell biology studies that examine the considerable ability of individual sperm cells to respond to *in situ* encountered chemical, surface or other conditions (Bahat & Eisenbach 2006, Friedrich & Jülicher 2007, Alvarez et al. 2012, 2014, Babcock et al. 2014).

The relative size of G, mt and E effects on the sperm phenotype

Surprisingly few studies estimated the relative effects size of G and E simultaneously in the same system (and possibly none have separated G, mt, and E effects). One study used female gene expression after sperm receipt as a parameter of sperm fitness parameter, and varied sperm age (E effect) within three different sperm genotypes (populations) (Otti et al. 2015). Approximately sixteen times as many female genes were differentially expressed in response to E effects (79

genes) as to G effects (5 genes). If the comparison was restricted to genes with substantial differences, there were still 5 times as many genes expressed in response to E than G (Otti et al. 2015). However, while quantifying the number of differentially expressed genes in females may be useful, the number of genes does not necessarily directly translate into differences in sperm fitness. The statistics table in the *Drosophila melanogaster* study by Clark et al. (1999) suggests less than 1% of the variation in paternity was explained by E (laboratory) effects, compared to 6-11% by male G effects. Both studies have the limitation that they include seminal fluid effects. More closely related to sperm function *per se* 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 the genotype explained 3 times as much variance as pH, whereas temperature explained 1.3 times as much variance as genotype.

We conclude that E effects on sperm function are ubiquitous, take many forms and may be as large as G effects, or even larger. Our randomised literature search gives reason to suggest that it is not tenable to assume the sperm phenotype is almost exclusively shaped by G effects. E effects can be direct or 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 E effects when examining variation in reproductive success.

CONSEQUENCES OF ENVIRONMENTAL EFFECTS ON THE SPERM PHENOTYPE

Reduced significance and hampered predictability of sperm competition

The observation that sperm functions vary between environments and that sperm can accumulate damage and information during their passage through the environment has important consequences. The first and foremost is that for many species a sperm genotype can occupy a very large phenotypic space (**Figure 3**). This fact severely hampers the predictability of sperm function,

based on the male genotype (G), both in 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 the 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. Figure 1 in Reinhardt 2007). Therefore, the loaded raffle in sperm competition (Parker 2009) cannot be expressed by a loading coefficient that is independent of time and the environment. In nature, there will hardly be two males that have had an identical lifestyle, habitat utilization or age at the time of mating (when their sperm compete), and hence have sperm with an identical E component. We therefore predict that such 'carry-over' E 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 E effects on postejaculatory reproductive success (Almbro et al. 2011, Mehlis & Bakker 2014, Breckles & Neef 2014, Vasudeva et al. 2014, Gasparini et al. 2014).

Sperm function may determine species ranges

Sperm velocity was either 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), or that environmental variation affected males differently in such a way that consistent population variation did not emerge. Examples include sperm motility in a polychaete (Schlegel et al. 2014) and a sea urchin (Schlegel et al. 2012) in response to ocean acidification. These examples show that sperm phenotypic plasticity (at the male or 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, E

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 can be expected under the predicted higher UV radiation doses (stickleback - Rick et al. 2014; sea urchins – Lu & Wu 2005, Nahon et al. 2009), under predicted increased CO2 concentration in the water (sea star - Uthicke et al. 2013; mussels – Vihtakari et al. 2013; oyster - Barros et al. 2013, coral and sea cucumber – Morita et al. 2010) and under predicted increased water temperature (guppy - Breckels & Neff 2013). Sperm longevity may be reduced under increased water temperatures (sea urchin – Binet & Doyle 2013), or 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 E effects, may not shift thermal critical limits of sperm velocity (Adriaenssens et al. 2012).

Intra-ejaculate 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 were exposed to different treatments. As different environments will cause different patterns of ejaculate heterogeneity, ejaculate heterogeneity may contribute to phenotypic and genetic divergence of populations that live in different habitats.

The Brynhild effect

It has been suggested by some sexual selection models that females benefit from creating barriers to sperm that only the best sperm can pass and fertilize the 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 stronger damage to sperm, similar to the female character of Brynhild in the Nordic epic saga, the Nibelungenlied (Song of the Nibelungs) who resided inside a ring of fire. Noble men, aiming to get across to marry her, found death or injury. The man who succeeded needed magical power to cross the fire.

Sperm viability is not a good fitness indicator

Some environments may be so stressful that sperm apoptosis is initiated, including attacks by retroviruses that inject foreign RNA or DNA into sperm DNA. Aitken & Baker (2014) point 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." Even though phrased in a group selectionist view, this remark illustrates that 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 to outcompeting his own genetically undamaged sperm (Aitken et al. 2013; Aitken & Koppers 2011). Aitken & Baker (2014) also point out 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."

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 (E) or a

positive indicator of the ability of a genotype to respond to the environment (G x E), rather than exclusively reflecting a negative genotype (G).

Variance effects in numerical sperm competition

Sperm competition predicts a numerical advantage for males delivering more sperm. Because of the E component of the sperm phenotype, sperm ecology is able to also specify this prediction of a male advantage. Continuous, and constant, sperm production will automatically lead to the fact that ejaculates with more sperm also contain more recently produced sperm, i.e. sperm that have been exposed to the environment for a shorter period (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.

Mean ejaculate traits may be non-informative

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

Trade-offs in sperm function can hamper comparability

Sperm function can decline within seconds of activation (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 Figure 2 of Reinhardt & Otti 2012).

Adaptive habitat choice

Given the E 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.

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 it was the ovarian fluid chemistry that allowed sticklebacks to overcome the osmotic constraints on sperm imposed by the new freshwater habitat. The most obvious sperm niche is seminal fluid, an evolutionarily very diverse character (Poiani 2006, Avila et al. 2012). Seminal fluid fulfills niche functions by buffering the pH for sperm, reducing oxidative stress to sperm, supporting motility, longevity and improving offspring development across a variety of taxa (e.g. Aitken & Clarkson 1988, Scaggiante et al. 1999, Kang et al. 2008, Shaliutina-Kolesova et al. 2014, Rickard et al. 2014, Bromfield et al. 2014, Heise et al. 2010). Specific examples include seminal antioxidants (Poiani 2006, Avila et al. 2012) or antimicrobial properties (Poiani 2006, Otti et al. 2009, 2013).

Other niches may transiently reduce sperm motility and save cellular energy resources by containment of sperm in bundles, spermatophores or with additional sperm types (Reinhardt 2007).

Many female traits also serve as niches by plastically improving sperm function. Reinhardt (2007), Holt & Lloyd (2010) and Heifetz et al. (2010) review female traits that reduce sperm metabolism, sperm oxidative stress and so extend sperm longevity during pre-fertilization storage. These traits include hypothermia at the sites of sperm storage, reduction of sperm motility by binding sperm to

epithelia, packing them tightly or organizing them in bundles. Sperm niche function are also noticeable by immunological and antioxidant protection, interference with sperm metabolism, reduction in the number or size of mitochondria or a delay of sperm activation. Recent work on insects suggests that reducing sperm metabolism happens in a way that is adaptive to females (by delaying infertility) (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013). Both studies support the idea that the effect was specifically directed towards sperm phenotypes not sperm genotypes because the sperm genotype could not be predicted based on sperm metabolism.

Selection may be directed against E, not G, components of the sperm phenotype

If E effects on sperm are often damaging, male and female traits are predicted to evolve that discriminate against sperm based on the sperm phenotype unrelated to the sperm genotype. In males, such traits include those that specifically disfavor aged sperm phenotypes as seen during repeated mating with the same female (mating with different females also does but can obviously function in sexual selection), 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 Reinhardt 2007). In females, repeated mating to the same male reduces representation of aged or environmentally damaged sperm phenotypes in the fertilization set, as can sperm dumping by females if it is related to time in storage. 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 Reinhardt 2007).

However, while these traits automatically alter ejaculate variability in terms of E effects, relatively few empirical tests exist. For example, in a cricket species, males expelled non-used sperm while simultaneously younger sperm was more successful in reaching the female sperm storage organ (Reinhardt & Siva-Jothy 2005). In bedbugs, Otti et al. (2013) demonstrated that the antibiotic activity in the seminal fluid transferred during one mating was sufficient to reduce the sperm

mortality caused by simultaneously transferred bacteria. Finally, as experimental scrotum insulation results in reduced sperm motility or DNA fragmentation (Brito et al. 2003, Banks et al. 2005), one may conclude that the scrotum evolved in order to reduce E effects on sperm.

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

NATURAL SELECTION AND DIVERSIFICATION IN THE SPERM PHENOTYPE

A model for phenotypic sperm evolution

There are two principal ways of sperm phenotypic diversification, via sperm environments and sperm phenotypic plasticity, or via sperm traits *per se*. Both ways can lead to adaptive population genetic changes and contribute to evolutionary change.

While selection can operate on increased male reproductive success by favoring sperm traits directly (**Figure 4**, *blue arrows*), sperm phenotypic plasticity opens another route for selection: males traits may be favored that create sperm niches in which phenotypically plastic sperm function better than in the absence of such niches (**Figure 4**, *brown area*), making niches adaptive paternal effects. An obvious example of the evolution of a sperm niche is seminal fluid. Sperm cells experiencing the newly evolved sperm niche may then allow further sperm diversification by providing an arena in which novel sperm traits *per se* evolve (**Figure 4**, *brown area*) (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**, *brown area*). This sperm phenotype may now benefit either from a male mutation that generates, say, thermal stability in the sperm environment (**Figure 4**, *brown area*) and so optimizes energy expenditure (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**, *blue arrows*). These kind of successive changes can explain environment-mediated sperm diversification if the male environment changes (e.g. generally increased sugar availability to the male) but do not require such changes (e.g. in the case of 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), the evolution of seminal fluid complexity (Poiani 2006, Avila et al. 2012) or provide a plausible mechanism for those examples of ecological speciation where divergence in diet specialization translated into postmating reproductive isolation (Nosil 2012). This model may also help to explain how males may adaptively alter fertilization ability of their sperm such that sperm, or ejaculates, function best under their paternal environments (Marshall 2015). And it can incorporate co-evolutionary interactions where male traits evolve that induce females to create sperm niches (such as male seminal substances that manipulate the sperm storage ability of females – Avila et al. 2011).

While G effects exist in terms of the sperm activating ability by seminal fluid of foreign males of the same or even other species (Morrell et al. 2014, Usinger 1966, den Boer et al. 2010), our model can generate co-evolution-like dynamics between sperm and males, where males evolve niches to harness their own sperm.

Genotype-by-environment interaction effects on the sperm phenotype

Generally, G x E effects on the sperm phenotype have been rarely addressed. For example, of the 178 articles of our literature research that contained relevant data, 6 articles presented the data separately for two, or more, breeds or separate populations (broadly equivalent to genotypes) (3%). This suggests that very few of the thousands of articles reporting effects on sperm cells by diet, parental smoking habit or caffeine consumption, pH or temperature effects, salinity, antioxidant or additions 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 x E analysis.

We extended our literature search in a second step and assessed the title and abstracts of all 7445 articles without search terms ('manually') for evidence of G x E interactions and of local adaptation. This resulted in a number of additional articles. Whereas no significant G x 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 x E interaction effects (**Table 1**).

→ **Table 1**

The common existence of species-specific maxima of sperm motility at different temperatures, osmolarities or pH values that correlate to habitat conditions (Table S2, Alavi & Cosson 2005) suggests that local adaptation in sperm is common.

CONCLUSIONS

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

The co-evolution-like dynamics between sperm and internal environments of males or females has evolutionary and medical consequences that can be summarized in one sentence: Don't take the sperm out of context. The lack of the co-evolved sperm niche for sperm tested in medical sperm function tests may provide an explanation for the poor predictive power of sperm function tests for conception and paternity (Aitken 2006, but see Froman & Feltman 1998, Birkhead et al. 1999). It is also consistent with this model that the predictive power of these sperm function tests is improved again if sperm function is measured after sperm 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 that has 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. As male and female environments (indirect sperm environment) can differ between populations or via ecological specialization within populations, natural selection may substantially contribute to the evolution of sperm diversity, via local adaptation.
4. This diversification may or may not be augmented by postejaculatory sexual selection, i.e. sperm competition or female sperm choice, but sexual selection will benefit from considering environmental influences on sperm.
5. Applying ecological concepts may add to the formalization of describing 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' (E effects) and via sperm traits *per se* (G 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 E effects on the sperm phenotype the reason why sperm competition ability has low heritability (Moore & Simmons 2009) and only explains a low proportion in reproductive success (Pischedda & Rice 2011)?

5. What are the extent and mechanism of sperm epigenetic alterations and offspring characters?

6. Is Marshall's idea (2015) supported that external fertilizers are more likely to show adaptive sperm phenotypic plasticity than internal fertilizers?

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Acronyms and Definitions

E: environmental effects (on the sperm phenotype).

G: genetic effects (on the sperm phenotype). The male's nuclear genome.

G x E, or genotype-by-environment interaction: different genotypes respond differently to various environmental conditions.

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

mt: mitochondrial effects (on the sperm phenotype).

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

ROS: reactive oxygen species. A by-product of normal metabolism, these chemically reactive molecules can cause damage to membranes and DNA. ROS also act as cellular signals.

Sperm trait: a sperm character that is explained by the male genotype. Sperm characters that are explained by haploid genome of the sperm are not covered in this review.

Table 1. Examples of studies examining G x E interaction effects or adaptive phenotypic plasticity on the sperm phenotype.

Type of evidence	Level of comparison	Group	Sperm function and environments examined	Reference
G x E	Interspecific	Sea urchin	The fertilization ability, but not motility, of sperm decreased with increasing CO_2 concentration in the water in only one species	Sung et al. 2014
G x E	Interspecific	Sea urchin	Species differed in their sperm motility response to temperature	Rahman et al. 2009
G x E	Interspecific	Bird	Species differed in their sperm motility response to cooling protocols	Blanco et al. 2000
G x E	Interspecific	Cichlid fish	Consistent differences in motility pattern between mouthbrooding and externally spawning species	Reinhardt & Otti 2012
G x E	SNP*	Ram	Temperature treatment increased DNA fragmentation in sperm only in bearers of one allele but not another	Ramon et al. 2014
G x E	Karyotype	Mice	After irradiation, sperm defragmentation was larger in Y-bearing than X-bearing sperm, leading to reduced egg binding ability	Kumar et al. 2013
G x E	Population	Cattle	Altitude and season produced changes in motility that differed between two breeds of cattle	Barros et al. 2006, Chacur et al. 2013
G x E	Population	Chicken	Sperm of four chicken breeds differed in their susceptibility to different freezing methods	Schramm 2008
G x E, phenotypic plasticity	Population	Guppies	Populations responded to experimental evolution under altered temperatures with an increase in sperm length, but not sperm motility. Populations also displayed phenotypic plasticity in sperm length and sperm motility	Breckles & Neff 2014
G x E	Genotype	Humans	Exposure of males to certain	Axelsson et al.

			environments only caused altered morphology or chromatin integrity in some male genotypes (review)	2010
G x E	Genotype	Fruit fly	Larval rearing density affected the sperm length of some genotypes only	Morrow et al. 2008
G x E	Isogenic line	Fruit fly	Laboratory x genotype interaction effect accounted for 12-19% of the variation in paternity	Clark et al. 1999
G x E	Genotype	Flour beetle	Different genotypes varied in their sperm defence ability (P1) in relation to nutritional manipulation	Lewis et al. 2012
G x E	Genotype	Honeybee	Colonies showed an age x genotype interaction effect on sperm viability	Stürup et al. 2013
G x E	Genotype	Cod	Significant, but likely small, G x E interaction effect on sperm velocity	Purchase et al. 2010
G x E	Genotype	Bedbug	Twice as many female genes were differentially expressed in response to G x E effects compared to G effects	Otti et al. 2015
Local adaptation	Interspecific	Sea urchin	Sperm motility, metabolism, and temperature-dependent motility differed across four closely related species such that sperm functions was highest under conditions resembling the native habitat.	Mita et al. 2002
Local adaptation	Interspecific	Fish	Sperm motility, metabolism, and temperature-dependent motility differed across four closely related species such that sperm functions was highest under conditions resembling the native habitat.	Lindberg 1948
Local adaptation	Population	Fruit fly	Local adaptation in male fertility to temperature	Rohmer et al. 2004
Local adaptation	Population	Cichlid fish	Populations from two different habitats differed in their activation threshold of sperm motility, and the threshold reflected the ionic concentrations in these	Morita et al. 2010

			habitats	
Local adaptation	Population	Stickleback fish	Sperm from males of a saltwater population were motile in saltwater but not sperm from freshwater or brackish water populations	Elofsson et al. 2003
Local adaptation	Individual	Bees	Ejaculates contained much more live sperm when males were reared at the natural temperature in a hive, compared to when reared at lower or higher temperatures	Bienowska et al. 2011
Adaptive phenotypic plasticity?	Individual	Bedbug, cricket	Adaptive variation in sperm metabolic rates between male and female sperm store	Reinhardt & Ribou 2012, Ribou & Reinhardt 2013
Adaptive phenotypic plasticity	Individual	sea squirt	Fertilization ability of sperm varied adaptively with population density	Crean & Marshall 2008, Crean et al. 2013
Adaptive phenotypic plasticity	Individual	tubeworm	Sperm kept under low salinity produced offspring that survived better under low salinity	Ritchie & Marshall 2013