

# **The investment into sperm depends on genetic compatibility between pair mates in a monogamous species**

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## **Abstract**

Sperm quality determines offspring fitness. However, investment into sperm being costly, it depends on the health or condition of males. According to the differential allocation hypothesis, males should decrease their investment into sperm when mated with a low-quality female. In this study, we show that, in the black-legged kittiwake, a monogamous seabird, sperm quality affects chick performance and that good-quality males produce a sperm of good quality. In addition, males mated with more genetically compatible females produce better quality sperm. This study suggests that the effects of sperm quality on offspring fitness may act as a natural selective pressure on males' differential allocation strategies.

**Keywords :** Black-legged kittiwake, ejaculate, Major Histocompatibility Complex, Fitness, Differential Allocation

## Introduction

Sexual reproduction is complex. From the competition for mates to zygote formation, numerous behavioural, physiological, and molecular interactions between males and females are critical to successful reproduction. One of the most important driving forces of these interactions is related to the genetic compatibility between mates. In many taxa, offspring of genetically compatible partners have higher fitness. Many species have therefore developed reproductive strategies to limit the negative effect due to poor genetic compatibility.

In vertebrates, one of the best examples of genetic incompatibility is incompatibility at the Major Histocompatibility Complex (MHC, and HLA in humans), a group of polymorphic genes that play a critical role in immunity and disease resistance. Individuals with higher MHC-diversity have better immune capacities and can resist to a wider range of pathogens. Species from a wide range of taxa have therefore developed reproductive strategies to avoid the production of offspring with low MHC-diversity. For instance, blue petrels (*Pachyptila desolata*), great frigatebirds (*Fregata minor*), mandrills (*Mandrillus sphinx*) and several human populations prefer mating with a MHC-compatible partner (Setchell et al. 2010; Juola and Dearborn 2012; Strandh et al. 2012; Dandine-Roulland et al. 2019).

To maximise the MHC diversity of their offspring, several species have evolved post-copulatory strategies such as differential allocation into sperm or selective fertilisation and abortion. However, most studies on MHC-dependent post-copulatory strategies have focused on polygamous or socially monogamous species. In these species, individuals copulate with multiple partners, and can therefore choose among several mates both before and after copulation. In red junglefowls (*Gallus gallus*), females mate with multiple males and then bias sperm use in favour

of MHC-compatible males (Løvlie et al. 2013). In horses (*Equus caballus*), males allocate more sperm to MHC-compatible females (Jeannerat et al. 2018).

In contrast to promiscuous species, individuals of genetically monogamous species mate with a single partner and post-pairing mate choice is thus not possible. Mate choice is thus suggested to be the only key reproductive strategy. However, mate choice can be constraint, because of limited search area or asynchrony in reproductive phenology. In several genetically monogamous species, individuals can divorce, and have thus the possibility to mate with a better breeding partner. In serial genetically monogamous species, an adjustment of the reproductive investment based on the characteristics of the partner might have evolved to limit the costs associated with a poor-quality partner and save energy for future breeding attempts (differential allocation hypothesis; Burley 1988). In humans, who are considered as sequentially genetically monogamous (Schacht and Kramer 2019), sperm viability is higher when it is exposed to the follicular fluid of MHC-compatible females (Magris et al. 2021), which might be a strategy to decrease the costs associated with a poor reproduction (i.e., a reproduction between poorly MHC-compatible partners).

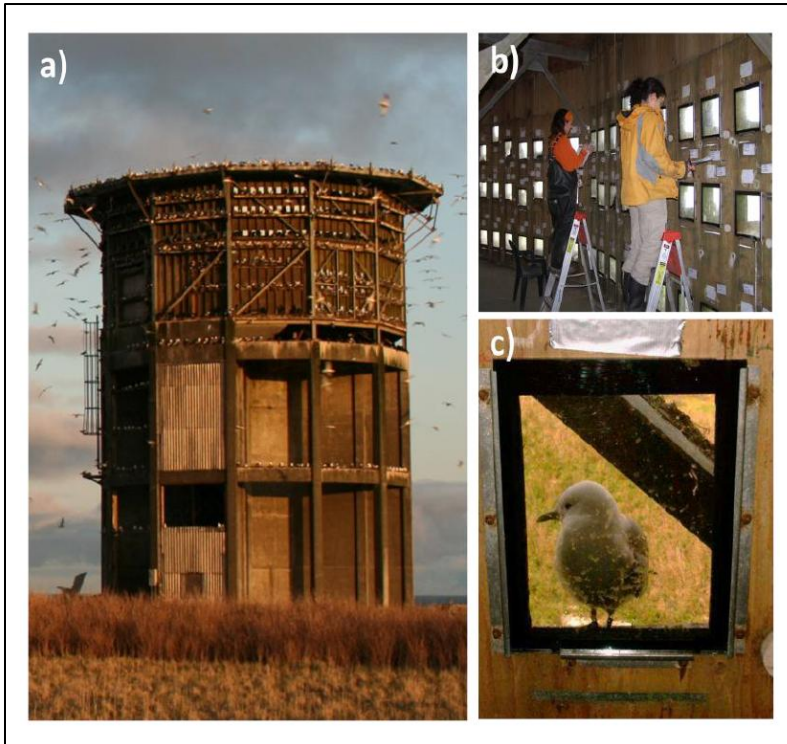
The black-legged kittiwake is a sequentially genetically monogamous seabird (Helfenstein et al. 2004; Naves et al. 2006). In this species, MHC diversity affects chick growth and survival (Pineaux et al. 2020), and parents are expected to have evolved strategies to decrease the costs associated with mating with poorly compatible partners (i.e., when they will produce chicks with low MHC diversity (i.e., when they will produce chicks with low MHC diversity; Pineaux et al. 2022)). In this study, we tested the hypothesis that males decreased their investment into sperm when mated with poorly compatible females. According to the differential allocation

hypothesis, individuals should base their reproductive investment according to a partner's trait that is associated with a decrease in offspring fitness. Therefore, we tested first whether sperm quality was associated with variation in chick performance and whether good-quality males (i.e., males with higher MHC-diversity) produced a higher quality sperm.

## **Material and methods**

### *Study site and species*

We studied a population of black-legged kittiwakes nesting on an abandoned US Air Force tower on Middleton island, Alaska (USA), during the 2017 and 2018 breeding seasons (from early May to mid-August). The nest sites created on the upper part of the tower can be observed from inside through sliding one-way windows and birds can be individually identified using color and metal bands (Fig. 1).



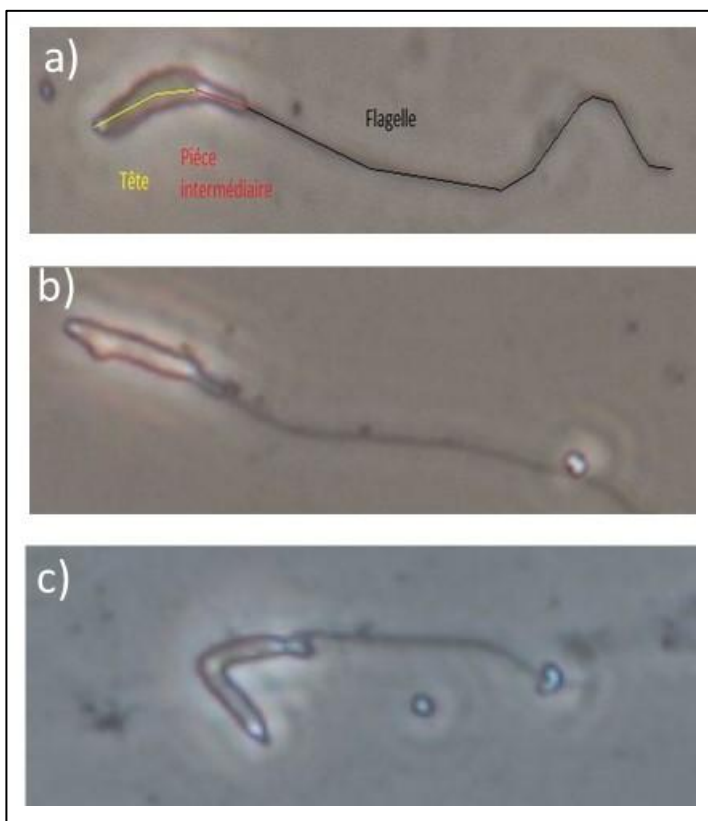
**Figure 1:** Pictures a) of the tower on Middleton island, Alaska, b) from inside the tower, and c) of a kittiwake viewed from inside the tower.

### *Sperm quality analyses*

Males were captured during the pre-laying period (early May to early June). Sperm was collected using a non-invasive massage described in Humann-Guillemot *et al.* (2018). Sperm was then collected with a capillary placed on top of the cloaca and immediately spread on a microscope slide. 5  $\mu$ L of formalin was added and the slide was left for 30 minutes in the open air to dry.

Sperm quality was described based on 3 morphological traits: 1) the proportion of normal spermatozoa, 2) the mean length of the spermatozoa and 3) the intra-ejaculate variation in sperm length. The slides were then analysed in the laboratory using an Olympus BX-51 light microscope at a magnification of 100 times. We classified the first 40 spermatozoa observed on a slide as normal or abnormal

according to the parameters We classified the first 40 spermatozoa observed on a slide as normal or abnormal. A spermatozoon considered as abnormal had certain types of deformations preventing its viability: a double or cut flagellum, an absent, curved, double or cut head, a deformation of the cytoplasm or the absence of an intermediate piece (Fig. 2). For each sample, we also measured the intra-individual variation in three morphometric parameters: the lengths of the head, the midpiece and the flagellum, using the first 5 to 10 normal spermatozoa (Fig. 2). The lengths of these three parameters were correlated. The length of a spermatozoon was thus calculated as the first axis of a principal component analysis on these three parameters. Intra-ejaculate variation in sperm length was calculated as the first axis of a principal component analysis on the coefficient of variation ( $100 \times \text{SD} / \text{mean}$ ) of each of these three parameters.



**Figure 2:** Pictures of a) a normal spermatozoon, b) a spermatozoon with a cytoplasmic droplet and c) a spermatozoon with a curved head.

### *Chick performance*

Nests were checked twice daily at 9:00 a.m. and 6:00 p.m. to record the presence or absence of chicks. Most pairs laid 2 eggs. Eggs and chicks were marked on the head with similar markers for identification shortly after hatching. Chicks were weighed using an electronic scale at the age of 10 days old and 35 days old.

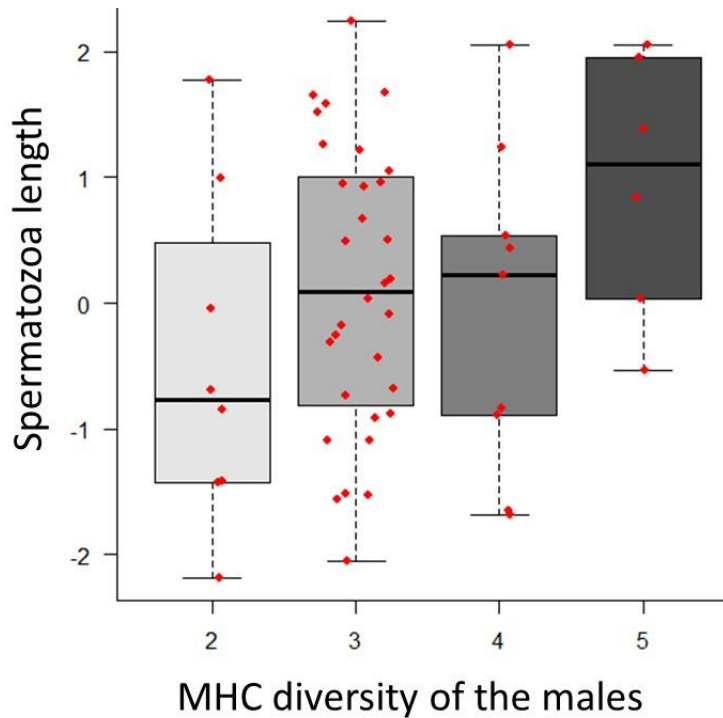
### *MHC genotype*

Blood from males and their female partners was collected from the alar vein using syringes and needles. DNA was extracted using « QIAGEN blood & tissue » extraction kit and birds were genotyped at the MHC Class II using high-throughput sequencing using the method described in Pineaux *et al.* (2020). MHC-diversity was estimated as the number of functional alleles. MHC-compatibility between the male and the female was 0 if the male and the female shared at least one functional allele, whereas it was 1 if they shared no functional allele.

## **Results**

### *Sperm quality and male MHC diversity*

Males with high MHC diversity had longer spermatozoa ( $F_{1,47} = 7.36$ ,  $P = 0.009$ ) and tended to have a lower intra-ejaculate variation in sperm length ( $F_{1,33} = 3.16$ ,  $P = 0.08$ ). However, they did not produce a higher percentage of normal spermatozoa than males with lower MHC-diversity ( $\chi^2_1 = 0.37$ ,  $P = 0.54$ ).

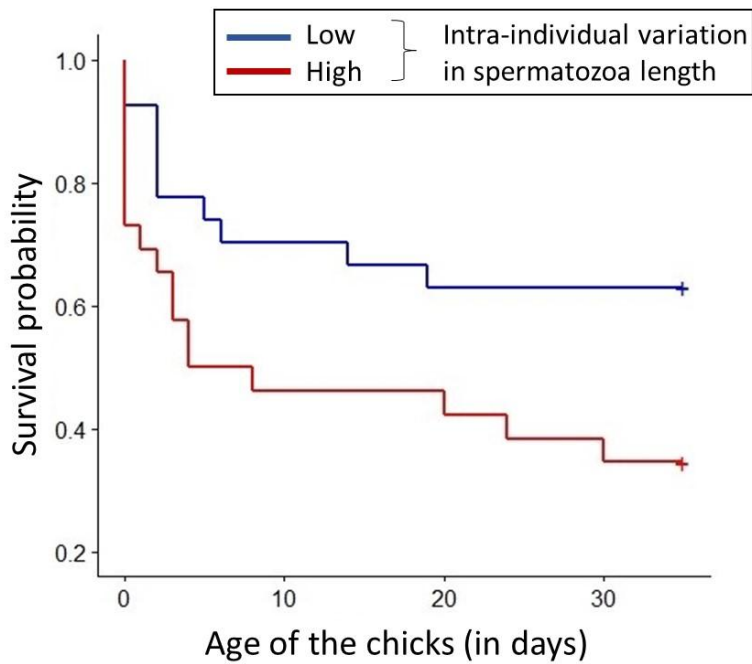


**Figure 3:** Mean length of the spermatozoa according to the MHC diversity of the male.

### Sperm quality and offspring performance

Chicks survived longer when their father had a higher percentage of normal spermatozoa and a lower intra-ejaculate variation in sperm length ( $\chi^2_1 = 4.17$ ,  $P = 0.041$  and  $\chi^2_1 = 6.11$ ,  $P = 0.01$ ; Fig. 4). This effect was however mainly driven by effects on hatching success (Fig. 4). 10-day-old chicks were also heavier when their father had a higher percentage of normal spermatozoa ( $F_{1, 41} = 5.0$ ,  $P = 0.03$ ).

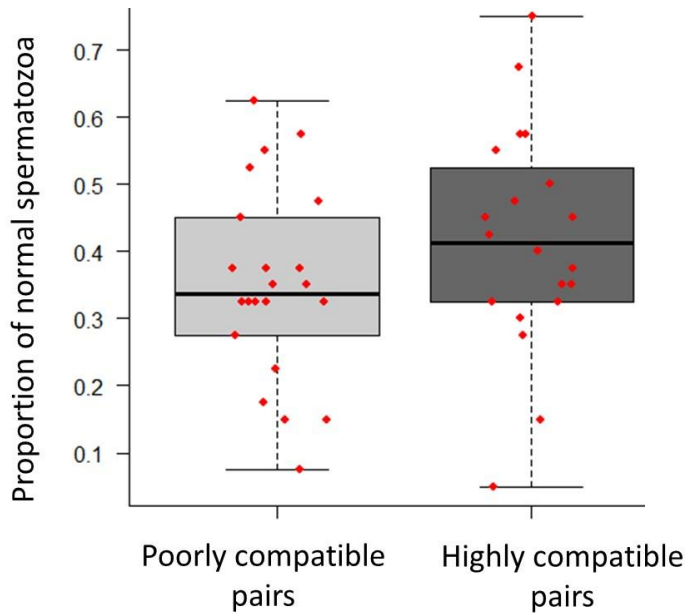




**Figure 4** : Survival probability of chicks between hatching and the age of 35 days, according to the intra-individual variability in sperm morphometry of their father.

### Sperm quality and MHC compatibility

Males mated with more MHC-compatible females produced a sperm with a higher percentage of normal spermatozoa ( $\chi^2_1 = 5.19$ ,  $P = 0.023$ ; Fig. 5). Neither sperm length nor the intra-ejaculate variation in sperm length were associated with the MHC compatibility between pair-mates (all  $P$ s > 0.05).



**Figure 5:** Proportion of normal spermatozoa in the sperm of males paired with poorly compatible females (who shared at least one MHC allele with the male) or highly compatible females (who shared no allele with the male).

## Discussion

Males, who produce a higher proportion of normal spermatozoa and more homogeneous sperm length, have eggs with better hatching success and chicks that are heavier at the age of 10 days. Although our study is correlative, it suggests that sperm quality may affect offspring performance. Our results are in line with studies in other species, which showed that sperm length or the proportion of normal spermatozoa is correlated with reproductive success (Parinaud et al. 1993; Snook 2005; Sætre et al. 2018). They further suggest that the intra-ejaculate variation in sperm length, which has been poorly considered by other studies, might be a sperm quality trait (Immler et al. 2008).

Intra-individual variation in sperm quality may be due to numerous factors, such as the age of the male, his diet, his exposure to pollutants or his health (Preston

et al. 2015; Meligy et al. 2019). In black-legged kittiwakes, we show that sperm quality is also associated with MHC-diversity. Males with higher MHC-diversity produce longer spermatozoa, and spermatozoa which are more homogenous in size. In numerous species, individuals with higher MHC-diversity are healthier or have a better reproductive success (Lenz et al. 2009; Agudo et al. 2012). Males with high MHC-diversity can also have a lower oxidative stress (Kurtz et al. 2006). Oxidative stress decreases sperm quality, by causing damages to DNA or sperm membrane, thereby altering sperm motility and the ability to fertilize the egg (Tremellen 2008; Aitken et al. 2014). In black-legged kittiwakes, males with high MHC-diversity might have a lower oxidative stress than males with a low MHC-diversity and thus might be able to produce better quality sperm.

The association between sperm quality and male MHC diversity may indicate that sperm production is costly. In kittiwakes, investment into sperm may therefore be adjusted according to the quality of the current reproduction, as shown in other species (Magris 2021). According to the differential allocation hypothesis, males should invest more into sperm quality when mated with good-quality females (Burley 1988). Accordingly, we found that males paired with MHC-compatible females produce a higher proportion of normal spermatozoa. Similarly, in Indian mealworms (*Plodia interpunctella*) and horses (*Equus caballus*), males produce higher quality sperm when mating with more genetically compatible females (Lewis and Wedell 2009; Jeannerat et al. 2018). Further studies are needed to determine whether this strategy allows males to save energy for future reproduction. In several species, including birds, individuals recognize MHC-diversity using olfactory cues (Leclaire et al. 2017). In kittiwakes, a recent study has shown that females are able to use olfaction to

recognize the MHC compatibility and diversity of the male (Pineaux et al. Soumis). However, this ability has not been tested in males yet.

The association between sperm quality and MHC compatibility between pair mates may be due to good-quality males being able to mate with MHC-compatible females, while poor-quality males are not. In kittiwakes, at the beginning of the breeding season, males compete with other males for access to nesting sites or females. Males with high MHC-diversity, probably being healthier, might have better fighting ability and therefore, may be able to choose among a wider range of females and secure the one who suits them best.

By limiting the risk of sperm competition, genetic monogamy has been suggested to reduce the selective pressures on sperm quality (van der Horst and Maree 2014). Our study shows however that in genetically monogamous species, sperm quality may be maintained by natural selection and might have exert selective pressure on the evolution of differential allocation strategy.

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