REPRODUCTION

Male reproductive ageing: a tale of the whole ejaculate

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Abstract

Ageing is nearly ubiquitous and encompasses all biological functions. We here focus on age-dependent changes in male reproductive capacity across a broad range of animal taxa. While there has been a long-standing focus on mating ability and overall reproductive success, we here highlight the underlying mechanisms that explain loss in fertilisation capacity in ageing males. Fertilisation is mediated by not only the presence of sperm, but also the cocktail of seminal fluid proteins that ensure sperm survival, capacitation and interaction with female physiology. Sperm ageing has received much attention in studies of male reproductive senescence; however, post-mating processes include a number of interlocked steps that together cumulate in successful fertilisation. As such we consider male ability to elicit female post mating responses such as uterine conformational changes, sperm storage and ovulation and the components within the ejaculate that mediate these post-mating processes. For the latter seminal fluid proteins are key and hence we reflect on age-dependent changes in quality of the entire ejaculate and its consequences for male reproductive capacity. While first studies accrue and highlight that changes in the non-sperm fraction can explain substantial variation in senescent male reproductive success and male ability to induce post-mating responses necessary for fertilisation many open questions still remain that warrant further investigations. One being what the potential age-dependent changes in composition are or whether there is a general decline and how this interacts with sperm to affect fertilisation success. Further, the impact females might have to ameliorate these changes will be an area of interest.

Ageing is a near ubiquitous phenomenon and observed as a decline in survival and reproductive performance with age (Nussey *et al.* 2013, Jones *et al.* 2014, Lemaitre *et al.* 2015, Maklakov & Immler 2016, Lemaitre & Gaillard 2017). The decline in survival (=actuarial ageing) is observed in a large number of organisms, both in the wild and in captivity (Nussey *et al.* 2013, Lemaitre *et al.* 2015). Its genetic basis as well as factors influencing the rate of ageing have been studied widely, with a whole body of theory explaining this phenomenon (for e.g. by Maklakov & Immler 2016, Gaillard & Lemaitre 2017, Lemaitre & Gaillard 2017). In this review we focus on age-dependent changes in male reproduction and the factors that affect the onset and rate of these changes. We have reviewed recent examples (from the past 10 years) across several animal taxa ranging from invertebrates to vertebrate species studied both in the wild and the laboratory. We focus on studies reporting senescent changes in the ejaculate, the tissues producing the ejaculate and the consequences for male reproductive success.

Reproductive senescence is characterised by a decline in sexual trait expression coupled with diminishing reproductive returns. Similar to actuarial ageing, reproductive ageing has also been observed in a large number of taxa and occurs in the wild, but here it is mainly studied in mammals and birds (reviewed in Lemaitre & Gaillard 2017, see Rodriguez-Munoz *et al.* 2019 for an insect example). It can be measured as a decline in mating activity and fertilisation success with increasing age and is often due to somatic changes, like decreased hormonal levels leading to increased dysfunction or less agile courtship resulting in lost mating opportunities. To give some brief examples, in fowl *Gallus gallus*, older males are less aggressive, resulting in fewer matings and reduced reproductive success as aggression is one important determinant of male mating success (McDonald *et al.* 2017). Apart from behaviour, a reduced investment in sexual ornaments, as observed in the decreased comb size in ageing fowl (Cornwallis *et al.* 2014) or duller foot colour in the blue-footed booby *Sula nebouxii* (Velando *et al.* 2011), can result in active discrimination by females. Senescent blue-footed booby males potentially signal their lower reproductive values, as those who carry more damaged DNA sites in their sperm also display a duller foot colour (Velando *et al.* 2011). However, a decline in mating performance is not inevitable or might be context dependent. Ageing male houbara bustards (*Chlamydotis undulata*) show no decline in sexual displays (Preston *et al.* 2011) and older

males gain a mating equally fast or faster than young males in *Drosophila pseudoobscura* (Dhole & Pfennig 2014) and *D. bipectinata* (Somashekar & Krishna 2011).

While we commonly think of an individual ageing with all traits declining synchronously, recent studies found asynchrony in ageing rates in for example wild Soay sheep (*Ovis aries*, Hayward *et al.* 2015) and for female but not male fowl (Cornwallis *et al.* 2014). This might be due to these traits having different energy requirements or incur different trade-off costs resulting in different ageing rates. This highlights that in studies of ageing we need to investigate traits separately to gain an integrated view on how an organism ages and to understand the factors that influence senescence rates across traits and subsequently reproductive output.

Senescence of male post-mating traits

Apart from the ability to gain a mating, often the reproductive gain from these mating interactions is reduced, when older males exhibit a declining ability to father offspring. Reproduction is a multi-step process and several of these steps occur post mating, culminating in successful fertilisation and offspring production. Several of these steps are mediated by components of the male ejaculate (Box 1). Decreases in male ability to elicit favourable female post-mating responses result in reduced paternity shares for older males (McDonald *et al.* 2017, Ruhmann *et al.* 2018).

Post-mating effects of male ageing are understudied in the wild (but see Kim *et al.* 2011), particularly in small, short-lived species, and most of our insight comes from captivity. There is accumulating evidence that males suffer from senescence of post-mating reproductive traits in some species (e.g. the cricket *Allonemobius socius*, Marshall *et al.* 2009; the blue-footed booby, Kim *et al.* 2011; the houbara bustard, Preston *et al.* 2015) but this is not universal (for flies e.g. Santhosh & Krishna 2013, Dhole & Pfennig 2014, Herrera-Cruz *et al.* 2018).

One possibility could be that studies not always tested males at the far end of a species age range. We aimed to include studies that span a significant age range across a male's lifespan, but a large number of studies do not report lifespan concurrently for their species under study. When possible we tried to find other papers reporting lifespan (Supplementary Table 1, see section on supplementary data given at the end of this article), though we are aware that differences in study conditions can affect lifespan estimates. This is problematic, when no effect is found and we cannot be sure this is due to a lack of including truly old males, when estimating effect sizes and when we want to compare across species to be able to identify similar stages of a male's life.

The most common measure of male post-mating success is the number of eggs or offspring a male's mating partner produces. In most species including birds, insects and mammals, these numbers are reduced after mating with a senescing male (Marshall *et al.* 2009, Kim *et al.* 2011, Rezaei *et al.* 2015, Koppik & Fricke 2017, Ruhmann *et al.* 2018; for a comprehensive review on vertebrates in the wild see Lemaitre & Gaillard 2017), but exceptions exist (Santhosh & Krishna 2013, Dhole & Pfennig 2014). Only few studies provide a more detailed view by reporting egg numbers and egg-to-adult survival/hatching success (Preston *et al.* 2015, Koppik & Fricke 2017, Ruhmann *et al.* 2018) or sperm competition success (Koppik & Fricke 2017, Ruhmann *et al.* 2018). These measurements though can give valuable insight into the mechanisms of male reproductive ageing.

In polyandrous species one male strategy to avoid post-mating competition between male ejaculates is to reduce the likelihood of female remating. Senescing *D. melanogaster* males can become increasingly poor in inducing this refractory period in females (Koppik & Fricke 2017, Koppik *et al.* 2018, Ruhmann *et al.* 2018). Cumulative losses occur when males additionally have a diminished ability to compete over fertilisations as seen in the fowl or the vinegar fly (Dean *et al.* 2010,

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Box 1: Seminal fluid proteins

Seminal fluid proteins (Sfps) occur in many internally and externally fertilising species, where they aid male reproductive success through their contributions to various processes, such as sperm capacitation, sperm competition and fertilisation (reviewed in Poiani 2006). Though the majority of Sfps is secreted by the various male accessory glands, other reproductive tract tissues can contribute to the non-sperm fraction of the ejaculate (McGraw *et al.* 2015). *Drosophila melanogaster* is an important model in research on the identification and function of Sfps (Box 2), but the number of species in which we have investigated Sfps is steadily increasing (for a review see Avila *et al.* 2011), whereby in insect taxa, they play an important role in a multitude of physiological and behavioural female post-mating changes. In mosquitoes Sfps influence for example, female egg maturation and host-seeking behaviour (reviewed in Klowden 1999), processes which are also important for their role as vectors of various diseases, and 198 respectively 93 Sfps have been identified in *Aedes albopictus* (Boes *et al.* 2014) and *A. aegypti* (Sirot *et al.* 2011). Studies on Sfp identity and function also extend to various vertebrate species (e.g. Druart *et al.* 2013 for a comparison across seven domestic mammalian species). In humans, 923 seminal fluid proteins have been identified through mass spectrometry (Pilch & Mann 2006). Common functions include sperm capacitation, contribution to sperm motility and survival within the female reproductive tract (Poiani 2006) and thus are important aids to male fertilisation success. Beyond fertilisation, seminal fluid can even impact offspring (metabolic) phenotype as recently shown in mice (Watkins *et al.* 2018). Investigations on Sfps in birds are relatively scarce, but a recent study in Red Junglefowl *Gallus gallus* reports a complex seminal fluid cocktail with 1141 Sfps (Borziak *et al.* 2016). Seminal fluid proteins are ubiquitous in sexually reproducing animals and, while their importance in reproduction already is apparent, many novel functions are likely to be discovered when we add more species to this list.

Koppik & Fricke 2017, McDonald *et al.* 2017, Koppik *et al.* 2018, Ruhmann *et al.* 2018). Since polyandry is common, considering senescing males' success in competitive situations is important to fully understand reproductive ageing in males, and thus calls for more studies integrating these aspects of male reproduction.

Mechanisms of male reproductive ageing

In males of advancing age, ejaculate size has been observed to decrease (e.g. the houbara bustard, Chantepie *et al.* 2015) as well as increase (e.g. the butterfly *Bicyclus anynana*, Kehl *et al.* 2015; the Glanville fritillary butterfly, Duplouy *et al.* 2018), with several conceivable explanations. Older males could actively alter their investment into each mating, possibly increasing it due to their lower residual reproductive value. Alternatively, the tissues producing the ejaculate ages, resulting in fewer products available and an overall smaller and less effective ejaculate which may have a variety of consequences for male reproductive success (see Fig. 1 for a graphical outline using *D. melanogaster* as an example). As the ejaculate is made up of sperm and the non-sperm components, we outline how somatic and germline senescence affect fertilisation success by considering age-dependent changes in sperm quality and the non-sperm fraction of an ejaculate separately for a comprehensive understanding of the consequences for male reproductive success. The two ejaculate fractions might show independent responses to male ageing as they are produced by different tissues. The testis and its germline are well protected with an expensive DNA repair and maintenance machinery (Maklakov & Immler 2016), which should at least slow germline ageing, while we would potentially expect less protection in the somatic reproductive tissues.

Sperm and testes ageing

To date theoretical considerations and empirical investigations into reproductive ageing of post-mating traits are mostly limited to sperm senescence (reviewed in Reinhardt 2007), distinguishing between two phases: pre- versus post-meiotic senescence (reviewed in Pizzari *et al.* 2008). Sperm ageing can be influenced by male actuarial ageing (pre-meiotic) but also be independent, when mature (post-meiotic) sperm age while being stored for extended periods, either within the male before ejaculation or within the female storage organs. Pre-meiotic ageing occurs due to the progressive deterioration of the spermatogenic machinery resulting in sperm not able to perform their primary goal of delivering their genetic contribution to the ova. Secondly, pre-meiotic sperm senescence is driven by the accumulation of mutations and might depend on the number of germline divisions. Deterioration of sperm genetic quality can have transgenerational effects and

lead to unhealthy offspring and/or offspring carrying compromised germlines. Despite Pizzari and colleagues having reviewed the basis for sperm ageing more than 10 years ago, the field has only slowly gained insight into the functional and genetic basis of sperm ageing and/or factors that increase or slow its progression.

Longitudinal studies following individual males allow repeated sampling of ejaculate characteristics and sperm traits across their lifespan. This approach provides great insight into dynamic changes with age at the individual level, but is less amenable to wild animals and so far has been done for captive animals only. In houbara bustards, this approach revealed severe deteriorations of male ejaculate quality with advancing age, for example, ejaculate size decreased, as did sperm viability (Chantepie *et al.* 2015). Additionally, ejaculated sperm numbers dropped by over 60% and the probability of producing sperm with severely impaired motility more than doubled (Preston *et al.* 2011). Similarly, following a group of captive zebrafish males (*Danio rerio*) over 31 months revealed fewer and fewer males to produce sperm. A decrease in sperm numbers was already observed early on, while a decline in motility occurred later in life (Johnson *et al.* 2018). With velocity being a key predictor of fertilisation success in external fertilisers, this should negatively impact male reproduction. In both cases, ejaculates could be sampled directly and repeatedly from individual males and revealed fewer and slower sperm to be produced with advancing male age. However, in both studies, males were abstinent between collections, thus ejaculate age was dependent on sampling interval. Ejaculate age affects sperm swimming speed in the guppy *Poecilia reticulate*, with sperm from a young ejaculate swimming faster (Gasparini *et al.* 2014), while the opposite effect was observed in the eastern mosquito fish *Gambusi holbrooki* (Vega-Trejo *et al.* 2019) and hence results from longitudinal studies might depend on the length of sampling intervals. Disentangling male age effects on ejaculate quality from ejaculate age thus would be informative, but is not trivial, particularly in animals where an ejaculate represents a fraction of the sperm and non-sperm components in storage. To clearly separate male age from ejaculate age effects, a male needs to be stripped first of his entire ejaculate to ensure a uniformly aged ejaculate and not a mix of freshly produced versus old products still in storage. Such an approach is promising in animals, where ongoing spermatogenesis is the norm and thus older males are as capable as young ones to deliver a 'fresh' ejaculate.

The observation for ejaculates of ageing males to contain fewer sperm is not uncommon (Supplementary Table 1) and indicates that also sperm production might be slowed (Vega-Trejo *et al.* 2019). Indeed in *D. melanogaster*, fewer germ line stem cells can be found in the testis stem cell niche with advancing male age (Boyle *et al.* 2007). The stem cells maintain their

A Male reproductive tract

Tissue senescence
might lead to
i) reduced
protein/sperm
production
ii) dysregulated
protein production
iii) pre-meiotic
sperm ageing

Ageing of
stored SFPs &
post-meiotic
sperm ageing

Reduced mating rate

Mating

Male senescence
might change
transfer of
sperm/SFPs

Female choice might
cause shorter transfer
times and/or increased
expel of sperm/SFPs

Ejaculate composition

Ejaculate transfer

Ejaculate quality & quantity

Sperm

Carrier for
several SFPs

Facilitate sperm
storage & usage

SFPs

Ejaculate receipt

immune system

metabolism

uterine conformational
changes

feeding and
sleeping patterns

**Female post-mating
response**

oviposition

sexual receptivity

ovulation

sperm storage

B

Survival vs Time graph with labels:
- [1]
- [3]
- Sperm transfer/storage
- [3]
- Uterine conformational changes
- [4]
- [3]
- female refractoriness
- [4]
- [3]
- [2]
- sperm competitiveness
- [3]
- [2]
- [1]
- offspring production
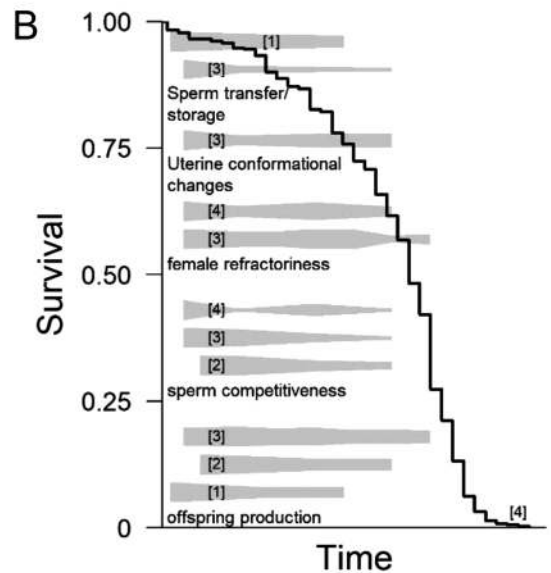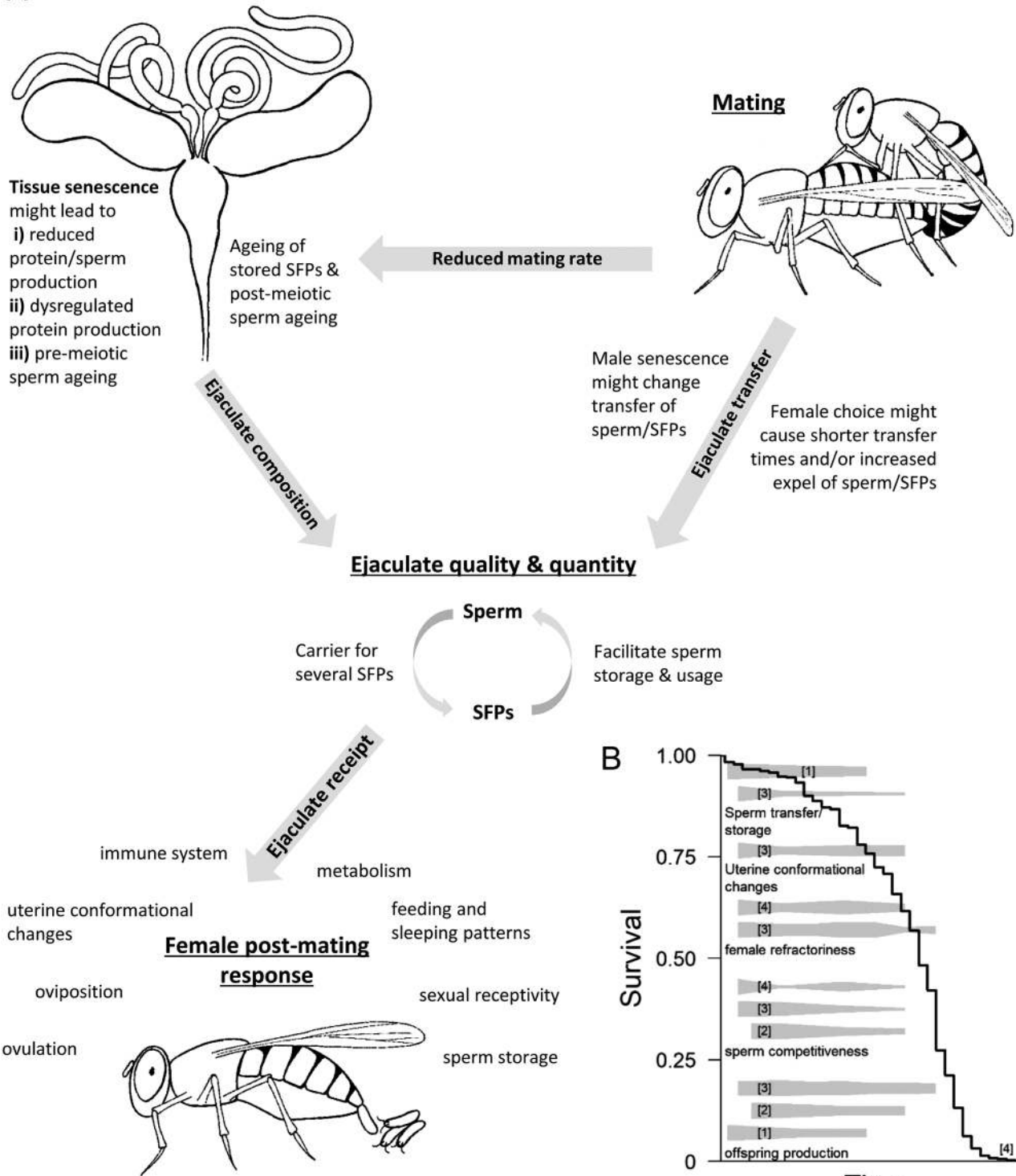- [4]

Survival axis: 1.00, 0.75, 0.50, 0.25, 0

Figure 1 (A) Schematic overview over possible routes via which Sfps and sperm in senescing *Drosophila melanogaster* males might influence female post-mating responses and thus male reproductive success. Information on the identity and function of various Sfps can be found in Box 2. (B) Graph shows survival (solid line) and expression of Sfp-mediated post-mating traits (in grey with changes in the magnitude of expression over time depicted by the thickness of the bar) in ageing *D. melanogaster* males over time. Mean survival time for males is assumed to be 65 days for data set [1] and 41.2 days for data sets [2], [3] and [4]. Data are derived from: [1] Rezaei *et al.* (2015), [2] Koppik and Fricke (2017), [3] Ruhmann *et al.* (2018), [4] Koppik *et al.* (2018).

ability to divide, but do so slower leading to a decreased spermatogenesis and thinner testes (Boyle *et al.* 2007). A reduced sperm production can translate into fewer sperm delivered to the female, matching observations of *D. melanogaster* females having fewer sperm in storage after mating to older males (Rezaei *et al.* 2015, Ruhmann *et al.* 2018). Transfer of less sperm though is not the rule, as the prospect of few remaining mating opportunities can enhance male investment in reproduction. Accordingly, older males transfer more sperm at mating than younger ones in for example *D. bipectinata* (Santhosh & Krishna 2013) or the butterfly *B. anynana*, in singly but not double mated males (Kehl *et al.* 2015). Also in humans, sperm concentration does not decline with age, but overall semen volume decreases (Johnson *et al.* 2015). However, increased or similar investments can be counteracted by other age-related changes as for example *B. anynana* females mated to younger males display higher fecundity than those mated to older ones despite older males' increased investment (Kehl *et al.* 2015), indicating that sperm numbers transferred is not a guarantee for reproductive success. Similarly in *Homo sapiens* sperm from older males were less motile and showed abnormal morphology (reviewed in Johnson *et al.* 2015).

Apart from the ability to produce and transfer sperm, viability is another commonly used measure of sperm ageing. So far, no consistent age-dependent pattern emerges for sperm viability (Supplementary Table 1). In general, sperm viability is higher in polyandrous species (Hunter & Birkhead 2002) due to selection pressures to maintain high fertilisation capacity under sperm competition. Hence, it is worthwhile to consider mating system in these assays as monandrous males might suffer stronger from an age-dependent decline in sperm viability than polyandrous ones and this might partly explain the variation in sperm viability data.

Increased accumulation of DNA damage with male age was reported in for example humans (Wyrobek *et al.* 2006, Kong *et al.* 2012) and old blue-footed booby males (Velando *et al.* 2011). The rate of mutation accumulation is predicted to be influenced by the number of germline stem cell divisions (Bartosch-Härlid *et al.* 2003). On a mechanistic level, sperm damage due to reactive oxygen species (ROS) has found much traction (reviewed in Reinhardt 2007). ROS production is tightly linked to metabolic activity, as it is a byproduct of mitochondrial energy production (Reinhardt 2007). In *B. anynana* both oxidative damage and antioxidative defence improved with male age (Kehl *et al.* 2015). In contrast, in the fowl, levels of seminal antioxidants were lower by 37% in old compared to young males potentially explaining the higher DNA damage found in older males (Noguera *et al.* 2012). Particularly old polyandrous males, who underwent multiple rounds of divisions, should possess more DNA damage, unless they also invest more in DNA repair and maintenance mechanisms. This damage has

evolutionary consequences due to its transgenerational effects and potential to persist in the germline, but to our knowledge a systematic investigation into age, male mating system/activity and DNA damage is lacking.

Sperm ageing has received much attention and while data are accumulating, we still have large gaps in our understanding. First, the distinction between pre-and post-meiotic sperm ageing is not trivial when length of post-meiotic storage inside the male is not controlled for. Also often single measures of age-dependent changes in sperm number or morphology are not linked to fertilisation ability (for e.g. by Moller *et al.* 2009, Gasparini *et al.* 2014, Herrera-Cruz *et al.* 2018). It is often implicitly assumed that age-dependent changes would result in a diminished fertilisation success; however, this is not necessarily the case as in some species like *D. bipectinata* and *D. pseudoobscura*, older males have higher reproductive success than younger males. Furthermore, the loss of male fecundity in *D. melanogaster* cannot always be explained by a decline in sperm numbers, as similarly a lack of seminal fluid proteins can lead to low fertility despite the presence of fertile sperm (Chow *et al.* 2015). Hence, in the next section, we will review the consequences of senescence of the non-sperm fraction of an ejaculate.

Ageing of the non-sperm fraction of the ejaculate

Across the animal kingdom, the non-sperm part of the seminal fluid contributes to reproductive success and shapes male–female interactions up until fertilisation. Some authors even suggest their absence might contribute to low implantation success in assisted reproductive technology (McGraw *et al.* 2015). The nature and function of seminal fluid proteins (Sfps) are well studied in *D. melanogaster* (Box 2), but are also known for other animals (Box 1). Ageing of the tissues that produces the non-sperm fraction and related quality changes has received far less attention in the study of male reproductive ageing. We here highlight that the onset and course of male reproductive senescence is significantly shaped by age-dependent changes in the production, transfer and action of Sfps (see also Bastos *et al.* 2017, Fig. 1).

While testis size often seems to decrease with male age (Supplementary Table 1), the opposite pattern is often found for the accessory gland (AG), the main Sfp producing tissue in insects (e.g. in flies, Rezaei *et al.* 2015, Reyes-Hernandez & Perez-Staples 2017; but see Mazeed and Mohanny, 2010 for honeybees). Whether these size changes improve male fecundity is unclear, as increased AGs contain more protein in some (e.g. *D. bipectinata* and *D. melanogaster*, Santhosh & Krishna 2013, Rezaei *et al.* 2015), but not all species (e.g. the Mexican fruit fly, Herrera-Cruz *et al.* 2018) and do not indicate how much protein is available at mating (Rezaei *et al.* 2015). The length of copulation duration

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Box 2: Seminal fluid proteins in *Drosophila melanogaster*

In the fruit fly *D. melanogaster*, the structure and function of seminal fluid proteins (Sfps) have been intensively studied (reviewed in Chapman & Davies 2004). A large fraction of these Sfps are produced and stored in the male accessory gland, and thus are referred to as accessory gland proteins (Acps, reviewed in Ravi Ram & Wolfner 2007). To date, about 200 different Sfps have been described in *D. melanogaster* and at least 138 of those are transferred to the female during mating (Findlay *et al*. 2008). Many Sfps have a profound effect on female behaviour and physiology (referred to as female post-mating response) elevating a male's reproductive success (Chapman & Davies 2004). The most prominently studied Sfp is sex peptide (SP), a short (36 amino acids long) peptide (Kubli 2003), for which the corresponding receptor in the female has been identified (SPR, Yapici *et al*. 2008). Upon ejaculate transfer, SP binds to sperm and is later gradually released in mated females, thus sustaining its effect long term (~1 week) compared to other Sfps (Peng *et al*. 2005). At the same time, other Sfps, for example Acp36DE, facilitate female sperm storage (Avila & Wolfner 2009), which highlights the intricate relationship between Sfps and sperm. Most Sfps act short term (~24 h) and ready the female reproductive system. Both Acp36DE and ovulin (Acp26Aa) facilitate the passage of the eggs. Acp36DE does so by mediating uterine conformational changes (Avila & Wolfner 2009), while ovulin simultaneously relaxes the oviduct musculature (Rubinstein & Wolfner 2013) causing a short-term boost in ovulation (Heifetz *et al*. 2005). SP influences female physiology in many ways, for example, by altering feeding intensity (Carvalho *et al*. 2006) and sleeping patterns (Isaac *et al*. 2010). Importantly, it enhances oogenesis leading to a substantial increase in egg laying after mating (Soller *et al*. 1997).

Apart from physiological changes also behaviour is affected and one important function of SP is the inhibition of female sexual receptivity (Chapman *et al*. 2003), which aids male reproductive success by avoiding sperm competition within the female reproductive tract. Short-term inhibition of female remating is further aided by PEBII, an Sfp associated with the mating plug produced by *D. melanogaster* males (Bretman *et al*. 2010). However, sperm competition within the female reproductive tract frequently occurs in *D. melanogaster* and the non-sperm fraction of the ejaculate is crucial to ensure that a male's sperm is represented in the fertilisation set to gain a significant paternity share (Fiumera *et al*. 2005, 2007). Due to their diverse roles in determining male reproductive success, we hypothesised several pathways through which Sfps might contribute to male reproductive ageing in *D. melanogaster* (summarised in Fig. 1).

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is often used as an indirect indication of investment into an ejaculate. In *D. pseudoobscura* copulation duration roughly doubled from 2–4 to 14–19-day-old males and these older males indeed induced a higher egg-laying rate (Dhole & Pfennig 2014), indicating that more of the relevant Sfps were transferred as observed in *D. bipectinata* (Santhosh & Krishna 2013). Ageing *D. melanogaster* males, on the other hand, transfer smaller ejaculates despite longer mating durations and larger AGs (Koppik *et al*. 2018). Hence, while copulation duration can be a good indicator of male investment, some caution is warranted. Additionally, senescing AGs in *D. melanogaster* lose their integrity and eventually individual cells at the tip of the gland exit the cell layer and entire delaminated cells are transferred to the female (Leiblich *et al*. 2012), bearing testimony to the profound structural changes of the tissue itself.

Senescence of the AG manifests at the phenotypic level as males failing to induce the appropriate female post-mating responses to Sfps. In the cricket *Allonemobius socius*, a reduced transfer of the Sfp ejaculate serine protease with advancing male age (8–40-day-old males) can be linked to a reduced egg-output in females (Marshall *et al*. 2009). Similarly, ageing *D. melanogaster* males (up to 6 weeks of age) decline in their ability to induce Sfp-mediated phenotypic post-mating changes, which together explain a large fraction of the observed lower reproductive success of older males, that was particularly evident when males encountered competition (Ruhmann *et al*. 2018). While only based on two Sfps, quantification of Sfp transfer revealed a mixed picture of age-dependent changes and no clear linear decline (Ruhmann *et al*. 2018), despite reduced expression for individual Sfp genes with age (Koppik & Fricke 2017). These are first steps and should

be complemented by larger scale transcriptome and/ or proteome studies. Assays of age-dependent changes in post-translation processes might prove equally informative, as this might explain the initial discrepancy between these first studies on Sfp expression, transfer and male phenotypic ability to elicit beneficial responses.

Different Sfps are often produced by distinct tissues or specialised cells within a tissue, with differential age-dependent declines in functionality potentially resulting in compositional changes. Particularly, senescence in regulatory mechanisms might lead to a dysregulated production of Sfps resulting in a sub-optimal seminal fluid composition. There is surprisingly little information on age-dependent compositional changes. One notable exception is the comparison of fowl ejaculates, and while compositional changes were found, again there were no simple age-dependent dynamics, as there was a strong distinction due to male age and sperm velocity (Borziak *et al*. 2016). Particularly, old males that retained high sperm velocity had a distinct Sfp signature, while those old males with slow sperm clustered closely with younger males (Borziak *et al*. 2016). While this is the first direct test, other studies hint at the possibility of compositional changes, but either have only explored a limited set of Sfps (Koppik & Fricke 2017, Ruhmann *et al*. 2018) or covered a limited age range (Simmons *et al*. 2014). Extending and broadening these studies using proteomics, allowing for direct quantification of proteins present and linking those insights with phenotypic data will be a fruitful avenue to our understanding of potential limitations ageing males encounter in producing a functional ejaculate. Here, longitudinal studies might prove particularly useful, by directly describing the trajectory of Sfp changes for individual males across their lifespan. This approach though can currently still

be technically and/or financially challenging. Species producing spermatophores might be a worthwhile study system as spermatophores are easily accessible and can be repeatedly sampled from the same individual.

Seminal fluid and sperm interactions

We so far considered the consequences of senescence of sperm and the non-sperm part of an ejaculate and the tissues that produce them separately. However, these two fractions interact, as the seminal fluid can have a protective function maintaining sperm viability (Simmons & Beveridge 2011) or aid sperm maturation (e.g. see Borziak *et al.* 2016 and literature within) and is also discussed as one possible explanatory factor in age-related declines in human fertility (Sartorius and Nieschlag 2010). Here we summarise the limited knowledge of the joint effects of age-related changes for both ejaculate fractions and highlight that this is a promising avenue for future research.

   As outlined in Box 2, Sfps aid sperm in reaching the sperm storage organs in *D. melanogaster* with older males less able to elicit the uterine transformational changes mediated by Acp36DE and as a consequence fewer sperm were found in storage shortly after mating (Ruhmann *et al.* 2018). Additionally, several of the Sfps are known to bind to the sperm tail (Peng *et al.* 2005), fewer sperm being transferred and/or stored thus also means fewer of those proteins being available to the female. The impact of senescent changes in these Sfp–sperm interactions on male reproductive success clearly need further investigations.

   The only other system, in which some of these dynamics between senescence in sperm traits and age-dependent changes in seminal fluid proteins have been investigated, is the fowl. Here, older males that retain high sperm velocity, a trait known to positively affect fertilisation success in this species, possessed a distinct Sf proteome (Borziak *et al.* 2016), indicating that males might be able to adjust their Sfp composition to maintain high sperm velocity and hence fertilisation rate. Though, clearly not all males were able to do so. In an independent study, older males displayed a low level of protective seminal antioxidants that was associated with a tendency to reduce sperm motility and sperm carried a signature of oxidative DNA damage (Noguera *et al.* 2012), indicating that the protection is leaky. As old males retained a high level of plasma antioxidants that was positively correlated with male comb size, a trait important for mating success, the authors speculated whether males trade-off post-mating success for pre-mating success as they age (Noguera *et al.* 2012). This opens up the possibility for condition-dependent ageing processes in this species which might explain why not all males in the previous study displayed a Sf proteome that facilitated high sperm velocity at old age. Given the high number of Sfps that are involved in sperm

capacitation, motility and viability at least in mammals (reviewed in Poiani 2006), Sfps are likely to play a key role in shaping these sperm traits in ageing males and thus deserve further investigation.

Interactions between the female reproductive tract and male ageing sperm

While male reproductive ageing and sperm ageing can compromise sperm genetic quality, females might take an active role and ameliorate damage. Support for this notion comes from the observation that *Drosophila* females can repair DNA damage in sperm (Vogel *et al.* 1985, Agrawal & Wang 2008). Apart from DNA repair, females can prevent negative effects of post-meiotic sperm ageing by eliciting extra copulations as seen in the black-legged kittiwake (*Rissa tridactyla*) (White *et al.* 2008) or reject copulations with old males, particularly if males reliably advertise the extent of their sperm damage as in the blue-footed booby (Velando *et al.* 2011). Additionally, female physiological adaptations aid sperm viability as observed in birds and the cricket *Gryllus bimaculatus* (Breque *et al.* 2003, Ribou & Reinhardt 2012) or sperm movement patterns in barn swallows *Hirundo rustica* (Moller *et al.* 2009) and alleviate the effects of post-meiotic sperm ageing. This indicates that females might actively alter sperm metabolism across a wide range of taxa and potentially protect sperm from degradation or exert cryptic female choice, whereby in zebrafish sperm selection can benefit offspring fitness (Alavioon *et al.* 2017).

Factors affecting reproductive ageing

A host of factors are known to alter the speed of actuarial ageing; however, we have limited insight, whether these factors have a similar effect on reproductive ageing. Rates of senescence are modulated by the lifespan – fecundity trade-off, whereby increased investment in early reproduction often accelerates somatic ageing (Kim *et al.* 2011, Cornwallis *et al.* 2014, Lemaitre *et al.* 2015), but also reproductive ageing (Preston *et al.* 2011). The long-standing underlying premise being that reproduction and somatic maintenance compete over resource allocation. More recently, hyperfunction (Blagosklonny 2012) – the idea that cellular processes optimal during development and early life continue to run at high levels during late life, where they cause harm and thus promote ageing – presents an alternative explanation for senescence (Gems & Partridge 2013). A recent study in *C. elegans* supports this idea: reduction of insulin/IGF-1 nutrient sensing signalling in adult nematodes simultaneously improved late-life reproduction (of mated worms) and longevity without any decline in total reproduction (Lind *et al.* 2019). Moreover, offspring of those treated worms had increased reproduction compared to worms from parents with a normal level of insulin/IGF-1 signalling,

thus showing that longevity and Darwinian fitness can be improved at the same time (Lind *et al.* 2019). Studying the genetic basis of reproductive ageing in *C. elegans* Wang *et al.* (2014) found 32 genes to extend reproductive lifespan, many of which interacted with either the insulin/IGF 1 or the TGF ß pathways. In this hermaphroditic animal equally many affected oocyte senescence (19 genes) as well as senescence of male function (11 genes), but curiously this did not affect spermatogenesis (Wang *et al.* 2014). These pathways also have a prominent role in affecting actuarial ageing and this might be part of a general and conserved link underlying the apparent fecundity–longevity trade-off. Illuminating the genetic factors of reproductive ageing will be fruitful future endeavours to pinpoint the pathways underpinning reproductive ageing.

Similar to actuarial ageing, reproductive ageing could potentially also decelerate under caloric restriction, when simultaneously the reproductive tissue is protected from physiological decline. Indeed, dietary restriction attenuated the age-related decline in male reproductive senescence in *D. melanogaster* by enhancing male germline stem cell maintenance and halting the age-related depletion (Mair *et al.* 2010). While at the phenotypic level, diet had little influence on age-dependent changes in insemination success in the Mexican fruit fly *A. ludens* (Harwood *et al.* 2015). The question whether males can delay reproduction and reallocate resources towards increased survival in times of scarcity and simultaneously decelerate reproductive ageing deserves further investigations.

Another fruitful avenue of potential modulators of reproductive ageing is the social environment whereby intra-sexual competition (Rodriguez-Munoz *et al.* 2019) or the mere perception of mates in the environment (Gendron *et al.* 2014) can cause increased mortality and senescence in pre-mating traits (Rodriguez-Munoz *et al.* 2019). This is in line with observations that the socio-sexual environment leads males to allocate more resources into reproduction when rivals are present (Bretman *et al.* 2011), with males held in isolation showing a slower reduction in lifespan (Leech *et al.* 2017). In *D. melanogaster*, male mating history did not accelerate reproductive ageing for pre-mating traits, but caused a distinct pattern for post-mating traits (Koppik *et al.* 2018). For these latter traits, prior mating opportunities proved beneficial in young males but neutral or negative in older males, potentially due to different reproductive investments and a trade-off between early and late reproductive efforts.

Open questions and outlook

One point became obvious from our review of male reproductive ageing across animal taxa: comparisons among different studies are often hampered because authors fail to report lifespans in their experimental population. This oversight makes it difficult to appropriately classify investigated age ranges. Hence, future studies should routinely report the average and maximal lifespan of their species. Ideally, measures of lifespan should be conducted simultaneously in the experimental cohort, as lifespan estimates can vary critically with experimental conditions. Additionally, cohort-based experiments might not be ideal when investigating senescence patterns. Since the selective appearance and disappearance of individuals with different reproductive strategies (Kim *et al.* 2011) and/or genetic quality (Torres *et al.* 2011) can mask or inflate ageing patterns when considering the entire cohort (Bouwhuis *et al.* 2009, Dugdale *et al.* 2011), calling for more studies following individuals across their lifespan. Longitudinal studies give greater insight into possible trade-offs, the shape and rate of age-dependent changes and allow better control for selective disappearance and the possible reasons behind it.

Here, we have outlined changes occurring in sperm and seminal fluid with male age and summarised reproductive trait expression in senescing males. While data are accumulating on each of the aforementioned aspects, studies integrating several or all facets of male reproduction in ageing individuals are still scarce (but see Marshall *et al.* 2009). Often, it is technically challenging to access all male components that contribute to reproductive success and the techniques required to thoroughly examine them might be very different for sperm and Sfps. However, given the intricate relationship between sperm and the non-sperm fraction of the ejaculate, we suggest that more integrated studies are necessary to fully understand male reproductive senescence. Reports on changes in sperm with male age outnumber those investigating a male's Sfp complement by far, but, with transcriptomics and proteomics becoming more accessible, this might soon start to change.

Another promising and exciting avenue is revealing how ejaculates of ageing males affect the multitude of interactions with female physiology. We already highlighted that females might actively alter ejaculate characteristics and through (cryptic) choice and investment decisions might mitigate or exaggerate effects of male senescence. Disentangling male age from female choice and elucidating the underlying mechanisms will be exciting future research programmes. Hence, many open questions remain into the ecological and evolutionary causes and consequences of male reproductive ageing.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/REP-18-0579.

Declaration of interest

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