Competition Between the Sperm of a Single Male Can Increase the Evolutionary Rate of Haploid Expressed Genes

Kiyoshi Ezawa and Hideki Innan[1]
The Graduate University for Advanced Studies, Hayama, Kanagawa 240-0193, Japan

ABSTRACT The population genetic behavior of mutations in sperm genes is theoretically investigated. We modeled the processes at two levels. One is the standard population genetic process, in which the population allele frequencies change generation by generation, depending on the difference in selective advantages. The other is the sperm competition during each genetic transmission from one generation to the next generation. For the sperm competition process, we formulate the situation where a huge number of sperm with alleles A and B, produced by a single heterozygous male, compete to fertilize a single egg. This "minimal model" demonstrates that a very slight difference in sperm performance amounts to quite a large difference between the alleles' winning probabilities. By incorporating this effect of paternity-sharing sperm competition into the standard population genetic process, we show that fierce sperm competition can enhance the fixation probability of a mutation with a very small phenotypic effect at the single-sperm level, suggesting a contribution of sperm competition to rapid amino acid substitutions in haploid-expressed sperm genes. Considering recent genome-wide demonstrations that a substantial fraction of the mammalian sperm genes are haploid expressed, our model could provide a potential explanation of rapid evolution of sperm genes with a wide variety of functions (as long as they are expressed in the haploid phase). Another advantage of our model is that it is applicable to a wide range of species, irrespective of whether the species is externally fertilizing, polygamous, or monogamous. The theoretical result was applied to mammalian data to estimate the selection intensity on nonsynonymous mutations in sperm genes.

FOR sexual organisms, reproduction is an essential process that allows an individual's genomic information to survive beyond its lifetime. Years ago, it was thought that the functional constraints on genes involved in reproduction should be as strong as those on functionally important genes such as histones, etc. (*e.g.*, Miyata and Yasunaga 1980; Li 1997); hence it was predicted that such genes should evolve much more slowly than average. Therefore, it was a surprise when the first molecular evolutionary analyses on reproduction-related genes (or proteins) revealed their faster than normal evolutionary rates (see, *e.g.*, Swanson *et al.* 2001; Swanson and Vacquier 2002a,b). Since then, analyses of additional reproductive genes in additional species continue to support the initial observation that reproductive genes evolve more rapidly than the genomic average (*e.g.*, Cutter and Ward 2005; Clark *et al.* 2006, 2009; Ramm *et al.* 2008; Turner and Hoekstra 2008; Findlay and Swanson 2010; Wong 2011). A common and particularly typical pattern for reproductive genes is a higher ratio, often denoted as d_N/d_S ($= \omega$), of the number of nonsynonymous nucleotide substitutions per nonsynonymous site (d_N) to the number of synonymous nucleotide substitutions per synonymous site (d_S). This pattern seems to be particularly remarkable among "sperm genes", namely, genes whose protein products are found in sperm (*e.g.*, Wyckoff *et al.* 2000; Torgerson *et al.* 2002; Swanson *et al.* 2003; Nielsen *et al.* 2005; Artieri *et al.* 2008; Dorus *et al.* 2010). When d_N/d_S is computed for the entire coding region of a gene, we call it a "gene-wide" d_N/d_S value. Sperm genes usually show higher gene-wide d_N/d_S values than the average over all genes in the genome. Furthermore, sperm genes commonly have local regions (or domains) whose d_N/d_S values significantly exceed 1.

There are a variety of potential explanations for this observation. Some of them are not suitable to explain the general trend that a wide variety of sperm genes exhibit

doi: 10.1534/genetics.113.152066
Manuscript received April 9, 2013; accepted for publication May 4, 2013
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.152066/-/DC1.
[1]Corresponding author: The Graduate University for Advanced Studies, Hayama, Kanagawa 240-0193, Japan. E-mail: innan_hideki@soken.ac.jp

high d_N/d_S values in various species. For example, (i) relaxation of selective constraints could account for elevated gene-wide d_N/d_S values (Swanson and Vacquier 2002a), but does not explain the common observation that many genes have local regions with $d_N/d_S \gg 1$ (*e.g.*, Ramm *et al.* 2008; Dorus *et al.* 2010; Wong 2011); (ii) defense against pathogens could explain the elevated d_N/d_S values in reproductive genes (*e.g.*, Vacquier *et al.* 1997), but this applies only to genes that are involved in battles against pathogens; and (iii) reinforcement of reproductive incompatibility in a speciation event can also accelerate the evolution of sperm genes (Dobzhansky 1940; Howard 1993), but this works only on special occasions where two closely related sympatric species coexist.

Most other models and hypotheses invoke either postcopulatory sexual selection, namely selection on reproductive genes after mating (reviewed, *e.g.*, in Birkhead and Pizzari 2002; Swanson and Vacquier 2002b; Clark *et al.* 2006; Turner and Hoekstra 2008), or sexual conflict (*e.g.*, Rice and Holland 1997; Frank 2000; Gavrilets 2000; Chapman *et al.* 2003; Hayashi *et al.* 2007). So far, these models have mainly focused on selection and/or competition at the individual level; when they consider a competition among sperm, it is almost always among sperm produced by different males. In these models, competition among sperm from a single male play only a secondary role, if any.

On the contrary, in real life, it is obvious that numerous sperm compete with each other even when a female mates with only a single male during a reproductive period (see, *e.g.*, Parker and Begon 1993; Manning and Chamberlain 1994). For a sperm to successfully fuse with an egg, it has to win a fierce competition with millions to billions of all the others, to be the only "winner"; the remaining 99.999...% of the sperm are destined not to be involved in fertilization. This process is quite complicated and involves many factors. For example, the rate of success depends on how fast it can swim in the right direction and how efficiently it can fuse with the egg. The former process may involve chemotaxis, and the latter may involve egg–sperm compatibility. Therefore, any kind of selection on sperm performance may potentially increase d_N/d_S values of a wide variety of sperm genes, irrespective of whether the species is externally fertilizing, polygamous, or monogamous.

The main goal of this study is to examine the effects of such competition among paternity-sharing sperm, which, as mentioned above, have almost always been neglected thus far. For this purpose, we here provide a "minimal model" of sperm gene population genetics that incorporates the intrinsic feature of the fertilization process. To be more specific, our minimal model focuses on the competition among sperm *introduced by a single mating event with a single male*. Even in this case, sperm can have different genotypes; for a single sperm gene, there would be two alleles if the male is heterozygous. With this minimal model, we demonstrate that even a very tiny phenotypic effect of a mutation at the level of a single sperm can amount to a substantial difference between allelic fitnesses at the level of a single inheritance, through fierce competition among millions to billions of sperm per each. This result implies that mutations with very weak effects at the molecular level can be a potential explanation of the widely observed high d_N/d_S ratios of sperm genes.

Because our theory applies only when each allele affects solely (or preferentially) haploid sperm genomes carrying it, the generality of our theory largely depends on how many sperm genes have haploid expression, namely, are expressed during the haploid phase of the sperm development. It used to be thought that such haploid expression should be very rare because the haploid phase spans only late stages of the sperm development, during which both DNA and cytoplasm are getting compactified (see, *e.g.*, Steger 1999). However, recent genome-wide expression analyses estimated that about several hundreds of sperm genes are haploid expressed in mammals (see, *e.g.*, Joseph and Kirkpatrick 2004). This number is comparable to that of genes examined in each of other proteome-scale analyses of mammalian sperm (see, *e.g.*, Good and Nachman 2005; Dorus *et al.* 2010). Furthermore, as Good and Nachman (2005) showed, it is among sperm genes expressed after the onset of meiosis, but not among those expressed before it, that high d_N/d_S regions were found significantly more frequently than the genome average. These results indicate that haploid expression seems to be indeed quite common among sperm genes. If so, the model we propose here could provide an important explanation of rapid evolution of sperm genes with a wide variety of functions, as long as they are haploid expressed.

Theory

Modeling sperm-competition process

In the process of fertilization, selection works in an essentially different way from that assumed in the standard population genetics, in which what usually matters is the number of offspring that mature enough to produce the next offspring. As illustrated in Figure 1A, suppose an autosomal locus with two alleles, *A* and *B*, that have different life span distributions. Assuming all else (such as fertility) being equal, selection should favor *B* because it has a higher possibility to mature and produce offspring.

In contrast, in the fertilization process, millions to billions of sperm compete for fertilizing an egg (or a handful of eggs), and selection should act on the fertilizing ability of sperm. In Figure 1B, we again consider two alleles at a single autosomal locus, *A* and *B*, that have different "velocities" in the direction to the egg. We suppose that sperm swim toward an egg, perhaps in response to pheromone-like chemoattractants. How fast a sperm can reach and fertilize the egg is determined by traits such as responsiveness to pheromone-like chemoattractants, swimming speed, ability to overcome obstacles surrounding the egg, and compatibility with the egg membrane.

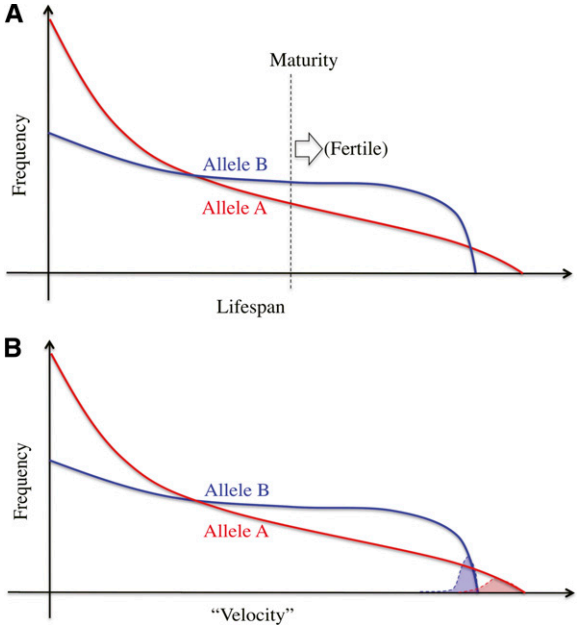

Figure 1 Illustration of how selection works in the standard population genetic framework (A) and in sperm competition (B). See text for details.

Here, to measure the performance of a sperm, we consider the total time from ejaculation till the completion of fertilization. Then, we define the velocity of the sperm, or the level of performance in fertilizing the egg, as the reciprocal of the total time, so that a sperm with a larger velocity is more likely to win the race for fertilization.

Sperm competition is usually extremely fierce, where only one (or a handful of) winner(s) among a huge number of competitors can pass on the genome(s) to the next generation, and the remaining 99.999...% of the sperm are eliminated. In this situation, the important factor is the right tail of each velocity distribution (red or blue shade in Figure 1B), to which the "fastest" sperm with each allele likely belongs. Given the distributions in Figure 1B, selection should favor A because it has a better chance to have the winner sperm among all competitors.

This intuitive expectation can be mathematically expressed as follows. Let x be the velocity as defined above. $f_Z(x)$ denotes the probability density function (PDF) of x for a single sperm with allele Z ($= A$ or B), and let X^Z ($Z = A$ or B) be a random variable following the PDF $f_Z(x)$. Then, the probability, $P[X^Z > x]$, that a sperm with allele Z has a velocity larger than x is given by: $P[X^Z > x] = \int_x^{+\infty} d\xi f_Z(\xi)$ (for $Z = A, B$).

Now, let us consider the situation where N_A sperm with allele A and N_B sperm with allele B compete to fertilize an egg. Suppose that all $N_A + N_B$ sperm start swimming for the egg at the same time and that there will be only a single sperm to win. If interactions between sperm are negligible, the distribution of the velocity of the fastest among N_Z sperm with allele Z ($= A$ or B) is given by the (cumulative) probability

$$P\left[\max\left\{X_1^Z,\ldots,X_{N_Z}^Z\right\}<x\right] = \left(P\left[X^Z<x\right]\right)^{N_Z} = \left(1-P\left[X^Z>x\right]\right)^{N_Z}, \tag{1}$$

because the maximum among $X_1^Z,\ldots,X_{N_Z}^Z$ is less than x if and only if *all of* $X_1^Z,\ldots,X_{N_Z}^Z$ are less than x.

The winner of the competition will have allele A if the fastest sperm with allele A outperforms the fastest with allele B and vice versa. Therefore, the probability that the winner has allele A is given by

$$\begin{aligned} &P[\text{winner} = A\,|N_A,N_B] \\ &= \int_0^{+\infty} dx \frac{d}{dx} P\left[\max\left\{X_1^A,\ldots,X_{N_A}^A\right\}<x\right] \\ &\quad\times\ P\left[\max\left\{X_1^B,\ldots,X_{N_B}^B\right\}<x\right] \\ &= \int_0^{+\infty} dx\, N_A f_A(x)\left(1-P\left[X^A>x\right]\right)^{N_A-1} \\ &\quad\times\left(1-P\left[X^B>x\right]\right)^{N_B} \\ &= 1-\int_0^{+\infty} dx\left(1-P\left[X^A>x\right]\right)^{N_A} \\ &\quad\times N_B f_B(x)\left(1-P\left[X^B>x\right]\right)^{N_B-1}. \end{aligned} \tag{2}$$

The last equation could be derived via partial integration. It should be noted that, as P [winner = A | N_A, N_B] + P [winner = $B|N_A, N_B$] = 1, the second term of the right-hand side of (2) is the probability that the winner has allele B. The "master equation", Equation 2, will provide the basis for the theory of sperm competition in general situations. When the mutation is exactly neutral, $f_A(x) \equiv f_B(x)$, this master equation can be easily integrated to give $P[\text{winner} = A\,|N_A,N_B] = N_A/(N_A+N_B)$, which faithfully reproduces the expectation in an exactly neutral situation.

Here, let us define $\delta f(x)$ as the difference between $f_A(x)$ and $f_B(x)$; that is, $\delta f(x) \equiv f_A(x) - f_B(x)$. In the following, we set $f(x) \equiv f_B(x)$, although the result is essentially the same even if we set $f(x) \equiv f_A(x)$. Suppose $\delta f(x)$ is small ($\int_{-\infty}^{+\infty} dx|\delta f(x)| \ll 1$); then the master equation, Equation 2 can be approximated up to $O(\delta f)$ as

$$\begin{aligned} P\left[\text{winner} = A\,|N_A\ A's\ \text{and}\ N_B\ B's\right] &\approx \frac{N_A}{N_A+N_B}\left\{1+\frac{N_B}{N_A+N_B}\psi_A\right\} \\ &\approx \frac{N_A}{N_A+N_B(1-\psi_A)} \approx \frac{N_A(1+\psi_A)}{N_A(1+\psi_A)+N_B}, \end{aligned} \tag{3}$$

where ψ_A is given by

$$\psi_A \approx (N_A+N_B) \times\int_0^{+\infty} dx\,\exp\left(-(N_A+N_B)\,P\left[X_f>x\right]\right)\delta f(x) \tag{4}$$

when $N_A + N_B \gg 1$ [For the derivation of (4) and the equation for a wide range of N_A and N_B, refer to Note 1 of

Supporting Information, File S1.] It is very interesting to note that the approximate Equation 3 demonstrates that the sperm-competition process we model here can be described similarly to the interindividual competition in the standard population genetics; that is, allele A has a selective advantage, or "competitive advantage", ψ_A over allele B in the "population" of competing sperm. This selective advantage can be easily incorporated into the basic framework of population genetics as we show in the next section.

It is known that in mammals "intercellular bridges" connect four haploid spermatids produced by the meiosis of a single diploid male germline cell (spermatocyte) (*e.g.*, Russell *et al.* 1990; de Rooji and Russell 2000; Yoshida 2010). Such intercellular bridges are believed to transport gene products among the connected spermatids and therefore could violate our theory's key assumption that the performance of each sperm is determined by (the gene product of) its own allele. However, the theory works as long as intercellular bridges are imperfect so that there is some difference in the composition of allelic gene products between sperm with different alleles. The only required adjustment is that the PDF of velocity should take account of the effect of intercellular bridges.

In Figure 2, we consider some typical cases where a mutation changes the PDF of the velocity ($f(x)$): (i) The PDF is exponential and the mutation from B to A increases the mean (Figure 2A), (ii) the PDF is a normal distribution and the mutation increases the mean (Figure 2B), (iii) the PDF is a normal distribution and the mutation increases the variance without changing the mean (Figure 2C), and (iv) the PDF has a finite maximum and the mutation increases the maximum (Figure 2D). As an example of case iv, we consider a power-law function with the exponent α and the maximum x_{M}:

$$f(x) = \begin{cases} \frac{\alpha+1}{x_{\mathrm{M}}}\left(1-\frac{x}{x_{\mathrm{M}}}\right)^{\alpha} & \text{for} \quad 0 < x < x_{\mathrm{M}}, \\ 0 & \text{for} \quad x \geq x_{\mathrm{M}}. \end{cases} \tag{5}$$

In all cases, the mutation is adaptive because the winning probability of A should be larger than that of B. These cases should cover quite a wide variety of changes in the PDF of the velocity, because what really matters in sperm competition is the change in the right tail (or end) of the PDF. Regarding the right tail, case iv may be the most natural because the sperm velocity should never reach infinity in nature. Other cases are considered because exponential and normal distributions are very popular functions. Each of the four cases specifies $f(x)$ and $\delta f(x)$, with which we can calculate ψ_A as a function of the numbers, N_A and N_B, of sperm with alleles A and B, respectively, by using Equation 4. Here, we assume $N_A = N_B \equiv N_{hSp}$ and investigate the enhancing effect of the number of sperm on the competitive advantage, ψ_A, by defining

$$R[\psi_A](N_{\mathrm{hSp}}) \equiv \frac{\psi_A(N_{\mathrm{hSp}})}{\psi_A(N_{\mathrm{hSp}}=1)}. \tag{6}$$

$R[\psi_A](N_{\mathrm{hSp}})$ represents how much the competitive advantage is enhanced in comparison with the extreme case with $N_{\mathrm{hSp}} = 1$, where only one sperm with allele A competes against only one with allele B. It should be noted that $R[\psi_A](N_{\mathrm{hSp}})$ does not depend on the magnitude of the PDF difference $\delta f(x)$ (as long as $|\delta f(x)| \ll 1$), because the scale factor of $\delta f(x)$ in the denominator cancels out that in the numerator. Figure 3 shows the dependence of $R[\psi_A](N_{\mathrm{hSp}})$ on N_{hSp} for the cases considered here.

In all cases, the sperm competitive advantage, ψ_A, increases as the half-number of sperm, N_{hSp}, increases (Figure 3) (for derivation, refer to Notes 2 and 3 of File S1). For cases i and ii, ψ_A increases roughly proportionally to the logarithm or its power of the total sperm number (cyan and green lines in Figure 3). In case iv, ψ_A is roughly proportional to $(N_{\mathrm{hSp}})^{1/(\alpha+1)}$ (red, blue, and purple lines in Figure 3), where α is the exponent used in Equation 5. The slope is determined by α. The quantitative difference among cases i, ii, and, iv depends on how the allelic difference in the PDF distributes along the velocity axis, especially near the right tail. Thus, regardless of the PDFs of velocity, Figure 3 shows that the efficacy of selection through a sperm competition increases dramatically with the number of mutually competing sperm.

Case iii is unique among the four cases because the PDFs for the two alleles have the same mean, while there is a difference in the shape of the right tail, which is the part that really matters. The result for this case is not shown in Figure 3 because the competitive advantage is zero in a one-on-one competition. Nevertheless, the competitive advantage is positive when $N_{\mathrm{hSp}} > 1$, and it increases roughly proportionally to $\ln(N_{\mathrm{hSp}})$ when $N_{\mathrm{hSp}} \gg 1$; its asymptotic behavior is quite similar to that of case i. In a sense, this case iii eloquently demonstrates the uniqueness of sperm competition: A mutant allele could gain a big advantage in a competition among numerous sperm *even if it has on average no advantage in a one-on-one competition between two sperm with different alleles*.

Fixation probability of a mutation

In the previous section, we showed that the advantage of allele A over allele B in sperm competition can be described by a single parameter, ψ_A, which can be readily incorporated into the basic population genetic framework. We are here interested in how the frequencies of alleles A and B change, from which we can derive the fixation probability of a mutant allele. We assume that A and B are selectively neutral except in sperm competition. In other words, it is assumed that the phenotypic effect at the sperm level determines the fate of the mutation.

Let us consider the expected frequency of allele A at generation $t + 1$ conditional on the frequency at generation t.

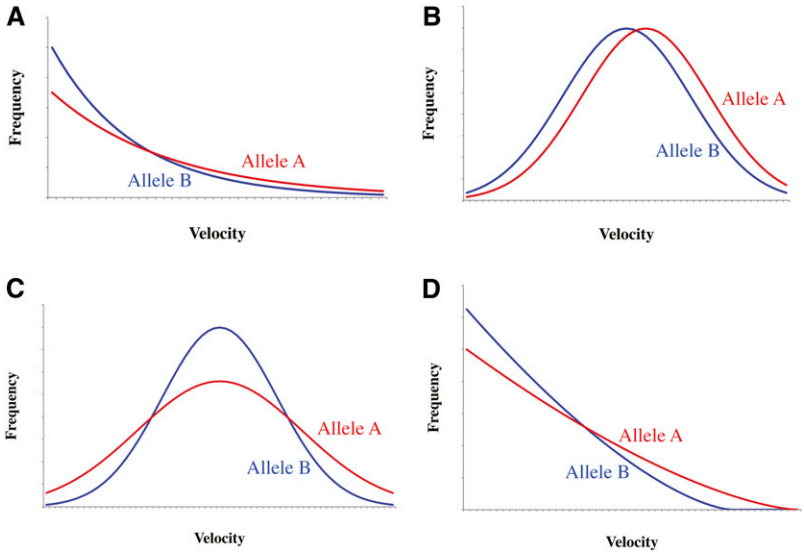

Figure 2 Illustration of typical patterns of mutational changes in the probability density function of the velocity. A, B, C, and D correspond to cases i, ii, iii, and iv, respectively, which are detailed in the text.

We ignore recurrent mutations between them. Here we focus on only "successful competition", where eggs are successfully fertilized by sperm, because unsuccessful competition does not contribute to the population genetic process. This should be reasonable under the assumption that the overall probability of successful fertilizations is independent of paternal and maternal genotypes. Let $P^{(P)}[Z \mid Z_1Z_2]$ denote the probability that a sperm with allele Z (= A or B) wins a successful competition among sperm ejaculated by a male individual with genotype Z_1Z_2 (Z_1, Z_2 = A or B). Obviously, for homozygous males, we have $P^{(P)}[A \mid AA] = P^{(P)}[B \mid BB] = 1$, and $P^{(P)}[A \mid BB] = P^{(P)}[B \mid AA] = 0$. For heterozygous males, we can use Equation 3. We also assume that there are as many sperm with allele A as those with allele B; *i.e.*, $N_A = N_B$. Note that this simplified assumption should not be used for mutations that directly change the numbers of active sperm with different alleles, such as mutations on Poisson-antidote type genes and apoptosis-related genes (*e.g.*, Da Fonseca *et al.* 2010).

Then, assuming a very small mutational effect [*i.e.*, $\delta f(x)$ is small], we have

$$P^{(P)}[A \mid AB] = \frac{1}{2}\left(1 + \frac{\psi_A}{2}\right), \tag{7}$$

$$P^{(P)}[B \mid AB] = \frac{1}{2}\left(1 - \frac{\psi_A}{2}\right). \tag{8}$$

Then, through some calculations detailed in Note 4 of File S1, we get a recursion relation,

$$p_{t+1}(A) = p_t(A) + \frac{\psi_A}{4} p_t(A)(1 - p_t(A)), \tag{9}$$

and

$$p_{t+1}(B) = 1 - p_{t+1}(A). \tag{10}$$

Here,

$$p_t(Z) \equiv \frac{1}{2}\left\{p_t^{(\mathrm{P})}(Z) + p_t^{(\mathrm{M})}(Z)\right\}$$

is the gender-averaged frequency of allele Z (= A or B) at the tth generation. The variables $p_t^{(\mathrm{P})}(Z)$ and $p_t^{(\mathrm{M})}(Z)$ are the frequencies of allele Z transmitted paternally and maternally, respectively. As long as we use this equation, our theory does not have to assume equal population sizes of males and females.

We can incorporate the above deterministic recursion equation directly into the standard population genetic theory and obtain the fixation probability by taking genetic drift into account. To do this, it is sufficient to note that our recursion equation, Equation 9, is equivalent to the standard deterministic recursion equation of the allele frequency,

$$p_{t+1}(A) = p_t(A) + s\, p_t(A)(1 - p_t(A)),$$

when allele A has a selective advantage of s ($\ll 1$) over allele B. Therefore, the diffusion theory framework as unfolded in section 8.8.3 of Crow and Kimura (1970) applies also to the current case, if s is replaced by $\psi_A/4$. Thus, we have the fixation probability $u(p)$ of allele A when its initial frequency is p,

$$u(p) = \frac{1 - \exp(-N_e\psi_A p)}{1 - \exp(-N_e\psi_A)}, \tag{11}$$

where N_e is the effective population size. The fixation probability of a single mutation with initial frequency $p = 1/(2N)$ is given by

$$u\frac{1}{2N} = \frac{\psi_A N_e/(2N)}{1 - \exp(-N_e\psi_A)}, \tag{12}$$

where N is the actual population size. Or, more simply, if $N_e = N$, Equation 11 is approximated as

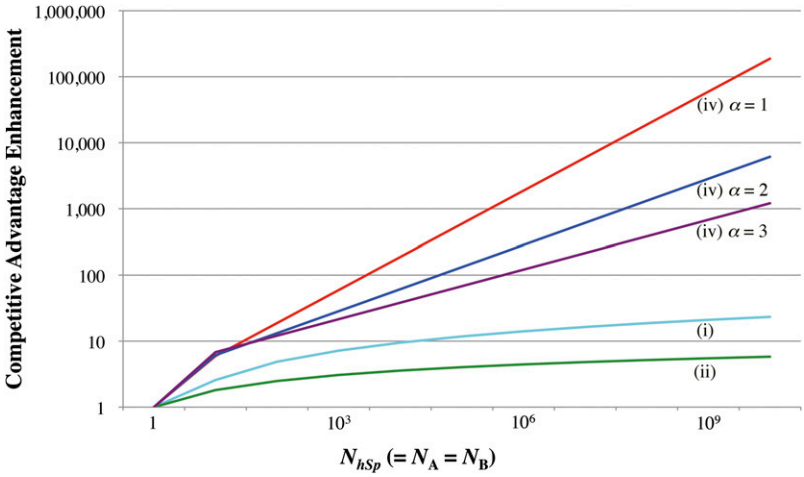

Figure 3 Enhancement, $R[\psi_A](N_{hSp})$, of sperm competitive advantage (ψ_A) for a range of the half-sperm number (N_{hSp}) compared to that in one-on-one competition. The cyan and green lines represent case i and case ii, respectively. All other lines represent case iv. The cases with α = 1, 2, and 3 are colored red, blue, and purple, respectively.

$$u\frac{1}{2N} = \frac{\psi_A/2}{1-\exp(-N\psi_A)}. \tag{13}$$

This in turn reduces to $u(p) \approx \psi_A/2$ when $N\psi_A \gg 1$.

It should be noted that, in this equation, we set the initial frequency of a mutant following the standard treatment in population genetic theory; that is, a single haploid mutant with frequency $p = 1/(2N)$ arises in the population at rate $2N\mu$, where μ is the mutation rate per generation per haploid locus. It should not be unreasonable to assume that this also works in sperm genes. There are many cell divisions in a single male individual, and a mutation that occurred in an early stage would result in a large number of sperm having the mutation. Mutations at later stages would occur more frequently but generate fewer mutant sperm each. The probability that the winner is one of such mutant sperm may be affected by selection on the mutation, but this effect should be very small in a single generation. If so, we can approximate the mutation process in a single (male) individual as a random process along a "genealogy" of competing selectively neutral sperm that originates from a single zygote. Then, μ (*i.e.*, the per-generation mutation rate) should be defined as the expected number of mutations from the top (*i.e.*, root) to any tip (*i.e.*, leaf) of the genealogy. This situation is analogous to the typical treatment in the standard theory of population genetics.

It would be intriguing to compare Equation 13 and the fixation probability of a normal adaptive mutation with selective coefficient s; *i.e.*, $u(1/2N) = 2s/(1-\exp(-4Ns))$. The two equations are identical when $s = \psi_A/4$ or the effect of the sperm competitive advantage is one-quarter as large as that of the standard additive selective advantage. There are two factors, each contributing one-half independently, that are multiplied together to give this ratio of 1/4. The first factor comes from the assumption that the transmission of alleles through females is selectively neutral. The other factor comes from the fact that competition among paternity-sharing sperm causes a selective bias only through *heterozygous* males.

Impact of paternity-sharing sperm competition on d_N/d_S ratios

Now that we have established the population genetics theory of sperm sharing the paternity, we can estimate their impact on the d_N/d_S ratios of sperm genes. First, assuming that synonymous mutations do not change the fertilization efficiency of sperm at all, the fixation probability of a synonymous mutation in a diploid population of size N is given by

$$\bar{P}[\text{fixed}|\text{synonymous}] = \frac{1}{2N},$$

according to the neutral theory (Kimura 1968, 1983).

Next we consider nonsynonymous sites. According to their impacts on the sperm fertilization efficiency, mutations on such sites are classified into three categories, namely, those that are competitively (a) disadvantageous, (b) neutral, and (c) advantageous. Then, roughly speaking, three parameters govern the average fixation probability of nonsynonymous mutations: (i) the proportion, p_N, of quite strictly neutral mutations among all nonsynonymous mutations; (ii) the proportion, p_{CA}, of competitively advantageous (CA) mutations; and (iii) the sperm competitive advantage (ψ_A) that follows a PDF, $F_{CA}(\psi_A)$, *over competitively advantageous mutations*. Note that because of the inflation in the competitive (dis)advantage deduced above, even slightly disadvantageous mutations will be immediately removed from the population. Therefore, the proportion of competitively disadvantageous mutations, p_{CD} (= $1 - p_N - p_{CA}$), contributes only negligibly little, if any, to the d_N/d_S ratio.

Given these three key parameters, applying Equation 13 will provide the average fixation probability:

$$\bar{P}[\text{fixed} \mid \text{nonsynonymous}] = p_{CA}\int_0^{+\infty} d\psi_A\, F_{CA}(\psi_A)\frac{\psi_A/2}{1-\exp(-N\psi_A)} + \frac{p_N}{2N}. \tag{14}$$

This is a theoretically rigorous expression, but the functional form of $F_{CA}(\psi_A)$ is usually unknown. In such a case, it is

more convenient and intuitive to define $\overline{\psi_A}$ as the "mean" of ψ_A, via the following equation:

$$\frac{\overline{\psi_A}/2}{1-\exp(-N\overline{\psi_A})} \equiv \int_0^{+\infty} d\psi_A F_{\mathrm{CA}}(\psi_A) \frac{\psi_A/2}{1-\exp(-N\psi_A)}. \tag{15}$$

Now, taking the ratio of the two fixation probabilities and assuming that the mutation rate is identical at synonymous and nonsynonymous sites, we get a formula for the average d_N/d_S ratio:

$$\begin{aligned}\overline{(d_N/d_S)} &= \frac{\bar{P}[\text{fixed} \mid \text{nonsynonymous}]}{\bar{P}[\text{fixed} \mid \text{synonymous}]} \\ &= p_{\mathrm{CA}}\overline{(d_N/d_S)}\big|_{\mathrm{CA}} + p_N.\end{aligned} \tag{16}$$

Here $\overline{(d_N/d_S)}|_{\mathrm{CA}}$ is the average contribution to the d_N/d_S ratio from competitively advantageous mutations:

$$\begin{aligned}\overline{(d_N/d_S)}\big|_{\mathrm{CA}} &\equiv \frac{\bar{P}[\text{fixed} \mid \text{nonsynonymous, CA}]}{\bar{P}[\text{fixed} \mid \text{synonymous}]} \\ &= \frac{N\overline{\psi_A}}{1-\exp(-N\,\overline{\psi_A})}.\end{aligned} \tag{17}$$

If the enhancement effect $R[\psi_A](N)$ is very large, say it exceeds 100, we may be able to assume that there are virtually no quite strictly neutral mutations, *i.e.*, $p_N \ll 1$, and that almost all nonsynonymous mutations could be more or less selected. In such a case, Equation 16 could be simplified as

$$\overline{(d_N/d_S)} \approx p_{\mathrm{CA}}\overline{(d_N/d_S)}\big|_{\mathrm{CA}}. \tag{18}$$

Thus, the average d_N/d_S value is now approximately determined by two parameters, *i.e.*, the proportion of competitively advantageous mutations (p_{CA}) and the mean competitive advantage of such mutations ($\overline{\psi_A}$).

The two colored curves in Figure 4 schematically illustrate the expected d_N/d_S ratio averaged over CA mutations ($\overline{(d_N/d_S)}|_{\mathrm{CA}}$) as functions of the winning probability, computed by Equation 17, with $N = 10^4$ (green line) and $N = 10^5$ (cyan line). In all cases, $\overline{(d_N/d_S)}|_{\mathrm{CA}}$ monotonically increases as the winning probability increases and becomes asymptotically proportional to the competitive advantage: $\overline{(d_N/d_S)}|_{\mathrm{CA}} \approx N\overline{\psi_A}$. Hence the gradient of the curve depends almost linearly on the population size N.

Application to data

Equation 16, or its approximation (18), could be used to estimate the intensity of selection on sperm genes or on any subregions within them. As an example, we use mammalian sperm protein genes, for many of which rapid amino acid substitutions have been demonstrated (Wyckoff *et al.* 2000; Torgerson *et al.* 2002; Swanson *et al.* 2003). We use the

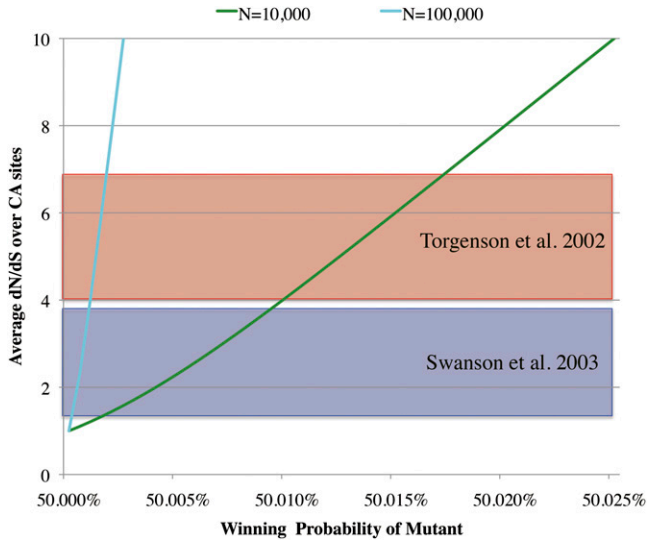

Figure 4 Average d_N/d_S as a function of the winning probability of a mutant allele. The colored curves show the dependence of $\overline{(d_N/d_S)}|_{\mathrm{CA}}$ on the winning probability ($\frac{1}{2}(1+\overline{\psi_A}/2)$) of the mutant allele A, computed from Equation 17. The diploid population size (N) is set to be 10^{+4} (green line) and 10^{+5} (cyan line). Red and blue shaded areas represent the ranges of such d_N/d_S values obtained by Torgerson *et al.* (2002) and by Swanson *et al.* (2003), respectively.

data in Torgerson *et al.* (2002) and in Swanson *et al.* (2003). In their maximum-likelihood analyses, the proportion of codon sites with elevated amino acid substitution rates and the d_N/d_S value averaged over such sites were inferred for each gene. Both studies compared two models of codon evolution under the framework of Yang *et al.* (2000). One is a purifying selection model known as "M7", in which the expected d_N/d_S is assumed to be beta-distributed between 0 and 1, and the other is a composite selection model known as "M8" that accommodates an additional class of sites with $d_N/d_S > 1$ on top of the classes of negatively selected sites as incorporated in M7. It was found that M8 fitted significantly better than M7 for some sperm genes (four genes in Torgerson *et al.* 2002 and six genes in Swanson *et al.* 2003), strongly indicating that these genes are likely involved in determining sperm performance. In the class of positively selected sites, estimates of $\overline{(d_N/d_S)}$ ranged from 4.0 to 6.9 for the former four genes and from 1.3 to 3.9 for the latter six genes (red and blue shaded areas, respectively, in Figure 4).

To estimate the mean winning probability, $\bar{P}^{(\mathrm{P})}[A\,|AB] = \frac{1}{2}(1+\overline{\psi_A}/2)$, corresponding to the above intervals, we use Equation 18. The proportion of advantageous mutations (p_{CA}) is usually unknown although it may not be very large as most mutations should be disadvantageous. Therefore, we conventionally assume $p_{\mathrm{CA}} = 1$, which should provide a possible lower bound of estimates of $\overline{\psi_A}$. Because this assumption of $p_{\mathrm{CA}} = 1$ means that almost all nonsynonymous mutations on the sites of interest are competitively advantageous, it obviously causes an underestimation of $\overline{\psi_A}$. A caveat in understanding this result is that the theory might

overestimate the role of positive selection in the haploid phase because it assumes neutrality of mutations in all other phases.

Assuming the population size to be $N = 10^4$ (a typical effective population size for humans, from Takahata 1993), we estimated that the winning probability ranged from 50.010% to 50.017% and from 50.0014% to 50.010%, corresponding to that in Torgerson *et al.* (2002) and Swanson *et al.* (2003), respectively. As demonstrated in Figure 3, competition among millions to billions of sperm is expected to enhance the effect of a mutation greatly, although the degree of enhancement differs, depending on the PDF of velocity and the type of its change. If we assume case iv with $\alpha = 3$ as an example, and given $N_{\mathrm{hSp}} \sim 10^8$ (an approximate number for humans, from, *e.g.*, Manning and Chamberlain 1994), we predict that the selective advantage *at the single-sperm level* should be, on average, from 4.7×10^{-8} to 5.7×10^{-7}. If these values are directly applied, they are equivalent to the population selection intensity, $2Ns$, of from 9.4×10^{-4} to 0.011. If we assume a bigger population size, *e.g.*, $N \sim 10^6$ for mice (Keightley *et al.* 2005), the obtained range of equivalent $2Ns$ is unchanged. This is because the population size, N, affects the average $d_{\mathrm{N}}/d_{\mathrm{S}}$ ratio only through the product $N\psi_A/4$ or Ns (see, *e.g.*, Equation 17). In contrast, if we are given a smaller number of sperm, *e.g.*, $N_{\mathrm{hSp}} \sim 10^6$ for mice (Manning and Chamberlain 1994), the single-sperm level selective advantage would be equivalent to $2Ns \sim 2.8 \times 10^{-3} - 0.034$, which is about three times larger than the range inferred for humans. This results from the positive correlation between N_{hSp} and ψ_A (Figure 3).

Thus, this result indicates that typical selective advantages of mutations at the single-sperm level should be very small; if selection acted on these mutations in the standard fashion (as in Figure 1A), the effect of selection would be negligible and they would behave almost as if they were neutral or nearly neutral. In other words, fierce competition among paternity-sharing sperm enables selection to act quite efficiently on mutations with such tiny phenotypic effects, which could cause $d_{\mathrm{N}}/d_{\mathrm{S}}$ values to significantly exceed 1 in some regions.

Discussion

In this article, we theoretically examined the population genetic behavior of mutations in sperm genes. We modeled the processes at two levels. One is the standard population genetic process, in which the population allele frequencies change generation by generation, depending on the difference in selective advantages. The other is the sperm competition during each genetic transmission from one generation to the next generation.

For the sperm competition process, we considered a very simple situation with monogamous mating, so that selection needs to be considered only in matings involving heterozygous males. In a single mating process involving a heterozygous male, a huge number of sperm with alleles A and B (approximately equal in number) compete to fertilize a single egg. Our theory demonstrates that a very slight difference in sperm performance (*i.e.*, velocity as we defined) amounts to quite a large difference in the winning probability. We found that this probability is given by a function of an important parameter, ψ_A, namely the competitive advantage of allele A over allele B in a single mating. We also demonstrated that ψ_A is much larger than it would be in a one-on-one competition between a pair of sperm, one with allele A and the other with allele B. This suggests that a very small phenotypic difference at the single-sperm level can be enhanced by fierce sperm competition.

For the generation-by-generation process, the standard population genetic theory can be directly applied with slight modifications. The only difference is that selection works only through heterozygous males. In a simple one-locus, two-allele model with alleles A and B, our theory shows that the fixation probability of a newly arisen mutant with A is given by Equation 13 if allele A has a competitive advantage of ψ_A over B. This equation indicates that $4 \times \psi_A$ in our model is equivalent to the selective advantage s in the standard model of additive selection. Of this reduction to one-quarter in the efficacy of selection, one-half is due to the neutrality (actually, absence) of the process for females, and the other half is due to selection operating only through heterozygous males.

While the efficacy of selection is reduced to one-quarter for sperm genes in the generation-by-generation process, the enhancement of selection in the sperm competition during each generation is much larger, potentially increasing adaptive amino acid substitutions in sperm genes. Indeed, as we demonstrated theoretically, the elevated $d_{\mathrm{N}}/d_{\mathrm{S}}$ values observed in sperm genes in mammals could be explained by mutations whose effects on individual sperm are so weak that they would be classified as "neutral" in a normal population genetic framework.

Our minimal model focuses on competition among sperm that share paternity. These competitions are ubiquitous and occur regardless of whether the mating system is monogamous, polygamous, or external. Thus, our result could provide a potentially important explanation of rapid evolution of sperm genes with a variety of functions in a wide range of species, as long as they are expressed in the haploid phase. The extent to which our model can be applied depends on how common haploid expression is among sperm genes. There are multiple lines of evidence that haploid expression in sperm is indeed quite common as mentioned in the Introduction (see, *e.g.*, Joseph and Kirkpatrick 2004; Good and Nachman 2005; Dorus *et al.* 2010). A notable work is the recent proteome-scale evolutionary analysis of mice by Good and Nachman (2005), which showed that sperm genes expressed after meiosis tend to have higher $d_{\mathrm{N}}/d_{\mathrm{S}}$ values than other sperm genes. Their study implies that, in such genes with haploid expression, the contribution of sperm competition to the rapid amino acid evolution should be large.

Our model may be well merged with previous models of postcopulatory sexual selection (*e.g.*, Birkhead and Pizzari 2002; Swanson and Vacquier 2002b; Clark *et al.* 2006; Turner and Hoekstra 2008) or sexual conflict (*e.g.*, Rice and Holland 1997; Frank 2000; Gavrilets 2000; Chapman *et al.* 2003; Hayashi *et al.* 2007), all of which focus mainly on competition or conflicts *among individuals*. Our results suggest that competition among paternity-sharing sperm can boost any mode of postcopulatory sexual selection and/or sexual conflict. For example, coupling our model with sexual conflict provides a more powerful explanation of the long-term acceleration of d_N/d_S in sperm genes. Our model alone does not guarantee that the acceleration of amino acid substitution lasts long. This is because each sperm gene eventually reaches the optimum of its fitness landscape, from which no adaptive mutations are expected. Thus a high d_N/d_S cannot be expected as long as the gene's fitness landscape keeps its shape (or optimum). This indicates that a long-term acceleration may require factors that shift the optimum.

Sexual conflict would be one example, which continuously shifts the optimum by a "coevolutionary chase" between the male and female genes (*e.g.*, in Rice and Holland 1997; Frank 2000; Gavrilets 2000; Chapman *et al.* 2003; Hayashi *et al.* 2007). An interesting question in this regard is why high d_N/d_S values are observed commonly among sperm genes but quite rarely among female-reproductive genes in many species (*e.g.*, Swanson and Vacquier 2002b; Clark *et al.* 2006). One important conclusion of our theory is that fierce sperm competition could enable even a mutation with a tiny phenotypic benefit to be fixed as if it were strongly selected for. This suggests that, even to a small shift in the female environment (*e.g.*, caused by only a single amino acid change), a sperm gene could respond via a large number of amino acid changes, each of which alters the sperm phenotype only slightly.

Another potentially important factor would be intermale competition through different phenotypes of sperm or other reproductive apparatuses (such as copulatory plugs). This could also change the environment in which sperm compete against one another and thus could shift the optimum of the fitness landscape of each sperm gene.

In this work, we focused only on the effects of mutations on sperm performance and ignored their effects on other phenotypes. If, however, a sperm gene is pleiotropic, it is obvious that the fitness at the sperm level is not the entire factor that determines the fate of the mutation (see, *e.g.*, Crow 2012). Nevertheless, such pleiotropic effects might not significantly affect the main conclusions of this study, for two reasons. First, a majority of sperm genes observed to have high d_N/d_S values seem to be specific to sperm (*e.g.*, Good and Nachman 2005; Turner and Hoekstra 2008). Second, even if a sperm gene is indeed pleiotropic, it is important to note, again, that fierce sperm competition could enhance the effect of a mutation on sperm functions but not its effect on other tissues. Therefore, there should be many occasions where sperm competition contributes to the accelerated d_N/d_S in sperm genes with haploid expression. This, combined with the observation that haploid expressed genes account for a substantial fraction of the sperm proteome (*e.g.*, Joseph and Kirkpatrick 2004; Good and Nachman 2005), could be one of the major explanations of the general trend that a wide variety of sperm genes show high d_N/d_S ratios in various taxonomic lineages.

Our theory may explain some previous observations that seemed enigmatic. One is on the role of postcopulatory sexual selection *among* (*male*) *individuals* as a potential major cause of the elevated d_N/d_S values. If this plays the major role, one would expect a positive correlation between d_N/d_S and the intensity of sexual selection. However, there was no significant correlation in a molecular evolutionary analysis of male sperm genes in rodents (Ramm *et al.* 2008). Why can d_N/d_S be elevated in a species with weak sexual selection as much as in species with intense sexual selection? One possible and simple answer could be that, at least in rodents, competition among paternity-sharing sperm is much more potent than intermale sperm competition. The former competition takes place irrespective of whether sexual selection occurs at the individual level or not, and our theory predicts that the former greatly enhances the selective advantage of a mutation. Thus, if the former's "baseline" effects are much larger than the effects influenced by individual-level sexual selection, the correlation between the protein evolution rate and the intensity of sexual selection would be relatively too small to be observed.

It should be noted here that our theory applies exclusively to sperm genes, especially haploid expressed ones, but that it does not apply to male reproductive genes lacking expression in sperm. Some genes of the latter type are known to have high d_N/d_S and show positive correlation between d_N/d_S and the intensity of (intermale) sexual selection. Among those showing the correlation is a seminal vesicle-derived protein, Svs2, which is a major component of the copulatory plug in rodents (Ramm *et al.* 2008). Similarly, semenogelins, SEMG1 and SEMG2, that are known to prevent other males' sperm from reaching the oocyte, also showed correlations in the primate lineages (*e.g.*, Ramm *et al.* 2008, and references therein). The rapidly evolving *Drosophila* male accessory gland proteins (*e.g.*, Swanson *et al.* 2001) also belong to this type. From their functions, it is obvious that these genes play important roles in intermale competition and that their rapid evolution is not caused by sperm competition. It is indicated that intermale sexual selection is an important mechanism to increase d_N/d_S especially of such male reproductive genes not expressed in sperm.

Flies are unique in that their sperm genes do not show significantly higher d_N/d_S values than the genome average (Dorus *et al.* 2006). Given that any kind of selection works very efficiently through fierce competition among sperm sharing the paternity, this unique observation should

indicate that such sperm competition may not be very intensive in flies. Indeed, there are several lines of evidence that flies have quite small numbers of competing sperm per egg and little haploid expression (see, *e.g.*, Erickson 1990; Manning and Chamberlain 1994). This agrees with our prediction that mutational effects on sperm genes should be enhanced in a manner positively correlated with the number of sperm per egg (*e.g.*, Figure 3).

Acknowledgements

We thank two anonymous reviewers for their helpful comments. This work is in part supported by grants from the Japan Society for the Promotion of Science (to H.I.).

Literature Cited

Artieri, C., W. Haerty, B. Gupta, and R. Singh, 2008 Sexual selection and maintenance of sex: evidence from comparisons of rates of genomic accumulation of mutations and divergence of sex-related genes in sexual and hermaphroditic species of *Caenorhabditis*. Mol. Biol. Evol. 25: 972–979.

Birkhead, T., and T. Pizzari, 2002 Postcopulatory sexual selection. Nat. Rev. Genet. 3: 262–273.

Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe, 2003 Sexual conflict. Trends Ecol. Evol. 18: 41–47.

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

Clark, N. L., J. Gasper, M. Sekino, and S. A. Springer, C. F. Aquadro *et al*., 2009 Coevolution of interacting fertilization proteins. PLoS Genet. 5: e1000570.

Crow, J., 2012 Upsetting the dogma: germline selection in human males. PLoS Genet. 8: e1002535.

Crow, J. F., and M. Kimura, 1970 *An Introduction to Population Genetics Theory*. The Blackburn Press, Caldwell, NJ.

Cutter, A., and S. Ward, 2005 Sexual and temporal dynamics of molecular evolution in *C. elegans* development. Mol. Biol. Evol. 22: 178–188.

Da Fonseca, R., C. Kosiol, T. Vinař, A. Siepel, and R. Nielsen, 2010 Positive selection on apoptosis related genes. FEBS Lett. 584: 469–476.

de Rooji, D., and L. Russell, 2000 All you wanted to know about spermatogonia but were afraid to ask. J. Androl. 21: 776–798.

Dobzhansky, T., 1940 Speciation as a stage in evolutionary divergence. Am. Nat. 74: 312–321.

Dorus, S., S. Busby, U. Gerike, J. Shabanowitz, D. Hunt *et al.*, 2006 Genomic and functional evolution of the *Drosophila malanogaster* sperm proteome. Nat. Genet. 38: 1440–1445.

Dorus, S., E. Wasbrough, J. Busby, E. Wilkin, and T. Karr, 2010 Sperm proteomics reveals intensified selection on mouse sperm membranes and acrosome genes. Mol. Biol. Evol. 27: 1235–1246.

Erickson, R., 1990 Post-meiotic gene expression. Trends Genet. 6: 264–269.

Findlay, G., and W. Swanson, 2010 Proteomics enhances evolutionary and functional analysis of reproductive proteins. Bioessays 32: 26–36.

Frank, S., 2000 Sperm competition and female avoidance of polyspermy mediated by sperm-egg biochemistry. Evol. Ecol. Res. 2: 613–625.

Gavrilets, S., 2000 Rapid evolution of reproductive barriers driven by sexual conflict. Nature 403: 886–889.

Good, J., and M. Nachman, 2005 Rates of protein evolution are positively correlated with developmental timing of expression during mouse spermatogenesis. Mol. Biol. Evol. 22: 1044–1052.

Hayashi, T., M. Vose, and S. Gavrilets, 2007 Genetic differentiation by sexual conflict. Evolution 61: 516–529.

Howard, D., 1993 Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis, pp. 46–69 in *Hybrid Zones and the Evolutionary Process*, edited by R. Harrison. Oxford University Press, New York.

Joseph, S., and M. Kirkpatrick, 2004 Haploid selection in animals. Trends Ecol. Evol. 19: 592–597.

Keightley, P., M. Lercher, and A. Eyre-Walker, 2005 Evidence for widespread degradation of gene control regions in hominid genomes. PLoS Biol. 3: e42.

Kimura, M., 1968 Evolutionary rate at the molecular level. Nature 217: 624–626.

Kimura, M., 1983 *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge, UK.

Li, W.-H., 1997 *Molecular Evolution*. Sinauer Associates, Sunderland, MA.

Manning, J., and A. Chamberlain, 1994 Sib competition and sperm competitiveness: an answer to 'why so many sperms?' and the recombination/sperm number correlation. Proc. Biol. Sci. 256: 177–182.

Miyata, T., and T. Yasunaga, 1980 Molecular evolution of mRNA: a method for estimating evolutionary rates of synonymous and amino acid substitutions from homologous nucleotide sequences and its application. J. Mol. Evol. 16: 23–36.

Nielsen, R., C. Bustamante, A. Clark, S. Glanowski, T. Sackton *et al.*, 2005 A scan for positively selected genes in the genomes of humans and chimpanzees. PLoS Biol. 3: e170.

Parker, G., and M. Begon, 1993 Sperm competition games: sperm size and number under gametic control. Proc. Biol. Sci. 253: 255–262.

Ramm, S., P. Oliver, C. Ponting, P. Stockley, and R. Emes, 2008 Sexual selection and the adaptive evolution of mammalian ejaculate proteins. Mol. Biol. Evol. 25: 207–219.

Rice, W., and B. Holland, 1997 The enemies within: intergenomic conflict, interlocus contest evolution, and the intraspecific red queen. Behav. Ecol. Sociobiol. 41: 1–10.

Russell, L., R. Ettlin, A. Hikim, and E. Clegg, 1990 Mammalian spermatogenesis, pp. 1–40 in *Histological and Histopathological Evaluation of the Testis*, edited by L. Russell, R. Ettlin, A. Hikim, and E. Clegg. Cache River Press, Clearwater, FL.

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

Swanson, W., and V. Vacquier, 2002a Reproductive protein evolution. Annu. Rev. Ecol. Syst. 33: 161–179.

Swanson, W., and V. Vacquier, 2002b The rapid evolution of reproductive proteins. Nat. Rev. Genet. 3: 137–144.

Swanson, W., A. Clark, M. Waldrip-Dail, M. Wolfner, and C. Aquadro, 2001 Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. Proc. Natl. Acad. Sci. USA 98: 7375–7379.

Swanson, W., R. Nielsen, and Q. Yang, 2003 Pervasive adaptive evolution in mammalian fertilization proteins. Mol. Biol. Evol. 20: 18–20.

Takahata, N., 1993 Allelic genealogy and human evolution. Mol. Biol. Evol. 10: 2–22.

Torgerson, D., R. Kulathinal, and R. Singh, 2002 Mammalian sperm proteins are rapidly evolving: evidence of positive selection in functionally diverse genes. Mol. Biol. Evol. 19: 1973–1980.

Turner, L., and H. Hoekstra, 2008 Causes and consequences of the evolution of reproductive proteins. Int. J. Dev. Biol. 52: 769–780.

Vacquier, V., W. Swanson, and Y. Lee, 1997 Positive Darwinian selection on two homologous fertilization proteins: What is the selective pressure driving their divergence? J. Mol. Evol. 44: S15–S22.

Wong, A., 2011 The molecular evolution of animal reproductive tract proteins: What have we learned from mating-system comparisons? Int. J. Evol. Biol. 2011: 908735.

Wyckoff, G., W. Wang, and C. Wu, 2000 Rapid evolution of male reproductive genes in the descent of man. Nature 403: 304–309.

Yang, Z., R. Nielsen, N. Goldman, and A. Pedersen, 2000 Codon-substitution models for heterogeneous selection pressure at amino acid sites. Genetics 155: 431–449.

Yoshida, S., 2010 Stem cells in mammalian spermatogenesis. Dev. Growth Differ. 52: 311–317.

Communicating editor: L. M. Wahl

GENETICS

Supporting Information
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.152066/-/DC1

Competition Between the Sperm of a Single Male Can Increase the Evolutionary Rate of Haploid Expressed Genes

Kiyoshi Ezawa and Hideki Innan

DOI: 10.1534/genetics.113.152066

File S1. Supplementary Notes

Note 1. Competitive Advantage of a Mutant in Sperm Competition

In the main text, we derived a master equation for the probability that a mutant sperm with allele A at a focal locus wins in a competition among N_A mutant sperm and N_B wild-type sperm (with allele B):

$$\begin{aligned} & P\ [\text{winner} = A \mid N_A\ A's\ \&\ N_B\ B's\ ] \\ = & \int_{-\infty}^{+\infty} dx \frac{d}{dx} \left\{ P\ [\max\{X_1^A, ..., X_{N_A}^A\} < x] \right\}\ P\ [\max\{X_1^B, ..., X_{N_B}^B\} < x] \\ = & \int_{-\infty}^{+\infty} dx\ N_A f_A(x) \left(1 - P\ [X^A > x]\right)^{N_A - 1} \left(1 - P\ [X^B > x]\right)^{N_B} \\ = & \ 1\ -\ \int_{-\infty}^{+\infty} dx\ \left(1 - P\ [X^A > x]\right)^{N_A} N_B f_B(x) \left(1 - P\ [X^B > x]\right)^{N_B - 1}\ , \end{aligned} \tag{S1}$$

which is identical to Eq. 2 in the main text. Here $f_A(x)$ and $f_B(x)$ are the probability density functions of a sperm competitiveness measure x for allele A and allele B, respectively. When $N_A, N_B \gg 1$, it can be approximated as:

$$\begin{aligned} & P\ [\text{winner} = A \mid N_A\ A's\ \&\ N_B\ B's\ ] \\ \approx & \int_{-\infty}^{+\infty} dx\ N_A f_A(x) \exp\left\{ - \left(N_A P\ [X^A > x]\ +\ N_B P\ [X^B > x] \right) \right\} \\ \approx & \ 1\ -\ \int_{-\infty}^{+\infty} dx\ N_B f_B(x) \exp\left\{ - \left(N_A P\ [X^A > x]\ +\ N_B P\ [X^B > x] \right) \right\}\ . \end{aligned} \tag{S2}$$

The exact master equation Eq. S1 provides the winning probability:

$$P\ [\text{winner} = A \mid N_A\ A's\ \&\ N_B\ B's\ ] = \frac{N_A}{N_A + N_B}\ ,$$

when the mutation (from B to A) is exactly neutral, i.e. $f_A(x) \equiv f_B(x)$.

(1-0) Perturbation formula in general case

In population genetics, it is very common to deal with a situation where the effect of the mutation is fairly small at a generation but could become large when accumulated through

generations. To deal with such situations, let us assume that the probability density functions (PDFs) for the two alleles are almost identical:

$$f_A(x) \equiv f(x) + \delta f(x) \ , \quad f_B(x) \equiv f(x) \ , \quad \text{with} \quad \int_{-\infty}^{+\infty} dx |\delta f(x)| \ll 1.$$

And let X_f denote a random variable conforming to the PDF $f(x)$. Then the probability, Eq. S1, that the winner has allele A is rewritten and approximated up to $O(\delta f)$ as:

$$\begin{aligned}
& P\ [\text{winner} = A \mid N_A\ A's\ \&\ N_B\ B's\ ] \\
= \ & 1 - \int_{-\infty}^{+\infty} dx\ N_B f(x) \left[1 - \int_x^{+\infty} d\xi\ (f+\delta f)(\xi)\right]^{N_A} \left[1 - \int_x^{+\infty} d\xi\ f(\xi)\right]^{N_B - 1} \\
\approx \ & 1 - N_B \int_{-\infty}^{+\infty} dx\ f(x) \left[1 - \int_x^{+\infty} d\xi\ f(\xi)\right]^{N_A+N_B-1} \\
& + N_B \int_{-\infty}^{+\infty} dx\ \left\{ f(x) \left[1 - \int_x^{+\infty} d\xi\ f(\xi)\right]^{N_A+N_B-2} N_A \int_x^{+\infty} d\xi\ \delta f(\xi) \right\} \\
= \ & 1 - \frac{N_B}{N_A+N_B} + \frac{N_A\ N_B}{N_A+N_B-1} \int_{-\infty}^{+\infty} dx\ \left\{ \frac{d}{dx} \left[1 - \int_x^{+\infty} d\xi\ f(\xi)\right]^{N_A+N_B-1} \int_x^{+\infty} d\xi\ \delta f(\xi) \right\} \\
= \ & \frac{N_A}{N_A+N_B} + \frac{N_A\ N_B}{N_A+N_B-1} \int_{-\infty}^{+\infty} dx\ \left[1 - \int_x^{+\infty} d\xi f(\xi)\right]^{N_A+N_B-1} \delta f(x) \ . \qquad \text{(S3)}
\end{aligned}$$

Partial integration was used to achieve the last equation. Now, define a quantity:

$$\begin{aligned}
\psi_A(N_A,\ N_B) \ &\equiv \ \frac{(N_A+N_B)^2}{N_A+N_B-1} \int_{-\infty}^{+\infty} dx\ \left[1 - \int_x^{+\infty} d\xi f(\xi)\right]^{N_A+N_B-1} \delta f(x) \\
&= \ \frac{N_A+N_B}{N_A+N_B-1} \int_0^{N_A+N_B} dy\ \left(1 - \frac{y}{N_A+N_B}\right)^{N_A+N_B-1} \delta \ln f(x(y)) \qquad \text{(S4)}
\end{aligned}$$

The latter equation results from changing dummy integration variables from x to $y \equiv (N_A + N_B)P\ [X_f > x]$ and introducing the notation, $\delta \ln f(x) \equiv \frac{\delta f(x)}{f(x)}$. When $N_A + N_B \gg 1$, it is approximated as:

$$\begin{aligned}
\psi_A(N_A,\ N_B) \ &\approx \ (N_A+N_B) \int_{-\infty}^{+\infty} dx\ \exp\left(-(N_A+N_B)P\ [X_f > x]\right) \cdot \delta f(x) \\
&= \ \int_0^{+\infty} dy\ e^{-y}\ \delta \ln f(x(y)) \ . \qquad \text{(S5)}
\end{aligned}$$

The first approximate equation gives exactly Eq. 4 in the main text.

With Eq. S4 (or Eq. S5), Eq. S3 can be rewritten and rearranged as:

$$\begin{aligned} P\ [\text{winner} = A \mid N_A\ A's\ \&\ N_B\ B's\ ] &\approx \frac{N_A}{N_A+N_B}\left\{1+\frac{N_B}{N_A+N_B}\psi_A\right\} \\ &\approx \frac{N_A}{N_A+N_B(1-\psi_A)} \\ &\approx \frac{N_A(1+\psi_A)}{N_A(1+\psi_A)+N_B}\ , \end{aligned} \tag{S6}$$

which is referred to as Eq. 3 in the main text. Here we omitted the dependence of ψ_A on N_A and N_B for notational convenience. The approximate equations Eq. S6 demonstrate that allele A has an advantage as much as ψ_A, as defined in Eq. S4, over allele B in the sperm competition. Thus the problem boils down to estimating the competitive advantage $\psi_A(N_A,\ N_B)$.

Let us now calculate the competitive advantage in several specific cases.

(1-1)Increased mean in exponential distribution

First we consider a simplest example, where the measure x follows an exponential distribution and the mutation slightly increases the mean:

$$f(x) = \exp(-x)\ , \quad \text{and} \quad f(x)+\delta f(x) = (1-\delta\tau)\exp(-(1-\delta\tau)x)\ .$$

Here we rescaled x so that the mean is 1 for the wild-type.

In this case, $y = (N_A+N_B)\exp(-x)$, and

$$\delta \ln f(x) \approx \delta\tau(x-1) = \delta\tau\left\{\ln\left(\frac{N_A+N_B}{y}\right)-1\right\}\ .$$

Substituting this into Eq. S5, we get:

$$\begin{aligned} \psi_A &\approx \int_0^{+\infty} dy\ e^{-y}\ \delta\tau\left\{\ln\left(\frac{N_A+N_B}{y}\right)-1\right\} \\ &= \delta\tau\left\{\ln(N_A+N_B)-1+\gamma\right\}\ , \end{aligned} \tag{S7}$$

where $\gamma \equiv -\int_0^{+\infty} dy e^{-y} \ln y = 0.57721...$ is Euler's constant. Thus, ψ_A in this case roughly scales as $\ln(N_A + N_B)$.

(1-2) Shift of normal distribution

Next let us consider a case where the measure x is governed by a normal distribution and the mutation shifts the mean of the distribution:

$$f(x) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{x^2}{2}\right) \ , \quad \text{and} \quad f(x) + \delta f(x) = f(x - \delta m) \ .$$

Here, we rescaled and shifted x so that its mean and variance become 0 and 1, respectively, for the wile-type.

In this case, $\delta \ln f(x) \approx \delta m \ x$, and

$$y(x) = (N_A + N_B) \int_x^{+\infty} \frac{dx}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} \approx \frac{N_A + N_B}{\sqrt{2\pi}\ x} e^{-\frac{x^2}{2}} \ .$$

The right-most hand side is the leading term of an asymptotic expansion for $x \gg 1$. Solving it for x iteratively, we get:

$$\begin{aligned}
x(y) &\approx \sqrt{2\ln\left(\frac{N_A + N_B}{\sqrt{2\pi}\ y\ x(y)}\right)} \\
&\approx \sqrt{2\ln\left(\frac{N_A + N_B}{\sqrt{2\pi \times 2\ln\left(\frac{N_A+N_B}{\sqrt{2\pi}\ y\ x(y)}\right)}}\right) - 2\ln y} \\
&\approx \sqrt{2\ln\left(\frac{N_A + N_B}{\sqrt{2\pi\left\{2\ln\left(\frac{N_A+N_B}{\sqrt{2\pi}\ x(y)}\right) - 2\ln y\right\}}}\right) - 2\ln y} \\
&\approx \sqrt{2\ln\left(\frac{N_A + N_B}{\sqrt{4\pi\ \ln\left(\frac{N_A+N_B}{\sqrt{4\pi}}\right)}}\right)} - \frac{\ln y}{\sqrt{2\ln\left(\frac{N_A+N_B}{\sqrt{4\pi\cdot\ln\left(\frac{N_A+N_B}{\sqrt{4\pi}}\right)}}\right)}} \ .
\end{aligned} \tag{S8}$$

Substituting the last approximation into Eq. S5, we have:

$$\begin{aligned}\psi_A &\approx \int_0^{+\infty} dy\ e^{-y}\ \delta m\ x(y) \\ &\approx \delta m \sqrt{2\ln\left(\frac{N_A+N_B}{\sqrt{4\pi\ \ln\left(\frac{N_A+N_B}{\sqrt{4\pi}}\right)}}\right)}\left\{1+\frac{\gamma}{2\ln\left(\frac{N_A+N_B}{\sqrt{4\pi\ \ln\left(\frac{N_A+N_B}{\sqrt{4\pi}}\right)}}\right)}\right\} . \end{aligned} \tag{S9}$$

Because $\ln(N_A+N_B)$ is fairly small compared to $N_A+N_B(\gg 1)$ yet considerably larger than 1, we see that the competitive advantage ψ_A roughly scales as $\sqrt{\ln(N_A+N_B)}$ in this case.

(1-3) Variance increase in normal distribution

Once again, we assume that the measure x behaves according to a normal distribution: $f(x)=\frac{1}{\sqrt{2\pi}}\exp(-\frac{x^2}{2})$. This time, we consider that the mutant increased the variance of the distribution:

$$f(x)+\delta f(x)=\frac{1-\delta\sigma}{\sqrt{2\pi}}\exp\left(-\frac{\{(1-\delta\sigma)x\}^2}{2}\right) .$$

In this case, the dummy variable y, and consequently the function $x(y)$ as well, are the same as in the last subsection. Regarding $\delta\ln f(x)$, we have:

$$\delta\ln f(x)\approx\delta\sigma(x^2-1) .$$

Substituting these approximations into Eq. S5, we get:

$$\begin{aligned}\psi_A &\approx \int_0^{+\infty} dy\ e^{-y}\ \delta\sigma\left[(x(y))^2-1\right] \\ &\approx \delta\sigma\left\{2\ln\left(\frac{N_A+N_B}{\sqrt{4\pi\ \ln\left(\frac{N_A+N_B}{\sqrt{4\pi}}\right)}}\right)+2\gamma-1\right\} . \end{aligned} \tag{S10}$$

Thus, the competitive advantage ψ_A roughly scales as $\ln(N_A+N_B)$ in this case.

(1-4) Increased maximum of an upper-bounded distribution

So far, the distribution of the measure x for the sperm performance was either exponential or normal, neither of which is bounded from above.

In actual sperm competitions, however, it may be more natural to assume a performance measure bounded from above by a positive maximum value. We could consider that all characters influencing the sperm competitiveness could be integrated into a single measure, which we call the "velocity", which is the reciprocal of the total time from the start (ejaculation) till the completion of the fertilization. No matter how good the sperm performance is, the total time remain finite and can never be zero, thus there must always be a finite non-zero upper bound in the "velocity". Considering this way, a natural form of the probability distribution $f(x)$ near the upper-boundary x_M would be:

$$f(x) \propto \begin{cases} (\alpha+1)(x_M - x)^\alpha & \text{for} \quad x < x_M \ , \\ 0 & \text{for} \quad x \geq x_M \ , \end{cases} \tag{S11}$$

where the exponent $\alpha > 0$ determines the steepness of the distribution. At this point, the asymptotic distribution (Eq. S11) still has a freedom of a multiplication factor. For later convenience, we choose such a factor that the functional form should be valid in the entire region, $0 \leq x$, and we also rescale x so that x_M will be 1:

$$f(x) = \begin{cases} (\alpha+1)(1 - x)^\alpha & \text{for} \quad 0 \leq x < 1 \ , \\ 0 & \text{for} \quad x \geq 1 \ . \end{cases} \tag{S12}$$

In this case, the dummy integration variable becomes:

$$y = \begin{cases} (N_A + N_B)(1 - x)^{\alpha+1} & \text{for} \quad x < 1 \ , \\ 0 & \text{for} \quad x \geq 1 \ . \end{cases}$$

Because y is zero all across $x \geq 1$, the second equation in Eq. S5 needs a slight modification if $\delta f(x) > 0$ in $x > 1$:

$$\psi_A = \int_0^{+\infty} dy \ e^{-y} \ \delta \ln f(x(y)) + (N_A + N_B) \int_1^{+\infty} dx \ (f(x) + \delta f(x)) \ . \tag{S13}$$

Now, let us consider a particular case where the mutation slightly widen the region of x by increasing the upper-bound:

$$f(x) + \delta f(x) = \frac{\alpha + 1}{1 + \delta x_M} \left(1 - \frac{x}{1 + \delta x_M}\right)^{\alpha}.$$

Then, for $x < 1$, we have

$$\delta \ln f(x) \approx \delta x_M \left(\frac{\alpha\ x}{1 - x} - 1\right) \approx \delta x_M \left[\alpha \left(\frac{N_A + N_B}{y}\right)^{\frac{1}{\alpha+1}} - (\alpha + 1)\right]. \tag{S14}$$

Substituting the above two equations into Eq. S13 yields:

$$\begin{aligned}
\psi_A &\approx \delta x_M \int_0^{+\infty} dy\ e^{-y} \left[\alpha \left(\frac{N_A + N_B}{y}\right)^{\frac{1}{\alpha+1}} - (\alpha + 1)\right] \\
&\quad + (N_A + N_B) \int_1^{1+\delta x_M} dx\ \frac{\alpha + 1}{1 + \delta x_M} \left(1 - \frac{x}{1 + \delta x_M}\right)^{\alpha} \\
&= \delta x_M \left[(N_A + N_B)^{\frac{1}{\alpha+1}}\ \alpha\ \Gamma\left(\frac{\alpha}{\alpha + 1}\right) - (\alpha + 1)\right] + (N_A + N_B) \left(\frac{\delta x_M}{1 + \delta x_M}\right)^{\alpha+1} \\
&\approx \delta x_M (N_A + N_B)^{\frac{1}{\alpha+1}}\ \alpha\ \Gamma\left(\frac{\alpha}{\alpha + 1}\right).
\end{aligned} \tag{S15}$$

The last approximation holds because we are now considering δx_M that is small enough to give $\delta x_M\ (N_A + N_B)^{\frac{1}{\alpha+1}} \ll 1$, and because we now consider $(N_A + N_B)^{\frac{1}{\alpha+1}} \gg 1$. The approximate equation Eq. S15 states that the competitive advantage ψ_A roughly scales as $(N_A + N_B)^{\frac{1}{\alpha+1}}$ in this case.

Note 2. Calculation of Advantage in One-on-One Competition

Here we derive a formula for competitive advantage in one-on-one competitions, then apply it to the aforementioned four particular cases. They will serve as a basis for assessing the enhancement of the advantage by fierce competitions among numerous sperm.

(2-0) General formula

As in the main text (or in Supplementary Materials and Methods), consider the case where the mutation from B to A changed the distribution only slightly:

$$f_B(x) = f(x) \ , \quad f_A(x) = f(x) + \delta f(x) \ .$$

Then, the probability that allele A wins in a one-on-one competition with B is:

$$\begin{aligned} P\ [\text{winner} = A \mid 1\ A\&\ 1\ B\ ] &= \int_{-\infty}^{+\infty} dx \left[(f(x) + \delta f(x)) \int_{-\infty}^{x} d\xi f(\xi) \right] \\ &= \frac{1}{2} + \int_{-\infty}^{+\infty} dx \left[\delta f(x) \int_{-\infty}^{x} d\xi f(\xi) \right] \ . \end{aligned} \tag{S16}$$

If we set

$$\psi_A \equiv 4 \int_{-\infty}^{+\infty} dx \left[\delta f(x) \int_{-\infty}^{x} d\xi f(\xi) \right] \ , \tag{S17}$$

the above equation is rearranged as:

$$\begin{aligned} P\ [\text{winner} = A \mid 1\ A\&\ 1\ B\ ] &= \frac{1}{2} \left(1 + \frac{1}{2} \psi_A \right) \\ &\approx \frac{1}{1 + (1 - \psi_A)} \\ &\approx \frac{1 + \psi_A}{(1 + \psi_A) + 1} \ . \end{aligned} \tag{S18}$$

Thus ψ_A is interpreted as the competitive advantage of allele A over allele B. [Actually, these equations are special cases of Eqs. S3, S4 and S6 when $N_A = N_B = 1$.] Now we will calculate Equation S17 for specific cases.

(2-1) Increased mean in exponential distribution

In this case, $f(x) = \exp(-x)$ and $\delta f(x) \approx \delta\tau\ (x-1)\exp(-x)$ (for $x \geq 0$). Substituting them into Equation S17, we have:

$$\begin{aligned}
\psi_A &\approx 4\int_0^{+\infty} dx \left[\delta\tau\ (x-1)\exp(-x)\int_0^x d\xi \exp(-\xi)\right] \\
&= 4\ \delta\tau \int_0^{+\infty} dx\,[(x-1)\exp(-x)(1-\exp(-x))] \\
&= 4\ \delta\tau \left[\Gamma(2) - \Gamma(1) - \left(\frac{1}{2}\right)^2 \Gamma(2) + \left(\frac{1}{2}\right)\Gamma(1)\right] \\
&= \delta\tau\ . \qquad \text{(S19)}
\end{aligned}$$

(2-2) Shift of normal distribution

In this case, $f(x) = \frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)$ and $\delta f(x) \approx \delta m\ x f(x) = -\delta m\ \frac{d}{dx} f(x)$. Substituting them into Equation S17, we have:

$$\begin{aligned}
\psi_A &\approx 4\int_{-\infty}^{+\infty} dx \left[-\delta m\ \frac{d}{dx} f(x)\int_{-\infty}^x d\xi f(\xi)\right] \\
&= -4\ \delta m \left[f(x)\int_{-\infty}^x d\xi f(\xi)\right]_{x=-\infty}^{x=+\infty} + 4\ \delta m \int_{-\infty}^{+\infty} dx\,(f(x))^2 \\
&= 4\ \delta m \int_{-\infty}^{+\infty} dx\,\{f(x)\}^2\ .
\end{aligned}$$

Actually, equations up to this point hold for an infinitesimal constant shift *of any distribution* that is differentiable in the interval $-\infty < x < +\infty$. Now, substituting $f(x) = \frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)$ into the rightmost hand side, we get:

$$\begin{aligned}
\psi_A &\approx 4\ \frac{\delta m}{2\pi}\int_{-\infty}^{+\infty} dx \exp(-x^2) \\
&= \frac{2}{\sqrt{\pi}}\ \delta m\ . \qquad \text{(S20)}
\end{aligned}$$

(2-3) Variance increase in normal distribution

In this case, $f(x) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{x^2}{2}\right)$ and $\delta f(x) \approx \delta\sigma(x^2-1)f(x) = \delta\sigma \left(\frac{d}{dx}\right)^2 f(x)$. Substituting them into Equation S17, we have:

$$\begin{aligned}
\psi_A &\approx 4\int_{-\infty}^{+\infty} dx \left[\delta\sigma \left(\frac{d}{dx}\right)^2 f(x) \int_{-\infty}^{x} d\xi f(\xi)\right] \\
&= 4\ \delta\sigma \left[\frac{d}{dx} f(x) \int_{-\infty}^{x} d\xi f(\xi)\right]_{x=-\infty}^{x=+\infty} - 4\ \delta\sigma \int_{-\infty}^{+\infty} dx \left(\frac{d}{dx} f(x)\right)\ f(x)\ . \\
&= -4\ \delta\sigma \int_{-\infty}^{+\infty} dx \left[\frac{1}{2}\frac{d}{dx}\left(f(x)\right)^2\right] \\
&= -4\ \frac{\delta\sigma}{2} \left[\left(f(x)\right)^2\right]_{x=-\infty}^{x=+\infty} \\
&= 0\ . \qquad \text{(S21)}
\end{aligned}$$

Therefore, just increasing the variance of a normal distribution gives no competitive advantage (of $O(\delta\sigma)$) to the mutant as far as one-on-one competitions are concerned.

(2-4) Increased maximum of an upper-bounded distribution

In this case, $f(x) = (\alpha+1)(1-x)^\alpha$ (for $0 \le x \le 1$) and $\delta f(x) \approx \delta x_M\ \left(\frac{\alpha x}{1-x} - 1\right) f(x)$. Substituting them into Equation S17, we have:

$$\begin{aligned}
\psi_A &\approx 4\int_0^1 dx \left[\delta x_M \left(\frac{\alpha x}{1-x} - 1\right)(\alpha+1)(1-x)^\alpha \int_0^x d\xi\ (\alpha+1)(1-\xi)^\alpha\right] \\
&= 4\ \delta x_M\ (\alpha+1) \int_0^1 dx \left\{\left[\alpha x(1-x)^{\alpha-1} - (1-x)^\alpha\right]\left[1-(1-x)^{\alpha+1}\right]\right\} \\
&= 4\ \delta x_M(\alpha+1) \left[\alpha\ B(2,\ \alpha) - \frac{1}{\alpha+1} - \alpha\ B(2, 2\alpha+1) + \frac{1}{2\alpha+2}\right] \\
&= 4\ \delta x_M \left[\alpha(\alpha+1)\frac{1}{\alpha(\alpha+1)} - 1 - \alpha(\alpha+1)\frac{1}{(2\alpha+1)(2\alpha+2)} + \frac{1}{2}\right] \\
&= \frac{2(\alpha+1)}{2\alpha+1}\ \delta x_M\ . \qquad \text{(S22)}
\end{aligned}$$

Note 3. Enhancement Factor of Sperm Competitive Advantage

Now that we know the competitive advantage both for one-on-one competition and for competition among numerous competitors, we can calculate the enhancement factor for the specific cases.

(3-1) Increased mean in exponential distribution

In this case, $\psi_A(N_A = N_B = 1) \approx \delta\tau$, and $\psi_A(N_A, N_B \gg 1) \approx \delta\tau \{\ln(N_A + N_B) - 1 + \gamma\}$. Thus, we have:

$$R[\psi_A](N_A, N_B) \equiv \frac{\psi_A(N_A, N_B)}{\psi_A(N_A = N_B = 1)} \approx \ln(N_A + N_B) - 1 + \gamma \ . \tag{S23}$$

(3-2) Shift of normal distribution

In this case, $\psi_A(N_A = N_B = 1) \approx (2\delta m)/\sqrt{\pi}$ and

$$\psi_A(N_A, N_B \gg 1) \approx \delta m \sqrt{2 \ln \left(\frac{N_A + N_B}{\sqrt{4\pi \ \ln \left(\frac{N_A + N_B}{\sqrt{4\pi}} \right)}} \right)} \quad .$$

Taking the ratio of these two yields:

$$R[\psi_A](N_A, N_B) \approx \sqrt{\frac{\pi}{2} \ \ln \left(\frac{N_A + N_B}{\sqrt{4\pi \ \ln \left(\frac{N_A + N_B}{\sqrt{4\pi}} \right)}} \right)} \quad . \tag{S24}$$

(3-3) Variance increase in normal distribution

In this case, $\psi_A(N_A = N_B = 1) \approx 0$ and

$$\psi_A(N_A, N_B \gg 1) \approx \delta\sigma \left\{ 2 \ln \left(\frac{N_A + N_B}{\sqrt{4\pi \ \ln \left(\frac{N_A + N_B}{\sqrt{4\pi}} \right)}} \right) + 2\gamma - 1 \right\} \ .$$

Thus, $R[\psi_A]$ is $+\infty$, because there is no advantage in an one-on-one competition.

(3-4) Increased maximum of an upper-bounded distribution

In this case, $\psi_A(N_A = N_B = 1) \approx \delta x_M \times 2(\alpha+1)/(2\alpha+1)$ and

$$\psi_A(N_A, N_B \gg 1) \approx \delta x_M \ (N_A + N_B)^{\frac{1}{\alpha+1}} \ \alpha \ \Gamma\left(\frac{\alpha}{\alpha+1}\right) \ .$$

Taking the ratio, we have:

$$\begin{aligned} R[\psi_A](N_A, N_B) &\approx (N_A + N_B)^{\frac{1}{\alpha+1}} \ \frac{\alpha(2\alpha+1)}{2(\alpha+1)} \ \Gamma\left(\frac{\alpha}{\alpha+1}\right) \\ &= (N_A + N_B)^{\frac{1}{\alpha+1}} \ \frac{2\alpha+1}{2} \ \Gamma\left(\frac{2\alpha+1}{\alpha+1}\right) \ . \end{aligned} \tag{S25}$$

Note 4. Population Genetic Behavior of Mutant Frequency under Paternity-Sharing Sperm Competition

In the previous subsection, we examined the effect of an allelic difference on each instance of sperm competition, which we expressed in terms of the probability that a better allele will win. In population genetics, such competitions will take place here and there throughout the population. Thus, we expect that even a small competitive advantage could accumulate through generations to make a big difference.

Here, we want to focus on the effect of competitions among sperm sharing paternity, which have been overlooked in the previous studies. For this purpose, we consider an extreme situation in which a population consists of individuals that are strictly monogamous (and especially mono-androus). In this situation, there will *never* be post-copulatory competitions, including sperm competitions, *between different males*.

As in the previous section (or in the main text), we focus on a single locus (or site) and assume that the locus has two alleles, A and B, which are selectively neutral except in sperm competition. Here the locus is assumed to be on an autosome. We also assume that there are no further mutations at the locus (or site) and that the two alleles were present from the beginning in the current generation. Let $P^{(P)}\ [Z \mid Z_1Z_2]$ denote the probability that a sperm with allele Z (= A or B) wins a successful competition among sperm ejaculated by a male individual with the genotype Z_1Z_2 ($Z_1,\ Z_2$ = A or B). Obviously, for homozygous males, we have:

$$\begin{aligned} P^{(P)}\ [A \mid AA] &= P^{(P)}\ [B \mid BB] = 1\ , \\ P^{(P)}\ [A \mid BB] &= P^{(P)}\ [B \mid AA] = 0\ . \end{aligned} \tag{S26}$$

For heterozygous males, we can use Eq. S6. Assuming that there are an equal number of sperm with alleles A and B , $N_A = N_B$, and assuming that the allele difference has only a

small effect, we have:

$$P^{(P)}\ [A \mid AB] = \frac{1}{2}\left(1 + \frac{\psi_A}{2}\right)\ , \quad P^{(P)}\ [B \mid AB] = \frac{1}{2}\left(1 - \frac{\psi_A}{2}\right)\ . \tag{S27}$$

Let $p_t^{(P)}(Z_1Z_2)$ be the frequency of paternal genomes with the genotype Z_1Z_2 ($Z_1,\ Z_2$ = A or B) at the locus in the current (*i.e.*, the t-th) generation. Then, the expected frequency, $p_{t+1}^{(P)}(Z)$, of allele Z (= A or B) *of paternal origin* at the next (*i.e.*, the $(t+1)$-th) generation is in general:

$$p_{t+1}^{(P)}(Z) = \sum_{Z_1Z_2=AA,AB,BB} P^{(P)}\ [Z \mid Z_1Z_2]\ p_t^{(P)}(Z_1Z_2)\ .$$

This equation, after substituting Eq. S26 and Eq. S27 into it, reduces to:

$$\begin{aligned} p_{t+1}^{(P)}(A) &= p_t^{(P)}(AA) + \frac{1}{2}\left(1 + \frac{\psi_A}{2}\right) p_t^{(P)}(AB)\ , \\ p_{t+1}^{(P)}(B) &= 1 - p_{t+1}^{(P)}(A)\ . \end{aligned} \tag{S28}$$

In the deterministic limit, the diploid frequencies $p_t^{(P)}(Z_1Z_2)$ are given by the Hardy-Weinberg principle (see e.g. section 2.2 of **?**):

$$\begin{aligned} p_t^{(P)}(AA) &= p_t^{(P)}(A)\ p_t^{(M)}(A)\ , \\ p_t^{(P)}(AB) &= p_t^{(P)}(A)\ p_t^{(M)}(B) + p_t^{(P)}(B)\ p_t^{(M)}(A)\ , \\ p_t^{(P)}(BB) &= p_t^{(P)}(B)\ p_t^{(M)}(B)\ , \end{aligned} \tag{S29}$$

where $p_t^{(M)}(Z)$ is the frequency of allele Z *of maternal origin* at the current (i.e. the t-th) generation. Substituting Eq. S29 into Eq. S28, we get:

$$p_{t+1}^{(P)}(A) = \frac{1}{2}\left\{p_t^{(P)}(A) + p_t^{(M)}(A)\right\} + \frac{\psi_A}{4}\left\{p_t^{(P)}(A)\ p_t^{(M)}(B) + p_t^{(P)}(B)\ p_t^{(M)}(A)\right\}\ , \tag{S30}$$

and $p_{t+1}^{(P)}(B) = 1 - p_{t+1}^{(P)}(A)$.

Let us next consider the evolution of the maternal allele frequency. If we assume that the alleles A and B have the same probability of transmission to the next generation, the reasoning leading to paternal allele frequency also applies here, with $\psi_A = 0$. The result is:

$$p_{t+1}^{(M)}(A) = \frac{1}{2}\left\{p_t^{(P)}(A) + p_t^{(M)}(A)\right\}\ , \tag{S31}$$

and $p_{t+1}^{(M)}(B) = 1 - p_{t+1}^{(M)}(A)$.

Taking the arithmetic mean of Eq. S30 and Eq. S31, and ignoring terms of $O((\psi_A)^2)$, we get a simple recursion relation:

$$p_{t+1}(A) = p_t(A) + \frac{\psi_A}{4} p_t(A)(1 - p_t(A)) \ , \tag{S32}$$

and $p_{t+1}(B) = 1 - p_{t+1}(A)$. In the main text, they are Eqs. 9 and 10, respectively. Here,

$$p_t(Z) \equiv \frac{1}{2}\left\{ p_t^{(P)}(Z) + p_t^{(M)}(Z) \right\} \ ,$$

is the gender-averaged frequency of allele Z (= A or B) at the t-th generation.

Although we have ignored genetic drift so far, taking account of genetic drift is not so difficult. For this purpose, it is sufficient to notice that our recursion equation, Eq. S32, is equivalent to the deterministic recursion equation of the allele frequency:

$$p_{t+1}(A) = p_t(A) + s\ p_t(A)(1 - p_t(A)) \ ,$$

when allele A has a selective advantage of s ($\ll 1$) over allele B. Therefore, the diffusion theory framework such as unfolded in section 8.8.3 of (**?**) applies also here, if s is replaced by $\frac{\psi_A}{4}$. Thus we have the fixation probability $u(p)$ of allele A when its initial frequency is p:

$$u(p) \approx \frac{1 - \exp(-N_e \psi_A\ p)}{1 - \exp(-N_e \psi_A)} \ , \tag{S33}$$

where N_e is the effective population size. This is Eq. 11 in the main text. The initial frequency of a new mutation should be $p = 1/(2N)$, where N is the actual population size. If $N_e = N$, the equation is approximated as:

$$u(p) \approx \frac{\psi_A/2}{1 - \exp(-N\psi_A)} \ , \tag{S34}$$

which in turn reduces to $u(p) \approx \psi_A/2$ when $\exp(N\psi_A) \gg 1$. The effect of sperm competitive advantage is 1/4-fold smaller than that of selective advantage of the same intensity (i.e. when $s = \psi_A$). A multiplicative factor of 1/2 comes from the neutrality of the alleles in the maternal transmission, and the other multiplicative factor of 1/2 originates from the fact that thecompetition among paternity-sharing sperm is effective only when the male is heterozygous.