Symposium

The Evolutionary Consequences of Selection at the Haploid Gametic Stage*

Simone Immler[1,†] and Sarah P. Otto[2]

1. School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom; 2. Biodiversity Research Centre and Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

Submitted October 30, 2017; Accepted March 26, 2018; Electronically published June 8, 2018

abstract: As an immediate consequence of sexual reproduction, biphasic life cycles with alternating diploid and haploid phases are a common characteristic of sexually reproducing eukaryotes. Much of our focus in evolutionary biology has been directed toward dynamics in diploid or haploid populations, but we rarely consider selection occurring during both phases when studying evolutionary processes. One of the reasons for this apparent omission is the fact that many flowering plants and metazoans are predominantly diploid with a very short haploid gametic phase. While this gametic phase may be short, it can play a crucial role in fundamental processes including the rate of adaptation, the load of mutation, and the evolution of features such as recombination. In addition, if selection acts in different directions between the two phases, a genetic conflict will occur, impacting the maintenance of genetic variation. Here we provide an overview of theoretical and empirical studies investigating the importance of selection at the haploid gametic phase in predominantly diploid organisms and discuss future directions to improve our understanding of the underlying dynamics and the general implications of haploid selection.

Keywords: biphasic life cycle, sexual reproduction, eukaryotes, haploid selection.

Introduction

Sexual reproduction is the most common form of reproduction in eukaryotes. A basic consequence of sex is an alternation between a diploid phase (following syngamy) and a haploid phase (following meiosis). The relative duration of these phases varies substantially across taxonomic groups and ranges from diplontic life cycles, where somatic growth and development occurs in diploid cells, to haplontic life cycles, where growth and development are limited to haploid cells (for review, see Mable and Otto 1998). While the existence of two alternating ploidy levels has been known for over a century (Strasburger 1894), the actual consequences for evolutionary processes are still relatively poorly understood. Much of the past and present research in evolutionary genetics assumes that a population is either haploid or diploid (e.g., Crow and Kimura 1970; Ewens 1979); only rarely are the evolutionary consequences of selection in both phases considered. Taking both phases into account when studying evolutionary processes may be particularly important when selection is acting in both phases but not necessarily in the same direction (Ewing 1977; Immler et al. 2012; Otto et al. 2015). While theoretical work predicts such a genetic conflict across ploidy levels to have a significant impact on major evolutionary dynamics, including the maintenance of genetic variation (Ewing 1977; Immler et al. 2012; Otto et al. 2015) and the rate of adaptation (Orr and Otto 1994), empirical testing of these ideas is lacking far behind. In the following sections, we present an overview of our current understanding of the importance of haploid selection from both a theoretical and an empirical point of view.

Part of the reason for the limited focus on alternating generations is that the haploid phase is substantially shorter in both animals and plants, which can mislead us into assuming that selection during this phase is weak. In addition, in animals the general idea is that gene transcripts and proteins are shared among cells during the development of sperm, limiting the scope for selection on the haploid genome (but see Joseph and Kirkpatrick 2004). However, empirical evidence for selection at the haploid stage in plants and animals is mounting, and it is therefore time to reconsider some of our assumptions and investigate the role of alternating haploid and diploid phases for evolutionary processes more carefully. We here present an overview of the evidence for and role of selection during both haploid and diploid phases of sexual life cycles, discussing the wider implications for evolution, even in predominantly diploid organisms.

The dynamics of selection acting on a haploid genome are profoundly different from those acting on a diploid genome (Crow and Kimura 1965). In a diploid genome, any allele whose expression is less than perfectly dominant will experi-

* These articles originated as the 2017 Vice Presidential Symposium presented at the annual meetings of the American Society of Naturalists in Portland, Oregon.

† Corresponding author; email: s.immler@uea.ac.uk.

ORCIDs: Immler, http://orcid.org/0000-0003-1234-935X; Otto, http://orcid.org/0000-0003-3042-0818.

Am. Nat. 2018. Vol. 192, pp. 000–000.
DOI: 10.1086/698483

ence a masking effect, where its impact is altered by the expression of its sister allele. In terms of fitness, the masking effect can reduce the selection experienced by an allele. In contrast, any allele that is expressed in a haploid genome is fully exposed to selection. This difference in exposure to selection affects the spread and fixation of newly occurring alleles. In particular, deleterious de novo mutations are more effectively purged if expressed in a haploid genome due to the lack of masking (Crow and Kimura 1965), while beneficial alleles are more likely to establish (Orr and Otto 1994). In addition, haploids and diploids differ in their chance of carrying a new allele, with the theoretical expectation that this rate is doubled in diploids (but see Sharp et al. 2018). The net result of having both haploid and diploid selection can thus impact a wide variety of evolutionary processes, including mutation load, rates of adaptation, and the evolution of life cycles.

Haploid Selection in Male Gametes

Most of the existing empirical work aimed at revealing selection during the haploid phase has been performed in male gametes. The reason for this is that male gametes are easier to collect, manipulate, and sequence. In flowering plants, although the duration of the haploid gametic phase is limited, many genes are expressed in haploid male gametophytes (Haldane 1932; Mascarenhas 1990; Borg et al. 2009; Walbot and Evans 2003 estimated that up to 60% of genes have haploid expression). Interestingly, while many of these genes are expressed in the sporophytic stage as well as the gametophytic stage, about 10% of genes are pollen specific (e.g., Honys and Twell 2004; Pina et al. 2005; Borges et al. 2008; Borg et al. 2009; Arunkumar et al. 2013). Despite this, we know relatively little about the evolutionary consequences of haploid gene expression in plants.

In diplontic animals, the presence of gene expression in the haploid genome of gametes is much debated (Joseph and Kirkpatrick 2004). Several lines of evidence have led many to conclude that the scope for haploid selection in animals is minimal, including the highly compact nucleus in sperm (e.g., Steger 1999), the existence of cytoplasmic bridges allowing RNA and proteins to be shared among haploid spermatids after meiosis (Dadoune et al. 2004), and the fact that DNA-free sperm in *Drosophila* can enter an egg (Lindsley and Grell 1969). However, even if haploid expression is suppressed for many genes, those genes that are expressed in the haploid phase may experience substantial selection (Joseph and Kirkpatrick 2004; Immler 2008). This idea is supported by the finding of unequal sharing of the Spam gene in house mice (e.g., Zheng et al. 2001), the observation of de novo transcription in postmeiotic spermatids in *Drosophila* (Vibranovski et al. 2010), and more recently by a study in zebrafish showing a link between sperm genotype and sperm phenotype (Alavioon et al. 2017). Although the exact extent of haploid-specific transcription remains unclear, there are significant levels of mRNA in sperm (Alcivar et al. 1989; Ostermeier et al. 2002; Yang et al. 2009; Bonache et al. 2012), with evidence of transcription within postmeiotic sperm (Naz 1998; Zheng et al. 2001; Welch et al. 2006; Barreau et al. 2008). Translation also occurs, surprisingly not by the standard nuclear ribosome but by a mitochondrial-type ribosome (Gur and Breitbart 2006, 2008). Furthermore, variation in the gene-expression profile among haploid sperm from a single ejaculate has been shown to correlate with motility and fertility in humans (Lambard et al. 2004), consistent with selection in the haploid phase. In addition, long-term sperm storage has led to a unique network of sperm-specific genes in the proteome of honeybees *Apis mellifera* (Zareie et al. 2013). These genes code not only for proteins known to be involved in sperm metabolism and motility but also for genes involved in transcription, translation, and enzyme regulation (Zareie et al. 2013), as well as for specialized proteins for adenosine triphosphate production using acidifying glycolytic metabolism (Paynter et al. 2017). This altered proteomic network suggests long-term selection for haploid sperm survival in the anaerobic spermatheca, a result that would be valuable to replicate in other species with long-term sperm storage.

Beyond haploid selection on gene expression, there is also substantial scope for haploid selection on the process of DNA condensation. DNA condensation in sperm involves a highly organized repackaging of the genome, replacing histones with transitional proteins and ultimately protamines, a process that allows the nucleus to shrink by an order of magnitude in sperm (Ioannou and Tempest 2018). Yet the haploid genome must also be primed to restore transcriptional activity following fertilization, particularly at genes important in early development. Consequently, condensation is not uniform across the haploid genome, and histones plus a more open chromatin configuration remain at several sites associated with genes involved in early embryonic development as well as zygotic origins of replication (Ward et al. 2010; Champroux et al. 2016; Ioannou and Tempest 2018). These findings suggest that haploid selection may act on the DNA sequence itself, altering the degree to which the DNA is locally compacted versus remains open and primed for reexpression in the diploid phase. Future work is needed to shed light on how these patterns are governed by the haploid genome itself and how they vary among sperm.

Regardless of the mechanism, a recent study in the zebrafish *Danio rerio* suggests that haploid selection does play an important role, with far-reaching implications (Alavioon et al. 2017). In this study, selection on sperm phenotypes (short- vs. long-lived sperm) showed a 7% difference in offspring fitness, and variation in sperm phenotype was clearly linked to sperm genotype. Sequencing of sperm pools selected by phenotype suggested that regions across the entire genome were involved in determining genetic differences between short- and long-lived sperm pools.

Haploid Selection in Female Gametes

Much of the empirical evidence described in this review is limited to male gametes such as pollen and sperm, whereas evidence from female gametes is generally lacking. Three main reasons can explain this discrepancy: (i) male gametes are generally produced in abundance and are exposed to extreme levels of selection due to the fact that only a few out of potentially millions of male gametes succeed in fertilizing an egg (Cruden 1976; Birkhead et al. 1993; Keller and Reeve 1995); (ii) male gametes are generally released from the male into environments that are often extremely different, including exposure to aquatic environments of varying salinity, altered temperatures, ultraviolet light, and/or acidic conditions inside the female reproductive tract, while female gametes remain inside the female until shortly before or even after fertilization, reducing the exposure of female gametes to selection; (iii) while the timing and completion of meiotic divisions in female gametes vary strongly across taxa (Gilbert 2013), in many vertebrates the completion of meiosis happens late (often during or after fertilization), leaving the female gamete effectively diploid throughout the life cycle.

Nevertheless, the female haploid genome may play a role during fertilization and can actively cause nonrandom fusions among male and female pronuclei. A striking example has been described in the ctenophore *Beroe ovata*, where polyspermy is the rule rather than the exception and female pronuclei have been observed to actively migrate between male pronuclei that impacted on the egg surface (Carré and Sardet 1984). The mechanisms and scope underlying this observation remain unknown, but such processes could be more common than thought. A suggestive pattern has been described in the house mouse *Mus musculus*, where nonrandom fusion among gametes appears to be based on major histocompatibility complex (MHC) loci (Wedekind et al. 1996). Whether this is the result of egg choice among different sperm or a mechanism involving the second meiotic division (i.e., after fertilization) is currently unclear. Similar studies in whitefish *Coregonus* sp. and Atlantic salmon *Salmo salar*, however, failed to provide evidence for nonrandom fusion based on MHC haplotypes in male and female gametes (Wedekind et al. 2004; Promerová et al. 2017). Nonrandom fusion among gametes has also recently been discussed as a possible explanation for the observation of non-Mendelian inheritance in mouse mutants (Nadeau 2017). An interesting idea mentioned in this context is the possibility that such mechanisms could be condition dependent, making them challenging to detect. A carefully planned genome-wide approach to study gamete fusion and the association of haploid gametic genomes would be a great way to expand these studies beyond MHC to detect the extent of selection on haploid gametes during the fertilization process.

While the examples described above demonstrate the potential for haploid gene expression and selection, more work is needed to document variation among species in the extent of haploid gene expression and its implications for evolution. In the following sections, we summarize the theoretical predictions for the main evolutionary processes potentially affected by selection at the haploid gametic stage in otherwise diplontic organisms, discuss these predictions in the light of empirical results, and make suggestions about how such predictions could be tested.

Rate of Adaptation

The rate of adaptation depends to a large degree on the rate at which beneficial mutations arise in a population. Beneficial mutations are predicted to occur at twice the rate in diploid organisms compared to haploid organisms, offering a potential advantage in adaptive processes (e.g., Charlesworth 1983; Paquin and Adams 1983; Valero et al. 1992). However, because beneficial mutations are partially masked in diploids, they can be lost more easily when they first arise and spread more slowly than in haploids, slowing the rate of adaptation in theoretical models of both sexual and asexual populations (Orr and Otto 1994). In fact, an experimental study in the budding yeast *Saccharomyces cerevisiae*, where haploid and diploid lines were evolved over 2,000 generations, showed that in large, asexual populations, haploids adapt faster than diploids (Zeyl et al. 2003). A follow-up study confirmed this observation by showing that haploid lines adapting for 187 generations across a variety of stressful environments evolved faster than their isogenic diploid sister lines (Gerstein et al. 2011). Interestingly, the mutation rate has recently been shown to be higher per basepair in haploid than in diploid yeast (Sharp et al. 2018), which would also hasten the relative rate of haploid adaptation to the extent that this result generalizes to other species and conditions. Similarly, the effect size of beneficial mutations in haploid strains appears to be much larger than in homozygous diploid strains, which would further contribute to selection being more efficient in haploids than in diploids (Gerstein 2013). The relative advantage of haploid versus diploid genotypes varies greatly among environments, however, as found across a variety of yeast strains (Zörgö et al. 2013), which may also shape the ability of haploid and diploid organisms to proliferate and adapt to different conditions.

But what about the rate of adaptation in genes expressed at the haploid gametic stage compared to genes expressed only in the diploid phase of diplontic species? Empirical evidence for selection on the haploid phase is strongest in flowering plants, coming from both experimental mating trials and molecular sequence analysis. For example, manipulating the placement of pollen on stigmas, the number

of pollen donors, or the amount of pollen deposited alters the strength of pollen competition, often with measurable effects on offspring fitness (Snow and Spira 1996; Skogsmyr and Lankinen 1999; Aronen et al. 2002; Lankinen and Skogsmyr 2002; Lankinen et al. 2009). Furthermore, directly exposing pollen to selection (e.g., altered temperatures, herbicides, or other stressors) can hasten the breeding of plant strains adapted to those conditions (Searcy and Mulcahy 1985; Frascaroli and Songstad 2001; Ravikumar et al. 2003, 2012; Clarke et al. 2004; Hedhly et al. 2004; Scott 2016). At the sequence level, pollen-specific oleosin-like proteins (Schein et al. 2004) and glycine-rich pollen surface proteins (Fiebig et al. 2004) show signs of rapid evolution, supporting the notion that selection on haploid-expressed genes can be effective. Similarly, in *Arabidopsis thaliana* and the moss *Funaria hygrometrica*, haploid-specific genes exhibited faster evolution than diploid-specific genes (Szövényi et al. 2013). By comparing to levels of polymorphism in *Arabidopsis*, the authors suggest that relaxed purifying selection, rather than more efficient positive selection, is responsible for the faster rate of evolution at haploid-specific genes (Szövényi et al. 2013), but this may be a consequence of the high selfing rate—and low degree of pollen competition—of *A. thaliana*. An explicit test for the extent of positive selection in *Capsella grandiflora* convincingly showed that pollen-specific genes exhibited higher proportions of adaptive substitutions and a higher rate of adaptive nonsynonymous to synonymous substitutions than genes expressed in sperm or seedling tissues, and genes expressed in all three tissues showed signs of elevated positive selection compared to genes expressed in either of the diploid tissues (Arunkumar et al. 2013).

Empirical testing of such ideas in animals is lagging far behind the research performed in plants. We currently know little about which genes are expressed at the postmeiotic stages and even less about the extent of positive selection. A study comparing orthologous protein-encoding genes in humans and rodents showed that proteins involved in reproduction, and more specifically in sperm-egg interactions, are among the most rapidly evolving of all studied proteins (Makalowski and Boguski 1998). Similar patterns were described in a wide range of species and taxa (reviewed extensively in Swanson and Vacquier 2002*a*, 2002*b*; Clark et al. 2006). A plausible explanation for the rapid evolution of these genes is that they are under sexually antagonistic selection and the rapid evolution is a good example for the arms race between the sexes. While these findings support the idea that selection on these genes must be strong, the question of the extent to which these genes are expressed in a haploid fashion requires further investigation. If any of these genes were expressed in a haploid manner in sperm, then an alternative to sexually antagonistic selection is conflict between haploid and diploid phases. Yet another alternative is conflict across both sexes and ploidy levels, as discussed further below (Immler et al. 2012).

Mutation Load and Mutation Rate

While de novo mutations are one of the key factors contributing to genetic variation, only a few are advantageous, and most are effectively neutral or mildly deleterious (Simmons and Crow 1977; Crow 1993; Eyre-Walker and Keightley 2007). Mutations that are deleterious to an organism's fitness (Muller 1932) cause a wide variety of diseases in humans (Cavalli-Sforza and Bodmer 1971) and are thought to shape a variety of features, from sex (Barton and Charlesworth 1998) to genetic canalization (Wagner et al. 1997). Any mechanism that purges such mutations should thus be favored. One such mechanism that has been explored theoretically is selection in the haploid phase (Gordo and Charlesworth 2000; Hough et al. 2013; Otto et al. 2015), because deleterious mutations are better exposed to selection without masking and are eliminated at a faster rate. Females in particular are selected to favor haploid selection among the gametes received, as this raises the mean fitness of her offspring (Otto et al. 2015). In addition, selection in the short-lived haploid phase of diplontic organisms allows purging without the substantial risk of somatic mutations and cancer that would plague multicellular haploids and are thought to maintain diploidy in multicellular organisms (Orr 1995). Similarly, haploid selection can mitigate the negative effects of inbreeding depression (Charlesworth and Charlesworth 1987) through the efficient removal of deleterious mutations even in small populations.

Empirical evidence for purifying selection in genes expressed during the haploid gametic phase is still scarce and primarily limited to studies in flowering plants. Haploid purifying selection in *Silene latofolia* has slowed down the degeneration of the Y chromosome (Chibalina and Filatov 2011), and in *Capsella grandiflora*, purifying selection was most evident in genes that were exclusively expressed in pollen or in all tissues compared to genes exclusively expressed in sperm or diploid seedling tissue (Arunkumar et al. 2013). In the selfing *Arabidopsis thaliana* a higher accumulation of deleterious mutations in pollen-specific genes has been reported (Harrison et al. 2015), and the authors note that selfing in this species increases homozygosity in the diploid sporophyte and reduces competition among pollen and hence selection on pollen-specific genes. It would be worthwhile to gather more such data across a variety of species to look at the impact of the mating system on haploid selection and vice versa.

Recombination Rate

Haploid selection is also a potentially important factor affecting the evolution of recombination. First, haploid selection would influence the nature of epistasis and the degree of selective interference among loci, both of which shape the evolution of recombination rates (e.g., Barton 2009; Otto 2009). Second, because haploid selection typically differs between males and females, it can drive the evolution of

reduced recombination on sex chromosomes (e.g., coupling alleles favored in pollen with the Y chromosome; Scott and Otto 2017).

Furthermore, theoretical models have also found that haploid selection is key to sex differences in recombination rates (heterochiasmy), a common phenomenon in both animals and plants (Burt et al. 1991). Lenormand (2003) rejected several forms of diploid selection as explanations for heterochiasmy but found that epistasis in the haploid phase can drive differences in the rate of recombination between males and females. Indeed, examining data from plants, Lenormand and Dutheil (2005) found lower recombination in females than in males when selection is stronger in female gametophytes and similarly when selection is weaker in male gametophytes. Meiotic drive is similar in nature to haploid selection, affecting the differential success of haploid chromosomes, and it too can drive sex differences in recombination (Brandvain and Coop 2012), although the sex that will evolve the higher rate of recombination depends on the location of the modifier of recombination (linked or unlinked) and the details of the drive (whether it acts in males or females and, if in females, whether it acts during meiosis I or II). The increasing availability of genomic sequence data promises to improve our understanding of heterochiasmy and to allow direct tests of whether organisms with stronger genomic evidence of haploid selection display greater heterochiasmy.

Maintenance of Variation in the Haploid Phase

For selection to be effective in haploids, there must be variation in haploid expressed genes. On average, gametes produced by one diploid individual share 50% of their genes, but this genetic relatedness may vary from 0% to 100% due to the processes of segregation and recombination. These two processes reshuffle the chromosomes and the alleles present in the diploid genome, allowing for an almost unlimited number of haploid gametes (Cohen 1967, 1973). But such reshuffling generates variation only if loci expressed in the haploid phase are polymorphic in the first place. Below, we discuss three nonmutually exclusive hypotheses for the maintenance of genetic variation during the haploid phase.

De Novo Mutations

Ultimately, de novo mutations are the source of genetic variation and would also contribute to variation in performance among haploid gametes. While mutations that reduce gamete performance would be removed, recurrent mutation would regenerate variation. Estimates of the deleterious mutation rate are generally less than one per generation (Bataillon 2000), but many persist for several generations, making it plausible that gametes carry several deleterious mutations. These will be relevant to haploid selection, however, only if expressed in the gamete via haploid transcripts or if they impact the efficacy of DNA condensation. If only a small fraction of genes are relevant in the haploid phase, as is thought to be the case in animals (Dadoune et al. 2004), we might not expect mutation-selection balance to contribute much phenotypic variation among sperm.

Balancing Selection

An alternative to mutation-selection balance that can maintain higher levels of variation at a locus is balancing selection, where different alleles are favored in different life stages. Loci expressed during the diploid and haploid phases are potentially subject to differential or even antagonistic selection where different alleles are favored in the two phases. Such "ploidally antagonistic selection" can maintain stable genetic polymorphisms (Ewing 1977) and is more likely to do so than sexually antagonistic selection when the dominance of an allele is the same in the two sexes (Immler et al. 2012). The conditions maintaining variation are even more readily met if selection is antagonistic not only across ploidy levels but also between sexes, for example, if an allele is beneficial when expressed in a haploid male gamete but deleterious when expressed in a diploid female offspring (Immler et al. 2012). Empirical tests of these predictions do not currently exist, but the increasing availability of genomic and transcriptomic data sets that include haploid-specific expression data could offer a perfect opportunity to fill this gap, particularly in flowering plants where haploid expressed genes are known.

An alternative scenario of balancing selection maintaining genetic variation may arise if male gametes experience different environments prior to fertilization, similar to Levene's model with multiple ecological niches (Levene 1953). For insect-pollinated plants, for example, there may be different selective pressures acting on pollen transported by different species of insect, depending on the insect's foraging behavior, pollen placement, and grooming (Hasegawa et al. 2015). A similar scenario could be found in externally fertilizing organisms, where the locations and timing of sperm and egg release may vary spatially, favoring different haplotypes (e.g., sometimes fast-swimming and sometimes long-lived sperm win).

Frequency-Dependent Selection

Finally, selection based on the relative frequency of specific gamete genotypes can maintain genetic variation when types are more successful when rare than when common. Frequency-dependent selection related to the detection of pathogens is thought to maintain high levels of variation at MHC loci (Takahata and Nei 1990). While it is unclear whether MHC loci are expressed in the haploid phase, the very nature of gamete competition—many compete but

few succeed—makes it plausible that frequency- and/or density-dependent selection act on genes expressed in the haploid phase. In particular, gamete recognition proteins (e.g., bindins) have been shown to experience frequency-dependent selection, with rare types being favored when sperm compete strongly (Levitan and Ferrell 2006). Although the extent of haploid versus diploid expression of these proteins in the sperm is not well understood (requiring single-sperm phenotyping), polymorphism is observed in different species of sea urchin and is predicted theoretically when sperm are overabundant, using models with either haploid or diploid expression (Tomaiuolo and Levitan 2010). In plants, gametophytic self-incompatibility is a well-known form of frequency dependence that maintains extensive polymorphism involving selection in the haploid phase, whereby only pollen with a different genotype than the maternal plant succeed in fertilization (Newbigin et al. 1993).

Genetic Interactions in the Haploid Phase

Beside the mechanisms maintaining genetic variation in the haploid phase described above, the impact on gamete phenotype and selection may also depend on how genes interact in the haploid phase and their additive effects and possible epistatic interactions. Such a scenario currently seems to be the most plausible explanation for the finding of genome-wide differences in allele frequencies observed among selected sperm pools in the zebrafish (Alavioon et al. 2017). However, whether these patterns are the result of additive effects or epistatic interactions between genes is currently unclear. In any case, the effects and possible interactions between fundamental housekeeping and regulatory genes may contribute to the sperm phenotype being favored by haploid selection and resulting in fitter offspring.

Epistatic interactions among alleles during the haploid gametic phase can also provide a possible explanation for the recent surprising observation that males and females differ in allele frequency at numerous autosomal loci in humans and *Drosophila* (Cheng and Kirkpatrick 2016; Lucotte et al. 2016). This finding can be explained only by selection within the generation, but this includes very early selection—among the gametes. In particular, epistasis between loci on the sex chromosomes and autosomal loci, where specific alleles on the sex chromosome combined with alleles on autosomes yield favorable gamete phenotypes, could drive observed differences in the resulting offspring. In other words, selection among gametes might cause alleles on the Y chromosome to become genetically associated with a different set of autosomal alleles than alleles on the X chromosome, generating differences in allele frequency between daughters and sons. While speculative, selection at the haploid stage might help alleviate the severe segregation load that appears to be implied by these studies.

Future Directions

Understanding the extent of selection at the haploid gametic phase in predominantly diploid organisms may provide important insights into the dynamics of major evolutionary processes. Despite the seemingly short amount of time spent haploid, any selection occurring at that stage may have a disproportionate effect on the speed of adaptation and, given the fundamental tie to mating, on the development of reproductive isolation between species. Previous authors (e.g., Swanson and Vacquier 2002*a*, 2002*b*) have suggested that both purifying selection and positive selection can explain the rapid evolution of reproductive proteins—but the extent to which this selection depends on the diploid genome of the parent versus the haploid genome of the gamete remains unclear.

Also unclear is the extent to which haploid selection purges deleterious mutations from diplontic organisms. Certainly, expression of fundamental housekeeping genes responsible for the general fitness of an organism during the haploid phase could offer a unique opportunity for "proofreading" a genome and eliminating deleterious mutations. But the extent to which haploid selection is an effective selective sieve—and in which organisms—is virtually unknown at present.

With the availability of ever more sophisticated sequencing tools and the opportunity to sequence even individual cells, we expect that the extent of haploid selection among diploid animals and plants will become better understood. Experimentally, a promising approach in plants would be to perform genomic sequencing of a paternal and distantly related maternal plant (distant enough that short-read sequences can be distinguished), alongside sequencing of styles after fertilization (as distant from the stigma as possible); after distinguishing maternal and paternal reads, analyses of the SNPs from the father that depart from a 50:50 expectation would indicate haploid selection. In animals, we recommend that more studies of sperm competition be conducted that focus on the gametic products of a single individual, not just competing sperm from different males. Studies of pollen or sperm from single males, such as that conducted by Alavioon et al. (2017), are needed from a much broader array of organisms—plants and animals—to elucidate the functional importance of haploid selection. The assumption that haploid selection is absent in primarily diploid organisms has, in our opinion, prevented scientists from investigating its importance. The genomic tools and accumulating evidence indicate that it is time to take a more serious look for haploid selection.

Acknowledgments

We are grateful to Michael Scott for invaluable discussions on the topic and for collating an extensive collection of rel-

evant literature in his PhD thesis. This project was funded by a Starting Grant from the European Research Council to S.I. and a Discovery Grant from the Natural Sciences and Engineering Research Council to S.P.O.

Literature Cited

Alavioon, G., H. Hotzy, K. Nakhro, S. Rudolf, D. G. Scofield, S. Zajitschek, A. A. Maklakov, and S. Immler. 2017. Haploid selection within a single ejaculate increases offspring fitness. Proceedings of the National Academy of Sciences of the USA 118:8053–8058.

Alcivar, A. A., L. E. Hake, C. F. Millette, J. M. Trasler, and N. B. Hecht. 1989. Mitochondrial gene expression in male germ cells of the mouse. Developmental Biology 135:263–271.

Aronen, T., T. Nikkanen, A. Harju, H. Tiimonen, and H. Häggman. 2002. Pollen competition and seed-siring success in *Picea abies*. Theoretical and Applied Genetics 104:638–642.

Arunkumar, R., E. B. Josephs, R. J. Williamson, and S. I. Wright. 2013. Pollen-specific, but not sperm-specific, genes show stronger purifying selection and higher rates of positive selection than sporophytic genes in *Capsella grandiflora*. Molecular Biology and Evolution 30:2475–2486.

Barreau, C., E. Benson, E. Gudmannsdottir, F. Newton, and H. White-Cooper. 2008. Post-meiotic transcription in *Drosophila* testes. Development 135:1897–1902.

Barton, N. H. 2009. Why sex and recombination? Pages 187–195 *in* Cold Spring Harbor Symposia on Quantitative Biology. Vol. 74. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science 281:1986–1990.

Bataillon, T. 2000. Estimation of spontaneous genome-wide mutation rate parameters: whither beneficial mutations? Heredity 84:497–501.

Birkhead, T. R., A. P. Møller, and W. J. Sutherland. 1993. Why do females make it so difficult for males to fertilize their eggs? Journal of Theoretical Biology 161:51–60.

Bonache, S., A. Mata, M. D. Ramos, L. Bassas, and S. Larriba. 2012. Sperm gene expression profile is related to pregnancy rate after insemination and is predictive of low fecundity in normozoospermic men. Human Reproduction 27:1556–1567.

Borg, M., L. Brownfield, and D. Twell. 2009. Male gametophyte development: a molecular perspective. Journal of Experimental Botany 60:1465–1478.

Borges, F., G. Gomes, R. Gardner, N. Moreno, S. McCormick, J. A. Feijó, and J. D. Becker. 2008. Comparative transcriptomics of *Arabidopsis* sperm cells. Plant Physiological Genomics 148:1168–1181.

Brandvain, Y., and G. Coop. 2012. Scrambling eggs: meiotic drive and the evolution of female recombination rates. Genetics 190:709–723.

Burt, A., G. Bell, and P. H. Harvey. 1991. Sex differences in recombination. Journal of Evolutionary Biology 4:259–277.

Carré, D., and C. Sardet. 1984. Fertilization and early development in *Beroe ovata*. Developmental Biology 105:188–195.

Cavalli-Sforza, L. L., and W. F. Bodmer. 1971. The genetics of human populations. W. H. Freeman, San Francisco.

Champroux, A., J. Torres-Crreira, P. Gharagozloo, J. R. Drevet, and A. Kocer. 2016. Mammalian sperm nuclear organization: resiliencies and vulnerabilities. Basic and Clinical Andrology 26:17–39.

Charlesworth, B. 1983. Adaptive evolution in the laboratory. Nature 302:479–480.

Charlesworth, B., and D. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237–268.

Cheng, C., and M. Kirkpatrick. 2016. Sex-specific selection and sex-biased gene expression in humans and flies. PLoS Genetics 12:e1006170.

Chibalina, M. V., and D. A. Filatov. 2011. Plant y chromosome degeneration is retarded by haploid purifying selection. Current Biology 21:1475–1479.

Clark, N. L., J. E. Aagaard, and W. J. Swanson. 2006. Evolution of reproductive proteins from animals and plants. Reproduction 131:11–22.

Clarke, H. J., T. N. Khan, and K. H. M. Siddique. 2004. Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. Euphytica 139:65–74.

Cohen, J. 1967. Correlation between sperm "redundancy" and chiasma frequency. Nature 215:362–363.

———. 1973. Cross-overs, sperm redundancy and their close association. Heredity 31:408–413.

Crow, J. F. 1993. Mutation, mean fitness, and genetic load. Oxford Survey of Evolutionary Biology 9:3–42.

Crow, J. F., and M. Kimura. 1965. Evolution of sexual and asexual populations. American Naturalist 99:439–450.

———. 1970. An introduction to population genetics theory. Harper & Row, New York.

Cruden, R. W. 1976. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31:32–46.

Dadoune, J.-P., J.-P. Siffroi, and M.-F. Alfonsi. 2004. Transcription in haploid germ cells. International Review of Cytology 237:1–56.

Ewens, W. J. 1979. Mathematical population genetics. Springer, Berlin.

Ewing, E. 1977. Selection at the haploid and diploid phases: cyclical variation. Genetics 87:195–208.

Eyre-Walker, A., and P. D. Keightley. 2007. The distribution of fitness effects of new mutations. Nature Reviews Genetics 8:610–618.

Fiebig, A., R. Kimport, and D. Preuss. 2004. Comparisons of pollen coat genes across Brassicaceae species reveal rapid evolution by repeat expansion and diversification. Proceedings of the National Academy of Sciences of the USA 101:3286–3291.

Frascaroli, E., and D. Songstad. 2001. Pollen genotype selection for a simply inherited qualitative factor determining resistance to chlorsulfuron in maize. Theoretical and Applied Genetics 102:342–346.

Gerstein, A. C. 2013. Mutational effects depend on ploidy level: all else is not equal. Biology Letters 9:20120614.

Gerstein, A. C., L. A. Cleathero, M. A. Mandegar, and S. P. Otto. 2011. Haploids adapt faster than diploids across a range of environments. Journal of Evolutionary Biology 24:531–540.

Gilbert, S. F. 2013. Developmental biology. Sinauer, Sunderland, MA.

Gordo, I., and B. Charlesworth. 2000. The degeneration of asexual haploid populations and the speed of Muller's ratchet. Genetics 154:1379–1387.

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

———. 2008. Protein synthesis in sperm: dialog between mitochondria and cytoplasm. Molecular and Cellular Endocrinology 282:45–55.

Haldane, J. 1932. The causes of evolution. Harper, New York.

Harrison, M. C., E. B. Mallon, D. Twell, and R. L. Hammond. 2015. Deleterious mutation accumulation in *Arabidopsis thaliana* pollen genes: a role for recent relaxation of selection. bioRxiv. doi:10.1101/016626.

Hasegawa, Y., Y. Suyama, and K. Seiwa. 2015. Variation in pollen-donor composition among pollinators in an entomophilous tree

species, *Castanea crenata*, revealed by single-pollen genotyping. PLoS ONE 10:e0120393.

Hedhly, A., J. I. Hormaza, and M. Herrero. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). American Journal of Botany 91:558–564.

Honys, D., and D. Twell. 2004. Transcriptome analyses of haploid male gametophyte development in *Arabidopsis*. Genome Biology 5:R85.

Hough, J., S. Immler, S. C. H. Barrett, and S. P. Otto. 2013. Evolutionarily stable sex ratios and mutation load. Evolution 67:1915–1925.

Immler, S. 2008. Sperm competition and sperm cooperation: the potential role of diploid and haploid expression. Reproduction 135:275–283.

Immler, S., G. Arnqvist, and S. P. Otto. 2012. Ploidally antagonistic selection maintains stable genetic polymorphism. Evolution 66:55–65.

Ioannou, D., and H. G. Tempest. 2018. Does genome organization matter in spermatozoa? a refined hypothesis to awaken the silent vessel. Systems Biology in Reproductive Medicine (forthcoming).

Joseph, S. B., and M. Kirkpatrick. 2004. Haploid selection in animals. Trends in Ecology and Evolution 19:592–597.

Keller, L., and H. K. Reeve. 1995. Why do females mate with multiple males? the sexually selected sperm hypothesis. Advances in the Study of Behavior 24:291–315.

Lambard, S., I. Galeraud-Denis, G. Martin, R. Levy, A. Chocat, and S. Carreau. 2004. Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. Molecular Human Reproduction 10:535–541.

Lankinen, A., J. Maad, and W. S. Armbruster. 2009. Pollen-tube growth rates in *Collinsia heterophylla* (Plantaginaceae): one-donor crosses reveal heritability but no effect on sporophytic-offspring fitness. Annals of Botany 103:941–950.

Lankinen, A., and I. Skogsmyr. 2002. Pollen competitive ability: the effect of proportion in two-donor crosses. Evolution and Ecology Research 4:687–700.

Lenormand, T. 2003. The evolution of sex dimorphism in recombination. Genetics 163:811–822.

Lenormand, T., and J. Dutheil. 2005. Recombination difference between sexes: a role for haploid selection. PLoS Biology 3:e63.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. American Naturalist 87:331–333.

Levitan, D. R., and D. L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. Science 312:267–269.

Lindsley, D., and E. Grell. 1969. Spermiogenesis without chromosomes in *Drosophila melanogaster*. Genetics 61:69–78.

Lucotte, E. A., R. Laurent, E. Heyer, L. Ségurel, and B. Toupance. 2016. Detection of allelic frequency differences between the sexes in humans: a signature of sexually antagonistic selection. Genome Biology and Evolution 8:1489–1500.

Mable, B., and S. Otto. 1998. The evolution of life cycles with haploid and diploid phases. BioEssays 20:453–462.

Makalowski, W., and M. S. Boguski. 1998. Evolutionary parameters of the transcribed mammalian genome: an analysis of 2,820 orthologous rodent and human sequences. Proceedings of the National Academy of Sciences of the USA 95:9407–9412.

Mascarenhas, J. 1990. Gene activity during pollen development. Annual Review of Plant Physiology and Plant Molecular Biology 41:317–338.

Muller, H. 1932. Some genetic aspects of sex. American Naturalist 66:118–138.

Nadeau, J. H. 2017. Do gametes woo? evidence for non-random unions at fertilization. bioRxiv. doi:10.1101/127134.

Naz, R. K. 1998. Effect of actinomycine D and cycloheximide on human sperm function. Archives of Andrology 41:135–142.

Newbigin, E., M. A. Anderson, and A. E. Clarke. 1993. Gametophytic self-incompatibility systems. Plant Cell 5:1315–1324.

Orr, H. A. 1995. Somatic mutation favors the evolution of diploidy. Genetics 139:1441–1447.

Orr, H. A., and S. P. Otto. 1994. Does diploidy increase the rate of adaptation? Genetics 136:1475–1480.

Ostermeier, G. C., D. J. Dix, D. Miller, P. Khatri, and S. A. Krawetz. 2002. Spermatozoal RNA profiles of normal fertile men. Lancet 360:772–777.

Otto, S. P. 2009. The evolutionary enigma of sex. American Naturalist 174(suppl.):S1–S14.

Otto, S. P., M. Scott, and S. Immler. 2015. Evolution of haploid selection in predominantly diploid organisms. Proceedings of the National Academy of Sciences of the USA 112:15952–15957.

Paquin, C., and J. Adams. 1983. Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations. Nature 302:495–500.

Paynter, E., A. H. Millar, M. Welch, B. Baer-Imhoof, D. Cao, and B. Baer. 2017. Insights into the molecular basis of long-term storage and survival of sperm in the honeybee (*Apis mellifera*). Scientific Reports 7:40236.

Pina, C., F. Pinto, J. A. Feijó, and J. D. Becker. 2005. Gene family analysis of the *Arabidopsis* pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. Plant Physiological Genomics 138:744–756.

Promerová, M., G. Alavioon, S. Tusso, R. Burri, and S. Immler. 2017. No evidence for MHC class II-based non-random mating at the gametic haplotype in Atlantic salmon. Heredity 118:563–567.

Ravikumar, R. L., G. N. Chaitra, A. M. Choukimath, and C. D. Soregaon. 2012. Gametophytic selection for wilt resistance and its impact on the segregation of wilt resistance alleles in chickpea (*Cicer arietinum* L.). Euphytica 189:173–181.

Ravikumar, R. L., B. S. Patil, and P. M. Salimath. 2003. Drought tolerance in sorghum by pollen selection using osmotic stress. Euphytica 133:371–376.

Schein, M., Z. Yang, T. Mitchell-Olds, and K. J. Schmid. 2004. Rapid evolution of a pollen-specific oleosin-like gene family from *Arabidopsis thaliana* and closely related species. Molecular Biology and Evolution 21:659–669.

Scott, M. F. 2016. Mathematical models of life cycle evolution. PhD diss. University of British Columbia, Vancouver.

Scott, M. F., and S. P. Otto. 2017. Haploid selection favors suppressed recombination between sex chromosomes despite causing biased sex ratios. Genetics 207:1631–1649.

Searcy, K., and D. Mulcahy. 1985. Pollen selection and the gametophytic expression of metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). American Journal of Botany 72:1700–1702.

Sharp, N. P., L. Sandell, C. G. James, and S. P. Otto. 2018. The genome-wide rate and spectrum of spontaneous mutations differs between haploid and diploid yeast. Proceedings of the National Academy of Sciences of the USA 115:E5046–E5055. doi:10.1073/pnas.1801040115.

Simmons, M. J., and J. F. Crow. 1977. Mutations affecting fitness in *Drosophila* populations. Annual Review of Genetics 11:49–78.

Skogsmyr, I., and A. Lankinen. 1999. Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. Evolution and Ecology Research 1:971–985.

Snow, A. A., and T. P. Spira. 1996. Pollen-tube competition and male fitness in *Hibiscus moscheutos*. Evolution 50:1866–1870.

Steger, K. 1999. Transcriptional and translational regulation of gene expression in haploid spermatids. Anatomical Embryology 199:471–487.

Strasburger, E. 1894. The periodic reduction of the number of the chromosomes in the life-history of living organisms. Annals of Botany 8:281–316.

Swanson, W. J., and V. D. Vacquier. 2002*a*. Reproductive protein evolution. Annual Review of Ecology and Systematics 33:161–179.

———. 2002*b*. The rapid evolution of reproductive proteins. Nature Reviews Genetics 3:137–144.

Szövényi, P., M. Ricca, Z. Hock, J. Shaw, K. K. Shimizu, and A. Wagner. 2013. Selection is no more efficient in haploid than in diploid life stages of an angiosperm and a moss. Molecular Biology and Evolution 30:1929–1939.

Takahata, N., and M. Nei. 1990. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics 124:967–978.

Tomaiuolo, M., and D. R. Levitan. 2010. Modeling how reproductive ecology can drive protein diversification and result in linkage disequilibrium between sperm and egg proteins. American Naturalist 176:14–25.

Valero, M., S. Richerd, V. Perrot, and C. Desombe. 1992. Evolution of alternation of haploid and diploid phases in life cycles. Trends in Ecology and Evolution 7:25–29.

Vibranovski, M., D. Chalopin, H. Lopes, M. Long, and T. Karr. 2010. Direct evidence for postmeiotic transcription during *Drosophila melanogaster* spermatogenesis. Genetics 186:431–433.

Wagner, G., G. Booth, and H. Bagheri. 1997. A population genetic theory of canalization. Evolution 51:329–347.

Walbot, V., and M. M. Evans. 2003. Unique features of the plant life cycle and their consequences. Nature Reviews Genetics 4:369–379.

Ward, W. S. 2010. Function of sperm chromatin structural elements in fertilization and development. Molecular Human Reproduction 16:30–36.

Wedekind, C., M. Chapuisat, E. Macas, and T. Rülicke. 1996. Nonrandom fertilization in mice correlates with the MHC and something else. Heredity 77:400–409.

Wedekind, C., M. Walker, J. Portmann, B. Cenni, R. Muller, and T. Binz. 2004. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. Journal of Evolutionary Biology 17:11–18.

Welch, J. E., R. R. Barbee, P. L. Magyar, D. O. Bunch, and D. A. O'Brien. 2006. Expression of the spermatogenic cell-specific glyceraldehyde 3-phosphate dehydrogenase (GAPDS) in rat testis. Molecular Reproduction and Development 73:1052–1060.

Yang, C. C., Y. S. Lin, C. C. Hsu, S. C. Wu, E. C. Lin, and W. T. K. Cheng. 2009. Identification and sequencing of remnant messenger RNAs found in domestic swine (*Sus scrofa*) fresh ejaculated spermatozoa. Animal Reproduction Science 113:143–155.

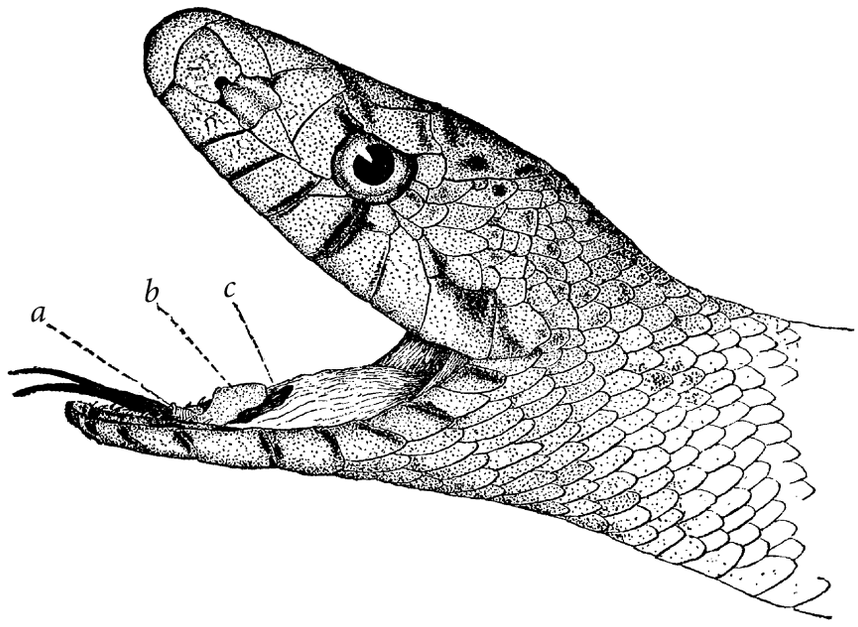
Zareie, R., H. Eubel, A. H. Millar, and B. Baer. 2013. Long-term survival of high quality sperm: insights into the sperm proteome of the honeybee *Apis mellifera*. Journal of Proteome Research 12:5180–5188.

Zeyl, C., T. Vanderford, and M. Carter. 2003. An evolutionary advantage of haploidy in large yeast populations. Science 299:555–558.

Zheng, Y., X. Deng, and P. Martin-DeLeon. 2001. Lack of sharing of Spam1 (Ph-20) among mouse spermatids and transmission ratio distortion. Biology of Reproduction 64:1730–1738.

Zörgö, E., K. Chwialkowska, A. Gjuvsland, E. Garré, P. Sunnerhagen, G. Liti, A. Blomberg, S. Omholt, and J. Warringer. 2013. Ancient evolutionary trade-offs between yeast ploidy states. PLoS Genetics 9:e1003388.

Symposium Editor: Locke Rowe



"To all these species the popular name of bull-snake is applied, and the more common eastern variety is also popularly called the pine-snake. They are great, good-natured fellows, always getting out of man's way when they can, but occasionally, if they are pressed, they will throw themselves into a somewhat threatening attitude and emit a peculiar hiss. . . . The tongue-sheath is represented at *a*; the epiglottis at *b*; and the rima-glotidis (aperture of the windpipe) at *c*." From "On the Character and Function of the Epiglottis in the Bull-Snake (*Pityophis*)" by Charles A. White (*The American Naturalist*, 1884, 18:19–21).