UPPSALA
UNIVERSITET

Haploid selection in animals

Exploring the fitness consequences and underlying mechanisms

GHAZAL ALAVIOON

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, Norbyvagen 18d, Uppsala, Friday, 14 September 2018 at 13:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Professor Tommaso Pizzari (University of Oxford).

Abstract
Alavioon, G. 2018. Haploid selection in animals. *Exploring the fitness consequences and underlying mechanisms*. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1681. 41 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0358-1.

A consequence of sexual reproduction in eukaryotes is the evolution of a biphasic life cycle with alternating diploid and haploid gametic phases. While our focus in evolutionary biology is on selection during the diploid phase, we know relatively little about selection occurring during the haploid gametic stage. This is particularly true in predominantly diploid animals, where gene expression and hence selection have long been thought to be absent in haploid cells like gametes and particularly sperm. During my PhD, I tested the idea of selection during the haploid gametic phase using zebrafish *Danio rario* as a study species. I combined a large-scale selection experiment over three generations with fitness assays and next-generation sequencing to assess the importance of haploid selection. We measured offspring fitness in all three generations. In addition, we compared gene expression in brain and testes of F1 and F3 adult male from each treatment by RNA sequencing. We found that offspring sired by longer-lived sperm showed higher survival rate and higher early- and late-life reproductive fitness compared to offspring sired by shorter-lived sperm. We also found differentially expressed genes between the two treatments with functions in metabolic and developmental pathways. These findings suggest that the observed fitness differences to be caused by small expression changes in many basic genes. We also tested for a genetic underpinning of the selected sperm phenotypes and identified allelic differences across the entire genome. Finally, we investigated the additive genetic component and parental effect of different sperm phenotypes. We found generally low additive genetic variation and high parental effects on sperm performance traits. In conclusion, this thesis provides evidence that the phenotypic variation among intact fertile sperm within an ejaculate affects offspring fitness throughout life and provides a clear link between sperm phenotype and offspring fitness and between sperm phenotype and sperm genotype.

Keywords: sperm, evolution, haploid selection, reproductive aging, fitness

Ghazal Alavioon, Department of Ecology and Genetics, Norbyvägen 18 D, Uppsala University, SE-752 36 Uppsala, Sweden.

Maybe, not every sperm is sacred!

Cover illustration name "Fertilization" by Roy M. Francis based on the photo taken from zebrafish sperm entering the papilla of an egg (Hoechst Staining x100 fluorescent microscope). Photo credit Ghazal Alavioon & Roy M.Francis.

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I.   **Alavioon, G**.**,** Hotzy, C.*, Rudolf, S., Zajitscheck, S., Maklakov, A.A., Immler, S. (2017) Sperm selection within a single ejaculate increases offspring fitness. *PNAS,* 10(2), p.20131040.

II.   **Alavioon, G**., Cabrera, A., Le Chatelier, M., Maklakov, A.A., Immler, S. Within-ejaculate selection for sperm longevity reduces male reproductive ageing. *Manuscript*

III. **Alavioon. G.**, Francis, R.M.*, Wyszkowska, J., Immler, S. The fitness consequences of selection in haploid sperm across generations. *Manuscript*

IV.   **Alavioon, G.**, Baños-Villalba, A., Schielzeth, H., Immler, S. Sperm performance traits exhibit low heritability and strong parental effects in external fertilizer. *Manuscript*.

* These authors contributed equally to the study.

Reprints were made with permission from the respective publishers.

Additional Papers

The following papers were published/in publishing process during the course of my doctoral studies but are not part of the thesis.

Immler, S., Hotzy, C., **Alavioon, G.**, Petersson, E., Arnqvist, G. (2014) Sperm variation within a single ejaculate affects offspring development in Atlantic salmon. *Biology Letters,* 10(2), p.20131040.

C.Berg, E., I. Lind, Martin., **Alavioon, G.**, Maklakov, A.A. (2014) Evolution of differential maternal age effects on male and female offspring development and longevity. *Functional Ecology.* 29(1), pp.104–110

Promerova, M., **Alavioon, G.**, Tusso Gomez, S., Burri, R., Immler, S. (2017) No evidence for MHC class II-based disassortative mating at the gamete level in Atlantic salmon. *Heredity.* 118(6), p.563

Silva*, WTAF., Jolly*, C., **Alavioon, G.**, Kiehl, B., Immler, S. Perceived sperm competition intensity in zebrafish males affects gene expression in early offspring. *Manuscript*

* These authors contributed equally to the study.

Contents

Abbreviations

DNA	deoxyribonucleic acid
dpf	days post fertilization
hpf	hours post fertilization
IVF	*in vitro* fertilization
LAT	long activation time
RNA	ribonucleic acid
SAT	short activation time
spa	seconds post activation

1 Introduction

1.1 Sexual reproduction and the evolution of a biphasic life cycle

Sexual reproduction is the most common form of reproduction in eukaryotes. In order for sexual reproduction to occur two individuals of two different sexes are needed to reproduce and contribute to the next generation. In animals, a sperm from a male and an ovum from a female are required to produce a zygote. The ubiquity of sexual reproduction is still somewhat unclear, as it bears substantial costs (Daly 1978; Lloyd 1980). However, sexual reproduction and the resulting combination of genomes from different individuals also have many potential benefits. One benefit is the effective removal of deleterious mutations. For example if mutations are more harmful to males than females as a result of sexual selection then those deleterious mutations have lower equilibrium frequency in sexual populations compare to asexual populations (Agrawal 2001). This means that if an individual is reproducing asexually, it will, with high probability pass the mutation on to its progeny but when the same individual reproduces sexually then the frequency of the mutation is potentially lost, which results in the dilution across generations and in the population (Agrawal 2001). In the 19th century, it was suggested that due to the fact that there are no obvious direct fitness advantages to sexual reproduction, other evolutionary forces must be behind the phenomenon of sexual reproduction (Crow 1994). One hypothesis was that sexual reproduction provides the possibility of recombination that can help populations to adapt to the new environments more efficiently (Crow 1994) and potentially survive better, therefore despite the costly process of sexual reproduction, it outcompeted the asexual form and became a dominant way of reproduction among eukaryotic taxa. As a consequence, the biphasic life cycle can be found in all sexually reproducing eukaryotes.

In 1851, Hofmeister discovered that plants have two distinct phases in their life cycle and that they constantly alternate between the two across generations (Mable and Otto 1998). In 1894, Strasburger suggested that this alternation in fact is the alternation between diploid and haploid phases (Roe 1975).

It has since been shown that sexual eukaryotes in general exhibit a life cycle with alternating haploid (gametic or gametophytic) and diploid (sporophytic)

phases (Mable and Otto 1998; Hughes and Otto 1999). The relative duration of each phase varies across taxa and may range from organisms with growth and development occurring largely during the haploid phase (haplontic; species of green algae (Otto and Marks 1996), brown algae (Bell 1997) and yeast (Fowell 1969) to those where growth and development occurs mainly during the diploid phase (diplontic; most animals) (Richerd *et al*., 1993) [Figure 1]. In some organisms such as the red alga *Gracilaria verrucosa*, growth and development processes may occur in both phases and they can co-exist potentially occupying different ecological niches (Hughes and Otto 1999). In this alga, both diploid and haploid spores can co-exist. Both spore types survive better than male gametes and haploid spores have even higher survival (Destombe *et al*., 1992). In green algae *Ulva* spp., vegetation and growth happens in both, the haploid and diploid phase (Mable and Otto 1998).
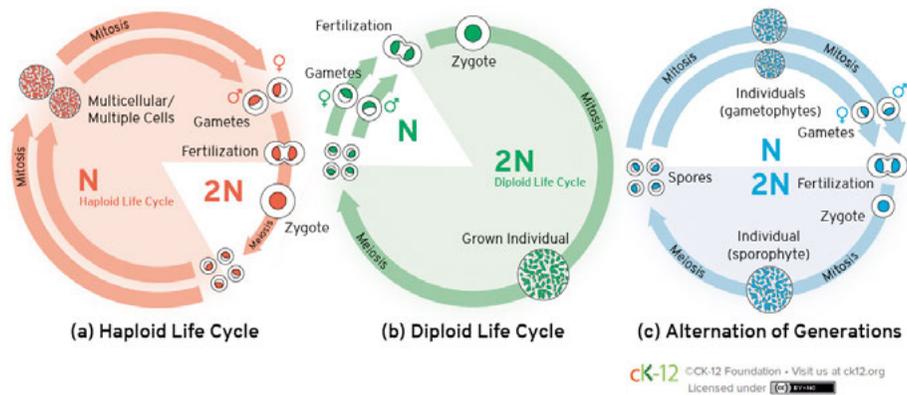

Figure 1. Three form of life cycles, from left to right: haploid, diploid and haplo-diploid.

1.2 Haploid and diploid selection

The mechanisms and outcomes of selection occurring during diploid and haploid phases differ substantially (Crow and Kimura 1965). Because diploids have two copies of each allele, they can mask recessive mutations, which are therefore less exposed to selection. In contrast, when an allele is expressed in a haploid state, it is entirely exposed to selection since there is no masking effect of a sister allele. This can in fact facilitate the rate of spreading and fixation of beneficial alleles while reducing the accumulation of deleterious mutations in a population by efficiently eliminating deleterious mutations (Haldane 1924; Crow and Kimura 1965; Mable and Otto 1998). Diploid populations have a higher chance of carrying a new mutation,

and while this can be an advantage when the mutation is beneficial, it is a disadvantage when the new mutation is deleterious. Diploid populations also have the advantage of survival due to the masking effect of recessive alleles given that the majority of mutations affecting fitness are partially recessive (Simmons and Crow 1977), while the occurrence of such mutations is generally low in haploid populations (Mable and Otto 1998). Haploid populations on the other hand, may have lower survival (Mable and Otto 1998) due to the lack of masking effect for deleterious mutations but those who survive can carry beneficial alleles and pass them on to the next generations more efficiently, therefore haploid populations tend to have higher average fitness in equilibrium (Crow and Kimura 1965).

Haploid selection is a situation in which a phenotype under selection is determined by a haploid allele (Joseph and Kirkpatrick 2004).

Haploid gene expression and haploid selection is proven and well studied in plants (Mascarenhas 1990). In 1982, Zamir *et al.* showed that gametophytic selection for low temperature tolerance of tomato pollen is at least partially determined by genes expressed in the haploid pollen (Zamir *et al.*, 1982). Several other studies also showed the expression of many genes in haploid male gametophytes in flowering plants (Mascarenhas 1990; Walbot and Evans 2003; Borg *et al.*, 2009).

In contrast, in animals, haploid selection is understudied due to an existing dogma that gene expression at the post-meiotic haploid phase is largely absent. Many researchers dismissed the possibility of haploid selection in animals for several reasons. The first reason was the fact that most animals spend the majority of their life cycle as diploids followed by a very short haploid stage. The other reason was that the DNA in sperm/gametes is densely packed and almost entirely lacking a cytoplasm, therefore haploid gene expression and translation (Kettaneh and Hartl 1976) are impossible and sperm in basically transcriptionally silent (Steger 1999; Joseph and Kirkpatrick 2004). Although later on, researchers found evidence of post-meiotic DNA transcription in sperm, they believed that the newly made transcriptomic products and other molecules could be shared between sperm cells through cytoplasmic bridges (Jeon 2004), therefore, all sperm cells benefit from the similarly defined sperm traits and none of the sperm develops advantages over others. Later on researchers found more proofs of DNA transcription (Erickson *et al.*, 1981) and even small amounts of protein translation in sperm cells (Gur and Breitbart 2006; Gur and Breitbart 2007; Gur and Breitbart 2008), and evidence that showed not all of the transcriptomes and proteins can be passed through cytoplasmic bridges (Erickson *et al.*, 1981). They also found that the alterations of the epigenome of sperm after meiosis (Teperek *et al.*, 2016) cause individual sperm to vary and to affect the next generation offspring differently. All these post-meiotic changes may form a basis for differences between individual sperm and create a potential for haploid selection to occur. In 2004 a review on a few studies showed several loci in animals' genome experience haploid selection and it empha-

sized that such selection might potentially affect several evolutionary processes. Antagonistic adaptation between haploid and diploid phases, sex specific recombination rates and genome imprinting, loads of deleterious mutations and extent of inbreeding depression are a few, among the many ways that haploid selection can affect evolutionary processes (Charlesworth and Charlesworth 1987; Charlesworth *et al.*, 1993; Joseph and Kirkpatrick 2004; Wyman and Wyman 2013).

1.3 Selection at the gametic stage

Throughout evolution gametes have been under strong selection. Natural selection drove the ova to be big and immobile while sperm became tiny and mobile (Parker *et al.*, 1972; Lehtonen and Parker 2014). Sexual selection, which is one form of natural selection acting on sperm, strongly contributed to the unique phenotypic diversity of sperm. From amoeboid sperm of nematodes lacking a tail entirely (Ward and Carrel, 1979) to sperm of an Australian termites species *Mastotermes darwiniensis* with 100 tails (Baccetti and Dallai, 1978), they all have one function only: to fertilize an ovum.

From mating to producing offspring, sexual selection can act at each and every step of the way (Kamimura 2014). The high number of spermatozoa produced by each male and the relatively low number of ova produced by each female force males to compete for access to females, whereas females are thought to choose among males (Andersson 1994; Parker and Simmons 1996). The two processes of male-male competition and female choice are driving the evolution of primary and secondary sexually selected traits such as male courtship behavior, male ornamentation and male weaponry, and these might can alter corresponding preferences in females (Kamimura 2014).

Sexual selection was thought to be limited to pre-mating stages until Geoff Parker discovered that sexual selection continues after mating (Parker 1970). He discovered that in polyandrous species, sperm from multiple males compete for the access to a limited number of ova in a process called sperm competition. A little later it was recognized that females are not passive at the postcopulatory level either, but that they evolved mechanisms that allow them to choose among sperm of different males (cryptic female choice; Andersson 1994). Similar to male-male competition and female mate choice during pre-copulatory sexual selection (Kvarnemo and Simmons 2013), sperm competition and cryptic female choice are the respective equivalents occurring during post-copulatory sexual selection (Parker 1970). Post-copulatory sexual selection has driven the evolution of many primary and secondary sexual traits such as mate guarding (Poole 1989), copulatory plugs (Sutter and Lindholm 2016) and sperm displacement mechanisms (Birkhead and Møller 1998; Simmons 2001). As processes involved in cryptic female

choice, females developed mechanisms such as sperm ejection (Pizzari and Birkhead 2000; Wagner *et al*., 2004; Córdoba-Aguilar 2006; Droge-Young *et al*., 2016), sperm utilization, and oviposition after copulation with fitter males meaning the sperm of certain males are favored over those of other males based on their phenotype or genotype (Choe and Crespi 1997; Firman *et al*., 2017).

Both pre- and post-copulatory selection and the processes involved as described above exposed sperm to continuous strong selection, which resulted in the unique diversity among sperm phenotypes (Snook 2005) despite the fact that this cell type has essentially the same function across all taxa (Baccetti and Afzelius 1976; Birkhead *et al*., 2008). Over generations, sperm competition and cryptic female choice lead to the rapid evolution of sperm traits. In some *Drosophila* species, longer sperm have the advantage to displace their small rival sperm in female sperm storage organs, therefore achieving a higher fertilization success (Lüpold *et al*., 2016). Moreover, the female reproductive tract selectively biased paternity in favor of males with longer sperm (Miller and Pitnick 2002), which led males to produce extraordinarily long sperm (Miller and Pitnick 2002; Lüpold *et al*., 2016). In the field cricket *Gryllus bimaculatus* on the other hand, males selected for longer sperm in an artificial selection experiment, had no greater fertilization success over those selected for shorter sperm (Morrow and Gage 2001b).

The main cause of the variation in sperm size (LaMunyon and Samuel 1999), sperm velocity (Gage *et al*., 2004) and other sperm traits, are similar processes involved in sperm competition (Snook 2005).

As mentioned above the concept of sperm competition was defined, as two or more males competing over the ova of a female (Parker 1970; Parker 1982) and the concept of intra-ejaculate sperm competition was not considered because scientists believed that there is no sperm competition unless there is a rival male or ejaculate in the picture (Simmons 2001). However, the great number of spermatozoa produced by each male in most species induces potentially strong sperm competition not only between sperm from different males but also between the sperm of a male in a single ejaculate and that is because sperm, either within the female reproductive tract or the harsh surroundings have to face numerous physical, chemical and immunological barriers to reach and fertilize the ova therefore in each copulation only a small fraction of sperm will reach and fertilize an egg (Cohen and McNAUGHTON 1974; Birkhead *et al*., 1993).

Beside all the mechanisms and behavioral adaptations described above that are found in both sexes that act as a selection force on male and female gametes, the shear number of sperm produced in the ejaculate of a male and the competition among them may act as a selective force to produce high quality sperm. However, while sperm competition between males is well studied, sperm competition within a single ejaculate has not received much attention. In Chapters I, II and III of this thesis I have focused on identifying different sperm cohorts by their varying phenotypes within a single ejaculate of a

male. Using artificial selection on sperm phenotype, I assessed the consequences of sperm phenotype on fitness traits of offspring from early embryo to adulthood.

1.4 Sperm research

Ancient Greek philosophers believed the generation of a new life originated from male semen and female menstrual blood (Birkhead *et al.*, 2008). Hipppocrates (ca.460-370 BC), Aristotle (ca.384-322 BC) and Galen (129-ca.200AD) considered that both sexes are important for reproduction. It was also assumed that semen of a male is a crucial component of reproduction and the female body is nourishing the seed from the male (Birkhead *et al.*, 2008). As a consequence, the word "spermatozoa" is derived from the Greek word "spermein" which means, "to sow" (Birkhead *et al.*, 2008). In 1676, sperm were named 'animalcules' (little animals) when Anton Leeuwenhoek first observed wiggling particles in a drop of a rabbit, a dog and his own semen using his own constructed microscope (Leeuwenhoek, 1678; Howards, 1997) [Figure 2].

Leuwenhook's finding, initiated a wave of interest in sperm biology. In the eighteenth century, some naturalists believed that spermatozoa only exist in semen to keep it fluid and that they have no role in reproduction (Cobb 2007), whereas others considered sperm as an organism that should be classified along with animals (Birkhead *et al.*, 2008). In 1780, Lazzaro Spallanzani discovered the crucial role of semen in fertilization by designing contraceptive trousers for male frogs preventing them from achieving fertilizations. Although Spallanzani was unable to assess and separate the role of spermatozoa and semen, in 1824, two other naturalists resolved the issue of whether the spermatozoa or seminal fluid triggers the development of ova (see also Wagner 1837). Finally in 1841, Albrecht von Kölliker suggested that sperm are actually cells and a few years later, three publications documented the interaction between spermatozoa and ova during fertilization in the sea urchin; (Von Baer 1847; Dufossé 1847; Derbès 1847).
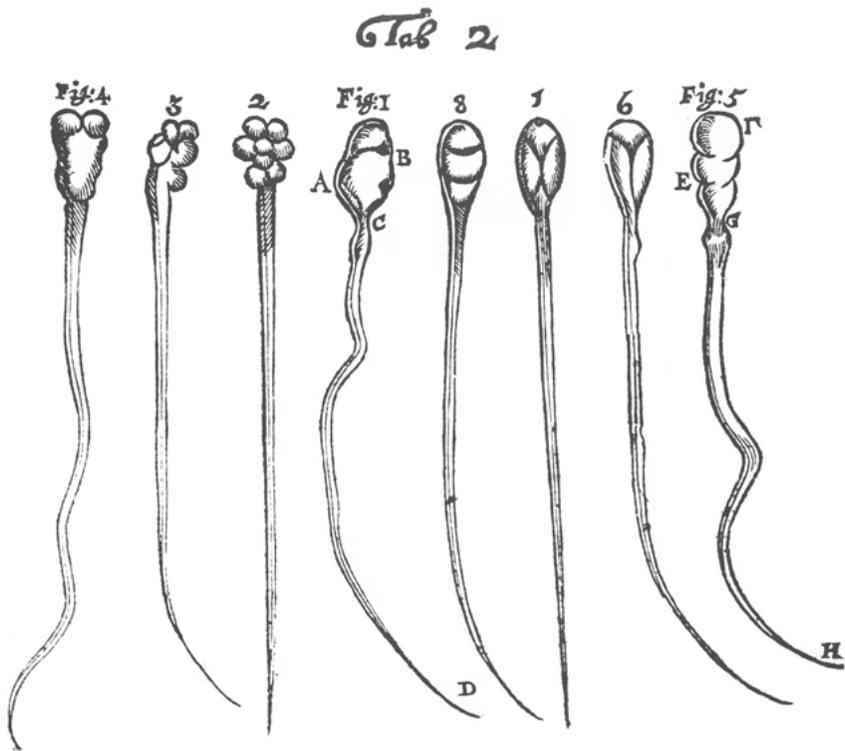
Figure 2. 1-4 *Human spermatozoids*, 5-8. *Canine spematozoids* drawn by Anton Leeuwenhoek. Source: (Chemes and Rawe 2003), (see also Phil. Trans. XII, 1678, no. 142, Table 2, drawings sent by Leeuwenhoek.)

For many years, sperm were believed to be only the DNA courier to the egg without having any additional role in fertilization or embryo development. In the early 1990s, researchers confirmed that beside DNA, sperm can transfer RNAs, protein, centrioles and epigenetic marks (DNA methylation and histone modification) (Parrington *et al*., 1996; Miller *et al*., 2005; Miller *et al*., 2010; Marshall 2015).

Our knowledge in sperm biology has come a long way and although there are still many more aspects and unanswered questions in sperm biology, many areas of basic and applied research including ecology and evolution, reproductive biology, human fertility, animal breeding and conservation biology have already benefited from around 340 years of sperm biology research (Birkhead *et al*., 2008).

1.5 Heritability of reproductive traits

Heredity is defined as a process by which parental traits are transmitted to the offspring through DNA. Based on individual variation within populations, the "constantly changing environment" forms a platform for natural selection to act on, and gives some individuals benefits or disadvantages both in survival and reproduction (achieving higher or lower fertilization success) and in general fitness. If these traits are to some extent heritable, then the next generations will continue to have similar traits. Charles Darwin (1859) reasoned that if parents with better fitness traits (traits resulting in higher survival and reproductive success) pass on these traits at a higher rate to the next generation, these traits would become more common in the population.

Traits vary substantially in their heritability. It has previously been shown that the traits more closely associated with fitness of an organism are generally more likely to have less heritability than the traits which are less associated to fitness, like morphology (Falconer 1960; Gavrus-Ion *et al.*, 2017). Rapid fixation of alleles leads to natural selection conferring highest fitness and not allowing many changes, therefore traits related to fitness are expected to have lower additive genetic variance (Houle 1992; Kruuk *et al.*, 2000). In other words, life history traits such as longevity and fecundity tend to have lower heritability than morphological, physiological and behavioural traits (Gustafsson 1986; Price and Schluter 1991). It had been shown that in crickets, ovary weight had higher additive genetic variance than embryo survival (García-González and Simmons 2007). Similarly, in the Glanville fritillary butterfly *Melitaea cinxia*, body size, female age at first reproduction and egg mass were all moderately to highly heritable, whereas heritabilities were low or non-existent in clutch size and lifetime egg production (Saastamoinen 2008). In free-ranging snakes, traits like egg mass showed high maternal heritability, but other traits like clutch size, clutch mass and post-partum maternal mass have very low heritability (Brown and Shine 2007).

Understanding the underlying genetic basis and degree of heritability of life-history traits is critical because it determines the effects and gradation of selection on such traits (Brown and Shine 2007). This understanding can be very informative and beneficial in animal breeding programs to select for desired traits. For example in cattle males, the testicular measurements are moderately to highly heritable, while heritability estimates for calving rate in females is ranked low to moderate. If for example selection for increased testicular size would result in an improvement in female reproduction, particularly an increase in calving rate and a decrease in first breeding age which are critical traits in measuring cattle reproductive performance, then to produce females with higher reproductive performance it would be sufficient to perform a selection on males testicular size without the need for selecting

on female desired traits directly (Meyer *et al*., 1990; Goyache and Gutiérrez 2001).

Research on heritability of sperm traits in animals is quite controversial. Several studies showed a substantial additive genetic component in heritability of sperm traits from fathers to sons while others showed very low additive genetic component. In promiscuous animals, sperm traits that are positively correlated with fertilization ability are reported to be heritable (by additive genetic component). Several studies show that sperm design traits (e.g head, mid-piece and flagellum length) are generally heritable in several species including the zebrafinch, *Taeniopygia guttata* (Birkhead *et al*., 2005), cricket *Gryllus bimaculatus* (Morrow and Gage 2001a) and In the dung beetle *Onthophagus taurus* (Simmons and Kotiaho 2007). There are several other studies reporting the high heritability of other sperm phenotypes like sperm functions (motility, velocity and viability) in the domestic fowl, *Gallus gallus domesticus* (Froman *et al*., 2002), in the cockroach *Nauphoeta cinerea* (Moore *et al.,* 2004) and in White leghorn (Bongalhardo *et al*., 2000). In Canadian Holstein bulls, heritability of semen volume, concentration, sperm motility, number of sperm, and number of motile sperm per ejaculate were heritable in young bulls and the heritability scores increased in mature bulls (Mathevon *et al*., 1998).

In contrast, other studies show low heritability of sperm competition success in bulb mite *Rhizoglyphus robini* (Konior *et al*., 2005), low heritability of sperm motility and no heritability of sperm concentration in *Nellore bulls* (Sarreiro *et al*., 2002) and low to no heritability of sperm viability in yearling Angus bulls (Knights *et al*., 1984).

So if not heritability, what is causing the variation in fitness related traits? Variation in fitness related traits like survival and reproductive traits in many species should be caused by factors other than direct genetic component. Parental effects are no longer neglected and counted as troublesome factors when assessing genetic heritability of traits (Mousseau and Fox 1998). It has been shown that parental effects are shaped by natural selection and act as efficient factors in adaptive phenotypic responses (Mousseau and Fox 1998) thereby affecting selection outcomes. Parental effects can lead to variation in offspring phenotypes and might also affect grand offspring development, fitness and reproductive traits (Curley *et al*., 2011). In the African cichlid *Neolamprologus pulcher*, a cooperativley breeding fish, helping behaviors had very low heritability, therefore the high within-individual consistencies in these behaviors were mainly due to maternal and permanent environment effects (Chervet *et al*., 2011). It also has been shown that in crickets, embryo survival is more affected by parental indirect genetic effects than an additive genetic component (García-González and Simmons 2007).

Although there are several studies on heritability of traits in breeding animals, there have been very few studies on parental effect and the heritability of sperm traits. In one study, Birkhead *et al*. (2005) showed that while sperm morphology traits like sperm head, mid-piece and flagellum length are herit-

able, significant indirect (maternal) genetic effects is also existing. Studies like this are important because understanding the genetic or non-genetic mechanisms behind the heritability of reproductive traits can greatly affect the degree of selection and successful production in breeding programs (Goyache and Gutiérrez 2001), assisted fertilization techniques and it also gives us insights to several evolutionary processes. Indirect genetic effects and parental effects collectively can have extensive implications for evolutionary processes due to the fact that they allow selection to act on traits for which there is no or very little additive genetic variation (García-González and Simmons 2007). In chapter IV of this thesis, I therefore focused on heritability of sperm traits in the zebrafish.

1.6 Reproductive Aging

Aging or senescence is the process of physiological deterioration of an organism over time, which results in the reduction of reproductive output and of the probability to survive (Partridge and Barton 1993). Oxidative damage to DNA, proteins, and other macromolecules that accumulate in the cells and tissues over time are thought to be one of the most important factors that lead to senescence (Ames *et al*., 1993). Also mutations may occur over time and those mutations that affect late-life traits can accumulate in a population when the individuals have a long life span (Wachter *et al*., 2013; Maklakov *et al*., 2015). Mutation load increases stress on soma and that can cause reduced reproductive performance, which results in the putative trade-off between lifespan and reproduction (Briga and Verhulst 2015). However, in some situations where life span extension has strong negative effects on one sex, it can have negligible or even positive effects on reproduction of the other sex (Berg and Maklakov 2012; Maklakov and Immler 2016). Several studies showed different effects of aging on reproduction in both human and animals. In human and subhuman primate species, the oocyte quality and implantation ability in females are reduced with increased age (Meldrum 1993). In female cockroaches *Nauphoeta cinerea*, mating after the optimum age (6 days postadult molt) resulted not only in fewer clutches and fewer eggs in each clutch but also reduced choosiness of the females, and therefore less investment into courtship before mating (Moore and Moore 2001). Males in most mammals also go through physiological and behavioral changes as a result of aging, which can cause a decrease in reproductive capacity (vom Saal *et al*., 1994; Strauss *et al*., 2013). In the brown Norway rat (also referred to as common rat), *Rattus norvegicus*, that exhibit a longer life span without suffering from age-related dysfunctions as Sprague-Dawley rats do, testicular sperm concentration and total sperm production declines in older males (Wang *et al*., 1993).

Reproductive aging affects both males and females but the ways in which the two sexes age may differ (Chen and Maklakov 2014; Griffin *et al*., 2018). While some previous studies showed the evolution of more rapid aging rate in male than in female in vertebrates (Clutton-Brock and Isvaran 2007) including natural populations of mammals (Promislow 1992; Carranza *et al*., 2004; Nussey *et al*., 2009), some others showed a higher mortality in females than males (McCulloch and Gems 2003). One of the important factors for male-biased mortality across taxa is the high cost of production and maintenance of male sexually selected traits (Promislow 1992) and intense male competition in polygamous species as we observe a female-biased mortality in monogamous species (Clutton-Brock and Isvaran 2007).

The antagonistic effect of aging and in particular reproductive aging on different sexes and the potential effect of haploid selection in the process pursued us to investigate the question further. In chapter II of my thesis, I investigated the effect of sperm selection within a single ejaculate on reproductive aging as an important trait reflecting overall fitness of the offspring sired by different sperm cohorts within a single ejaculate.

2 Study system

2.1 Zebrafish

The zebrafish *Danio rerio* is a tropical fresh water fish native to the South-eastern Himalayan region and belongs to Cyprinidae of the order Cypriniformes. Zebrafish habitats mostly consist of shady, slow flowing water bodies such as the slow-flowing streams and flood-plains of the Indian subcontinent (Ganguly *et al*., 2012). The zebrafish got its name from the uniform, dark blue-pigmented, horizontal stripes that run along the length of its body (Ganguly *et al*., 2012).

In captivity and with optimal feeding conditions, zebrafish can reach sexual maturity around 3-4 months. They can grow up to 7 cm but they are usually between 3-4 cm long (Spence *et al*., 2008). Sexually mature females can be identified by their slightly enlarged belly and a yellowish dorsal and anal fin. They also have a prominent genital papilla (Ganguly *et al*., 2012; Yossa *et al., 2013)*, while males are generally smaller, slimmer and have a reddish tone to their fins (Spence *et al*., 2008) [Figure 3].

Females can spawn every 2-3 day and each clutch may contain up to several hundred eggs. The eggs are relatively large (~0.7mm in diameter) and transparent (Spence *et al*., 2008). Male spermatogenesis in zebrafish lasts for around 6 days and males constantly produce sperm (Leal *et al*., 2009). Embryo development is relatively fast with precursors to all major organs within 36 hours post fertilization (Kimmel *et al*., 1995). The embryo maintains the transparency throughout the development and therefore all embryo developmental stages are clearly visible and traceable under a normal dissection microscope (Spence *et al*., 2008).

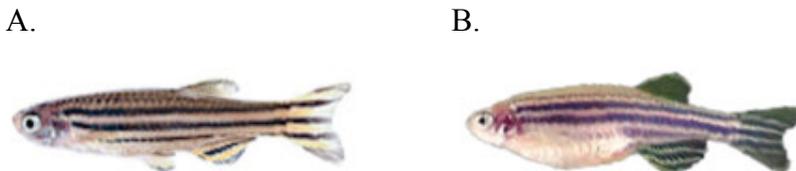A.   B.

Figure 3. From left to right, zebrafish male (A) and zebrafish female (B).

High fecundity, regeneration abilities, small size (adult 3-5 cm), low maintenance, relatively short generation time (sexual maturity 3-4 months) and optical transparency during early embryogenesis are a few characteristics that made zebrafish a compatible and popular animal model in a wide range of research fields throughout the years (Lawrence 2007). Zebrafish are a well established model organism in a variety of research topics such as developmental biology (Lele and Krone 1996), morphological and behavioral biology (Vascotto *et al.,* 1997; Grunwald and Eisen 2002), neurobiology (Rinkwitz *et al*., 2011), behavioral genetic study (Norton and Bally-Cuif 2010), cancer (Feitsma and Cuppen 2008) and other diseases and also drug developments (Sprague *et al*., 2006). More over the large scale genetic screen of zebrafish helped identification and characterization of genes and pathways and also mutations resembling human clinical disorders (Sprague *et al*., 2006). It has been one of the first species that has been cloned and modified to produce many transgenic strains.

Zebrafish are external fertilizers, which means the fertilization happens outside the female's body. Zebrafish sperm and eggs are activated upon contact with water. Being able to do *in vitro* fertilization assays under semi-natural conditions was crucial for the success of my experiments. The fact that zebrafish are external fertilizers in combination with their short generation time made them an ideal study organism for my projects.

2.2 Zebrafish used in my studies

The zebrafish used in all experiments in this thesis belonged to the AB wild-type strain and were originally obtained from the Zebrafish International Research Centre (ZIRC), Oregon, US and maintained at the SciLifeLab zebrafish facility at the Evolutionary Biology Center (EBC) of Uppsala University, Sweden. All adult fish were kept in small groups of 15 (3-L tanks) or 45 fish (10-L tanks) in a system equipped with an automated filtering cycle and constant water flow. Fish were kept at a water temperature of 28.5 °C and with 12:12 dark-light cycle. Fish were fed three (adults) to five (juvenile) times per day on a mixed diet consisting of high protein dried food and live brine shrimp. In all studies, we used zebrafish at the age between 8 to 12 months. Only in chapter II our experimental fish were older (12-24 months) due to the aim of the study to assess reproductive aging.

3 Main methods

3.1 Gamete collection

Before gamete collection, males were separated and kept in smaller unisex groups for 24 hours and without food to allow the replenishment of gametes and to avoid contamination with feces. We kept males in groups of 5-7 males per tank (1.5 liter) and females in company of one non-experimental male in the tank separated by a tank divider before in *vitro fertilization* (IVF).

Zebrafish initiate breeding at the onset of light (Eaton and Farley 1974; Avdesh *et al.*, 2012). In order to postpone egg laying, we covered the fish tanks with a black cloth until they were used in IVFs (collecting eggs). For gamete collection both males and females were anesthetized using MS-222 and Aquacalm^TM for up to maximum two minutes. Anaesthetized fish were then rinsed in fresh tank water and the area around the papilla carefully blotted dry to avoid premature activation of gametes. Males were placed in a wet, soft sponge with genital region up. We used blunt-edged forceps to gently press along the belly and collected the ejaculate into a microcapillary glass tube with a gentle suction (If necessary). The ejaculate was then transferred into an Eppendorf tube containing Hank's buffer and kept on ice until use. Females were rinsed, wiped dry and placed in a dry Petri dish. We gently squeezed the belly with damp fingers while avoiding touching the eggs. Only good quality clutches (yellowish intact eggs) were used for IVF and milky and disturbed clutches were discarded. Both males and females were returned to a recovery tank after squeezing until they fully recovered. They were then returned into their tank of origin and carefully monitored over the coming days to ensure full recovery.

3.2 In vitro fertilization

In vitro fertilization (IVF) is an assisted reproduction technique of artificially fertilizing an egg with sperm. IVF is widely used in animal breeding (Foote 1987), humans with fertility problems and laboratory animals (mouse, rabbit, etc.). In externally fertilizing fish, IVF occurs under semi-natural conditions that allow sperm to be activated and actively swim to the eggs. By adjusting

the dilution, we strived for as natural conditions as possible. The reason why we used IVF for our experiments was that it allows us the control gamete numbers, gamete quality and the exact timing of activation and fertilization. Moreover, using IVF not only gave us the ability to exert selection on sperm longevity to create two selection regimes within one ejaculate but also helped us to eliminate possible pre-copulatory sexual selection and mate choice.

3.3 Split clutch design in vitro fertilization

Split clutch design is a common design in biological experiments to obtain high statistical power and confidence. In our design, the eggs of a female and the ejaculate of a male were split into two or more parts and each part was exposed to a specific treatment. Using this design has the benefit of lowering individual variation (background noise) using one male and one female instead of several individuals. In other words, it allows comparison across treatments while controlling for genetic variation, gamete variation, quality and quantity (Agrawal *et al*., 2001) We used split clutch designs in *in-vitro* fertilizations in all four chapters.

3.4 DNA and RNA extraction, preparation, sequencing and analysis

Molecular work was done for chapters I and III in this thesis.
In paper I, we were interested in genetic differences (allele frequency differences) between two cohorts of sperm within a single ejaculate. Two pools of sperm were artificially selected based on their ability to swim out and reach a certain distance within a certain amount of time. DNA of selected and non-selected sperm pools and finclips were extracted and sequenced. The non-selected ejaculate and a fin clip from the same males were used as a reference to control for individual variation, sequencing biases due to technical issues and meiotic drive. Due to the low cell number present in the selected sperm pools, library preparations were performed in the DNA lab using a PCR-free library preparation kit from Illumina
In paper III, RNA was extracted from brain and testes of F1 and F3 males and gene expression levels were compared between the same tissues in two treatment lines (Short and Long sperm activation lines). The aim of this study was to investigate the effects of sperm selection on the offspring transcriptome.

RNA libraries were prepared using Illumina NEBNext Ultra Directional RNA library prep kit.

Extracted DNA from sperm pools and RNA from brain and testes were sent to the SNP&Seq ScilifeLab facility in Uppsala, Sweden for sequencing using Illumina HiSeq 2500 system.

4 Research aims

The overall aim of my PhD studies was to investigate the possibility of haploid selection in animals, the short-term and long-term effects of selection on sperm phenotype on the fitness of the resulting offspring and to assess heritability of sperm phenotypes more generally. The specific aim of each chapter of this thesis is as explained below:

I. Investigating the role of haploid selection in zebrafish *Danio rerio* and studying the effects of selection on sperm phenotype on offspring fitness and test for a link between sperm phenotype, sperm genotype and offspring phenotype.

II. Investigating the long-term effects of selection on sperm phenotype on the reproductive ageing in the resulting offspring and compare the effects on males and females.

III. Studying the effects of selection on a sperm phenotype, on offspring fitness for three subsequent generations and how this selection affects gene expression in the brain and testes of adult males.

IV. Investigating the heritability of performance sperm traits using an extensive pedigree and quantitative genetic analyses.

5 Summary of the papers and future prospects

An inescapable consequence of sex in eukaryotes is the evolution of a biphasic life cycle with alternating diploid and haploid phases. The occurrence of selection during the haploid phase can have far reaching consequences for several fundamental evolutionary processes. While haploid selection is well established in plants, little is known about haploid selection in animals. This is particularly true in predominantly diploid animals, where gene expression and hence selection have long been thought to be absent in haploid cells like gametes and particularly sperm. A current dogma assumes that in animals, intact fertile sperm within a single ejaculate are equivalent at siring viable offspring. During my PhD, I tested the idea of selection during the haploid gametic phase using zebrafish *Danio rario* as a model system.

In paper I, I combined a large-scale selection experiment with fitness assays and next-generation sequencing to assess the importance of haploid selection. We performed artificial selection on sperm longevity. We created two selection lines with longer-lived (LAT) and shorter-lived (SAT) sperm by manipulating the time between sperm activation and fertilization. Then we compared several fitness and reproductive traits in offspring early and late life in both treatment lines.

We showed that selection on phenotypic variation among intact fertile sperm within an ejaculate affects the fitness of offspring both in early and late life. We found that longer-lived sperm sired embryos with increased survival, reduced number of apoptotic cells and lower larval abnormality rate. Adult LAT offspring also exhibited higher reproductive fitness. We also tested for potential effects of pre- and post-ejaculate sperm aging on the same traits on the offspring but found no evidence for any influence of sperm ageing on our results.

In order to determine whether within-ejaculate sperm phenotypic variation is based on genetic mechanisms, we tested for genetic differences between sperm pools selected for different phenotypes. We performed *in vitro* assays to separate sperm within an ejaculate according to their ability to survive and swim to a certain distance. Sperm pools selected by motile phenotypes differed genetically at numerous sites throughout the genome. Our results in paper I showed that the phenotypic variation among intact fertile sperm within an ejaculate affects offspring fitness and that the phenotypic variation

of the two selected sperm cohorts are based on genetic variation, indicating a link between sperm phenotype, sperm genotype, and offspring phenotype. Our findings suggest the possibility of haploid selection and emphasize on the consequences and the important role of haploid selection in evolutionary processes and dynamics.

In paper II, we investigated the late life consequences and patterns of reproductive aging in response to haploid selection in the F1 offspring. F1 males and females from SAT and LAT lines were mated with non-experimental females and males four times from 12 to 24 months and reproductive traits (fertilization success, embryo survival and fecundity) were measured at each time point. While both LAT males and females generally exhibited higher reproductive fitness, and slower ageing, the effect of sperm selection at early and late ages differed between males and females and also across the different traits.

In paper III, we looked at SAT and LAT offspring fitness in F1, F2, and F3 and also in each generation after outcrossing the experimental fish with non-experimental fish (stopping the selection force). Our findings showed the persistence of the high fitness in LAT fish throughout the three generations with lower variance within treatment lines. Similarly, the high fitness effects were present in all generations even after stopping the selection force. We examined sperm viability and longevity as two additional sperm traits in males in all three generations. Similar to previously observed sperm traits, LAT males had higher sperm viability in all three generations. Sperm longevity on the other hand, was lower in LAT compare to SAT males in all generations; this trade off may be explained by resource allocation between sperm traits as a male's strategy to optimize fertilization success.

Furthermore, we looked at tissue specific gene expression patterns in F1 and F3 males in both treatment lines. We found that several fundamental genes which play important roles in basic and primary functions such as respiratory chain, cellular respiration, electron transport chain, oxidative phosphorylation, metabolic processes, mitochondrial processes, etc., were differentially expressed in brain and testes of LAT and SAT males. These differences in gene expressions of genes with primary functions are in line with the phenotypic and fitness differences we observed in the offspring of all three generations in response to our selection regime.

In paper IV, we investigated the heritability of sperm performance traits measured in our selection experiment by using the pedigree produced from the experiment for three generations. We also assessed the phenotypic correlations between those traits to test for potential trade offs between different sperm traits. We assessed heritability of sperm velocity, sperm motility, sperm viability and sperm longevity and found that while the additive genetic component plays a role in heritability of sperm traits, the parental effect component showed stronger effects. We found that sperm concentration and sperm longevity had negative associations with both, sperm velocity and

initial motility (sperm viability). On the contrary, there was a positive correlation between sperm velocity and initial motility (sperm viability). Similarly, sperm concentration and sperm longevity had a positive correlation. These findings suggest different allocation strategies in males to achieve a high fertilization success.

In conclusion, we showed that gametes can be the target of haploid selection and that such selection has far reaching consequences for general fitness in the resulting offspring throughout life. The finding of differences in tissue specific gene expression between males sired by different sperm types further confirms the fundamental effects of haploid selection on offspring performance. Additionally, we provide a clear link between sperm phenotype and sperm genotype, and between sperm phenotype and offspring fitness.

Furthermore, we sequenced F1 and F3 offspring and are currently scanning the genomes for treatment-specific SNPs.

Overall, my PhD provides comprehensive and pioneering evidence for the role and importance of the haploid genome in determining sperm phenotype and in turn for selection acting at the haploid gametic stage and the importance of gamete selection on next generation's short- and long-term fitness.

6 Sammanfattning på svenska

En oundviklig konsekvens av sex hos eukaryoter är utvecklingen av en bifasisk livscykel som alternerar mellan diploida och haploida faser. Förekomsten av selektion under den haploida fasen kan ha rejäla konsekvenser för ett flertal evolutionära processer. Till skillnad från haploid selektion hos växter finns det enbart liten kunskap om haploid selektion bland djur. Detta gäller i extra stor utsträckning djur som primärt är diploida, där genuttryck och därmed selektion under lång tid ansetts vara frånvarande hos haploida celler som könsceller, och framförallt hos spermier. Enligt en rådande dogm antas det att intakta och fertila spermier från ett enskilt ejakulat är likvärdiga när det kommer till att producera livskraftig avkomma. Under mina doktorandstudier har jag undersökt selektion i den haploida könscellsfasen med zebrafisken *Danio rario* som modellsystem.

I artikel I, kombinerade jag ett storskaligt selektionsexperiment med duglighetsanalys samt *Next Generation Sequencing* (NGS) för att utvärdera vikten av haploid selektion. Vi utförde artificiell selektion baserad på livslängd hos spermier. Vi skapade två selektionslinjer med långlivade (LAT) och kortlivade (SAT) spermier genom att manipulera tiden mellan spermieaktivering och fertilisering. Sedan jämförde vi ett flertal reproduktiva samt duglighetsegenskaper, tidigt och sent i livet hos avkomma från båda linjerna. Vi kunde visa på att selektion på fenotypisk variation bland intakta och fertila spermier inom ett ejakulat påverkar livsdugligheten hos avkomma, både tidigt och sent i livet. Vi fann att spermier med längre livslängd producerade embryon med ökad överlevnadsgrad, minskat antal apoptosa celler samt *en lägre grad av missbildningar i larvstadiet,* medan vuxen LAT-avkomma uppvisade ökad reproduktiv duglighet. Vi undersökte även samma egenskaper med pre- och post-ejakulat av spermier, men fann inga bevis för att spermieåldrande hade påverkan på resultaten. För att avgöra om fenotypisk variation inom ett ejakulat beror på genetiska mekanismer sökte vi efter genetiska skillnader mellan grupper av spermier som valts för olika fenotyper. Vi utförde *in vitro*-analys för att separera spermier inom ett ejakulat baserat på dess överlevnadsförmåga och förmåga att simma en viss distans. Spermiegrupper som valts baserat på rörliga fenotyper uppvisade genetiska skilnader på ett flertal platser inom *genomet*. Resultaten i artikel I visar på att fenotypisk variation bland intakt fertil sperma inom ett ejakulat påverkar livsdugligheten hos avkomma. Vidare visar resultaten att den fenotypiska variationen mellan två valda grupper av spermier beror på genetisk variation vilket indikerar en länk mellan spermiers fenotyp, spermiers genotyp samt avkommans fenotyp. Våra upptäckter visar på

möjligheten för haploid selektion och understryker konsekvenserna och vikten av haploid selektion för evolutionära konsekvenser och dynamik.

I arkikel II, undersökte vi konsekvenserna sent i livet samt mönster för reproduktivt åldrande baserat på haploid selektion av F1-avkomma. F1-hanar och honor från SAT- och LAT-linjer parades med icke-experimentella honor och hanar fyra gånger från 12 till 24 månader; reproduktiva egenskaper (lyckad fertilisering, embryoöverlevnad samt fruktbarhet) mättes vid varje tidpunkt. Medan både LAT-hanar och honor generellt uppvisade ökad reproduktiv form samt långsammare åldrande, skiljde sig effekten av spermieselektion vid tidiga och sena åldrar mellan hanar och honor över de olika egenskaperna.

I artikel III, undersökte vi formen hos SAT- och LAT-avkomma från F1, F2 och F3 samt i varje generation efter att ha korsat den experimentella fisken med icke-experimentell fisk (för att stoppa *selektionskraften*). Våra upptäckter visar att god form består hos LAT-fisk över tre generationer med lägre varians inom *linjerna*. Vidare uppvisades god form i alla generationer, även efter att selektionskraften hade stoppats.

Vi undersökte spermiers livsduglighet samt livslängd som ytterligare två spermie-egenskaper hos hanar i alla tre generationer. I likhet med tidigare observerade egenskaper hos spermier visade spermier från LAT-hanar högre livsduglighet i alla tre generationer. Spermier från LAT-hanar visade å andra sidan kortare livslängd jämfört med spermier från SAT-hanar i alla generationer. Detta kan möjligen förklaras som en resursallokering mellan spermiers egenskaper som en del av en hanes strategi för att uppnå optimal fertilisering. Vidare undersökte vi specifika mönster bland genuttryck hos F1 och F4 hanar i båda linjerna. Vi fann att ett flertal fundamentala gener som har viktiga roller i grundläggande och primära funktioner som exempelvis respiratorisk kedja, cellulär respiration, elektrontransportkedja, *oxidativ fosforylation*, metaboliska processer, mitokondriska processer et cetera, hade olika uttryck i hjärnan samt testiklar hos LAT- och SAT-hanar. Skillnaderna i genuttryck hos gener med primära funktioner stämmer överens med skillnaderna i fenotyp och duglighet vi observerade i avkomman hos alla tre generationer baserat på vårt urval.

I artikel IV undersökte vi ärftligheten av spermas prestationsegenskaper som uppmätts i selektionsexperiment genom att använda den stamtavla som producerades av ett experiment över tre generationer. Vi utvärderade även de fenotypiska korrelationerna mellan olika egenskaper hos spermier för att undersöka eventuella avvägningar mellan olika egenskaper. Vi utvärderade ärftligheten av spermas hastighet, mobilitet, livsduglighet samt livslängd och fann att föräldra-effekten på ärftlighet av spermas egenskaper var större än den additiva genetiska komponenten. Vi fann att spermakoncentration och livslängd hade negativa assiociationer med både hastighet och initial rörlighet (spermas livsduglighet). Å andra sidan fanns det en positiv korrelation mellan hastighet och initial rörlighet. Liknande fanns en positiv korrelation mellan koncentration av sperma och livslängd. Dessa upptäckter tyder på olika allokeringsstrategier hos hanar för att uppnå hög fertiliseringsgrad.

Slutsatsen är att vi visat att könsceller kan vara ett mål för haploid selektion och att sådan selektion har vidsträckta konsekvenser för dugligheten hos avkomman genom ett helt livsspann. Upptäckten av skillnader i vävnadsspecifika genuttryck mellan hanar producerade av olika typer av sperma stärker bekräftandet av att haploid selektion har inverkan på avkommans prestationsförmåga.

Vidare visar vi på ett tydligt samband mellan spermie-fenotyp och spermie-genotyp samt mellan spermie-fenotyp och dugligheten hos avkomma.

Översättning av/translation by Marcus Ihlar

7 Acknowledgements

My great interest in biology and animals life was discovered watching "One by One" which is a British TV series made by the BBC between 1984 and 1987. Its followed the career of international veterinary surgeon David Taylor (called Donald Turner in the series) and his work caring for exotic animals at zoos in Britain. "You have started watching it when you were about two years. Iran was in the middle of a war with Iraq back in the days. Every Tuesday night before the episode starts we had a little two year old jumping up and down and pointing at the TV, some weeks we had no electricity due to the war but seeing your excitement, your dad connected a portable little black and white TV to the car battery using a cable so you could watch the show" saying my parents.

Since then I have not been without animals and science books and I had this passion for knowing more about animal realm. I studied Natural Science in high school and did my Bachelors' in Microbiology. During my Bachelors' I attended the course Evolution and was totally fascinated by it and knew that is the way I wanted to go. I moved to Uppsala to do my Masters in Molecular and cell biology. At the end of my undergrad studies since I was looking for a practical course to do which was a requirement before starting my master thesis project, I have found the newly started Immler group at Evolutionary Biology Center (EBC), I sent Simone an email and asked to join the group for a short project. Started working with Simone in 2011 and I found the most wonderful mentor someone could possibly ask for. She wasn't only one of the smartest women I knew but also one of the most patient and kindest one. Started my PhD project with Simone I put my heart and soul in to my work and Simone pushed me through obstacles and believed in me even when I myself did not think I could succeed at times.

My mom **Giti**, my dad **Hessein** and my sister **Samin** also supported all my decisions and bare with me each step of the way, they have always believed in me more than I believed in myself. Thank you mom for reminding me of my passion for science and learning and having my back at all times and supporting me every time I needed it. Thank you dad for all the experiences you shared with me, those I would need 50 more years to learn. Thank you for trusting me on all my decisions and lighting up my way.

Thank you my little sister whom I shared with ups and downs and excitements and sadness, I appreciate you all for going through sacrifices so I can enjoy life at its greatest and thrive as a woman and for all your love and support.

Thank you all my friends and family, everyone who has supported me emotionally when the time got rough.

Thank you my lab assistants and visiting students; **Joachim**, **Andea**, **Magali**, **Mathilde**, **Sylvan**, **Maria** and **Julia** for all the hard work in the lab. Special thanks to **Cosima**, I have learnt many many thing from you and you are one of the nicest people I have ever known, also, **Berrit**, **RoysRoys** and **Cecile**, working with you and being friends with you is so priceless for me, you were by my side whenever I needed you. **Mark** you have always been a supportive and a great great friend, **Alireza**, **Azi**, **Tutu**, **Vahid** we shared many good memories and experienced cool events together over these years. **Malte** you gave me great support during the last days of my thesis deadline ☺, I want to thank you all for being such awesome friends and family.

I also want to thank all the rest of the current and previous Immler group members, **Will**, **Hwei-yen**, **Berenice**, **Sergio**, **Bart**, **Marta**, **Khriezha**, **João** and **Susi** and my dear friends and colleagues **Shadi**, **Homa**, **Foteini**, **Lorena**, **Aeron**, **Alex**, the other **Alex** ;) **Venkat**, **Tobi** and **Caro**, it was my pleasure to get to know, work with you and party with you guys. Thank you **Alexei** for all the advice and great discussions we had. It was very nice to collaborate with you.

Many thanks to my co-authors that I have not mentioned yet, **Göran**, **Martin Lind**, **Elena Berg**, **Holger**, **Sandra** and **Doug** for all the feedbacks and support. Thank you everyone in Evolutionary Biology department and whole EBC for hosting me for all these years and for being such amazing collogues.

And my very special appreciation and thanks to **Simone**, who is my supervisor and my friend. I consider myself among the lucky ones to have such an amazing person with great knowledge and one of the kindest hearts as my mentor. Simone you have always been a supportive and enthusiastic mentor for me, someone who had always taken care of me and at the same time pushing me to break limits and be confident. You gave me all the support and hope every time I felt down and I appreciate it. I could have never asked for a better supervisor because you were the best. Thank you for giving me the opportunity to do my PhD in your group and under your supervision.

At the end I would like to mention that the four year of my PhD was one of the most enjoyable and exciting periods of my life and it was not because of anything other than my great mentor Simone, my mom Giti, my dad Hossein, my sister Samin and all my friends and family whom without, this would not be possible.

Thank you all.

8 References

Agrawal AF (2001). Sexual selection and the maintenance of sexual reproduction. *Nature* **411**: 692–695.

Agrawal AF, Brodie ED, Brown J (2001). Parent-Offspring Coadaptation and the Dual Genetic Control of Maternal Care. *Science* **292**: 1710–1712.

Ames BN, Shigenaga MK, Hagen TM (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci* **90**: 7915–7922.

Andersson MB (1994). *Sexual Selection*. Princeton University Press.

Avdesh A, Chen M, Martin-Iverson MT, Mondal A, Ong D, Rainey-Smith S, *et al.* (2012). Regular Care and Maintenance of a Zebrafish (Danio rerio) Laboratory: An Introduction. *J Vis Exp JoVE*.

Baccetti B, Afzelius BA (1976). The biology of the sperm cell. *Monogr Dev Biol*: 1–254.

Bell G (1997). The evolution of the life cycle of brown seaweeds. *Biol J Linn Soc* **60**: 21–38.

Berg EC, Maklakov AA (2012). Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. *Proc R Soc Lond B Biol Sci* **279**: 4296–4302.

Birkhead TR, Hosken DJ, Pitnick SS (2008). *Sperm Biology: An Evolutionary Perspective*. Academic Press.

Birkhead TR, Møller AP (1998). *Sperm Competition and Sexual Selection*. Academic Press.

Birkhead TR, Møller AP, Sutherland WJ (1993). Why do Females Make it so Difficult for Males to Fertilize their Eggs? *J Theor Biol* **161**: 51–60.

Birkhead TR, Pellatt EJ, Brekke P, Yeates R, Castillo-Juarez H (2005). Genetic effects on sperm design in the zebra finch. *Nature* **434**: 383–387.

Bongalhardo DC, Dionello NJL, Ledur MC (2000). Genetic parameters for semen traits in a White Leghorn strain.: 1. Heritabilities and correlations. *Rev Bras Zootec* **29**: 1320–1326.

Borg M, Brownfield L, Twell D (2009). Male gametophyte development: a molecular perspective. *J Exp Bot* **60**: 1465–1478.

Briga M, Verhulst S (2015). What can long-lived mutants tell us about mechanisms causing aging and lifespan variation in natural environments? *Exp Gerontol* **71**: 21–26.

Brown GP, Shine R (2007). Repeatability and heritability of reproductive traits in free-ranging snakes. *J Evol Biol* **20**: 588–596.

Carranza J, Alarcos S, Sánchez-Prieto CB, Valencia J, Mateos C (2004). Disposable-soma senescence mediated by sexual selection in an ungulate. *Nature* **432**: 215–218.

Charlesworth B, Morgan MT, Charlesworth D (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**: 1289–1303.

Chemes HE, Rawe VY (2003). Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. *Hum Reprod Update* **9**: 405–428.

Chen H, Maklakov AA (2014). Condition Dependence of Male Mortality Drives the Evolution of Sex Differences in Longevity. *Curr Biol* **24**: 2423–2427.

Chervet N, Zöttl M, Schürch R, Taborsky M, Heg D (2011). Repeatability and Heritability of Behavioural Types in a Social Cichlid. *Int J Evol Biol*.

Choe JC, Crespi BJ (1997). *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press.

Clutton-Brock TH, Isvaran K (2007). Sex differences in ageing in natural populations of vertebrates. *Proc R Soc Lond B Biol Sci* **274**: 3097–3104.

Cobb M (2007). The Egg & Sperm Race: The Seventeenth-Century Scientists Who Unravelled the Secrets of Sex, Life, and Growth. *eweb:303086*.

Cohen J, McNAUGHTON DC (1974). Spermatozoa: The Probable Selection of a Small Population by the Genital Tract of the Female Rabbit. *J Reprod Fertil* **39**: 297–310.

Córdoba-Aguilar A (2006). Sperm ejection as a possible cryptic female choice mechanism in Odonata (Insecta). *Physiol Entomol* **31**: 146–153.

Crow JF (1994). Advantages of sexual reproduction. *Dev Genet* **15**: 205–213.

Crow JF, Kimura M (1965). Evolution in Sexual and Asexual Populations. *Am Nat* **99**: 439–450.

Curley JP, Mashoodh R, Champagne FA (2011). Epigenetics and the origins of paternal effects. *Horm Behav* **59**: 306–314.

Daly M (1978). The Cost of Mating. *Am Nat* **112**: 771–774.

Derbès, AA (1847). Observations sur le Méchanisme et les Phenomènes qui Accompagnent la Formation de l'Embryon chez l'Oursin Comestible. *Ann Sci Nat Zool*: 80–98.

Destombe C, Godin J, Lefebvre C, Dehorter O, Vernet P (1992). Differences in Dispersal Abilities of Haploid and Diploid Spores of Gracilaria verrucosa (Gracilariales, Rhodophyta). *Bot Mar* **35**: 93–98.

Droge-Young EM, Belote JM, Perez GS, Pitnick S (2016). Resolving mechanisms of short-term competitive fertilization success in the red flour beetle. *J Insect Physiol* **93–94**: 1–10.

Dufossé A (1847). Observations sur le developpement des oursins (Echinus esculentus). Comptes Rendus Hebd. *Seances Acad Sci*: 15–18.

Eaton RC, Farley RD (1974). Spawning Cycle and Egg Production of Zebrafish, Brachydanio rerio, in the Laboratory. *Copeia* **1974**: 195–204.

Erickson RP, Lewis SE, Butley M (1981). Is haploid gene expression possible for sperm antigens? *J Reprod Immunol* **3**: 195–217.

Falconer DS (1960). *Introduction to quantitative genetics*. Oliver And Boyd: Edinburgh; London.

Feitsma H, Cuppen E (2008). Zebrafish as a Cancer Model. *Mol Cancer Res* **6**: 685–694.

Firman RC, Gasparini C, Manier MK, Pizzari T (2017). Postmating Female Control: 20 Years of Cryptic Female Choice. *Trends Ecol Evol* **32**: 368–382.

Foote RH (1987). In vitro fertilization and embryo transfer in domestic animals: Applications in animals and implications for humans. *J In Vitro Fert Embryo Transf* **4**: 73–88.

Fowell RR (1969). Life cycles in yeasts. *The yeasts*: 303–383.

Froman DP, Pizzari T, Feltmann AJ, Castillo-Juarez H, Birkhead TR (2002). Sperm mobility: mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with male status in the domestic fowl, Gallus gallus domesticus. *Proc R Soc Lond B Biol Sci* **269**: 607–612.

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA (2004). Spermatozoal Traits and Sperm Competition in Atlantic Salmon: Relative Sperm Velocity Is the Primary Determinant of Fertilization Success. *Curr Biol* **14**: 44–47.

Ganguly S, C Dora K, Sarkar S, Chowdhury S, Ahmed S (2012). ZEBRA FISH-GENERAL BIOLOGY AND A BIOMEDICAL RESEARCH TOOL. *Int J Sci Environ Technol* **1**: 193–96.

García-González F, Simmons LW (2007). Paternal Indirect Genetic Effects on Offspring Viability and the Benefits of Polyandry. *Curr Biol* **17**: 32–36.

Gavrus-Ion A, Sjøvold T, Hernández M, González-José R, Esteban Torné ME, Martínez-Abadías N, *et al.* (2017). Measuring fitness heritability: Life history traits versus morphological traits in humans. *Am J Phys Anthropol* **164**: 321–330.

Goyache F, Gutiérrez JP (2001). Heritability of reproductive traits in Asturiana de los Valles beef cattle breed. *Arch Tierz* **44**: 489–496.

Griffin RM., Hayward AD, Bolund E, Maklakov AA, Lummaa V, Gaillard JM (2018). Sex differences in adult mortality rate mediated by early-life environmental conditions. *Ecol Lett* **21**: 235–242.

Grunwald DJ, Eisen JS (2002). Headwaters of the zebrafish — emergence of a new model vertebrate. *Nat Rev Genet* **3**: 717–724.

Gur Y, Breitbart H (2006). Mammalian sperm translate nuclear-encoded proteins by mitochondrial-type ribosomes. *Genes Dev* **20**: 411–416.

Gur Y, Breitbart H (2007). Protein translation in mammalian sperm. *Soc Reprod Fertil Suppl* **65**: 391–397.

Gur Y, Breitbart H (2008). Protein synthesis in sperm: dialog between mitochondria and cytoplasm. *Mol Cell Endocrinol* **282**: 45–55.

Gustafsson L (1986). Lifetime Reproductive Success and Heritability: Empirical Support for Fisher's Fundamental Theorem. *Am Nat* **128**: 761–764.

Haldane JBS (1924). A Mathematical Theory of Natural and Artificial Selection. Part Ii the Influence of Partial Self-Fertilisation, Inbreeding, Assortative Mating, and Selective Fertilisation on the Composition of Mendelian Populations, and on Natural Selection. *Biol Rev* **1**: 158–163.

Houle D (1992). Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.

Howards SS (1997). Antoine van Leeuwenhoek and the discovery of sperm. *Fertil Steril* **67**: 16–17.

Hughes JS, Otto SP (1999). Ecology and the Evolution of Biphasic Life Cycles. *Am Nat* **154**: 306–320.

Jeon KW (2004). *International Review of Cytology: A Survey of Cell Biology*. Elsevier.

Joseph SB, Kirkpatrick M (2004). Haploid selection in animals. *Trends Ecol Evol* **19**: 592–597.

Kamimura Y (2014). Pre- and postcopulatory sexual selection and the evolution of sexually dimorphic traits in earwigs (Dermaptera). *Entomol Sci* **17**: 139–166.

Kettaneh NP, Hartl DL (1976). Histone transition during spermiogenesis is absent in segregation distorter males of Drosophila melanogaster. *Science* **193**: 1020–1021.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995). Stages of embryonic development of the zebrafish. *Dev Dyn* **203**: 253–310.

Knights SA, Baker RL, Gianola D, Gibb JB (1984). Estimates of heritabilities and of genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. *J Anim Sci* **58**: 887–893.

Konior M, Keller L, Radwan J (2005). Effect of inbreeding and heritability of sperm competition success in the bulb mite *Rhizoglyphus robini*. *Heredity* **94**: 577–581.

Kruuk LEB, Clutton-Brock TH, Slate J, Pemberton JM, Brotherstone S, Guinness FE (2000). Heritability of fitness in a wild mammal population. *Proc Natl Acad Sci* **97**: 698–703.

Kvarnemo C, Simmons LW (2013). Polyandry as a mediator of sexual selection before and after mating. *Phil Trans R Soc B* **368**: 20120042.

LaMunyon CW, Samuel W (1999). Evolution of sperm size in nematodes: sperm competition favours larger sperm. *Proc R Soc Lond B Biol Sci* **266**: 263–267.

Lawrence C (2007). The husbandry of zebrafish (Danio rerio): A review. *Aquaculture* **269**: 1–20.

Leal MC, Cardoso ER, Nóbrega RH, Batlouni SR, Bogerd J, França LR, *et al.* (2009). Histological and Stereological Evaluation of Zebrafish (Danio rerio) Spermatogenesis with an Emphasis on Spermatogonial Generations. *Biol Reprod* **81**: 177–187.

Leeuwenhoek DA (1678). Observationes D. Antonii Lewenhoeck, de Natis e semine genitali Animalculis. *R SocLond Philos Trans 12*: 1040–1043.

Lehtonen J, Parker GA (2014). Gamete competition, gamete limitation, and the evolution of the two sexes. *Mol Hum Reprod* **20**: 1161–1168.

Lele Z, Krone PH (1996). The zebrafish as a model system in developmental, toxicological and transgenic research. *Biotechnol Adv* **14**: 57–72.

Lloyd DG (1980). Benefits and Handicaps of Sexual Reproduction. In: *Evolutionary Biology*, Springer, Boston, MA, pp 69–111.

Lüpold S, Manier MK, Puniamoorthy N, Schoff C, Starmer WT, Luepold SHB, *et al.* (2016). How sexual selection can drive the evolution of costly sperm ornamentation. *Nature* **533**: 535–538.

M J Simmons, Crow and JF (1977). Mutations Affecting Fitness in Drosophila Populations. *Annu Rev Genet* **11**: 49–78.

Mable, B, Otto, S (1998). The evolution of life cycles with haploid and diploid phases. *BioEssays*: 453–462.

Maklakov AA, Rowe L, Friberg U (2015). Why organisms age: Evolution of senescence under positive pleiotropy? *BioEssays* **37**: 802–807.

Maklakov AA, Immler S (2016). The Expensive Germline and the Evolution of Ageing. *Curr Biol* **26**: R577–R586.

Marshall DJ (2015). Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects | Journal of Experimental Biology. *J Exp Biol* **218.1**: 107–113.

Mascarenhas JP (1990). Gene Activity During Pollen Development. *Annu Rev Plant Physiol Plant Mol Biol* **41**: 317–338.

Mathevon M, Buhr MM, Dekkers JCM (1998). Environmental, Management, and Genetic Factors Affecting Semen Production in Holstein Bulls. *J Dairy Sci* **81**: 3321–3330.

McCulloch Diana, Gems David (2003). Evolution of male longevity bias in nematodes. *Aging Cell* **2**: 165–173.

Meldrum DR (1993). Female reproductive aging—ovarian and uterine factors. *Fertil Steril* **59**: 1–5.

Meyer K, Hammond K, Parnell PF, MacKinnon MJ, Sivarajasingam S (1990). Estimates of heritability and repeatability for reproductive traits in Australian beef cattle. *Livest Prod Sci* **25**: 15–30.

Miller D, Brinkworth M, Iles D (2010). Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. *Reproduction* **139**: 287–301.

Miller D, Ostermeier GC, Krawetz SA (2005). The controversy, potential and roles of spermatozoal RNA. *Trends Mol Med* **11**: 156–163.

Miller GT, Pitnick S (2002). Sperm-Female Coevolution in Drosophila. *Science* **298**: 1230–1233.

Moore PJ, Edwin Harris W, Tamara Montrose V, Levin D, Moore AJ, Rowe L (2004). Constraints on evolution and postcopulatory sexual selection: trade-offs among ejaculate characteristics. *Evolution* **58**: 1773–1780.

Moore PJ, Moore AJ (2001). Reproductive aging and mating: The ticking of the biological clock in female cockroaches. *Proc Natl Acad Sci* **98**: 9171–9176.

Morrow EH, Gage MJG (2001a). Artificial selection and heritability of sperm length in Gryllus bimaculatus. *Heredity* **87**: 356–362.

Morrow EH, Gage MJG (2001b). Sperm competition experiments between lines of crickets producing different sperm lengths. *Proc R Soc Lond B Biol Sci* **268**: 2281–2286.

Mousseau TA, Fox CW (1998). The adaptive significance of maternal effects. *Trends Ecol Evol* **13**: 403–407.

Norton W, Bally-Cuif L (2010). Adult zebrafish as a model organism for behavioural genetics. *BMC Neurosci* **11**: 90.

Nussey DH, Kruuk LEB, Morris A, Clements MN, Pemberton JM, Clutton-Brock TH (2009). Inter- and Intrasexual Variation in Aging Patterns across Reproductive Traits in a Wild Red Deer Population. *Am Nat* **174**: 342–357.

Otto SP, Marks JC (1996). Mating systems and the evolutionary transition between haploidy and diploidy. *Biol J Linn Soc* **57**: 197–218.

Parker GA (1970). SPERM COMPETITION AND ITS EVOLUTIONARY CONSEQUENCES IN THE INSECTS. *Biol Rev* **45**.

Parker GA (1982). Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J Theor Biol* **96**: 281–294.

Parker GA, Baker RR, Smith VGF (1972). The origin and evolution of gamete dimorphism and the male-female phenomenon. *J Theor Biol* **36**: 529–553.

Parker GA, Simmons LW (1996). Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proc R Soc Lond B* **263**: 315–321.

Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA (1996). Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. *Nature* **379**.

Partridge L, Barton NH (1993). Optimally, mutation and the evolution of ageing. *Nature* **362**: 305–311.

Pizzari T, Birkhead TR (2000). Female feral fowl eject sperm of subdominant males. *Nature* **405**: 787–790.

Poole JH (1989). Mate guarding, reproductive success and female choice in African elephants. *Anim Behav* **37**: 842–849.

Price T, Schluter D (1991). On the Low Heritability of Life-History Traits. *Evolution* **45**: 853–861.

Promislow DEL (1992). Costs of sexual selection in natural populations of mammals. *Proc R Soc Lond B* **247**: 203–210.

Richerd S, Couvet D, Valéro M (1993). Evolution of the alternation of haploid and diploid phases in life cycles. II. Maintenance of the haplodiplontic cycle. *J Evol Biol* **6**: 263–280.

Rinkwitz S, Mourrain P, Becker TS (2011). Zebrafish: An integrative system for neurogenomics and neurosciences. *Prog Neurobiol* **93**: 231–243.

Roe KE (1975). Origin of the alternation of generations in plants:reconsideration of the traditional theories. *Biologist*: 1–13.

vom Saal FS, Finch CE, Nelson JF (1994). Natural history and mechanisms of reproductive aging in humans, laboratory rodents, and other selected vertebrates. *Physiol Reprod* **2**: 1213–1314.

Saastamoinen M (2008). Heritability of dispersal rate and other life history traits in the Glanville fritillary butterfly. *Heredity* **100**: 39–46.

Sarreiro LC, Bergmann J a. G, Quirino CR, Pineda NR, Ferreira VCP, Silva MA (2002). Heritability and genetic correlation between scrotal circumference, libido and seminal traits in Nellore bulls. *Arq Bras Med Veterinária E Zootec* **54**: 602–608.

Simmons LW (2001). *Sperm Competition and Its Evolutionary Consequences in the Insects*. Princeton University Press.

Simmons LW, Kotiaho JS (2007). Quantitative genetic correlation between trait and preference supports a sexually selected sperm process. *Proc Natl Acad Sci* **104**: 16604–16608.

Snook RR (2005). Sperm in competition: not playing by the numbers. *Trends Ecol Evol* **20**: 46–53.

Spence R, Gerlach G, Lawrence C, Smith C (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biol Rev* **83**: 13–34.

Sprague J, Bayraktaroglu L, Clements D, Conlin T, Fashena D, Frazer K, *et al.* (2006). The Zebrafish Information Network: the zebrafish model organism database. *Nucleic Acids Res* **34**: D581–D585.

Steger K (1999). Transcriptional and translational regulation of gene expression in haploid spermatids. *Anat Embryol (Berl)* **199**: 471–487.

Strauss JF, Barbieri RL. (2013). *Yen and Jaffe's Reproductive Endocrinology*. Elsevier Health Sciences.

Sutter A, Lindholm AK (2016). The copulatory plug delays ejaculation by rival males and affects sperm competition outcome in house mice. *J Evol Biol* **29**: 1617–1630.

Teperek M, Simeone A, Gaggioli V, Miyamoto K, Allen GE, Erkek S, *et al.* (2016). Sperm is epigenetically programmed to regulate gene transcription in embryos. *Genome Res* **26**: 1034–1046.

Vascotto SG, Beckham Y, Kelly GM (1997). The zebrafish's swim to fame as an experimental model in biology. *Biochem Cell Biol* **75**: 479–485.

Von Baer KE (1847). Auszug aus einem Berichte des Akademikers v. Baer, aus Triest vom 1.(13.) November 1845. *Bull Cl Phys-Math Acad Imp Sci St Pétersbourg*: 231–240.

Wachter KW, Evans SN, Steinsaltz D (2013). The age-specific force of natural selection and biodemographic walls of death. *Proc Natl Acad Sci* **110**: 10141–10146.

Wagner RH, Helfenstein F, Danchin E (2004). Female choice of young sperm in a genetically monogamous bird. *Proc R Soc Lond B Biol Sci* **271**: S134–S137.

Walbot V, Evans MMS (2003). Unique features of the plant life cycle and their consequences. *Nat Rev Genet* **4**: 369–379.

Wang C, Leung A, Sinha-Hikim AP (1993). Reproductive aging in the male brown-Norway rat: a model for the human. *Endocrinology* **133**: 2773–2781.

Wyman MJ, Wyman MC (2013). Sex-specific recombination rates and allele frequencies affect the invasion of sexually antagonistic variation on autosomes. *J Evol Biol* **26**: 2428–2437.

Yossa R, Sarker PK, Proulx E, Saxena V, Ekker M, Vandenberg GW (2013). A Practical Approach for Sexing Zebrafish, Danio rerio. *J Appl Aquac* **25**: 148–153.

Zamir D, Tanksley SD, Jones RA (1982). Haploid Selection for Low Temperature Tolerance of Tomato Pollen. *Genetics* **101**: 129–137.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Science and Technology **1681**

Editor: The Dean of the Faculty of Science and Technology