Essay

The 'faulty male' hypothesis for sex-biased mutation and disease

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Biological differences between males and females lead to many differences in physiology, disease, and overall health. One of the most prominent disparities is in the number of germline mutations passed to offspring: human males transmit three times as many mutations as do females. While the classic explanation for this pattern invokes differences in post-puberty germline replication between the sexes, recent whole-genome evidence in humans and other mammals has cast doubt on this mechanism. Here, we review recent work that is inconsistent with a replication-driven model of male-biased mutation, and propose an alternative, 'faulty male' hypothesis. This model proposes that males are less able to repair and/or protect DNA from damage compared to females. Importantly, we suggest that this new model for male-biased mutation may also help to explain several pronounced differences between the sexes in cancer, aging, and DNA repair. Although the detailed contributions of genetic, epigenetic, and hormonal influences of biological sex on mutation remain to be fully understood, a reconsideration of the mechanisms underlying these differences will lead to a deeper understanding of evolution and disease.

Haldane[1] was the first to suggest a higher per-generation mutation rate in males compared to females, using data on the appearance of hemophilia in the offspring of unaffected parents. This paper is also often cited for its proposed explanation for the observed male bias: since the male germline is continuously dividing and the female germline is not, "if mutation is due to faulty copying of genes at a nuclear division, we might expect it to be commoner in males than females"[1]. Haldane's germline-replication hypothesis is also consistent with a paternal age effect, whereby older males leave more mutations to their offspring. Both male-biased mutation and a paternal age effect have been firmly established by whole-genome sequencing of human pedigrees[2–6] and pedigrees of multiple mammals[7–16]. Male-biased mutation across mammals is also supported by data from comparative studies[17,18], though it is not possible to detect a paternal age effect from phylogenetic data.

What is often overlooked is that Haldane[1] proposed a second hypothesis to explain male-biased mutation: male chromosomes may not be as well protected as female chromosomes. If the female germline was "relatively invulnerable to radiation and other influences, the difference is explicable". Unfortunately, Haldane did not know of any biological mechanism that could offer such protection, and could only note in the end: "On either of these hypotheses we should expect higher mutability in the male to be a general property of human and perhaps other vertebrate genes. It is difficult to see how this could be proved or disproved for many years to come."

In this essay, we consider the data on mutation rates that have accrued in the 75+ years since Haldane's original hypotheses. We focus on many aspects of recent whole-genome sequencing projects that are inconsistent with the germline-replication hypothesis, with respect to both male bias and the paternal age effect. In order to reconcile these observations, we introduce a new hypothesis — the 'faulty male' model — that proposes a reduced ability of males to protect their germline relative to females. This model and associated mechanistic data reflect a modern interpretation of Haldane's overlooked hypothesis for differences in germline mutation between the sexes. Further, we highlight patterns of male-biased DNA repair, cancer, and aging that are consistent with the somatic tissues of males also being more liable to damage. These data suggest the possibility of a shared basis for male-biased mutation in the germline and soma.

Genome-scale data are inconsistent with the germline-replication model

The germline-replication hypothesis originally proposed by Haldane[1] focuses on the mitotic cell divisions needed to maintain continuous spermatogenesis, and the errors that result from these replication events. While this framework has occasionally been questioned[17,19,20], post-puberty mitotic cell division in the male germline has become *the* textbook explanation for male-biased mutation (e.g. Lynch[21], Jobling *et al.*[22], and Strachan and Read[23]). However, multiple results from recent whole-genome studies are inconsistent with the germline-replication hypothesis. Below we consider six observations that strongly conflict with this model.

A maternal age effect

While much weaker than the paternal age effect (Figure 1A), studies with large numbers of sequenced pedigrees have now been able to detect an effect of maternal age on the number of transmitted *de novo* mutations[2,3,6]. In the absence of ongoing replication in the female germline, this pattern points to accumulating exogenous damage as a likely source of mutations. Presumably, such damage must also accumulate in the male germline and must contribute in part to the paternal age effect.

Mutation is male-biased just after puberty

Pedigree-based studies can only observe transmitted *de novo* mutations among individuals that have had children: this means that our knowledge about male bias typically begins at the age of reproductive maturity. Nevertheless, studies including young parents reveal that many more paternally inherited mutations are already present shortly after puberty, in both humans (Figure 1A) and domestic cats[14]. In humans, this bias is present for both point mutations[19] and microsatellites[24], suggesting similar mechanisms for both. Under the assumption that the male and female germlines have approximately the same number of cell divisions before puberty[25], the same degree of male bias at this stage is not consistent with an important role for post-puberty mitotic cell division.

Spermatogenic cycle length is not predictive of mutation accumulation rates

The production of sperm follows a highly synchronized cycle of cell division, with a duration that varies between species[26]. If mutations in the male germline are largely driven by mitotic replication, we would expect the number of mutations to increase at a rate that is proportional to the length of the spermatogenic cycle (i.e. the slope of the line after puberty in Figure 1B). However, comparisons between species have revealed highly similar rates of paternal mutation accumulation (~1.5 mutations/year), even when there is a twofold difference in the spermatogenic cycle length[3,15,16]. While there are a number of reasons why cycle length might not exactly correspond to the degree of male bias[27,28], the observation of a relatively constant bias across species (see also de Manuel *et al.*[17]) suggests that replication rate is unlikely to be the major factor influencing this bias.

CpG mutations accumulate in a male-biased fashion

C-to-T mutations at CpG dinucleotides occur an order of magnitude more frequently than other mutations due to the deamination of methylated cytosines[29–31]. Importantly, deamination occurs spontaneously and is not driven by polymerase errors during replication. This suggests that mutations at CpG sites should be free from the male bias and paternal age effect that would be observed in replication-driven mutations. However, C-to-T mutations at CpG sites demonstrate both of these effects (Figure 2). These patterns at CpGs are difficult to reconcile with a replication-driven model for mutation.

Hibernating species do not show a lower degree of male bias

Many seasonally breeding animals undergo testicular regression, whereby testis size decreases by up to 95% (Young and Nelson[32]). In addition to an overall reduction in size, spermatogenesis is greatly reduced or absent for a large fraction of the year[32,33]. A reasonable prediction from the germline-replication model might then be a reduction in the degree of male bias and a diminution of the paternal age effect among seasonal breeders. However, a study of germline mutation rates in grizzly bears found the same level of male bias as in non-hibernating species, as well as a match with the predicted number of transmitted mutations given paternal ages[13]. These results further suggest a disconnect between male mutation bias and spermatogenic cycling, although additional mechanisms could still reconcile such a relationship in hibernating species[13].

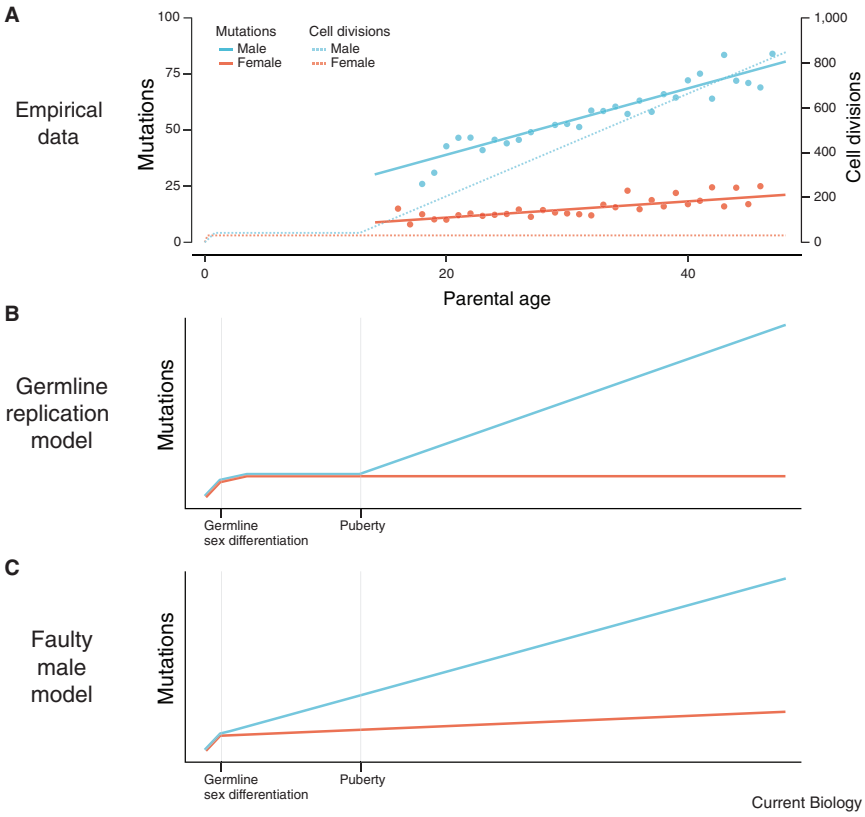

Figure 1. Models and data for germline mutation.
(A) The accumulation of germline mutations in human males and females (colored points and solid lines) as a function of parental age (from Jónsson *et al.*[3]). Each point represents the average number of total mutations transmitted from male and female parents of the specified age. Lines show the linear regression of these points with parental age. Cell divisions with age (dashed lines) come from calculations in Ségurel *et al.*[28]. For clarity, dashed lines pre-puberty are slightly shifted between males and females so as not to overlap. (B) The germline replication model proposes that the number of mutations transmitted by a parent should be proportional to the number of germline cell divisions. Here, the expected number of mutations from male and female parents is drawn to exactly track the cell divisions shown in (A). (C) The faulty male hypothesis proposes that the number of mutations is consistently higher in males than females after germline sex differentiation, but that mutations accumulate in both sexes through time. Here, the slopes of the mutation-accumulation lines after differentiation are identical to those from the linear regression of mutation data in (A).

Somatic mutation accumulation is not correlated with number of cell divisions

The somatic mutation rate varies greatly among tissues and is consistently higher in all somatic cell types than the germline mutation rate[34,35]. Nonetheless, variation in somatic mutation rate among tissues is not associated with replication activity[36]. For example, mutation rates in neurons and smooth muscle, two cell types that are thought to rarely divide, are similar to those in frequently dividing cells. In fact, for many tissues there is no observed difference in the rate of mutation accumulation between terminally differentiated cells and their progenitor stem cells[36]. Although there are clearly differences in mutation rates in the germline and soma, the limited effect of differences

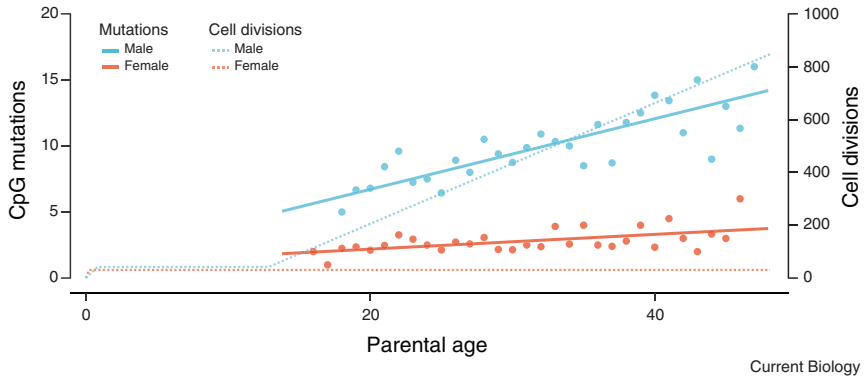

Figure 2. Patterns of CpG mutations in human males and females.
The accumulation of germline mutations in human males and females as a function of parental age, only for mutations at CpG sites. All elements of the plot are the same as in Figure 1A (based on data from Jónsson *et al.*[3] and Ségurel *et al.*[28]), except that only mutations at CpG sites are included.

in replication rates between somatic tissues suggests that replication may be playing a more limited role in the germline as well.

The 'faulty male' hypothesis for higher male germline mutation rates

Biological sex influences many different aspects of phenotype and physiology[37]. These effects are driven by genetic, epigenetic, hormonal, and exogenous mechanisms, or some combination of all these[38,39]. Here, we propose that mutation rates in male mammals are higher than in females because males are generally worse at protecting and repairing DNA. This 'faulty male' hypothesis invokes physiological and molecular differences between the sexes as the main cause of the difference in mutation rates, rather than post-puberty germline replication. While this does not preclude a role for continuing cell division in the male germline as a source of mutation, it reduces the explanatory role that it plays.

The faulty male hypothesis follows the logic laid out by Haldane's alternative model[1]: males are worse at protecting and/or repairing their gametes from DNA damage, resulting in male-biased mutation and a paternal age effect. While these general patterns are also predicted by the germline-replication hypothesis (Figure 1B), only the faulty male model — in which mutation is uncoupled from cell division — accounts for the additional patterns laid out in the previous section (Figure 1C).

What mechanism(s) might explain differences in the germline mutation rate between the sexes? There is some direct evidence for the differential action of DNA repair machinery between males and females. For example, researchers have found that polymerase theta is more effective in the female germline; this is likely explained by the inaccessibility of mature sperm to repair by this polymerase due to chromatin structure[40]. Indeed, DNA in sperm is packaged in a distinct manner from oocyte DNA, using protamines rather than histones[41]. There is, however, much indirect evidence for different mechanisms of mutation between the sexes[42]. For instance, the frequency of each type of single-nucleotide mutation differs in the male and female germlines[2,3,43], as does the amount of gross DNA damage experienced[44,45]. In addition, multiple tumor suppressor genes on the X chromosome escape inactivation, providing females with additional protection against cancer[46]; if these genes also act in germline DNA repair, this could lead to mutational differences between males and females.

More generally, a number of molecular mechanisms that differ between the sexes, many of which are modulated by sex hormone regulation, likely contribute to the disparity in mutation rates. Almost 37% of human genes show sex-biased expression in at least one tissue[47], including many genes in the germline. Differences in germline gene expression are likely due in part to DNA/chromatin modifications, especially differential methylation[48], and chromatin accessibility is known to be sex-biased in many tissues[49]. Additionally, sex-biased differences in resting metabolism[50], metabolite concentrations[51] and macroscopic differences in protective organs (such as skin; Giacomoni *et al.*[52]) could plausibly contribute to sex-biased mutation.

Finally, note that levels of sex hormones vary throughout mammalian development and adulthood — for both males and females — and are absent prior to embryonic sexual differentiation[42]. The absence of male–female hormonal differences early in development might explain why there is no male bias in the mutations arising during this period[5,53].

Is the male soma 'faulty'?

By de-emphasizing the role of germline replication as a major driver of male-biased mutation, we raise the possibility that the underlying causes of male bias may be acting similarly outside the germline. Many of the mechanisms invoked in the previous section to explain differences in mutation rates between the sexes are not specific to the germline and may have similar effects on somatic mutation rates. Common mutational variants identified in germline and somatic datasets support the idea of a mechanistic link[54]. Such a connection between germline mutation rates and somatic mutation rates would open many new avenues of research.

Male-biased somatic mutation rates

The most straightforward question to ask is whether somatic nucleotide mutation rates are male biased. However, this question is surprisingly difficult to answer, as many studies either do not have the power to address the question or do not consider the possibility of a difference between male and female samples. The largest source of data is from studies of cancer tissues. A male bias in the number of nucleotide mutations (often referred to as 'mutation load') is observed across cancer datasets. This is consistent across whole-genome sequencing data — which include both coding and non-coding changes[55,56] — and the targeted sequencing of protein-coding genes[57]. While data from cancer sequencing supports the generality of male bias in mutation, it is possible that such samples do not represent mutation processes in healthy somatic tissues. It is

also important to note that the observed somatic male bias is both weaker than that observed among germline mutations and is not observed in every tissue[55,56]. This could suggest that different mechanisms explain germline and somatic mutation differences between the sexes.

Male-biased cancer

The vast majority of cancers in tissues present in both sexes are male biased[58]. Figure 3 summarizes recent worldwide data on cancer incidence[59], illustrating a higher incidence of most cancers in males. While lifestyle choices associated with gender roles may explain some of these disparities, differences between the sexes remain after controlling for multiple risk factors[60]. In addition, childhood cancers are also highly male biased[61,62], which suggests sex as the fundamental biological factor driving this pattern.

Several biological mechanisms have been proposed to explain male-biased cancer, including differences between the sexes in hormones, metabolism, immunity, X-linked tumor suppressors, and general DNA repair[46,63–65]. There is increasing evidence for sex-specific differences in the DNA damage response pathway, defects in which are thought to fuel carcinogenesis[66]. In addition, some experimental evidence points to differing responses in DNA double-strand break repair[67]. Finally, there is indirect evidence that DNA repair in males is relatively inferior in populations already susceptible to DNA damage. For instance, males are more likely to develop secondary cancers when radiation is used to treat a primary cancer and are more likely than females to develop cancers when they have inherited germline mutations in tumor suppressor genes[65].

Given the evidence presented above for higher somatic mutation rates in males, we propose that a faulty male soma may also play a role in driving the variation in cancer rates between the sexes. Differences in somatic mutation rates should not be considered the only cause of differences in cancer rates, especially if we consider that, under the multiple-hit hypothesis, the rate of oncogenic transformation is not linear with mutation rate. If male-biased somatic rates were solely responsible, we would expect differences in cancer rates to be much higher. Instead, a higher somatic mutation rate in males should be considered alongside existing mechanisms proposed in the literature. While the invocation of sex bias in somatic mutation rates overlaps with previous hypotheses about differences in DNA repair between the sexes, the underlying causes may be quite different; this also suggests that approaches used to study this mechanism could be expanded, for instance by whole-genome sequencing of somatic tissues.

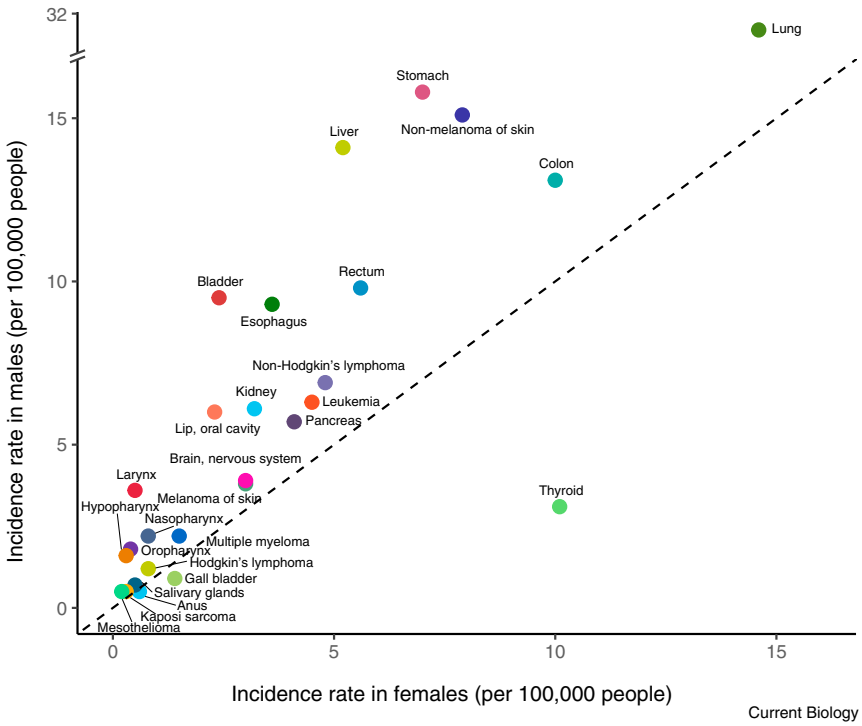

Figure 3. Male bias in the incidence of cancer.
Male and female age-standardized incidence rates for 27 types of cancer per 100,000 people. Rates reported are worldwide numbers for the year 2020 (graph based on data from Sung *et al.*[59]). The dashed line represents equal incidence rates in males and females (i.e. no sex bias); points above the line are male-biased, while those below are female-biased. Note that the y-axis is artificially shortened to be able to include results for lung cancer incidence on the plot.

Male-biased aging

As with cancer, there is a clear sex bias in human aging, with females consistently living longer than males[68,69]. A higher mortality rate in males is present from birth and extends well into old age: only 10% of super-centenarians are male[68]. Lower longevity in males arises from many causes, with male bias in 14 of the top 15 causes of death in the United States[70] — only Alzheimer's disease has an age-adjusted death rate that is female biased.

There are multiple proposed mechanisms to explain sex differences in aging and senescence[69,71]. These mechanisms include differences in sex hormones, mitochondria, telomeres, epigenetic marks, proteostasis, cellular senescence, metabolism, immunological factors, and general genomic instability. The term 'genomic instability' covers many different types of mutations, and generally minimizes the role of point mutations, but is commonly invoked as a factor driving sex differences in aging[72]. On the other hand, there is now a large literature on the accumulation of somatic single-nucleotide variants with age, regardless of sex (see Ren *et al.*[73] for a review). Indeed, the somatic DNA damage theory of aging[74] posits that deleterious mutations occurring throughout a lifetime are a major determinant of mortality and senescence[75–77].

We propose that a faulty male soma contributes to male-biased aging. Somatic nucleotide mutations would not explain all differences in aging

between the sexes, but are perhaps one important contributor to faster aging and higher mortality in males. An additional intriguing link between somatic mutation and aging comes from a study that found reduced longevity in families with higher germline mutation rates[78]. If, as we have posited here, there is an underappreciated relationship between germline and somatic mutation rates, then the aging process may be amenable to study via more easily measured germline mutations.

Discussion and conclusions

Uncovering the molecular basis for evolution and disease is key to understanding the mechanisms driving both. Here, we have proposed that differences in germline mutation rates between the sexes are driven by 'faulty' males, i.e. the reduced ability of males to repair and/or protect germline cells from mutation. There are multiple lines of evidence that favor this model over the dominant germline-replication model, though germline differences may be explained by elements from both models acting in unison. More speculative is the proposal that the male soma is similarly faulty. While such a model could explain many aspects of male-biased cancer and aging, we do not yet have enough data to properly evaluate it relative to previously proposed explanations. If mutational mechanisms act very differently between the sexes, this would significantly impact our study of human health, influencing the diagnosis and treatment of congenital disease, fertility management, and our understanding of the aging process. Such differences may also change how we think about the processes driving evolution, especially the molecular basis for many evolutionary differences among species.

Given that we do not yet know the molecular basis underlying faulty males in either the germline or soma, it will be important to explore possible mechanisms. One intriguing possibility is found in the DREAM complex, a repressor of DNA repair known to be active in somatic tissues[79]. DREAM is a cell-cycle regulator that directly or indirectly increases the number of mutations in tissues where it is active, such that its inhibition restores germline-like mutation rates to somatic tissues[79]. An obvious potential mechanism for sex-biased mutation is therefore sex-biased expression of the DREAM complex: higher expression in male tissues would lead to higher mutation rates.

Regardless of the specific actors, as the sequencing of somatic tissues becomes more prevalent it will be imperative to ensure that future studies include sex as a biological variable. Currently, patient cohorts in such studies are not selected with sex-specific effects in mind, but this will be crucial for uncovering the sources of mutational differences between males and females. Similarly, studies of methylation and other epigenetic marks that may drive differences in mutation rates must be carried out in cells or tissues from both sexes: simply knowing the methylation state in one sex is insufficient for understanding associated phenotypic differences. Such studies may also help us to understand the source of male bias in cancers (Figure 3) and many other diseases. In carrying out research for this essay, it also became clear that there have been considerably more studies on DNA repair and packaging in human sperm than in human oocytes. While much of this difference could be due to the relative accessibility of each cell type, a fundamental understanding of the differences in germline mutation will require additional efforts in studying the oocyte, as well as germline stem cells in both males and females.

Uncovering the mechanisms — and evolutionary causes — underlying sex differences will also likely require a comparative approach, both among species and among types of mutations. Comparisons between species allow us to observe variation in many biological parameters that do not vary within humans (e.g. average age at puberty, average age at reproduction, and maximum lifespan) or that show a different pattern than in humans (e.g. male *Caenorhabditis elegans* live longer than hermaphrodites[68]). Comparative sequencing has also revealed the degree to which germline mutational sex bias varies among vertebrates[8]. While comparative somatic sequencing studies have only recently appeared[80], future work that includes both sexes from each species will be invaluable. Such studies may also provide independent tests of the correlations discussed here. For instance, birds exhibit a less male-biased germline mutation rate than humans[17], as well as little-to-no male bias in either aging[69] or cancer[81]. Finally, understanding the mechanisms driving male-biased nucleotide mutations will be helped by studying different types of mutations. In humans, small insertions and deletions show the same major patterns as nucleotide mutations[3]. In contrast, evidence suggests that larger structural variants are male biased[82], but these do not appear to be age-dependent in either humans or macaques[82,83]. Understanding the differences between these mutation types, and whether the same patterns appear in somatic tissues and across species, will help us to uncover the processes leading to male-biased mutation.

ACKNOWLEDGEMENTS

We thank Jeff Rogers, Josh Rubin, and three reviewers for helpful comments on the manuscript. This work was supported by National Institutes of Health grant R01-HD107120.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Haldane, J.B.S. (1947). The mutation rate of the gene for haemophilia, and its segregation ratios in males and females. Ann. Eugen. *13*, 262–271.
2. Goldmann, J.M., Wong, W.S., Pinelli, M., Farrah, T., Bodian, D., Stittrich, A.B., Glusman, G., Vissers, L.E., Hoischen, A., Roach, J.C., *et al.* (2016). Parent-of-origin-specific signatures of *de novo* mutations. Nat. Genet. *48*, 935–939.
3. Jónsson, H., Sulem, P., Kehr, B., Kristmundsdottir, S., Zink, F., Hjartarson, E., Hardarson, M.T., Hjorleifsson, K.E., Eggertsson, H.P., Gudjonsson, S.A., *et al.* (2017). Parental influence on human germline *de novo* mutations in 1,548 trios from Iceland. Nature *549*, 519–522.
4. Kong, A., Frigge, M.L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., Gudjonsson, S.A., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., *et al.* (2012). Rate of *de novo* mutations and the importance of father's age to disease risk. Nature *488*, 471–475.
5. Rahbari, R., Wuster, A., Lindsay, S.J., Hardwick, R.J., Alexandrov, L.B., Al Turki, S., Dominiczak, A., Morris, A., Porteous, D., Smith, B., *et al.* (2016). Timing, rates and spectra of human germline mutation. Nat. Genet. *48*, 126–133.
6. Wong, W.S., Solomon, B.D., Bodian, D.L., Kothiyal, P., Eley, G., Huddleston, K.C., Baker, R., Thach, D.C., Iyer, R.K., Vockley, J.G., and Niederhuber, J.E. (2016). New observations on maternal age effect on germline *de novo* mutations. Nat. Commun. *7*, 10486.
7. Bergeron, L.A., Besenbacher, S., Bakker, J., Zheng, J., Li, P., Pacheco, G., Sinding, M.-H.S., Kamilari, M., Gilbert, M.T.P., Schierup, M.H., and Zhang, G. (2021). The germline mutational process in rhesus macaque and its implications for phylogenetic dating. Gigascience *10*, giab029.
8. Bergeron, L.A., Besenbacher, S., Zheng, J., Li, P., Bertelsen, M.F., Quintard, B., Hoffman, J.I., Li, Z., St. Leger, J., Shao, C., *et al.* (2023). Evolution of the germline mutation rate across vertebrates. Nature *615*, 285–291.

9. Besenbacher, S., Hvilsom, C., Marques-Bonet, T., Mailund, T., and Schierup, M.H. (2019). Direct estimation of mutations in great apes reconciles phylogenetic dating. Nat. Ecol. Evol. *3*, 286–292.
10. Lindsay, S.J., Rahbari, R., Kaplanis, J., Keane, T., and Hurles, M.E. (2019). Similarities and differences in patterns of germline mutation between mice and humans. Nat. Commun. *10*, 4053.
11. Thomas, G.W.C., Wang, R.J., Puri, A., Harris, R.A., Raveendran, M., Hughes, D.S.T., Murali, S.C., Williams, L.E., Doddapaneni, H., Muzny, D.M., *et al.* (2018). Reproductive longevity predicts mutation rates in primates. Curr. Biol. *28*, 3193–3197.
12. Venn, O., Turner, I., Mathieson, I., de Groot, N., Bontrop, R., and McVean, G. (2014). Strong male bias drives germline mutation in chimpanzees. Science *344*, 1272–1275.
13. Wang, R.J., Pena-Garcia, Y., Bibby, M., Raveendran, M., Harris, R.A., Jansen, H.T., Robbins, C.T., Rogers, J., Kelley, J.L., and Hahn, M.W. (2022). Examining the effect of hibernation on germline mutation rates in grizzly bears. Genome Biol. Evol. *14*, evac148.
14. Wang, R.J., Raveendran, M., Harris, R.A., Murphy, W.J., Lyons, L.A., Rogers, J., and Hahn, M.W. (2022). *De novo* mutations in domestic cat are consistent with an effect of reproductive longevity on both the rate and spectrum of mutations. Mol. Biol. Evol. *39*, msac147.
15. Wang, R.J., Thomas, G.W.C., Raveendran, M., Harris, R.A., Doddapaneni, H., Muzny, D.M., Capitanio, J.P., Radivojac, P., Rogers, J., and Hahn, M.W. (2020). Paternal age in rhesus macaques is positively associated with germline mutation accumulation but not with measures of offspring sociability. Genome Res. *30*, 826–834.
16. Wu, F.L., Strand, A.I., Cox, L.A., Ober, C., Wall, J.D., Moorjani, P., and Przeworski, M. (2020). A comparison of humans and baboons suggests germline mutation rates do not track cell divisions. PLoS Biol. *18*, e3000838.
17. de Manuel, M., Wu, F.L., and Przeworski, M. (2022). A paternal bias in germline mutation is widespread in amniotes and can arise independently of cell division numbers. eLife *11*, e80008.
18. Wilson Sayres, M.A., Venditti, C., Pagel, M., and Makova, K.D. (2011). Do variations in substitution rates and male mutation bias correlate with life-history traits? A study of 32 mammalian genomes. Evolution *65*, 2800–2815.
19. Gao, Z., Moorjani, P., Sasani, T.A., Pedersen, B.S., Quinlan, A.R., Jorde, L.B., Amster, G., and Przeworski, M. (2019). Overlooked roles of DNA damage and maternal age in generating human germline mutations. Proc. Natl. Acad. Sci. USA *116*, 9491–9500.
20. Hurst, L.D., and Ellegren, H. (1998). Sex biases in the mutation rate. Trends Genet. *14*, 446–452.
21. Lynch, M. (2007). The Origins of Genome Architecture (Sunderland, MA: Sinauer Associates).
22. Jobling, M., Hollox, E., Hurles, M., Kivisild, T., and Tyler-Smith, C. (2014). Human Evolutionary Genetics (New York: Garland Science).
23. Strachan, T., and Read, A. (2018). Human Molecular Genetics, 5th edition (New York, NY: Garland Science).
24. Forster, P., Hohoff, C., Dunkelmann, B., Schürenkamp, M., Pfeiffer, H., Neuhuber, F., and Brinkmann, B. (2015). Elevated germline mutation rate in teenage fathers. Proc. R. Soc. B Biol. Sci. *282*, 20142898.
25. Drost, J.B., and Lee, W.R. (1995). Biological basis of germline mutation: comparisons of spontaneous germline mutation rates among Drosophila, mouse, and human. Environ. Mol. Mutagen. *25*, 48–64.
26. Luetjens, C.M., Weinbauer, G.F., and Wistuba, J. (2005). Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol. Rev. Camb. Philos. Soc. *80*, 475–488.
27. Scally, A. (2016). Mutation rates and the evolution of germline structure. Philos. Trans. R. Soc. Lond. B Biol. Sci. *371*, 20150137.
28. Ségurel, L., Wyman, M.J., and Przeworski, M. (2014). Determinants of mutation rate variation in the human germline. Annu. Rev. Genomics Hum. Genet. *15*, 47–70.
29. Bird, A.P. (1980). DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Res. *8*, 1499–1504.
30. Coulondre, C., Miller, J.H., Farabaugh, P.J., and Gilbert, W. (1978). Molecular basis of base substitution hotspots in *Escherichia coli*. Nature *274*, 775–780.
31. Duncan, B.K., and Miller, J.H. (1980). Mutagenic deamination of cytosine residues in DNA. Nature *287*, 560–561.
32. Young, K.A., and Nelson, R.J. (2001). Mediation of seasonal testicular regression by apoptosis. Reproduction *122*, 677–685.
33. Tsubota, T., Howell-Skalla, L., Nitta, H., Osawa, Y., Mason, J., Meiers, P., Nelson, R., and Bahr, J. (1997). Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear *Ursus americanus*. Reproduction *109*, 21–27.
34. Milholland, B., Dong, X., Zhang, L., Hao, X., Suh, Y., and Vijg, J. (2017). Differences between germline and somatic mutation rates in humans and mice. Nat. Commun. *8*, 15183.
35. Moore, L., Cagan, A., Coorens, T.H.H., Neville, M.D.C., Sanghvi, R., Sanders, M.A., Oliver, T.R.W., Leongamornlert, D., Ellis, P., Noorani, A., *et al.* (2021). The mutational landscape of human somatic and germline cells. Nature *597*, 381–386.
36. Abascal, F., Harvey, L.M.R., Mitchell, E., Lawson, A.R.J., Lensing, S.V., Ellis, P., Russell, A.J.C., Alcantara, R.E., Baez-Ortega, A., Wang, Y., *et al.* (2021). Somatic mutation landscapes at single-molecule resolution. Nature *593*, 405–410.
37. Mauvais-Jarvis, F., Merz, N.B., Barnes, P.J., Brinton, R.D., Carrero, J.-J., DeMeo, D.L., De Vries, G.J., Epperson, C.N., Govindan, R., and Klein, S.L. (2020). Sex and gender: modifiers of health, disease, and medicine. Lancet *396*, 565–582.
38. Bernabeu, E., Canela-Xandri, O., Rawlik, K., Talenti, A., Prendergast, J., and Tenesa, A. (2021). Sex differences in genetic architecture in the UK Biobank. Nat. Genet. *53*, 1283–1289.
39. Khramtsova, E.A., Davis, L.K., and Stranger, B.E. (2019). The role of sex in the genomics of human complex traits. Nat. Rev. Genet. *20*, 173–190.
40. Wang, S., Meyer, D.H., and Schumacher, B. (2023). Inheritance of paternal DNA damage by histone-mediated repair restriction. Nature *613*, 365–374.
41. Moritz, L., and Hammoud, S.S. (2022). The art of packaging the sperm genome: Molecular and structural basis of the histone-to-protamine exchange. Front. Endocrinol. *13*, 895502.
42. Broestl, L., and Rubin, J.B. (2021). Sexual differentiation specifies cellular responses to DNA damage. Endocrinology *162*, bqab192.
43. Wang, R.J., Al-Saffar, S.I., Rogers, J., and Hahn, M.W. (2023). Human generation times across the past 250,000 years. Sci. Adv. *9*, eabm7047.
44. Bajpayee, M., Dhawan, A., Parmar, D., Pandey, A.K., Mathur, N., and Seth, P.K. (2002). Gender-related differences in basal DNA damage in lymphocytes of a healthy Indian population using the alkaline Comet assay. Mutat. Res. *520*, 83–91.
45. Slyskova, J., Naccarati, A., Polakova, V., Pardini, B., Vodickova, L., Stetina, R., Schmuczerova, J., Smerhovsky, Z., Lipska, L., and Vodicka, P. (2011). DNA damage and nucleotide excision repair capacity in healthy individuals. Environ. Mol. Mutagen. *52*, 511–517.
46. Dunford, A., Weinstock, D.M., Savova, V., Schumacher, S.E., Cleary, J.P., Yoda, A., Sullivan, T.J., Hess, J.M., Gimelbrant, A.A., Beroukhim, R., *et al.* (2017). Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias. Nat. Genet. *49*, 10–16.
47. Oliva, M., Muñoz-Aguirre, M., Kim-Hellmuth, S., Wucher, V., Gewirtz, A.D.H., Cotter, D.J., Parsana, P., Kasela, S., Balliu, B., Viñuela, A., *et al.* (2020). The impact of sex on gene expression across human tissues. Science *369*, eaba3066.
48. Stewart, K.R., Veselovska, L., and Kelsey, G. (2016). Establishment and functions of DNA methylation in the germline. Epigenomics *8*, 1399–1413.
49. Kukurba, K.R., Parsana, P., Balliu, B., Smith, K.S., Zappala, Z., Knowles, D.A., Favé, M.-J., Davis, J.R., Li, X., Zhu, X., *et al.* (2016). Impact of the X chromosome and sex on regulatory variation. Genome Res. *26*, 768–777.
50. Bowes, H.M., Burdon, C.A., and Taylor, N.A.S. (2021). The scaling of human basal and resting metabolic rates. Eur. J. Appl. Physiol. *121*, 193–208.
51. Mittelstrass, K., Ried, J.S., Yu, Z., Krumsiek, J., Gieger, C., Prehn, C., Roemisch-Margl, W., Polonikov, A., Peters, A., Theis, F.J., *et al.* (2011). Discovery of sexual dimorphisms in metabolic and genetic biomarkers. PLoS Genet. *7*, e1002215.
52. Giacomoni, P.U., Mammone, T., and Teri, M. (2009). Gender-linked differences in human skin. J. Dermatol. Sci. *55*, 144–149.
53. Sasani, T.A., Pedersen, B.S., Gao, Z., Baird, L., Przeworski, M., Jorde, L.B., and Quinlan, A.R. (2019). Large, three-generation human families reveal post-zygotic mosaicism and variability in germline mutation accumulation. eLife *8*, e46922.
54. Meyerson, W., Leisman, J., Navarro, F.C.P., and Gerstein, M. (2020). Origins and characterization of variants shared between databases of somatic and germline human mutations. BMC Bioinformatics *21*, 227.
55. Li, C.H., Prokopec, S.D., Sun, R.X., Yousif, F., Schmitz, N., PCAWG Tumour Subtypes and Clinical Translation, Boutros, P.C., and PCAWG Consortium (2020). Sex differences in oncogenic mutational processes. Nat. Commun. *11*, 4330.
56. Podolskiy, D.I., Lobanov, A.V., Kryukov, G.V., and Gladyshev, V.N. (2016). Analysis of cancer genomes reveals basic features of human aging and its role in cancer development. Nat. Commun. *7*, 12157.
57. Li, C.H., Haider, S., Shiah, Y.-J., Thai, K., and Boutros, P.C. (2018). Sex differences in cancer driver genes and biomarkers. Cancer Res. *78*, 5527–5537.
58. Lopes-Ramos, C.M., Quackenbush, J., and DeMeo, D.L. (2020). Genome-wide sex and gender differences in cancer. Front. Oncol. *10*, 597788.
59. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. *71*, 209–249.
60. Jackson, S.S., Marks, M.A., Katki, H.A., Cook, M.B., Hyun, N., Freedman, N.D., Kahle, L.L., Castle, P.E., Graubard, B.I., and Chaturvedi, A.K. (2022). Sex disparities in the incidence of 21 cancer types: Quantification of the contribution of risk factors. Cancer *128*, 3531–3540.
61. Liu, Z., Yang, Q., Cai, N., Jin, L., Zhang, T., and Chen, X. (2019). Enigmatic differences by sex in cancer incidence: evidence from childhood cancers. Am. J. Epidemiol. *188*, 1130–1135.
62. Radkiewicz, C., Bruchfeld, J.B., Weibull, C.E., Jeppesen, M.L., Frederiksen, H., Lambe, M., Jakobsen, L., El-Galaly, T.C., Smedby, K.E., and Wästerlid, T. (2023). Sex differences in lymphoma incidence and mortality by subtype: A population-based study. Am. J. Hematol. *98*, 23–30.
63. Clocchiatti, A., Cora, E., Zhang, Y., and Dotto, G.P. (2016). Sexual dimorphism in cancer. Nat. Rev. Cancer *16*, 330–339.
64. Dorak, M.T., and Karpuzoglu, E. (2012). Gender differences in cancer susceptibility: an inadequately addressed issue. Front. Genet. *3*, 268.
65. Rubin, J.B. (2022). The spectrum of sex differences in cancer. Trends Cancer *8*, 303–315.
66. Cardano, M., Buscemi, G., and Zannini, L. (2022). Sex disparities in DNA damage response

pathways: Novel determinants in cancer formation and therapy. iScience *25*, 103875.
67. Rall-Scharpf, M., Friedl, T.W.P., Biechonski, S., Denkinger, M., Milyavsky, M., and Wiesmüller, L. (2021). Sex-specific differences in DNA double-strand break repair of cycling human lymphocytes during aging. Aging *13*, 21066–21089.
68. Austad, S.N., and Fischer, K.E. (2016). Sex differences in lifespan. Cell Metab. *23*, 1022–1033.
69. Bronikowski, A.M., Meisel, R.P., Biga, P.R., Walters, J.R., Mank, J.E., Larschan, E., Wilkinson, G.S., Valenzuela, N., Conard, A.M., de Magalhães, J.P., *et al.* (2022). Sex-specific aging in animals: Perspective and future directions. Aging Cell *21*, e13542.
70. Xu, J., Murphy, S.L., Kochanek, K.D., and Arias, E. (2021). Deaths: Final data for 2019. Natl. Vital. Stat. Rep. *70*, 1–87.
71. Hägg, S., and Jylhävä, J. (2021). Sex differences in biological aging with a focus on human studies. eLife *10*, e63425.
72. Fischer, K.E., and Riddle, N.C. (2018). Sex differences in aging: genomic instability. J. Gerontol. Series A *73*, 166–174.
73. Ren, P., Dong, X., and Vijg, J. (2022). Age-related somatic mutation burden in human tissues. Front. Aging *3*, 1018119.
74. Jin, K. (2010). Modern biological theories of aging. Aging Dis. *1*, 72–74.
75. Kinzina, E.D., Podolskiy, D.I., Dmitriev, S.E., and Gladyshev, V.N. (2019). Patterns of aging biomarkers, mortality, and damaging mutations illuminate the beginning of aging and causes of early-life mortality. Cell Rep. *29*, 4276–4284.
76. Schumacher, B., Pothof, J., Vijg, J., and Hoeijmakers, J.H.J. (2021). The central role of DNA damage in the ageing process. Nature *592*, 695–703.
77. Vijg, J., and Dong, X. (2020). Pathogenic mechanisms of somatic mutation and genome mosaicism in aging. Cell *182*, 12–23.
78. Cawthon, R.M., Meeks, H.D., Sasani, T.A., Smith, K.R., Kerber, R.A., O'Brien, E., Baird, L., Dixon, M.M., Peiffer, A.P., Leppert, M.F., *et al.* (2020). Germline mutation rates in young adults predict longevity and reproductive lifespan. Sci. Rep. *10*, 10001.
79. Bujarrabal-Dueso, A., Sendtner, G., Meyer, D.H., Chatzinikolaou, G., Stratigi, K., Garinis, G.A., and Schumacher, B. (2023). The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. Nat. Struct. Mol. Biol. *30*, 475–488.
80. Cagan, A., Baez-Ortega, A., Brzozowska, N., Abascal, F., Coorens, T.H.H., Sanders, M.A., Lawson, A.R.J., Harvey, L.M.R., Bhosle, S., Jones, D., *et al.* (2022). Somatic mutation rates scale with lifespan across mammals. Nature *604*, 517–524.
81. Kapsetaki, S.E., Compton, Z., Dolan, J., Harris, V.K., Rupp, S.M., Duke, E.G., Harrison, T.M., Aksoy, S., Giraudeau, M., Vincze, O., *et al.* (2023). Life history and cancer in birds: clutch size predicts cancer. Preprint at bioRxiv, https://doi.org/10.1101/2023.02.11.528100.
82. Belyeu, J.R., Brand, H., Wang, H., Zhao, X., Pedersen, B.S., Feusier, J., Gupta, M., Nicholas, T.J., Brown, J., Baird, L. *et al.* (2021). *De novo* structural mutation rates and gamete-of-origin biases revealed through genome sequencing of 2,396 families. Am. J. Hum. Genet. *108*, 597–607.
83. Thomas, G.W.C., Wang, R.J., Nguyen, J., Harris, R.A., Raveendran, M., Rogers, J., and Hahn, M.W. (2021). Origins and long-term patterns of copy-number variation in rhesus macaques. Mol. Biol. Evol. *38*, 1460–1471.

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Q & A

Ykä Helariutta

Ykä Helariutta did his MSc and PhD from 1990 to 1995 at the University of Helsinki with Teemu Teeri. He then performed his postdoctoral training from 1995 to 1998 at New York University (also in affiliation with the Lewis B. and Dorothy Cullman Program at the New York Botanical Garden) with Philip Benfey. He established his own group in 1999 back at the University of Helsinki, where he became a Full Professor in 2005. Between 2014 and 2022 he acted as a Professor of Plant Developmental Biology at the Sainsbury Laboratory, University of Cambridge, before returning full time to his position at the University of Helsinki last year.

How did you become interested in plant science? I developed the spirit of a collector quite early. I started my own herbarium when I was around 11 and kept supplementing it every summer, when visiting various locations in Finland. The diversity of plants and the details of their anatomy fascinated me. Besides vascular plants, I also collected lichens — a botanical hobby compatible with the long Finnish winters. It thus felt quite natural to deepen my understanding of plants and study botany. While at university in the late 1980s I realized that my artistic skills were not compatible with a career in taxonomy, so I turned to genetics, which provided a different opportunity for a collector in the form of new gene functions.

You are a specialist in plant vascular biology. How did you define your focus? For my PhD I had the chance to work on flower development in *Gerbera*. After this experience I started to contemplate a research program on wood development, as wood is rich and economically significant in Finland. Through reading the literature, and after attending various meetings, I had also become familiar with *Arabidopsis* as a genetic model. Then I had the chance to join the Benfey laboratory, one of the leading labs on plant morphogenesis through their work on root development in *Arabidopsis*. At the time, it was situated in the vibrant Greenwich Village section of Manhattan. After my *Gerbera* work, with its emphasis on plant molecular biology rather than genetics, I remember thinking that the first Benfey lab meetings were rich in genetic vocabulary. It felt like learning to walk again. But it paid off. In the Benfey lab I had the opportunity to participate in one of its mainstream projects: understanding the mechanisms of patterning in plant tissue. After having first worked during my PhD on an emerging model and then becoming immersed in *Arabidopsis* genetics, I had developed a good skill set for initiating my independent research program on wood development by combining *Arabidopsis* genetics and transgenic work on *Populus*.

Why did you make a lengthy visit to the Sainsbury Laboratory? During the early years in my own lab we made steady progress on understanding genetic mechanisms governing vascular morphogenesis in the *Arabidopsis* root. We started to contribute conceptually on how plant hormones regulate the transcriptional networks governing vascular development. It was inspiring as many extremely talented young scientists visited the group, and we uncovered many new concepts on various cellular aspects, such as