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Mother's curse neutralizes natural selection against a human genetic disease over three centuries

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According to evolutionary theory, mitochondria could be poisoned gifts that mothers transmit to their sons. This is because mutations harmful to males are expected to accumulate in the mitochondrial genome, the so-called 'mother's curse'. However, the contribution of the mother's curse to the mutation load in nature remains largely unknown and hard to predict, because compensatory mechanisms could impede the spread of deleterious mitochondria. Here we provide evidence for the mother's curse in action over 290 years in a human population. We studied a mutation causing Leber's hereditary optical neuropathy, a disease with male-biased prevalence and which has long been suspected to be maintained in populations by the mother's curse. Male carriers showed a low fitness relative to non-carriers and to females, mostly explained by their high rate of infant mortality. Despite poor male fitness, selection analysis predicted a slight (albeit non-significant) increase in frequency, which sharply contrasts with the 35.5% per-generation decrease predicted if mitochondrial DNA transmission had been through males instead of females. Our results are therefore even suggestive of positive selection through the female line that may exacerbate effects of the mother's curse. This study supports a contribution of the mother's curse to the reduction of male lifespan, uncovering a large fitness effect associated with a single mitochondrial variant.

In most species maternal inheritance of mitochondrial DNA (mtDNA) is the norm[1]. This implies that natural selection should not restrict the transmission of mtDNA mutations that reduce the fitness of males, but are neutral or advantageous in females[2], leading to intersexual intragenomic conflicts[3]. In principle, deleterious mutations could accumulate in the mitochondrial genome at high cost to males, limiting their fecundity, health and lifespan[4–6]. This selective sieve, known as the mother's curse[7] could be a widespread phenomenon shaping male life history and fitness, whenever selection acting on mtDNA genes is asymmetric between the sexes[4].

As indisputable as this logic may seem[2], several factors may impede the build-up of such a male-specific load or counteract its adverse effects. These include mitonuclear epistasis, paternal leakage of mitochondria during fertilization and the purging of harmful mutations through selection through female lines[8–13]. In theory, mitonuclear epistasis can have large effects on the evolutionary rate of mutations compensating for adverse mitochondrial effects in males[14]. Purging can occur when non-random mating (that is, assortative mating or inbreeding) brings together partners sharing both their mitotype and (part of) their fitness. When epistasis and non-random mating interact, they can strongly limit genomic conflicts[14]. Kin selection can also act to purge male-deleterious mitochondrial variants, for instance when males help their sisters and contribute to their fitness. It has also been suggested that selection should have favoured mitochondrial symbionts with limited capacity to develop mutations harmful to males, either during the evolution of vertical (maternal) transmission or as a result of competitive coexistence with free-living pathogens[13].

These possible mechanisms for the attenuation or reversal of the mother's curse remain to be fully tested, but some evidence exists for the impact of the mother's curse in evolution. Experiments on captive animals have identified specific mitochondrial lines associated with faster ageing or lower fecundity[15,16], some showing a strong male bias[17,18]. Perhaps the best evidence for the cumulative effect of the mother's curse comes from a previously published transcriptome study[19]. In this study, it was predicted that the male bias in the mitochondrial mutation load should be stronger for traits exhibiting greater sexual dimorphism (for which the two sexes have different optima) and be apparent in the expression of nuclear genes, because the effects of mtDNA variation on traits is mostly mediated through interactions with the nuclear genome[19]. They show that naturally occurring specific mitochondrial lines in *Drosophila melanogaster* are associated with sex asymmetry in nuclear gene expression: mtDNA variation had little impact in females but modified the expression of approximately 10% of nuclear genes in males, with a particular bias in favour of genes that are known to correlate with fitness and genes for which the expression is greater in tissues involved in male-specific functions, such as testes.

These studies support the cumulative exposition to effects of the mother's curse during evolutionary history, but did not measure selection per se (although some experimental studies have used artificial selection to document sex-specific mtDNA effects on life

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history (for example, ref. [12])). Therefore, the reported patterns may not mirror sex-specific differences in fitness, as the two sexes may have different optima for traits such as lifespan[20], so that the values associated with the highest fitness may differ between the sexes. Yet, traits that exhibit sex-specific optima should be exposed the most to the mother's curse, because they are exactly those for which natural selection on mtDNA cannot optimize values in males[19]. This underscores the importance of having individual fitness data to assess whether different mitochondrial lines are equally adaptive in both sexes.

mtDNA has a major role in complex diseases, in particular metabolic and neurodegenerative diseases[21]. Evolutionary theory has suggested that male-biased disruptions of healthy mitonuclear interactions contribute to disease expression[22]. Leber's hereditary optical neuropathy (LHON) is the paradigmatic example put forward to uphold the theory of the mother's curse owing to its unusual gender-biased penetrance[2,11,23,24]. LHON causes degeneration of the optic nerve, inducing bilateral vision loss, with typical onset at teenage or adult age[25,26], although symptoms may occur at earlier ages[27,28]. This disease is caused by mutations in mitochondrial genes encoding the respiratory chain[26] (Supplementary Fig. 1). However, whether LHON affects fitness or did so in the past and, consequently, truly qualifies as a disease subject to the mother's curse is unknown. In fact, a study[29] recently argued against the idea of a fitness cost to males by pointing out that there was a lack of reports of reduced male fertility in "male patients with pathogenic mtDNA mutations, such as LHON". Using individual fitness data for a modern human population since its foundation, here we show the mother's curse 'in action' and its impact on the fate of an mtDNA mutation that causes LHON.

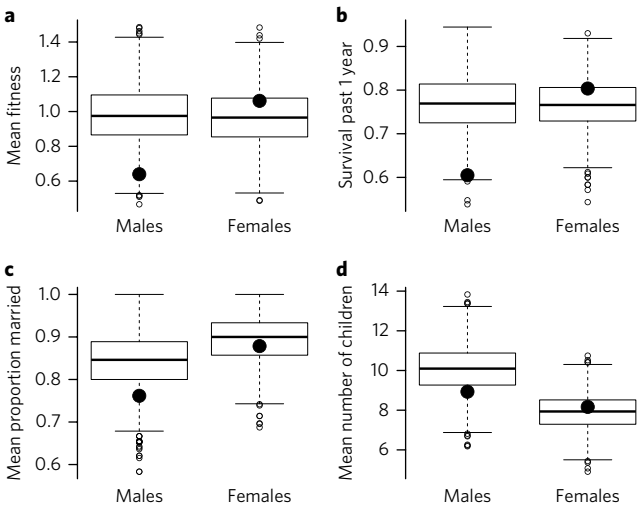

Fig. 1 | Fitness components in 97 individuals carrying the T14484C mitochondrial variant in the French-Canadian population of Québec between 1670 and 1750. a–d, Relative fitness (**a**) and infant survival (**b**) (in both cases $n=52$ females and 38 males with known death date), proportion married (**c**) ($n=33$ women and 21 men who survived to maturity) and fertility or number of children ever born (**d**) ($n=29$ married women and 16 married men). Dots show average trait values for male and female carriers, respectively; box plots illustrate the distribution of mean trait in 1,000 randomly drawn sets of matched non-carriers in males and females, respectively. See Methods for a description of box plots.

Results

During the 17th century, an immigrant woman introduced LHON to New France (now Québec). This woman was among the commoners known as the 'Filles du Roy', sent by the King of France as brides to compensate for the unbalanced sex ratio in the French colony[30]. Analysis of ascending genealogies of present day LHON patients has previously shown that one of the Filles du Roy carried the T14484C mutation[31], one of the three main mutations causing LHON[26] (Supplementary Fig. 1). She got married in 1669 and subsequently gave birth to ten children, of which six were daughters. Nowadays, the T14484C mutation is the most common among affected French-Canadians, contributing 89% of cases in the overall Québec population. The expression of LHON is strongly gender-biased, with eight males affected for every one female among modern T14484C carriers in Québec[25]. Age at onset (median in 43 males = 19 years, range = 6–48) and penetrance varies greatly among carriers, with effects ranging from mild to severe vision loss[25].

Genealogical imputation of T14484C. We used two datasets to document the temporal trajectory of T14484C in the population. The 'Registre de population du Québec ancien' (RPQA, or Early Québec Population Register)[32] contains the complete life history of French-Canadians born in Québec between the foundation of New France (1608) and 1800 ($n=755,575$). The BALSAC register contains record of French-Canadians married between 1621 and 1960 (n = around 5 million individuals; http://balsac.uqac.ca). Genealogical analysis using the RPQA allowed us to impute T14484C to all 190 maternal line descendants of the Fille du Roy that were born between 1670, that is, the birth year of her first child, and 1775 (see Methods and Supplementary Table 1). BALSAC data allowed us to impute T14484C to 2,038 maternal line descendants of the Fille du Roy that were married between 1670 and 1960.

Selection on the LHON-causing mutation. We measured natural selection on T14484C in males and females in the French-Canadian population. RPQA data contains information about complete family size including married and non-married offspring born in Québec at least up to 1750, that allows calculation of their relative fitness[33] ($n=120,998$; see Methods). Average fitness for the entire population was higher for females (mean relative fitness ($\overline{w}$) = 1.10) than males ($\overline{w}=0.98$), and much lower for male carriers than non-carriers (see below). The difference between sexes is explained by the fact that our fitness parameter integrates reproductive timing and, in New France, the average age at marriage was several years earlier for women than men[34] (a male-biased sex ratio also contributed to the sex difference in mean fitness). The importance of fitness differences between carriers ($n=97$) and non-carriers ($n=120,901$ available for resampling; see Methods) was evaluated using a matching and resampling procedure controlling for birth year and birth location. The fitness of female carriers was higher ($\overline{w}=1.06$) than that of matched female non-carriers ($\overline{w}=0.97$), indicating no negative selection for T14484C among females (Fig. 1a; $P=0.7$), but rather a slight, albeit non-significant, positive selection. By contrast, the fitness of male carriers was only 65.3% that of male non-carriers ($\overline{w}_{\text{carriers}}=0.64$, $\overline{w}_{\text{non-carriers}}=0.97$, $P=0.03$; Fig. 1a), indicating strong selection against the variant. The corresponding selection coefficients predicted a 2.7% per-generation increase in T14484C frequency due to the female transmission of mtDNA (Table 1). This is in sharp contrast to the 35.5% predicted decrease if transmission had been through male lines instead (Table 1).

Effect of the mutation on fitness components. Remarkably, we found that the fitness cost to male carriers was mostly mediated through a strong association between T14484C and infant mortality. This was unexpected, considering the typical age of onset of LHON. Survival between 0 and 1 years old was 20.8% lower in male carriers (mean = 0.61) than non-carriers (mean = 0.77, $P=0.004$; Fig. 1b). This pattern holds when we extended the infant mortality analysis to 186 carriers born up to 1775 (after excluding 4 of unknown sex; Methods, Supplementary Information and Supplementary Fig. 2).

Table 1 | Natural selection on the mitochondrial variant T14484C causing the Leber's hereditary optical neuropathy

	Model*		Males			Females		
Life stage	***S***	**ΔP**	***S***	**ΔP**	**$100\times\Delta P/P_0$**	***S***	**ΔP**	**$100\times\Delta P/P_0$**
Infant	$S_{inf}=P_0(1-P_0)(\overline{w}_c-\overline{w}_{nc})$	$\Delta P_{inf\to juv}=S_{inf}-S_{juv}$	-2.84×10^{-4}	-1.66×10^{-4}	−20.8%	2.13×10^{-5}	3.11×10^{-5}	3.9%
Juvenile	$S_{juv}=P_{juv}\left(1-P_{juv}\right)(\overline{w}_c-\overline{w}_{nc})f_{inf}$	$\Delta P_{juv\to mar}=S_{juv}-S_{mar}$	-1.18×10^{-4}	-6.03×10^{-5}	−7.5%	-9.88×10^{-6}	-1.87×10^{-5}	−2.3%
Married	$S_{mar}=P_{mar}(1-P_{mar})(\overline{w}_c-\overline{w}_{nc})f_{inf}f_{juv}$	$\Delta P_{mar\to off}=S_{mar}$	-5.73×10^{-5}	-5.73×10^{-5}	−7.2%	8.78×10^{-6}	8.78×10^{-6}	1.1%
Total selection	$S_{total}=P_0(1-P_0)(\overline{w}_c-\overline{w}_{nc})S_{inf}$	$\Delta P_{total}=\Delta P_{inf\to juv}+\Delta P_{juv\to mar}+\Delta P_{mar\to off}=S_{inf}$	-2.84×10^{-4}	-2.84×10^{-4}	−35.5%	2.13×10^{-5}	2.13×10^{-5}	2.7%

Selection acting through the two sexes was measured as the genetic covariance between carrier status and relative fitness. Carrier frequency in the parental generation (P_0) was set at the highest value (0.0008) observed between 1670 and 1775. The selection (S) acting independently on each life-history stage, as well as total selection, are reported along with the corresponding change (ΔP) in T14484C frequency expected between two life-history stages (or per generation for total selection). The per generation (total) change through males is, however, only theoretical, because mtDNA is transmitted only by mothers. The percentage change in carrier frequency relative to the parental generation ($100\times\Delta P/P_0$) is also shown. Subscripts indicate the stage to which parameters apply, that is, the infant ('inf'; 0–1-year-old), juvenile ('juv') or married ('mar') stage. Fitness values used in the model were averages for carriers ($\overline{w}_c$) and matched non-carriers ($\overline{w}_{nc}$) who survived up to the beginning of a given stage. *Carrier frequency (P) refers to that prior to selection, that is, at the beginning of a given stage; f_{inf} is the fraction of infants born surviving to become a juvenile, f_{juv} is the fraction of juveniles who marry. Selection coefficients for each stage do not sum up to total selection, because the covariance sums up the effects of current and future stage-specific selection.

Independent selection at the infant stage contributed most of the total predicted per-generation decrease in T14484C frequency, had the transmission been through male instead of female lines (20.8% out of 35.5%; Table 1). Such a population-level association between a LHON-causing mutation and infant mortality is unknown in the medical literature (see Discussion).

Conditional on their survival to age 1, male carriers also tended to have a lower probability of getting married (mean = 0.74 and 0.86 in carriers and non-carriers, respectively, $P=0.1$; Fig. 1c) and, for those who married, to produce fewer children (mean fertility = 8.9 and 10.1 in carriers and non-carriers, respectively, $P=0.2$; Fig. 1d). Although these two differences are not significant when taken individually, which may plausibly reflect the reduced 'sample' size for male carriers surviving to maturity, they nonetheless contribute to lower their overall fitness. Independent selection in males at the juvenile stage, as well as through fertility in married individuals, both contributed about one fifth of the total predicted per-generation decrease in frequency (around 7% out of 35.5%; Table 1).

In contrast to males, the survival rate of female carriers was not smaller (mean = 0.80, $P=0.7$; Fig. 1b) than that of non-carriers (mean = 0.77). Female carriers married at a slightly lower proportion than non-carriers, but those who did had a slightly higher fertility, albeit these differences were not significant (Fig. 1c,d and Table 1). Again, the exact numbers obtained for these stages should be interpreted with caution, some differences were not significant but nevertheless contributed to the overall selection as measured from fitness. And as mentioned above, we have data for all known carriers in the population but, in absolute numbers, few carriers were left once viability selection at the infant stage had taken place.

The association between T14484C and infant mortality did not result from the potentially confounding effect of biodemographic factors that are known to be related with survival in this population, such as birth rank or mother's age at birth[35,36], or variation in death registration consistency (Supplementary Information, Supplementary Table 2 and Supplementary Figs. 3–5). Likewise, recurrent disease outbreaks (for example, measles, smallpox) in preindustrial societies might have affected the sexes differentially (for example, ref. [37]). However, the robustness of our results is insured by our matching procedure and was confirmed by a separate analysis controlling for disease outbreaks (Supplementary Information, Supplementary Table 3 and Supplementary Fig. 3). Finally, the strong selection against male carriers was still detected when not controlling at all for birth year and locality (albeit not the positive selection in females; Supplementary Table 4).

Consequences for the sex ratio in carrier lineages. We used the BALSAC data to test whether selection on T14484C continued beyond 1750. However, because total fitness could not be calculated from this dataset owing to the absence of information on infant mortality and non-married offspring (hence on full fertility), we adopted a different approach. If the effect of the mutation on fitness lasted beyond 1750 (or beyond 1775 for infant survival), we predicted that the ratio of the number of married men over the total number of married individuals would be lower in the carrier lineage

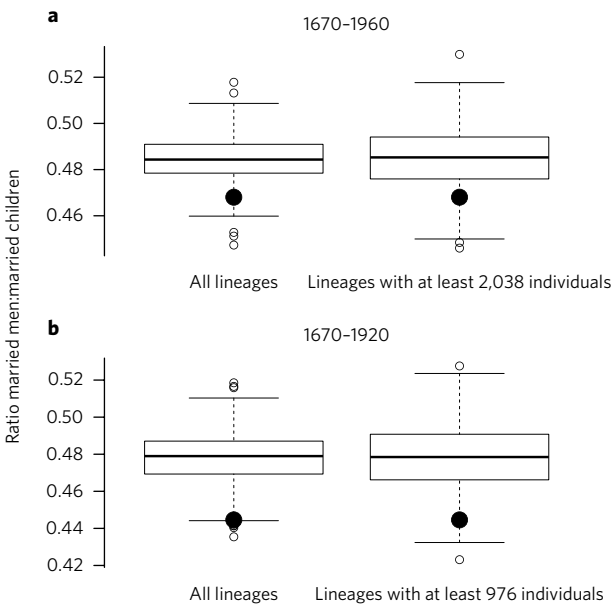

Fig. 2 | Sex ratio of married individuals in French-Canadian lineages between 1670 and 1960. Plots show the distribution across all lineages or lineages with the same number of individuals as the T14484C carrier lineage. Solid dots indicate the proportion in the T14484C carrier lineage. **a**, Lineages for the full 1670–1960 period. **b**, Lineages for the 1670–1920 period. Sample sizes are as follow: 1670–1960: 3,815,905 married individuals from 279,485 lineages (and 2,448,292 individuals from 364 lineages having at least 2,038 individuals each); 1670–1920: 1,901,977 married individuals from 203,001 lineages (and 1,253,242 individuals from 399 lineages having at least 976 individuals each). See Methods for a description of box plots.

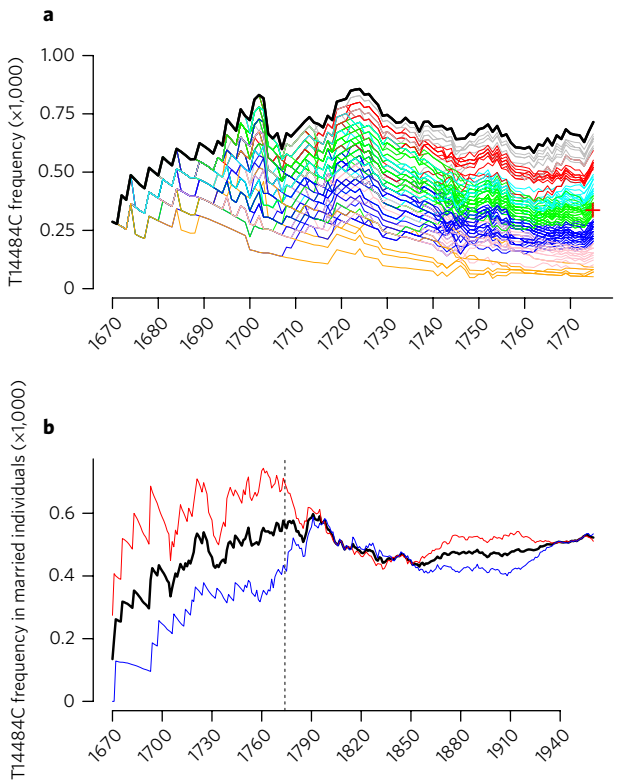

Fig. 3 | Change in the frequency of the LHON causing T14484C variant between 1670 and 1775 (RPQA data) and between 1670 and 1960 (BALSAC data). a, RPQA data (1670–1775). **b**, BALSAC data (1670–1960). The thick black line illustrates the observed frequency (based on 185 and 2,038 carriers in **a** and **b**, respectively). **a**, Thin coloured lines show trajectories of the expected frequency without an effect of the mother's curse (that is, if selection was as strong in females as in males). These expected trajectories were estimated from 1,000 simulations (100 trajectories randomly selected for illustrative purposes). **b**, The red and blue lines show the LHON mutation frequency in married females and males, respectively, and the vertical dotted line indicates the end of the period covered in **a**. The mean frequency in 1775 among the 1,000 simulations is indicated by the red cross. In both panels the initial increases in T14484C frequency is due to the reproduction of the woman who first introduced the mutation. Frequency calculations with RPQA data are based on 243,231 individuals born between 1670 and 1775 and with known birth date. Frequency calculations with BALSAC data are based on 3,803,643 individuals married between 1670 and 1960 and assumed to be alive at a given year (by assigning them the average age at marriage and lifespan after marriage for married people in the historical French-Canadian population).

(1,084 married women, 954 married men) than in other lineages. This was the case, whether we compared the T14484C lineage to all other remaining lineages or only to those lineages that have each left at least as many descendants as the T14484C lineage (Fig. 2). We also looked at the sex ratio pattern when excluding the period beyond 1920, as about half of T14484C carriers were born after 1920 and plausibly experienced better health care than the other half with the advent of modernization. Supporting this hypothesis, the difference between carriers and non-carriers was greater for the 1670–1920 period than for the broader 1670–1960 period (Fig. 2).

Temporal trend in the frequency of the LHON-causing mutation. The frequency of T14484C did not decrease in response to the strong negative effect of the mutation on male fitness, which is the exact definition of the mother's curse. After its introduction by the Fille du Roy and subsequent increase due to her reproduction, T14484C frequency oscillated between 0.6 and 0.8 per thousand living individuals (of all ages), ending at a value of 0.73 in 1775 (RPQA data, Fig. 3a). To assess the decrease that is expected for T14484C in the absence of the effect of the mother's curse, we performed simulations assuming that the allele was equally deleterious in females as in males. While the simulated trajectories of T14484C show notable variation due to stochasticity (Fig. 3a), the expected frequency of the mutation in 1775 was on average less than half as high (mean ± s.d. of 0.33 ± 0.15 per thousand individuals) as the observed frequency. This indicates that a fast purging of the genetic variant would be expected without the effect of the mother's curse. The trend observed with the marriage records from BALSAC data clearly shows that T14484C was maintained in the French-Canadian population at least up to 1960 (Fig. 3b).

Between 1670 and 1790, T14484C frequency in BALSAC data was higher in women than men, consistent with the poor survival of male carriers detected with RPQA data. Likewise, T14484C frequency was also higher in married women than men between 1850 and 1930 (Fig. 3b), suggesting that the mother's curse continued through the 19th and 20th centuries. This is also consistent with the biased sex ratio of married carriers (Fig. 2). The mother's curse was perhaps not constant, though, since for two other time periods (1790–1850 and 1930–1960), T14484C frequency in both sexes is nearly identical (and even slightly higher in married males for some years (for example, 1830; Fig. 3b). Finally, the lack of a clear sex difference in T14484C frequency after 1930 suggests that there was a decrease in the mortality of male carriers with the improvement of health care.

Reinforcement of the mother's curse through the female lineage. There was a tendency for female carriers to have a higher fitness than matched non-carriers, mainly owing to their higher 0–1 year survival (Fig. 1b, Table 1 and Supplementary Fig. 2). Statistical analyses do not allow us to reject the null hypothesis of no intrinsic (for example, biological) differences in survival between these two groups ($P = 0.23$ for the 1670–1750 period and $P = 0.1$ for the 1670–1775 period). Nevertheless, this pattern deserves some discussion, because the fitness difference observed here is based on the whole population of carriers, not a sample of them (that is, we used the full genealogy of the population to identify them). Consequently, these female carriers constitute the total pool of individuals that determined the fate of T14484C across generations. Simulating a survival rate of female carriers identical to that of non-carrier females resulted in a 22% lower expected frequency of T14484C after 105 years than the one observed (5.7×10^{-4} instead of 7.3×10^{-4}; Supplementary Fig. 6). Therefore, notwithstanding whether the higher survival of female carriers is the mere effect of one random realization of a population of carriers that is not fundamentally different from the remaining female population, or was owing to some undetected intrinsic difference with non-carriers (implying an intersexual genetic conflict), the better realized survival of female carriers appears to have exacerbated effects of the mother's curse. This may partly explain another pattern in the results. Between 1730 and 1775, T14484C frequency was relatively stable, if not decreasing, in the total population when calculated from RPQA data, thus including some infants that were deemed to die before the end of their first year, but increases in the marriage records from BALSAC, that is, after selection (Fig. 3). The better survival of female carriers relative to non-carrier females may have contributed to this increase.

Potential effects of assortative mating and kin selection. One mechanism by which the effect of the mother's curse might be partially alleviated is when carriers mate together in a greater proportion than expected by chance, either through assortative mating or inbreeding[8,9,38]. This could cause an association between mitotype and fitness in females, allowing a response to selection through

females. From mid-19th century onwards, there was an increasing departure from expectations for the frequency of assorted (two-carrier) mating (Supplementary Fig. 7). However this must be interpreted with caution, because the trend is explained by the observation of one or a few assorted mating for a few given time windows, when none are actually expected. Moreover, this purging mechanism is only effective with fecundity and not viability selection (see ref. [13]), whereas the association of T14484C with male fitness found here was mostly mediated though their lower viability. While kin selection could induce a female response to viability selection in male carriers, it must have had a limited effect on T14484C frequency: applying our parameter estimates to Wade's[13] model without accounting for sibling competition suggests that female carriers would have to lose approximately 10% of their fitness in that way for the frequency of T14484C to decrease by at least around 1% of its average value (approximately 0.0007) per generation.

Discussion

Our study shows that the mother's curse has contributed to the maintenance of a deleterious mitochondrial variant over centuries in a human population. Typically, the mother's curse is hypothesized as an accumulating load of mutations decreasing fecundity or accelerating senescence in male adults[4]; here we show an effect on infant mortality. Because infant mortality has a strong impact on fitness (as individuals dying before sexual maturity cannot leave any descendants), the mother's curse on a single mitochondrial mutation had a remarkably high impact on the fitness of male carriers in this population.

A major and unexpected finding of this study is the association between T14484C and infant mortality. Some patients developing LHON show other anomalies such as arrhythmia[39], hearing loss[27], spinal cord impairment[40], encephalopathy (the latter two in T14484C carriers[27]) and other cardiac and neurological symptoms[23,41]. These so-called 'Leber's plus' phenotypes[42] could result in death in some cases[43]. A report from the 1960s exists of three child deaths (ages unspecified) in a LHON-affected lineage in Australia[43]. A possible explanation for infant mortality in early Québec carriers is the development of Leigh syndrome. This normally fatal neurogenerative disease has been documented in a few modern individuals carrying various LHON-causing mutations and was the reported cause of death of a few (less than 5) children, including two infants under the age of 1 (ref. [44]). However, the strong population-level effect uncovered here was unsuspected and raises intriguing possibilities: (i) that T14484C continues to cause important health problems in young males, unbeknownst to medical practitioners; (ii) that gene–environment interactions underlie the mortality phenotype and the phenotype has disappeared with changing environment and health care, a hypothesis supported here by the patterns observed in married individuals; or (iii) that selection on compensatory nuclear genes has successfully counteracted this effect over the last several hundred years. Regarding this last possibility, a model of nuclear–mitochondrial epistasis[14] applied to our data indicates that it would require more than 150 generations for a nuclear variant that has a fully compensatory effect to reach fixation, a timescale an order of magnitude greater than that of our study.

One limitation of our study is that we cannot completely exclude that the T14484C variant was introduced more than once in the population. If so, other carrier lineages are likely to be extinct nowadays, otherwise they would presumably have been detected in modern LHON patients. Therefore, the relative fitness in these undetected, less successful carrier lineages (if they existed) was possibly lower than that of the single extant lineage that was detected, leading us to overestimate the fitness of carriers and making our results conservative. To avoid this problem we could have included in our analyses only those non-carriers belonging to lineages that were extant at the time when probands where genotyped, but we chose not to do so as we identified potential issues with the representativeness and interpretation of such a comparison. Finally, several genetic factors could modify the expression of LHON in T14484C carriers (Supplementary Fig. 1). Heteroplasmy, that is, intracellular variation in mitochondrial genomes, is known to affect the penetrance of LHON[45,46], although it has not been detected in T14484C carriers in Québec[25]. The mtDNA haplogroup J hosting T14484C in Québec carriers is known to be associated with a greater penetrance of LHON[47]. Therefore, other variants at unmeasured mitochondrial genes may contribute to the low male fitness in T14484C carriers and, consequently, to the mother's curse.

Wade[13] concluded that "despite the indisputable evolutionary logic of [the mother's curse] there are no reported cases of sperm-killing or son-killing mitochondria". Here we provide evidence for what could well be the first known son-killing mitochondria. This takes on a singular interest given the birth in 2016 of the first baby (a boy) carrying DNA from three parents, having received a mtDNA donation (that is, 'mitochondrial replacement') to avoid inheriting the Leigh syndrome from his mother[48]. It is worth mentioning here that evolutionary models related to sex biases in mitochondrial disease expression have met with scepticism from part of the medical community (including for LHON) within the debate surrounding mitochondrial replacement[29]. However, as shown here, evolutionary approaches can provide insights into the prevalence of genetic diseases in modern populations, because Darwinian fitness is ultimately the important parameter determining the demographic fate of genetic variants across generations, not their clinical symptoms per se.

Methods

Data. We used family reconstitution data compiled from the Catholic parish registers of birth, marriage and burial in the RPQA from the Programme de recherche en démographie historique (Université de Montréal), which comprises comprehensive data on life history and descending genealogy for the first centuries of settlement of the French-Canadian population[32]. We assigned T14484C to all descendants of the woman who introduced the variant in the population by identifying those who inherited her mitochondrial line, with low probability of incorrect mitochondrial assignment due to genealogical errors (see 'Estimating mitochondrial haplotype misassignment rate'). We calculated the fertility (complete family size) of individuals born up to 1750. This insured we had their full fertility data, because some life history events may be missing for individuals born later. A total of 98 T14484C carriers born up to 1750 were identified (45 males, 52 females, 1 newborn with unspecified sex). To substantially increase the temporal coverage of the data, we used the BALSAC register, which contains the genealogy of people married between 1621 and 1960. Thus, the RPQA individuals who married before the 19th century are also included in BALSAC. We assigned T14484C as before to the (married) matrilineal descendants of the woman who introduced the variant (carriers: 1,084 women, 954 men).

Fitness. The relative fitness of each individual born in Québec between 1670 and 1750 was calculated as his/her residual reproductive value at birth[33] using RPQA data ($n = 120{,}998$). This method accounts for temporal variation in the demography of the population, as well as for parental age at childbirth, two factors generating variation in fitness that are not accounted for with simpler measures such as raw fertility[33]. Fitness was adjusted for the population growth rate of the cohort (defined by the year of birth of individuals), an approach that has been used before for human historical data[49]. Because BALSAC does not include non-married individuals for the whole Québec population, the full fertility of individuals was unavailable for this register. Therefore, we did not calculate fitness using BALSAC data, but ran a different set of analyses on the data (see below).

Selection. The per-sex fitness of carriers was compared to the fitness distribution in the general population of non-carriers. Thus, dyads were created by pairing each carrier with a non-carrier chosen randomly among those sharing the same birth location, year and sex. This provided a matched random sample of non-carriers of equal size as the number of carriers over the study period (with a total of 3,696 control individuals matching one or more carriers for the 1670–1750 period). This procedure was repeated 1,000 times to obtain the null distribution of the average relative fitness in the non-carrier matched population. Because we had data for the entire population, such statistical testing might seem unnecessary to confirm the significance of differences between carriers/non-carriers. However, our study spans a multi-generational timescale and, because factors shaping infant mortality (for example, resources, diseases outbreaks and parental life history) vary

across space and time, this procedure was used to ensure that we were comparing carriers to groups of non-carriers exposed to similar conditions. A comparison between carriers and non-carriers was done using the same approach for fitness components: infant 0–1 year survival; whether a surviving individual married or not; and fertility. We define the juvenile stage as the period spanning from one-year-old to marriage, or from one-year-old to death when death occurred before the individual had the opportunity to marry. Infant survival corresponded to that apparent from birth and death records. Unreported mortality may be a concern when working with parish registers (see ref. [36] for details), but the case–control matching procedure, as well as the comparison of factors associated with mortality risk in infants in carriers and non-carriers (Supplementary Information) largely limit its potential impact on our results. In accordance with the theory of the mother's curse, all tests were one-tailed, that is, to determine whether T14484C carriers had lower values for fitness or fitness components. Differences between carriers and non-carriers are reported in box plots using R defaults, where the thick horizontal line shows the median and the lower and upper limits of the box correspond, respectively, to the 25th and 75th percentiles; whiskers extent beyond box limits by a value equal to 1.5× the box height or by the maximum (upper whisker) or minimum (lower whisker) value in the data if that value is within 1.5× the box height. We then measured the selection coefficient as the covariance between trait (carrier status) and relative fitness, also equivalent to the per-generation expected change in T14484C frequency due to fitness differences between carriers and non-carriers. Consistent with our age-structured approach of relative fitness, we obtained selection coefficients for three life-history stages: (i) infants, with selection acting on 0–1 year survival; (ii) juvenile, with selection acting through both survival (between age 1 and eventual marriage) and mating success; and (iii) married, with selection acting through both adult survival and fertility. Equations to calculate selection at each stage are given in Table 1. Finally, to assess whether T14484C effects on infant male mortality continued across centuries up to 1960, we used BALSAC data to test the prediction that the male proportion in married individuals was smaller in the T14484C lineage than in other maternal lineages (see main text).

Genetic trend. In theory, there are two ways by which we could assess how the frequency of T14484C should respond to selection in males in the absence of an effect of the mother's curse. The first is by making selection 'efficient' in male carriers in simulations. However, this would imply changing the type of inheritance (for example, assuming a paternal instead of maternal transmission of T14484C). Doing so would be invalid, as it would cause the uncoupling of fitness data from mitochondrial haplotypes at the individual level. Second, we could simulate a scenario of equal fitness costs of T14484C in female and male carriers. This is the approach we adopted here. Therefore, we introduced additional mortality by randomly removing a proportion of the women surviving beyond age 1 from the genealogy, to end up with total mortality rates approximately equal in female (average of 1,000 simulations = 0.41 ± 0.08 (mean ± s.d.)) and male infant carriers (0.39). Therefore, when a woman died in the model, all her descendants were also removed. The procedure was repeated 1,000 times and, for each simulation, the temporal trajectory of T14484C frequency was calculated. The genetic trend was calculated up to 1775, namely the last birth year of individuals included in our infant mortality analyses. Despite this modest addition in the temporal coverage, this nearly doubled the number of analysable T14484C carriers (from 97 to 186 (95 females, 91 males), excluding 4 of unspecified sex) due to rapid population growth. The same simulation approach was used to assess the expected frequency of T14484C under the assumption that female carriers had the same infant survival rate as non-carrier females.

Estimating mitochondrial haplotype misassignment rate. Previous studies on the French-Canadian population report that the genealogies are generally quite reliable[50,51], although mistakes in the parish records or false parenthood (unreported adoption, false paternity) or even mistaken maternity cannot be excluded. Because an error at a specific link in a genealogy will have a cascading effect on the attribution of a genetic state to the descendants arising downstream of that link[51], we assessed the mitochondrial haplotype misassignment rate (MHMR) by using the mtDNA hypervariable control region I sequence (CRI; 16,090–16,365) data from 874 living individuals. The ascending genealogies for these individuals were retrieved from BALSAC. Therefore, the maternal lineage of each of the 874 individuals was traced back in time to identify the female founder of each lineage associated with a specific mtDNA CRI variant. Each woman along a path linking a founder to a living individual is an ancestor of the latter. To calculate MHMR, we used only maternal genealogies for which the female ancestor was related to at least two contemporary individuals with a known mtDNA CRI variant. In total, 609 of the 874 typed individuals were informative for this purpose, and were linked in pairs or larger groups through 5,005 female ancestors. A misassignment was identified when two different mtDNA CRI variants were attributed to the same female ancestor. Mitochondrial misassignments occurred for only 19 ancestors, corresponding to a MHMR of 0.38%. All misassignments involve haplogroup differences, not haplotype differences within haplogroups, thereby excluding that they resulted from de novo mutations. For instance, considering the assignment of T14484C to 52 female descendants in our analysis of fitness, the expected number of haplotype misassignments is $0.0038 \times 52 = 0.20$. Therefore, based on our error rate estimation, there is an approximately 20% probability that a single case of T14484C misassignment occurred. Moreover, 11 contemporary probands carrying the T14484C ascended from the same founder woman, through three of her daughters and four of her granddaughters[31]. Therefore, considering this observation and the low MHMR, it seems highly improbable that this woman and her first descendants were not carriers of T14484C.

Data availability. The data that support the findings of this study are available from the BALSAC register (http://balsac.uqac.ca) and RPQA (http://www.genealogy.umontreal.ca), but restrictions apply to the availability of these data, which were used under license for the current study. Data requests can be addressed to these third parties.

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Author contributions

D.L., E.M. and C.M. designed the study, C.M. performed the analyses, E.M. wrote the paper; B.B., A.A.C. and A.G. brought their expertise, respectively, in genetic medicine, evolution and ageing, and demography; all authors discussed the results and commented on the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Additional information

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