The Human Mutation Rate Is Increasing, Even As It Slows

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Abstract

Substitution rates vary between species, and many explanations regarding the causes of this variation have been proposed. Here we consider how new genomic data on the per-generation mutation rate impinge on proposed hypotheses for substitution rate variation in primates. We propose that the generation-time effect as it is usually understood cannot explain the observed rate variation, but instead that selection for decreased somatic mutation rates can. By considering the disparate causes underlying mutation rate changes in recent human history, we also show that the per-generation mutation rate is increasing even as the per-cell-division rate is decreasing.

Key words: **substitution rate, mutation rate, hominoid slowdown.**

Introduction

It is well documented that rates of nucleotide substitution vary between species (Wu and Li 1985; Britten 1986; Kumar and Subramanian 2002; Bromham 2009). By examining nucleotide changes at genomic positions that are not affected by natural selection, we can infer that this substitution rate variation is driven by differences in underlying mutation rates and not simply differences in the efficacy of selection between species (cf. Kimura 1983). Because the nucleotide mutation rate itself can be influenced by selection and drift (Kimura 1967; Kondrashov 1995; Sniegowski et al. 2000; Lynch 2010), understanding the relative impact of different evolutionary forces in driving changes in the mutation rate is key to understanding variation in substitution rates.

Many traits have been found to covary with substitution rates, and based on these trait correlations, many explanations for rate differences between species have been proposed. One of the most consistent relationships is between body size and substitution rate: larger organisms tend to have slower rates of molecular evolution (Bromham 2011; Lanfear et al. 2013). As there are many life history traits that are associated with body size, these are also often correlated with substitution rates. Some examples of such traits include metabolic rate (Martin and Palumbi 1993; Bleiweiss 1998), longevity (Nabholz et al. 2008), population size (Lynch 2010), and generation time (Li et al. 1996). Although many of these life history traits are correlated with one another, analyses of large data sets have to some extent been able to disentangle the contribution of each to variation in substitution rates (e.g., Bromham et al. 1996; Lanfear et al. 2007; Welch et al. 2008; Lourenco et al. 2013).

One of the most well-known examples of nucleotide substitution rate variation between species is known as the "hominoid slowdown" (Goodman 1985). The slowdown is based on the observation that the substitution rate in hominoids (Great Apes) is slower than that in Old World monkeys, which is again slower than that in New World monkeys (reviewed in Yi 2013). Within hominoids, humans show the slowest rate of all (Elango et al. 2006), and this rate may be continuing to fall (Scally and Durbin 2012). The general trend of lower substitution rates associated with longer generation times in the hominoids led to the proposal that this slowdown was directly due to differences in the generation time (Goodman 1962). Similar differences between rodents, artiodactyls, and primates have also been ascribed to the so-called "generation-time effect" (Laird et al. 1969; Wu and Li 1985).

The generation-time effect hypothesis proposes that shorter generation times lead to higher substitution rates "because in any arbitrary unit of time short-generation organisms will go through more generations and therefore more rounds of germ-cell divisions" (Li 1997, p. 229). The generation-time effect therefore assumes that there are either a fixed number of germline cell divisions per generation or that the rate of cell division per year decreases with increased generation time. Because germline cell divisions in many animals continue as an individual ages (Drost and Lee 1995), older males have gametes with more mutations (Haldane 1947; Crow 2000). Although it has long been known that the increased number of cell divisions with increased generation time will dampen any proposed generation-time effect (e.g., Wu and Li 1985; Hasegawa et al. 1987), until recently no quantitative estimates of this relationship were available.

Here, we show how new data on the per-generation mutation rate in humans directly contradict the generation-time hypothesis as an explanation for the hominoid slowdown. To understand why such data are relevant to the generation-time effect, we first discuss different ways in which the mutation rate can evolve and the effects of each of these on the substitution rate. In the supplementary

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Perspective

material S1 (Supplementary Material online), we formalize this discussion with a mathematical model.

Causes of Mutation Rate and Substitution Rate Variation

The per-generation mutation rate (μ_g) is a fundamental parameter in evolutionary biology, relevant to almost every aspect of the genetics of populations. This key trait is determined by the combined effects of DNA damage, repair, replication, and associated processes over the course of an individual's lifetime and therefore can be affected by a change in any one of these underlying systems. Here we focus on three major mechanisms that can affect the per-generation mutation rate (fig. 1).

Because mutations arise via DNA replication error and/or failure to repair those errors, one possible mechanism for rate variation is for the DNA replication and repair machinery to be more or less efficient in a particular species (fig. 1*a*; supplementary fig. S1*a*, Supplementary Material online). Changes in either the amino acid sequence of replication-associated proteins or the number and identity of proteins involved in replication and repair can affect the per-cell-division mutation rate (μ_c). This rate is known to vary among species, with the human germline per-cell-division rate being more than ten times lower than the mouse rate (Lynch 2010). Evolution of the per-cell-division rate affects both the per-generation mutation rate and the substitution rate between species (k) as the number of mutations per unit time increases or decreases. Assuming that changes to the replication machinery affect males and females equally, evolution of μ_c does not change the ratio of male-to-female mutations (α), which has a value greater than 1 in many species (e.g., Shimmin et al. 1993; Berlin et al. 2006; Wilson Sayres et al. 2011).

A second way to change the per-generation mutation rate is to change the rate at which germline cells divide (fig. 1*b*; supplementary fig. S1*b*, Supplementary Material online). With more cell divisions come more replication events, which leads to more mutation. Evidence suggests that closely related species differing in the intensity of sperm competition differ in the number of male germline cell divisions, with more competition leading to higher per-generation mutation rates (Bartosch-Harlid et al. 2003; Presgraves and Yi 2009). Because the changing cell-division rate leads to more or fewer mutations per unit time, the substitution rate is changed as a consequence. For instance, mouse male stem cells divide every 8.6 days, while human male stem cells divide every 16 days (Drost and Lee 1995). If cell-division rates show equivalent change in males and females, then α is not affected; however, changes biased to one sex will change the ratio of male-to-female mutations, resulting in changes to α (Presgraves and Yi 2009).

Finally, the generation time itself can directly affect the per-generation mutation rate (fig. 1*c*; supplementary fig. S1*c*, Supplementary Material online). Assuming that germline cell divisions continue throughout an individual's lifetime, increasing the generation time increases the number of mutations that accumulate (the "copy-error effect"; Bromham 2011). Indeed, the large-scale association between per-generation mutation rates and generation time may be a consequence of the greater opportunity for errors given longer generations (Lynch 2007, p. 86), as long as most mutations are derived from replication (which they seem to be; Kim et al. 2006). For species in which the number of mutations in offspring increases linearly with parental age, change in generation time should not affect the substitution rate (fig. 1*c*; supplementary fig. 1*c*, Supplementary Material

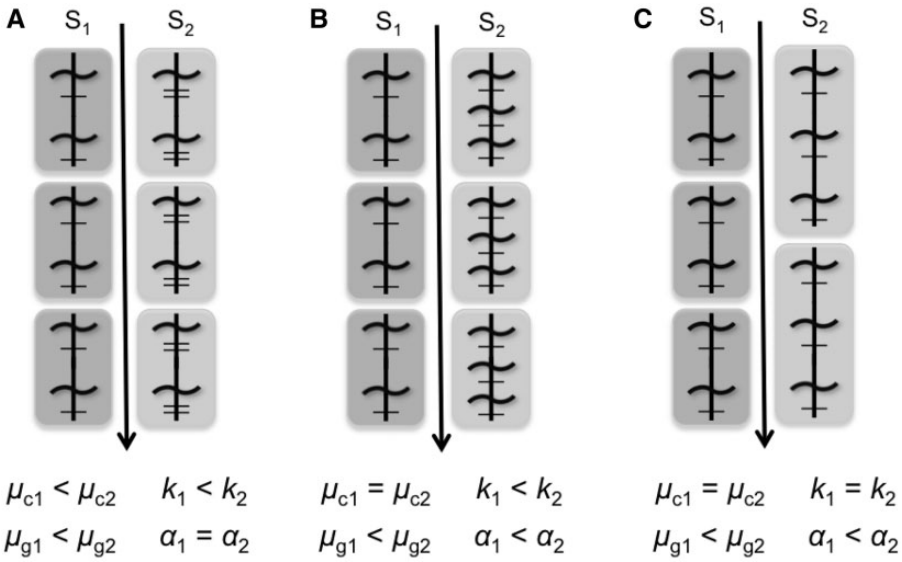

Fig. 1. Predictions about the per-cell-division (μ_c) and per-generation (μ_g) mutation rates, substitution rates (k), and male-to-female mutation ratio (α) between two species (S_1 and S_2) by varying (*A*) the efficiency of the DNA repair machinery, (*B*) the number of replications per unit time, or (*C*) the generation time. Note that α is only changed in (*B*) if the replication rate change occurs in males and not females. Each gray box represents one generation, while each wavy line indicates a germline replication event. Replications give rise to mutations, which are shown as notches. The arrow between the two species represents time.

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online). This claim assumes that the variation in generation time is occurring post-spermatogenesis, although the time to puberty can also evolve between species (e.g., Marson et al. 1991). Unfortunately, little is known about the relationship between the age of puberty and the number of pre-spermatogenesis cell divisions. Changes in the time to spermatogenesis between species could change the substitution rate if there are a fixed number of cell divisions that have to occur in this time; however, this number does not appear to be fixed among mammals (Drost and Lee 1995). Any difference in male and female mutation rates due to differences in germline cell differentiation will be further magnified by longer generations, and α is predicted to increase as a result.

Is There a Generation-Time Effect in Primates?

Given the earlier considerations, it is worthwhile considering whether the conditions necessary for the generation-time effect hold in primates. The generation-time effect hypothesis states that substitution rates slow when there is both an increase in the generation time and a decrease in the germline replication rate (Wu and Li 1985; Elango et al 2006). If a fixed number of germline cell divisions occur each generation in both sexes—as they do only in female primates—then longer generations result in a lower average rate of cell division per unit time (supplementary fig. S2, Supplementary Material online); as a consequence, substitution rates would indeed go down.

However, recent whole-genome data from humans show that the number of offspring mutations is a linear function of paternal age and is not correlated with maternal age (Kong et al. 2012; Sun et al. 2012). The children of fathers age 20 have approximately 40 de novo nucleotide mutations, of fathers age 30 have 60 mutations, and so on (Kong et al. 2012). Because the male mutation rate is so much higher than the female mutation rate, the former dominates the number of mutations found in offspring. Under these conditions, longer generation times have no effect on either the cell division rate per year or the per-cell-division mutation rate, and therefore there is no effect on substitution rates (fig. 1*c*; supplementary fig. S1*c*, Supplementary Material online). This does not mean that there should be no correlation between increased generation-time and decreased substitution rates, only that an associated factor is the cause of such correlations (see below). In addition, the absence of a direct effect of generation time on substitution rates can help to explain why the rate of DNA duplication can be increasing in hominoids (Hahn et al. 2007; Marques-Bonet et al. 2009). In this case, it is the repair machinery itself that is evolving—possibly in different ways for nucleotide and duplication mutations—not a common life-history trait.

Given the predictions laid out in the previous section, in primates there should also be a positive correlation between generation time and α because increased numbers of male germline cell divisions amplify differences between male and female mutation rates. Based on data from primates and other mammals, α does in fact scale positively with generation time (Presgraves and Yi 2009; Wilson Sayres et al. 2011).

The Human Nucleotide Mutation Rate Is Decreasing, and Increasing

In the absence of a generation-time effect, the observed decrease in hominoid substitution rates must be due to either a decrease in the per-cell-division mutation rate or a decrease in the germline cell division rate. The predictions laid out in figure 1 show that decreased rates of cell division would lead to lower values of α, which is contrary to the observed trends. Therefore, the data imply that there has been a decrease in the per-cell-division mutation rate (μ_c) in hominoids and that this rate is further decreasing in humans.

On the other hand, because the per-generation mutation rate (μ_g) is determined by the accumulation of mutations across many germline cell divisions, consideration of recent demographic shifts in human populations suggests that μ_g is actually increasing. In essence, the increased rate is simply a result of increases in the average human generation time, which is much longer now (25–30 years; Fenner 2005) than it was in archaic humans (18–19 years; Hemmer 2007). Even within the last 40 years, data from developed countries show an increasing average generation time for both females (Bongaarts 2001) and males (Svensson et al. 2011). Taken together with the fact that mutation rates increase with paternal age, these increases in generation time result in higher per-generation mutation rates.

Experimental manipulation of the age at reproduction in mutation-accumulation experiments has shown that increased generation times result in increased μ_g (Latta et al. 2013). In particular, increased generation times lead to increased per-generation deleterious mutation rates and increased variance in fitness among individuals. If similar increases in the variance in fitness among humans occur as a result of increases in μ_g, such changes may have important consequences for understanding the ongoing evolution of human health (cf. Nesse and Williams 1994; Stearns and Koella 2007).

Selection on Somatic Mutation Rate as an Explanation for the Hominoid Slowdown

Without the generation-time effect as an explanation for the observed slowdown in nucleotide substitution rates, it behooves us to ask whether there are other viable hypotheses for this pattern. Nonadaptive hypotheses would seem to predict a higher rate of mutation in humans as they have the lowest effective population size (Lynch et al. 2008, 2010). These predictions run counter to the observed patterns, at least within primates.

Multiple adaptive hypotheses have been proposed for the negative association between body size and substitution rate, many of which are concerned with the increased somatic mutation load experienced by long-lived, large-bodied organisms (Promislow 1994; Nabholz et al. 2008; Welch et al. 2008; Bromham 2011). We hypothesize that the hominoid DNA repair machinery has evolved to be more efficient in response to selection on the somatic mutation rate, which has in turn led to a lower germline mutation rate (cf., Britten 1986); this hypothesis assumes that the same repair proteins are used in

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the germline and soma (Marcon and Moens 2005; Galetzka et al. 2007). Although mutations in somatic cells do not affect offspring fitness, they do affect the fitness of the individual in which they occur and can therefore be a target of selection (Crow 1986; Lynch et al. 2008). Because the number of somatic cell divisions experienced by an organism is affected by both longevity and body size—and is generally correlated with generation time—all three of these measures may to some degree be associated with substitution rates (Welch et al. 2008), especially when measurements may be prone to error.

Conclusions

Until recently, measuring substitution rates between species was the only way to assess mutation rates on a large scale. Next-generation sequencing technologies now allow for whole-genome sequencing of parent–offspring trios (Conrad et al. 2011; Kong et al. 2012) and mutation-accumulation lines (Lynch et al. 2008; Denver et al. 2009; Keightley et al. 2009; Ossowski et al. 2010; Schrider et al. 2013). These methods have enabled the collection of per-generation mutation rates in various organisms (albeit for experimental individuals with particular generation times), and the hope is that we will be able to understand both divergence times and substitution rates in terms of these mutation rates. However, because μ_g will itself be affected by the generation time, summing a single value of this measure over a large number of generations to estimate divergence times is bound to be inaccurate; this will be especially true for lineages that undergo changes in the length of generations (e.g., Langergraber et al. 2012; Obbard et al. 2012) or when there is genetic variation in the mutation rate (e.g., Conrad et al. 2011; Schrider et al. 2013). As shown here, understanding differences in substitution rates first requires that we understand what aspect of the mutational process to measure. The implications of μ_g and μ_c for long-term evolutionary rates can be distinct, and radically different conclusions may be reached (e.g., increasing or decreasing mutation rates) depending on the measure used.

Supplementary Material

Supplementary material S1 is available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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Supplementary Information

Mutation rate model:

The mutation rate per generation (μ_g) of any organism can be simply calculated by multiplying the mutation rate per germline cell division (μ_c) by the number of germline cell divisions per generation (d_g):

$$\mu_g = (\mu_c)(d_g) \quad (1)$$

However, in dealing with organisms whose germlines go through different stages during the life cycle (such as mammals), a different mutation rate must be calculated for each stage. The mutation rates from each stage are then be averaged to determine the overall μ_g.

For mammals, there are three life stages in which germ cells can potentially experience different mutation rates, based on either a unique d_g or μ_c from that period. These stages are females per generation, males before puberty, and males after puberty. In both females and males before puberty, a fixed number of germ cell divisions occur (although this fixed number is likely not the same between the genders) and these cells use mitosis to replicate their DNA. In males after puberty, the number of germ cell divisions is continuous and is thought to relate linearly with generation time. Male germ cells after puberty also replicate their DNA with meiosis. These stages lead us to consider three separate mutation rates when determining the overall μ_g for any organism of interest: the mutation rate of females per generation (μ_{gF}), the mutation rate in males before puberty per generation (μ_{gMBP}), and the mutation rate in males after puberty per generation (μ_{gMAP}). Additionally, separate mutation rates per cell division may be considered for mitosis (μ_{cMIT}) and meiosis (μ_{cMEI}). We assume these to be equal, but include both terms in our model.

The calculation of μ_{gF} and μ_{gMBP} give constant terms based on the number of cell divisions in each stage:

$$\mu_{gF} = (\mu_{cMIT})(d_{gF}) \quad (2)$$

$$\mu_{gMBP} = (\mu_{cMIT})(d_{gMBP}) \quad (3)$$

Then, given that the number of cell divisions in males per generation after puberty (d_{gMAP}) is a linear relationship between the number of male cell divisions per year after puberty (d_{yMAP}) and generation time (GT) after the age of puberty (AP):

$$d_{gMAP} = (d_{yMAP})(GT - AP) \quad (4)$$

μ_{gMAP} is calculated as:

$$\mu_{gMAP} = (\mu_{cMEI})(d_{gMAP}) \quad (5)$$

The two terms for before and after puberty mutation rates in males can be averaged to give the overall male mutation rate per generation (μ_{gM}):

$$\mu_{gM} = \frac{(\mu_{gMBP} + \mu_{gMAP})}{2} \quad (6)$$

It then follows that the overall per-generation mutation rate (μ_g) of an organism is the average between the male and female contributions:

$$\mu_g = \frac{(\mu_{gM} + \mu_{gF})}{2} \quad (7)$$

Care must be taken when converting from μ_g to mutation rate per year (μ_y). Because each of the terms that contribute to μ_g occurs over a different period of absolute time, they must each be converted to mutation rates per year based on the amount of time they encompass, with the total male mutation rate per year (μ_{yM}) being the average of the per generation rates in the two male life stages:

$$\mu_{yF} = \frac{\mu_{gF}}{GT} \quad (8)$$

$$\mu_{yMBP} = \frac{\mu_{gMBP}}{AP} \quad (9)$$

$$\mu_{yMAP} = \frac{\mu_{gMAP}}{(GT - AP)} \quad (10)$$

$$\mu_{yM} = \frac{(\mu_{yMBP} + \mu_{yMAP})}{2} \quad (11)$$

Now the per-generation mutation rate can easily be converted to the per-year mutation rate by again averaging the male and female contributions:

$$\mu_y = \frac{(\mu_{yM} + \mu_{yF})}{2} \quad (12)$$

The substitution rate (k) is assumed to be equal to μ_y:

$$k = \mu_y \quad (13)$$

Finally, to calculate the male-to-female mutation ratio (α):

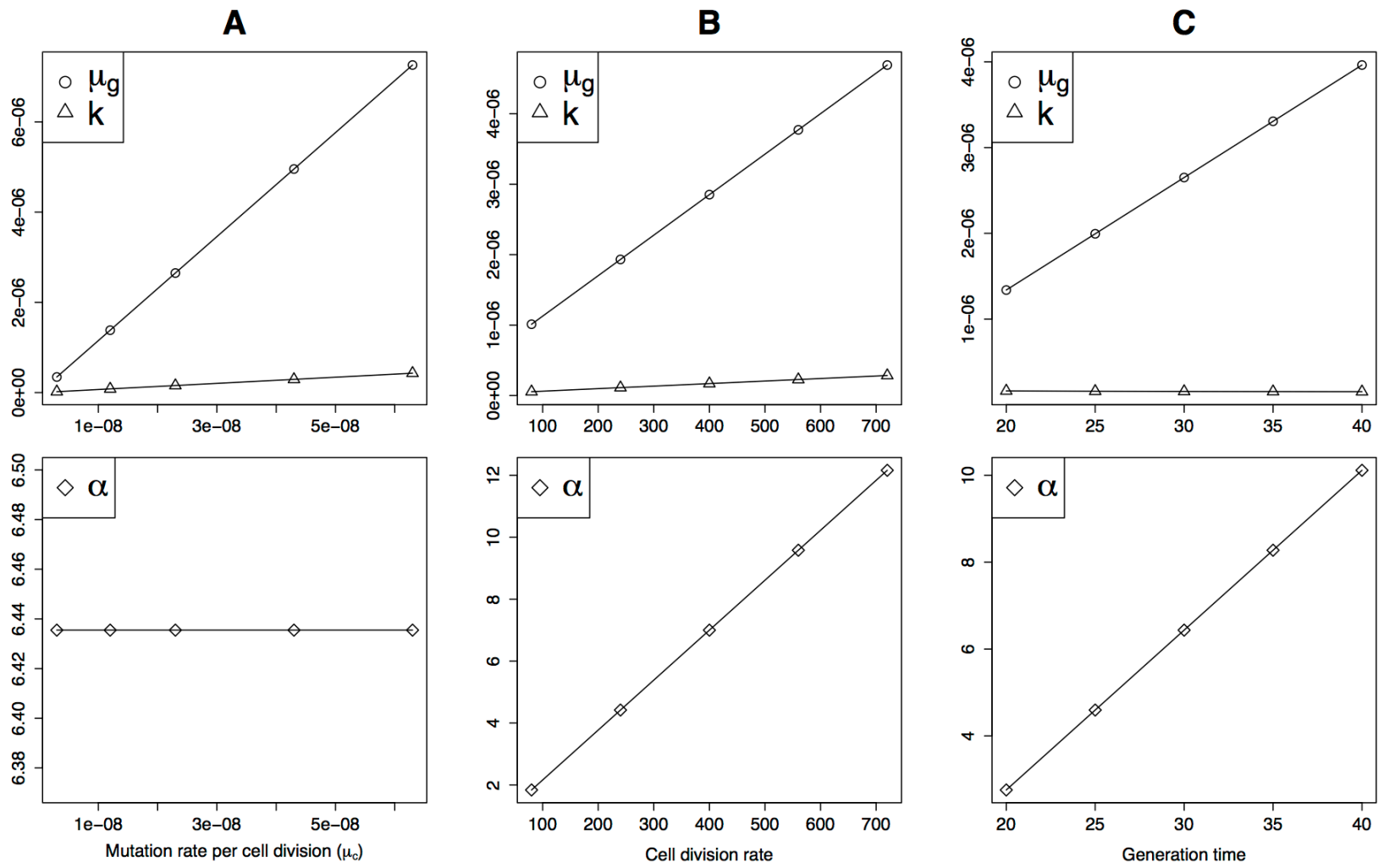
$$\alpha = \frac{\mu_{gM}}{\mu_{gF}} \qquad (14)$$

Supplementary Table S1: Life history and mutation rate parameters taken from Drost and Lee, 1995 and used in conjunction with equations 7, 13, and 14 in Figures S1 and S2.

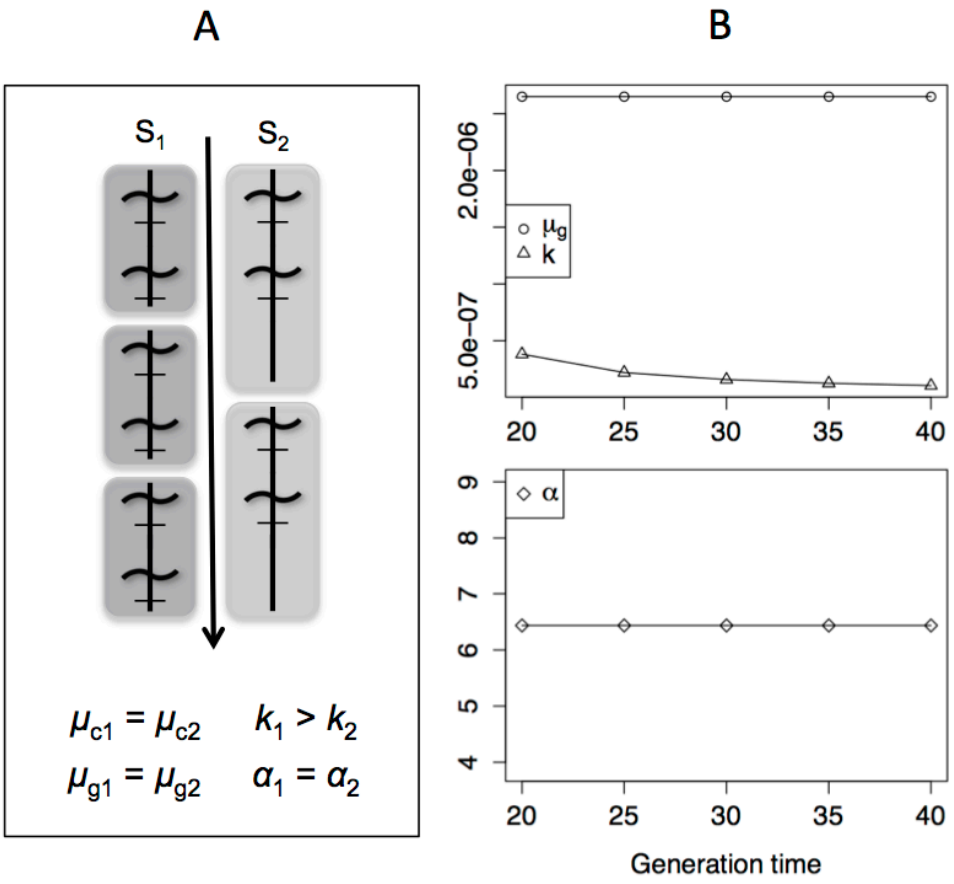
Generation Time	μ_c	Age of puberty	d_{gF}	d_{gMBP}	d_{yMAP}
30 years (20, 25, 30, 35, 40)[a]	2.3×10^{-8} (0.3, 1.2, 2.3, 4.3, 5.3)[b]	14 years	31	34	23 (5, 15, 25, 35, 45)

[a] The range of values used when a particular parameter was variable in Figure S1 are shown in parentheses

[b] All values for μ_c are x 10^{-8}



Supplementary Figure S1: The effect of changing the per cell division mutation rate (A), cell division rate per generation in males after puberty (B), or generation time (C) on the per generation mutation rate (Equation 7), substitution rate (Equation 13), and male-to-female mutation ratio (Equation 14). All parameters were taken from Drost and Lee, 1995 (Table S1).



Supplementary Figure S2: A demonstration of a "generation-time effect" in which generation time increases, but the rate of cell division does not. The left panel (A) demonstrates graphically how this occurs, while the right panel (B) uses values taken from Table S1 to calculate our model under this scenario.