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Abstract

Interest in mitochondrial influences on extended longevity has been mounting, as demonstrated by a growing literature. Such work has demonstrated that some haplogroups are associated with increased longevity and that such associations are population-specific. Most previous work however, suffers from the methodological shortcoming that long-lived individuals are compared with "controls" who are born decades after the aged individuals were. The only true controls of the elderly are people who were born on the same time period, but who did not have extended

longevity. Here we present results of a study in which we are able to test if longevity is independent of haplogroup type, controlling for time period, by using mitochondrial DNA genealogies. Since mtDNA does not recombine, we know the mtDNA haplogroup of the maternal ancestors of our living participants. Therefore, we compare the haplogroup of people with and without extended longevity, who were born during the same time period. Our sample is an admixed New World population which has haplogroups of Amerindian, European and African origin. We show that women who belong to Amerindian, European and African haplogroups do not differ in their mean longevity. Therefore, to the extent that ethnicity was tied in this population to mtDNA make up, such ethnicity did not impact longevity. In support of previous suggestions that the link between mtDNA haplogroups and longevity is specific to the population being studied, we found an association between haplogroup C and decreased longevity. Interestingly, the lifetime reproductive success and the number of grandchildren produced via a daughter of women with haplogroup C are not reduced. Our diachronic approach to the mtDNA and longevity link allowed us to determine that the same haplogroup is associated with decreased longevity during different time periods, and allowed us to compare the haplogroup of short and long-lived individuals born during the same time period. By controlling for time period, we minimize the effect of different cultural and ecological environments on differential longevity. With our diachronic approach, we investigate the mtDNA and longevity link with a biocultural perspective.

The possible role of the mitochondria in extending longevity has been investigated recently. Some researchers have chosen to focus on specific longevity-associated polymorphisms (LAPS) or have sequenced entire chromosomal regions (Castri et al., 2009; Lv et al., 2010; Nijiati et al., 2013; Raule et al., 2014; von Wurmb-Schwark et al., 2010; Yang et al., 2012), finding associations between markers and extended longevity. Others have chosen to focus on haplogroups, reporting that some haplogroups extend longevity (Cai et al., 2009; Courtenay et al., 2012; Dominguez-Garrido et al., 2009; Feng et al., 2011; Guney, Ak, Atay, Ozkaya, & Aydin, 2014; Nishigaki, Fuku, & Tanaka, 2010). A few papers report no association between mtDNA haplogroups and longevity (Costa et al., 2009; Pinos et al., 2012), and no paper reports a negative effect of mtDNA haplogroups on longevity. A review of these papers indicates that the association between markers such as the C150T SNP and extended longevity is well established [see (Castri et al., 2009) and references therein], while the association between haplogroups and longevity is population-specific (Raule et al., 2014; Zapico & Ubelaker, 2013). Unfortunately, haplogroup and longevity studies have been limited to a small number of populations, predominantly Chinese (Cai et al., 2009; Feng et al., 2011; Lv et al., 2010; Yang et al., 2012), Japanese (Nishigaki et al., 2010), and European (Courtenay et al., 2012; Dominguez-Garrido et al., 2009; Guney et al., 2014; Pinos et al., 2012) [see (Castri et al., 2009) for older citations] and have only worked with living subjects. It is striking that even though there is a large literature reporting Amerindian haplogroups (Carvajal-Carmona et al., 2003; Gomez-Carballa et al., 2012; Kashani et al., 2012; Kolman & Bermingham, 1997; Martinez-Cortes et al., 2013; Mizuno et al., 2014; Rickards, Martinez-Labarga, Lum, De Stefano, & Cann, 1999; Sandoval et al., 2009; Sans, Figueiro, & Hidalgo, 2012; Santos, Ward, & Barrantes, 1994), no study before us has determined if haplogroup affects longevity in an Amerindian-derived population. A cross-cultural and

biocultural approach to the study of the link between longevity and mtDNA haplogroups demands the inclusion of diverse populations. Otherwise, we do not have a complete view of the effects of cultural and genetic variation on human longevity.

A problem of studies on the link between mtDNA haplogroups and longevity is that they usually work with living subjects. Therefore, we do not know if the association between extended longevity and haplogroup reported by these papers would have been in place in previous centuries, under a different historical and ecological setting. As Lewis and Brunner note, any genetic influence on longevity is likely to vary by time and place (Lewis & Brunner, 2004).

A common methodological problem of these studies is the lack of correspondence between the generation of controls and long-lived subjects, because the controls are decades younger than are aged subjects (Cai et al., 2009; Nijiati et al., 2013; Pinos et al., 2012). The only true controls of long-lived subjects are those who were born during the same time period and lived in the same ecological and socio-cultural milieu as did aged subjects, but who did not have extended longevity (Castri et al., 2009; Lewis & Brunner, 2004).

A question left unanswered by most of the papers on the effect of mtDNA make up on longevity is whether long-lived individuals have increased lifetime reproductive success (LRS) and contribution to the next generation. If longevity is advantageous from a Darwinian-fitness perspective, we should expect to see a positive effect of extended longevity on the reproductive success of long-lived individuals. Therefore the advantageous mtDNA marker should be under positive selection and little additive genetic variance should be left in the gene pool (L. Madrigal, Relethford, & Crawford, 2003). Life time reproductive success has been used as a proxy for fitness among pre-industrial (Gillespie, Russell, & Lummaa, 2013) and industrial (Zietsch, Kuja-

Halkola, Walum, & Verweij, 2014) populations. In fact, Zietsch et al. (2014) note that LRS is a valid proxy for fitness in a population that overlaps in time with the one analyzed in this paper.

Here we determine if there are mitochondrial haplogroups associated with longevity, lifetime reproductive success and number of grandchildren in a historic admixed New World population, controlling for time period. By using mtDNA lineages and genealogical methods, we are able to compare the longevity of the haplogroups in previous centuries. In an effort to determine if ethnicity is associated with fitness, we also test if European, African and Amerindian haplogroups have higher lifetime reproductive success or longevity.

Materials and Methods

The data consist of maternal genealogies started from 152 living subjects, all of whom lived in Atenas, Costa Rica. These participants were enrolled in the study using a random sampling strategy. Only adults able to give informed consent were recruited. The project was approved by the committee on bioethics of the University of South Florida and the Universidad de Costa Rica. The genealogies were reconstructed with records obtained from the Church Chancery and the National Document Register. Fieldwork, data collection and genealogical methods for the reconstruction of the genealogies are described elsewhere (Lorena Madrigal & Melendez-Obando, 2008). MtDNA was extracted from 152 blood samples with standard procedures. The HVR-I of the mtDNA control region was amplified by PCR between nps 16024–16383 using primers H16401 and L15997. Both strands of the HVR-I were sequenced. In addition, seven binary PCR-restriction fragment length polymorphisms were typed, known to be specific to maternal lineages within America, Europe, and Africa, in order to help define the haplogroups,

namely 1663HaeIII, 13592HpaI, 110397AluI,110871MnlI, 211251 Tsp509I, 113262 AluI, 214766 MseI,and the COII/tRNAlys 9-bp deletion (Castri et al., 2009).

When genealogies coalesced on the same woman, she and her ancestors were counted only once for statistical analyses. Most genealogies are at least seven generations long, two are seventeen and thirteen generations long, and six are sixteen, fifteen, twelve and eleven generations long. Considering all individuals across all generations and all lineages, we have 1172 individuals in our data set. Several families extend back to the 1500's, as shown in figure 1.

Lifetime reproductive success is defined as the number of children produced who survived by the time of the woman's death. In their paper considering proxies of reproductive success, Strassman and Gillespie (2003) favor using the method we use here, that is, the number of offspring at time of censoring. In our study, censoring occurs with the woman's death (Strassmann & Gillespie, 2003). Number of grandchildren is defined as lifetime reproductive success (LRS) of one of the woman's daughters. We acknowledge that a better measure of grand maternal reproductive success would have been the number of children produced by all of the woman's daughters. However, we only have information on one of each woman's daughter's LRS, one of her daughter's LRS, and so forth, having started from the living participant and tracing her maternal genealogy back in time. The living women included in the present analysis were born in 1940 or before so that by the time of data collection (2003–2004) they were past their reproductive period.

The genealogical data were divided by twenty-year "generations", by century and half century, to control for time period. Long-lived individuals were defined as living 80 years or more, while short-lived individuals were defined as living 40 years or less. As De Benedictis et al (2001) note, any definition of longevity is quite arbitrary (De Benedictis et al., 2001).

However, the 80+ cut off point is frequently used in the longevity-mtDNA literature [see (Castri et al., 2009) and references therein]; (Dominguez-Garrido et al., 2009; Niemi et al., 2005; Yang et al., 2012). The ≤40 cut off point was chosen approximately following De Benedictis et al. (De Benedictis et al., 1999).

Frequency of haplogroups by longevity category (long-lived or short-lived) was tested with Fisher's exact test. Fisher's test is ideally suited for small sample sizes, as well as being extremely conservative (Niemi et al., 2005). Fisher's exact test is applied to a 2X2 table, which tests if longevity is significantly different in two haplogroups. We also compute the relative risk of dying in a certain longevity category by haplogroup. In the context of this paper, the relative risk (RR) is the ratio of the probability of an event occurring (death) in an exposed group (belonging to a haplogroup) to the probability of the event occurring in a comparison, non-exposed group (belonging to another haplogroup).

The mean longevity of haplogroups of Amerindian, European and African origin was compared with a non-parametric ANOVA. Normality of variables was tested with the Shapiro-Wilks test. Significance was declared with a Bonferroni-corrected value equal to 0.002 (Morrison, 1976). All statistical analysis was performed with SAS 9.4.

Results

As would be expected in an admixed New World population, our participants carry mitochondrial haplogroups from Native American, European and African origins, with an overwhelming preponderance of Amerindian haplogroups (88.82%). The most frequent Amerindian haplogroups are A (47.37%) and B (38.16%), whereas C and D are present at low frequencies (1.32 and 1.97% respectively). The remaining haplogroups are European, namely

haplogroups H (7.89%) and J (2.63%), while one haplogroup is African, namely haplogroup L4g (0.66%) [see (Castri et al., 2009) for more details].

Table 1 shows descriptive statistics for longevity, LRS and number of grandchildren for the entire period and divided by century of birth. From 1500's to the 1900's, longevity increased linearly, while LRS increased dramatically after the 1700's, possibly indicating an improvement in life conditions. The number of grandchildren for 1900–1940 is low, an indication that the daughter of the woman had not finished or was controlling her fertility. Tests of normality indicate that LRS, number of grandchildren and longevity are not normally-distributed for two of the three centuries and for the entire period. The sample size for the entire period does not equal the sample sizes by century because of missing observations.

Table 2 shows the sample size, mean longevity, lifetime reproductive success (LRS) and number of grandchildren by haplogroup for the entire time period. Although both LRS and number of grandchildren are extremely similar across all haplogroups ($p>0.05$), longevity is not. The mean longevity of haplogroup C is in stark contrast with that of all other haplogroups at 44.5 years of age, while the mean longevity of all the other haplogroups is at least 62 (see figure 2). The low mean longevity of haplogroup C is not due to an outlier, as all but one of the women had a longevity of less than 62 years. Neither is the decreased longevity of haplogroup C due to these women living during an earlier time period. In fact, the earliest year of birth of haplogroup C women was 1826. Therefore, the short mean longevity of these subjects is not due to their having been born in the 1500's, 1600's or 1700's. Because of the non-normality of the variables and the Bonferroni-corrected statistical cut-off point, , results of an ANOVA indicating that the mean longevity by haplogroup for the entire period is significantly different ($F=2.34$, $p=0.02$; Figure 2) are not taken too seriously. When the same comparison was performed with a non-

parametric ANOVA by century and for the entire period, the results were not significant (data not shown).

Results of Fisher's exact test indicate that haplogroup C has a greater probability of dying young (at or before 40) than does haplogroup A ($p = 0.0002$). Similar tests yielded non-significant results between haplogroup C and B, H, J and D. We obtained relative risks of dying young for haplogroup C in comparison with the other haplogroups. The relative risk was significant (did not overlap with one) for haplogroups C and A (RR=0.026, 95% CL=0.0065–0.1032) and for haplogroups C and B (RR=0.2475, 95% CL=0.1026–0.5969). These relative risks mean that exposure (being in haplogroup C) is significantly associated with decreased longevity (the category of interest).

It was of interest to determine if mtDNA haplogroups of European, Amerindian and African origin had significantly different longevity, LRS and number of grandchildren. A generation, half-century and century comparison of longevity, LRS and number of grandchildren by haplogroup of origin was not possible because of the small sample sizes from Europe and Africa. Therefore, we grouped women of all time periods into one of three groups: European, Amerindian and African according to their haplogroup. A non-parametric comparison of longevity, LRS and number of grandchildren yielded non-significant results (data not shown). These results indicate that to the extent that ethnicity was linked to haplogroup origin, it did not affect the longevity or reproductive outcome of the women.

Discussion

To what extent is longevity influenced by one's mtDNA make up? Researchers have shown that several mtDNA haplogroups are associated with increased longevity, but that these associations

are population-specific. Previously, Castrì et al. had shown the presence of longevity-associated polymorphisms (LAPS) in this data set (Castri et al., 2009). Here we focus on the question of haplogroup and ethnic effects on longevity and lifetime reproductive success. We collected data on living people and reconstructed their mitochondrial genealogies, reaching in a few cases to the 1500's. This allows us to look at longevity and reproductive success with a diachronic perspective. Indeed, whereas all previous research on mtDNA haplogroup and longevity focused on living people, we looked at the relationship between mtDNA haplogroup, longevity and reproductive success in living as well as in dead subjects. It is important to look at populations of different time periods because otherwise we do not know if results indicating a link between a mtDNA haplogroup and longevity are specific to a particular historical, cultural and ecological setting. A strength of this paper is its historical perspective.

We are also the first group to study the relationship between mtDNA haplogroup, longevity and reproductive success in a New World admixed population, which includes haplogroups from Amerindian, European and African origin. It is important to investigate populations of different origins so that we determine whether results indicating a link between a mtDNA haplogroup and longevity are population-specific. Our data gave us the opportunity of asking whether the haplogroups of European origin had an increased longevity, as would be expected if European sectors of the community had better access to resources. We found no such advantage, which suggests that **if** ethnicity was tied to mitochondrial origin, ethnicity did not affect longevity, LRS and the number of grandchildren produced. A strength of this paper is its admixed New World sample.

We found a difference of 25 years between the mean longevity of haplogroup C and the combined mean of all other haplogroups. When we compared the frequency of individuals who

died in the ≤40 age category by haplogroup, we obtained significant results when haplogroup C was compared with haplogroup A. The relative risk of dying young was significant for haplogroup C in comparison with haplogroups A and B. In sharp contrast with the decreased longevity of haplogroup C, its LRS and number of grandchildren did not differ when compared with the LRS and number of grandchildren of the other haplogroups. This indicates that the decreased longevity of individuals from haplogroup C did not compromise their reproductive output. Unfortunately, our data do not allow us to ascertain if the children of haplogroup C women experienced increased mortality after their mother's comparatively early deaths.

A limitation of our study is its sample size, particularly the small number of haplogroup C individuals. In similar admixed Latin American populations [the Central Valley of Costa Rica, Mexican Mestizos, Antioquia in Colombia (Carvajal-Carmona et al., 2003; Martinez-Cortes et al., 2013), but not in Venezuela (Gomez-Carballa et al., 2012)], the C haplogroup is much less frequent than are the A or B haplogroups. This is also the case for many (though not all) Latin American indigenous communities [(Mizuno et al., 2014) and references therein]; (Rickards et al., 1999; Sandoval et al., 2009). Indeed, in a study among Costa Rica's Huetar Indigenous population, the C haplogroup was absent (Santos et al., 1994). The fact that our sample has few haplogroup C participants simply reflects the variation of the population from which this sample was taken. The Atenas population, as well as the others cited above, has a low frequency of haplogroup C. Whether such low frequency is due to the shortened longevity we found in our study is an interesting question worth pursuing with a larger sample of living participants.

When we investigated the mtDNA haplogroups of aged individuals (>80) controlling for time period, we found that for all centuries, long-lived individuals did not differ significantly from controls in their mtDNA haplogroups. We are not the first group to find that the aged do

not differ in their mtDNA haplogroup, as no difference was reported in samples from Tunisia, Spain and North Europe (Benn, Schwartz, Nordestgaard, & Tybjaerg-Hansen, 2008; Costa et al., 2009; Pinos et al., 2012). Our results are particularly different from those reported in some European populations, in which the J haplogroup was found to be significantly associated with extended longevity (De Benedictis et al., 1999; De Benedictis et al., 2001; Niemi et al., 2003). In contrast, we found no association between extended longevity and the J haplogroup, a result also reported by others (Dato et al., 2004; Ross et al., 2001).

The fact that we found an association between decreased longevity and haplogroup C, which has not been reported before, might be explained by Raule et al.'s assertion that the effect of mitochondrial DNA variation on human longevity is influenced by the interaction between mutations in the mtDNA genome concomitantly inherited (Raule et al., 2014). Such inheritance may be at the root of the question why there are so many different longevity-mtDNA associations reported in the literature.

Most previous work on the mitochondrial bases of differential longevity has focused on individuals of unusually extended longevity rather than on individuals of unusually shortened longevity. We hope our results will spark interest in research on the mtDNA make up of short-lived individuals. We also hope to see other studies which, by using mtDNA, will study mitochondrial DNA of long-lived individuals who lived in previous centuries using controls born during the same time period.

Our study has evolutionary and bio-cultural approaches to the topic of differential longevity and mtDNA haplogroup. By investigating if differential longevity affects differential lifetime reproductive success, we have addressed the possible connection between longevity and fitness. By controlling for generation and century of birth, we controlled for a myriad of

environmental, historic and cultural factors which could have influenced longevity. We have contributed cross-cultural data to an ever-growing literature on mitochondrial influences on differential longevity by analyzing an admixed New World population. We suggest that rather than focusing on extended longevity, we should be focusing on differential longevity and differential reproduction of individuals with different mitochondrial DNA make up.

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Table 1

Summary statistics for longevity, life-time reproductive success, and number of grandchildren. Data for the entire period and by century.

Descriptive statistics for entire period			
	Longevity	LRS	Number of grandchildren
Mean	68.52	8	7.25
Standard deviation	17.65	3.68	3.96
Sample size	574	706	671
Shapiro-Wilk W p	p<0.0001	p<0.0001	p<0.0001
Birth date between 1900–1940			
	Longevity	LRS	Number of grandchildren
Mean	79.57	7.81	4.55
Standard deviation	13.52	3.9	3.63
Sample size	132	197	154
Shapiro-Wilk W p	p<0.0001	p<0.001	p<0.0001
Birth date between 1800–1899			
	Longevity	LRS	Number of grandchildren
Mean	67.58	8.2	8.15
Standard deviation	17.41	3.68	3.89
Sample size	321	350	350
Shapiro-Wilk W p	p<0.003	p<0.0005	0.0001
Birth date between 1700–1799			
	Longevity	LRS	Number of grandchildren
Mean	60	8.51	8.3
Standard deviation	14.2	3.13	3.25
Sample size	97	123	121
Shapiro-Wilk W p	P<0.92	P<0.1	0.12
Birth date between 1600–1699			
	Longevity	LRS	Number of grandchildren
Mean	54.87	5.31	6.3
Standard deviation	17.62	2.92	2.69
Sample size	15	19	20
Shapiro-Wilk W p	p<0.28	p<0.04	0.83
Birth date before 1600			
	Longevity	LRS	Number of grandchildren
Mean	54	6.22	7.08
Standard deviation	27.13	3.34	3.33
Sample size	9	23	26
Shapiro-Wilk W p	p<0.13	p<0.38	0.41

Table 2

Mean longevity, life-time reproductive success and number of grandchildren by haplogroup. 1500–1940.

HG	n	Longevity	LRS	Grandchildren
A	361	68.17	7.97	7.22
B	262	69.87	8.09	7.23
C	9	44.5	7.66	7
D	19	62.05	8.15	7.1
H	52	69.12	7.92	7.24
H1b1	12	67.83	8.66	7.45
J	26	72.85	7.43	6.7
L4g	3	74.33	8	8.5

Figure 1

A genealogy extending back to the 1500's. Names have been removed.

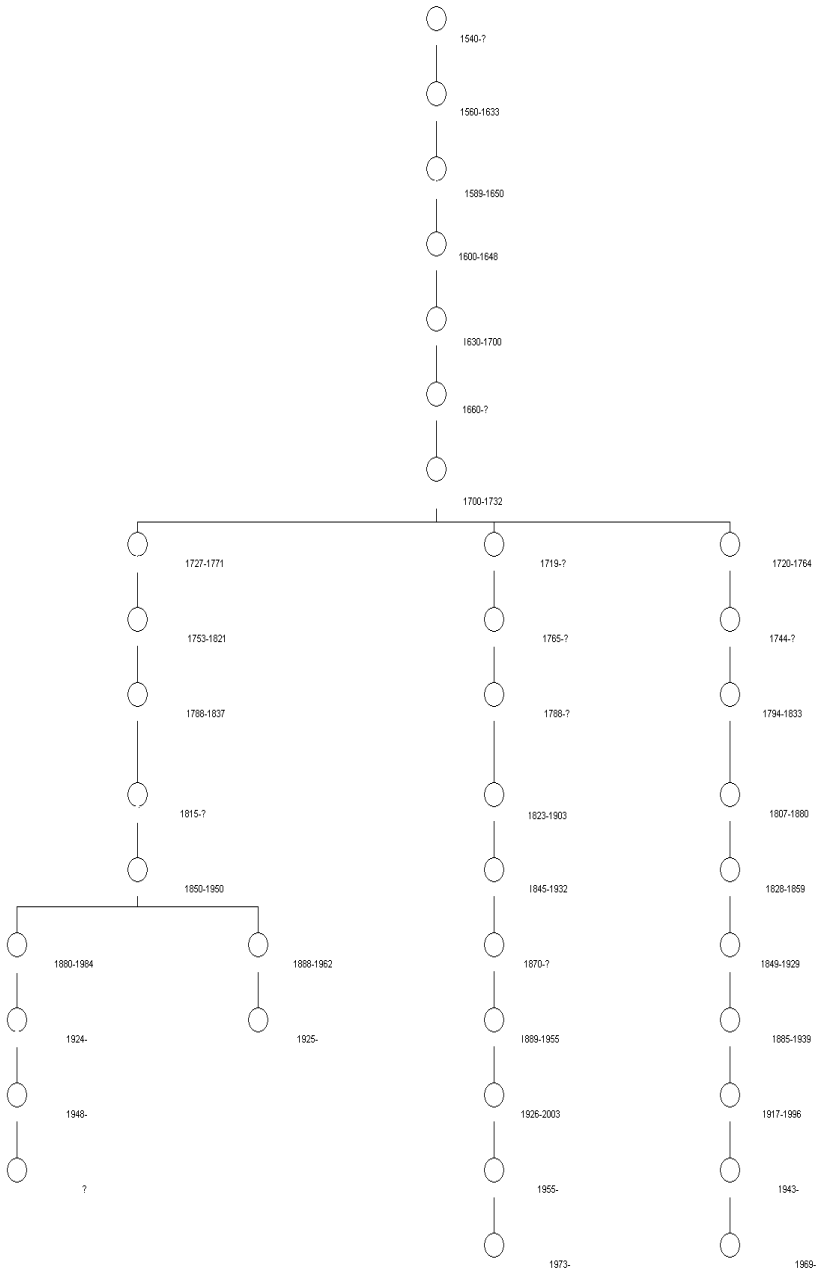

Figure 2

The mean longevity from 1500 to 1940 by haplogroup.