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X*Y females exhibit steeper reproductive senescence in the African pygmy mouse

Jean-François Lemaître^{a,1,2} , Yann Voituron^{b,1} , Léa Herpe^c , and Frédéric Veyrunes^{d,2}

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A wave of studies has recently emphasized the influence of sex chromosomes on both lifespan and actuarial senescence patterns across vertebrates and invertebrates. Basically, the heterogametic sex (XY males in XX/XY systems or ZW females in ZW/ZZ systems) typically displays a lower lifespan and a steeper rate of actuarial senescence than the homogametic sex. However, whether these effects extend to the senescence patterns of other phenotypic traits or physiological functions is yet to be determined. Here, we investigated whether sex chromosomes modulate reproductive senescence using females from the African pygmy mouse (*Mus minutoides*). This biological model exhibits an odd sex determining system with a third, feminizing sex chromosome, X*, resulting in three distinct female genotypes (XX, X*X, or X*Y) that coexist in natural populations. We found that the rate of senescence in litter size at birth is much more pronounced in heterogametic X*Y females than in homogametic XX or X*X females that may support the unguarded X or toxic Y hypotheses and can be directly linked to the complex and unique X*Y phenotype. A decrease in neonatal survival with mother's age has also been found, but this decline is not different between the three female genotypes.

reproductive ageing | *Mus minutoides* | litter size | neonatal survival

The occurrence of reproductive senescence (i.e., the decline in reproductive performance with increasing age) constitutes the rule rather than the exception, at least across mammals (1–3). However, empirical evidence accumulated over the last decades has revealed that reproductive senescence patterns are highly variable. Indeed, for a given reproductive trait (e.g., birth rate, litter size), the age at the onset of senescence and the rate of reproductive senescence display substantial variation both across and within species (2, 4). Identifying the ecological and biological drivers of this variability is currently a major scientific goal as reproductive senescence has detrimental effect on fitness (5, 6) and can be associated with multiple health-related issues in both males and females (7, 8).

Among the factors that could shape the variability in aging trajectories, a possible role played by sex chromosomes is currently attracting lots of attention (9, 10). However, all the investigations conducted so far in that context have focused on lifespan and actuarial senescence (i.e., the increase in mortality risk with advancing age) (9–11). Results from these studies have highlighted that across vertebrates, the homogametic sex—XX females in XX/XY genetic sex determination system (e.g., most mammals) or ZZ males in ZZ/ZW genetic determination system (e.g., birds)—typically show a longer lifespan than the heterogametic sex (XY males or ZW females) (12). Furthermore, in amphibians, a class of vertebrates where both XX/XY and ZZ/ZW species occur, the heterogametic sex consistently shows a steeper rate of actuarial senescence (i.e., the increase in mortality rate with age) than the homogametic sex (13). Currently, the survival advantage of the homogametic sex is attributed to the expression of recessive deleterious mutations in individuals carrying a single copy of the X (or Z) chromosome (i.e., unguarded X hypothesis) (9, 14) or a harmful effect of the Y (or W) chromosome caused in particular by its large content in transposable elements that can be progressively derepressed with increasing age and thus responsible for an increased mutation rates on autosomes and ultimately a faster aging (i.e., toxic Y hypothesis) (15).

Although the fine scale molecular mechanisms behind the homogametic sex advantage are yet to be deciphered, it is increasingly recognized that sex chromosomes can influence individual's fitness by modulating survival prospects (10, 12, 16). However, whether sex chromosomes drive other features of the aging phenotype is largely unknown. As such, the contribution of sex chromosomes to the reproductive senescence process—a major determinant of fitness (5) remains to be explored. In that context, mammalian species that have been described with unconventional sex-determining systems (17–19) may represent particularly valuable biological models. Among them is the African pygmy

Significance

How much sex chromosomes contribute to the aging process is a timely question. Here, using a long-term breeding colony of the African pygmy mouse (*Mus minutoides*)—a rodent characterized by a unique sex determination system where females can display three different sex genotypes (XX, X*X, or X*Y)—we showed that heterogametic females (i.e., X*Y) suffer from a much steeper reproductive senescence in litter size (measured at birth and weaning) than homogametic females (XX and X*X). This finding opens innovative avenues of research regarding the mechanisms shaping the evolution of aging.

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mouse, *Mus minutoides*, characterized by a third sex chromosome, a mutant of the X chromosome (called X*), enables to block the male program initiated by the Y chromosome. Hence, in that species, females with different types of sex chromosomes (XX, X*X, and X*Y) cohabit and produce living offspring throughout their lives, while all males are XY (20–22).

This unique system offers a relevant framework to investigate the influence of sex chromosomes on reproductive senescence, and we took advantage of a long-term dataset from a breeding colony. From an evolutionary point of view, the sex determination system of *M. Minutoides* is paradoxical because X*Y females must face a loss of 25% of nonviable YY embryos, but also meiotic issues arising from the absence of pairing with another X chromosome and the presence of a Y chromosome in meiotic prophase oocytes, which should generate substantial reproductive costs (22, 23). However, in the African pygmy mouse, the presence of a Y sex chromosome in females does not alter their reproductive capacities which are even increased. For instance, heterogametic (i.e., X*Y) females have more offspring over their lifetime, produce more pups per litter, reproduce earlier in life, display higher ovulation rates (21), and are more efficient in pup retrieval experiment (20) compared to other female genotypes. However, whether these evolutionary advantages described in heterogametic females are counterbalanced by an earlier or steeper reproductive senescence compared to homogametic females (XX or X*X) is unknown. Here, based on the recent accumulation of studies highlighting that the heterogametic sex suffers from a shortened lifespan (15, 24) possibly underpinned by a fastened biological aging, we predict that X*Y females in the African pygmy mouse should suffer from an earlier and/or faster reproductive senescence than homogametic females. Among homogametic females, we predict that similar reproductive senescence patterns between XX and X*X females, as previous studies (20, 21) did not detect any differences in life history strategies between these genotypes.

Interestingly, reproductive senescence pattern can differ among reproductive traits (25). Such asynchronous patterns of senescence between reproductive traits [likely molded by natural selection (26, 27)] have been repeatedly described in the literature (e.g., refs. 28–30). Therefore, a thorough assessment of the factors that putatively shape reproductive senescence patterns, such as the sex chromosomes, needs to encompass multiple reproductive traits (25). We thus focused our analyses of reproductive senescence patterns on three traits—litter size at birth, litter size at weaning,

and neonatal survival (i.e., pup survival between birth and weaning)—that taken together can provide a thorough understanding on the influence of sex chromosomes on reproductive success throughout the life course (25), as well as on the fitness consequences of carrying unusual sex chromosomes in females.

Results

The mean litter size at birth (computed over the lifespan) was higher for X*Y females (3.70 ± 0.38) than for XX (2.79 ± 0.30) or X*X females (3.27 ± 0.32) (Fig. 1A and Table 1a). Mean litter size at birth was also higher in X*X females compared to XX females (Fig. 1A and Table 1b). A similar pattern was observed for the mean litter size at weaning with a litter size higher for X*Y females (2.32 ± 0.40) than for XX (1.67 ± 0.32) or X*X females (2.00 ± 0.35) and higher for X*X than for XX females (Fig. 1B and Table 1b). On the contrary, the neonatal survival was not statistically different between the three genotypes (0.61 ± 0.09 , 0.58 ± 0.10 , and 0.61 ± 0.09 for X*Y, XX, and X*X females, respectively) (Fig. 1C and Table 1c).

The selected model describing the changes in litter size at birth as a function of maternal age across the whole dataset (irrespective of the female genotypes) was a threshold model with two slopes (*SI Appendix*, Table S1). Litter size at birth decreases linearly from the age at first reproduction to 165 d of age (slope of -0.0071 ± 0.0017 , Table 2a and *SI Appendix*, Fig. S3) and then from this threshold until the last age at reproduction, but with a much lower slope (slope of -0.0018 ± 0.0005 , Table 2a and *SI Appendix*, Fig. S3). A model representing a linear decline in litter size at birth until 311 d of age followed by a constant phase was selected for X*Y while the opposite pattern was found for X*X females. In these latter, litter size at birth remains constant until 338 d of age and then decline linearly (Table 2a and Fig. 2A and C). On the contrary, the linear model was selected for XX females (Table 2a and Fig. 2B). Although not retained, the linear model also provided a good fit to the data for the three female genotypes (*SI Appendix*, Table S1). Thus, to cover the period of decline of all females (XX, X*X and X*Y), we compared the rate of senescence in litter size at birth differs between the three genotypes over whole lifespan. We found that the rate of senescence is significantly higher for the X*Y females (slope of -0.0038 ± 0.0006) than for the X*X and XX females (slope of -0.0016 ± 0.0006 and slope of -0.0018 ± 0.001 , respectively, Fig. 2D and Table 3a). The rate of

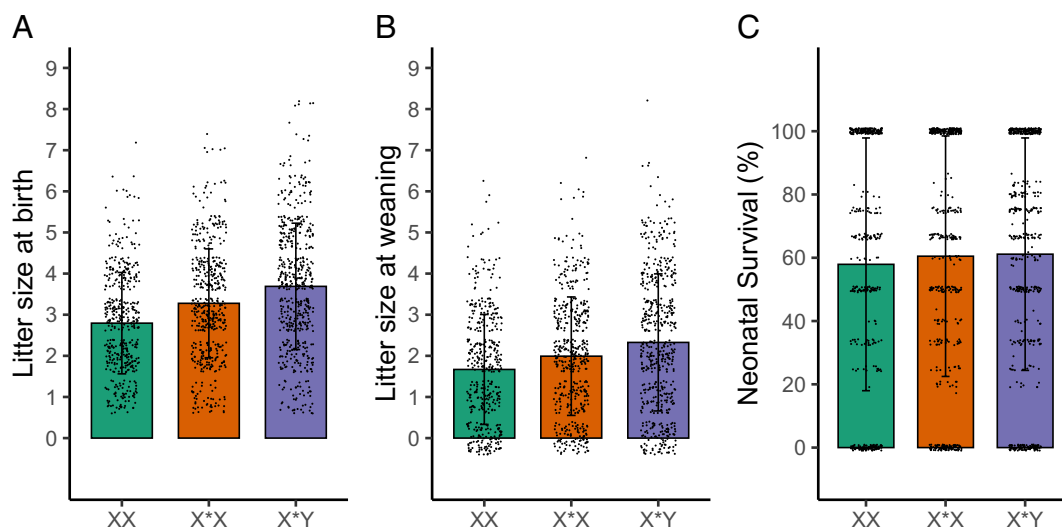


Fig. 1. Mean difference in litter size at birth (A), litter size at weaning (B), and neonatal survival (C) between females from the three genotypes.

Table 1. Parameter estimates of the models testing for an effect of the female genotype on litter size at birth (a), at weaning (d), and on neonatal survival (c)

(a)	Litter size at birth	Estimates	SE	t	P
	Intercept	3.68	0.07	49.17	<0.001
	Genotypic sex (XX)	-0.93	0.11	-8.35	<0.001
	Genotypic sex (X*X)	-0.43	0.11	-3.98	<0.001
(b)	Litter size at weaning	Estimates	SE	t	P
	Intercept	2.32	0.08	28.56	<0.001
	Genotypic sex (XX)	-0.69	0.12	-5.72	<0.001
	Genotypic sex (X*X)	-0.32	0.12	-2.75	<0.01
(c)	Neonatal survival	Estimates	SE	z-value	P
	Intercept	0.59	0.09	6.48	<0.001
	Genotypic sex (XX)	-0.19	0.14	-1.37	0.17
	Genotypic sex (X*X)	-0.02	0.13	-0.14	0.89

These models include the mother identity as a random effect (N = 432 females and 1,476 observations for a, N = 431 females and 1,463 observations for b and c). X*Y females are used as the reference group.

senescence in litter size was not statistically different between X*X and XX females (Fig. 2D and Table 3a).

Similar to litter size at birth, the selected model for litter size at weaning across the whole dataset was a 2-slope model with a threshold at 186 d of age (Appendix, Table S2 and Fig. S4). The decline in litter size at weaning was much more pronounced during the first phase (slope of -0.0072 ± 0.0015 between 47 and 186 d of age) than the second phase (slope of -0.0019 ± 0.0006 between 186 and 597 d of age) (Table 3b). For X*Y females, the litter size declines with age until 251 d of age (slope of -0.008 ± 0.001) and then remains constant (Fig. 3A). The pattern slightly differed for XX and X*X females which litter size declines linearly with age from the first reproduction to the age

at last reproduction (Table 2b and Appendix, Table S2 and Fig. 3B and C). We further tested for a difference in senescence rate in litter size at weaning between the three genotypes over the age range of 47 to 251 d (i.e., the range of age for which litter size declines in all female genotypes, see Fig. 3). We found that the rate of senescence was 7.6 times higher for the X*Y females (slope of -0.0091 ± 0.0017) than for the X*X (slope of -0.0012 ± 0.0019) and 5.1 times higher than for the XX females (slope of -0.0018 ± 0.0018) (Fig. 3D and Table 3b). The rate of senescence in litter size was similar between X*X and XX females (Fig. 3D and Table 3b).

We found a decrease in neonatal survival with maternal age (slope of -0.22 ± 0.04 , SI Appendix, Fig. S5 and Table S3) in the

Table 2. Parameter estimates of the best models describing the change in litter size at birth and litter size at weaning with maternal age for the whole dataset and for each genotype separately

(a)	Litter size at birth		Estimates	SE	t	P
	All females	Intercept	4.789	0.251	19.133	<0.001
	2 slopes model (threshold = 165 d)	Slope 1 [47 to 165 d]	-0.007	0.002	-4.062	<0.001
		Slope 2 [165 to 597 d]	-0.002	0.000	-3.746	<0.001
	XX females	Intercept	3.185	0.176	18.113	<0.001
	Linear model	Slope	-0.002	0.001	-2.729	<0.01
	X*X females	Intercept	3.307	0.078	42.656	<0.001
	Constant (threshold = 338 d), then 1 slope	Slope 1 [338 to 597 d]	-0.006	0.002	-3.265	<0.01
	X*Y females	Intercept	4.881	0.201	24.275	<0.001
	1 slope (threshold = 311 d), then constant	Slope 1 [47 to 311 d]	-0.005	0.001	-6.425	<0.001
(b)	Litter size at weaning		Estimates	SE	t	P
	All females	Intercept	3.708	0.230	16.145	<0.001
	2 slopes model (threshold = 186 d)	Slope 1 [47 to 186 d]	-0.007	0.001	-4.975	<0.001
		Slope 2 [186 to 597 d]	-0.002	0.001	-3.387	<0.001
	XX females	Intercept	2.136	0.19	11.20	<0.001
	Linear model	Slope	-0.002	0.00	-3.00	<0.01
	X*X females	Intercept	2.500	0.191	13.063	<0.001
	Linear model	Slope	-0.002	0.001	-2.895	<0.01
	X*Y females	Intercept	4.046	0.254	15.901	<0.001
	1 slope (threshold = 251 d), then constant	Slope 1 [47 to 251 d]	-0.008	0.001	-7.218	<0.001

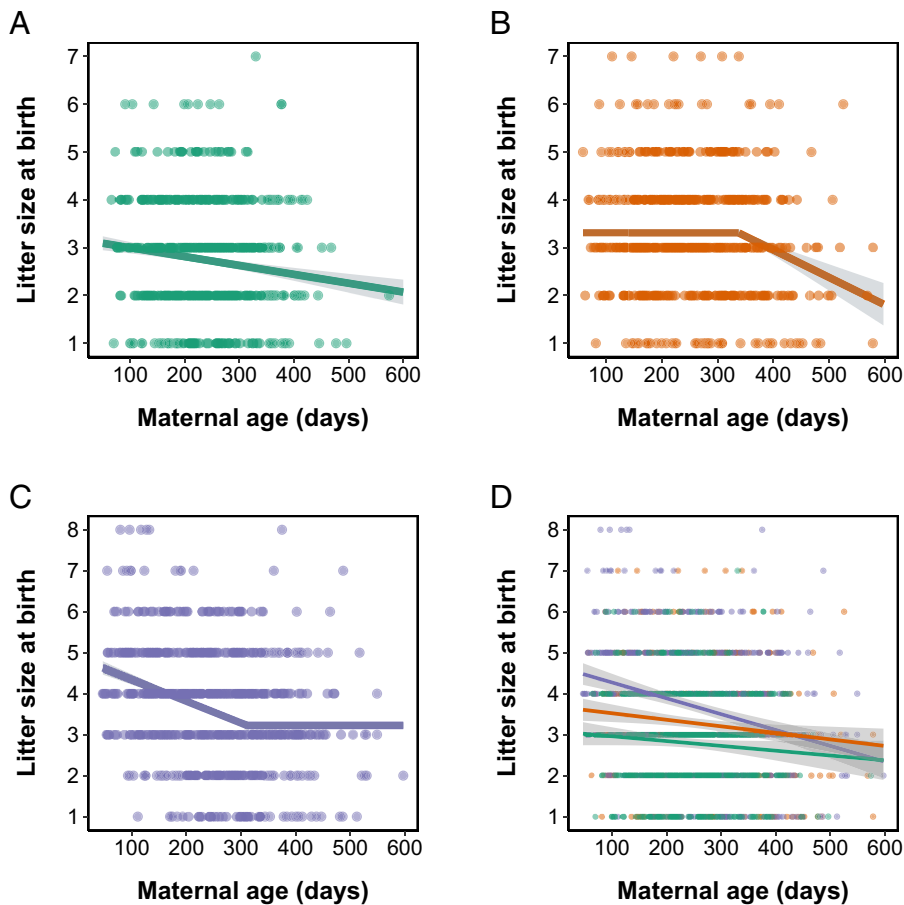


Fig. 2. Relationship between litter size at birth and maternal age for XX (A), X*X (B), and X*Y (C) females. The threshold value for X*Y females is 311 d and the threshold value for X*X females is 338 d. The figure (D) represents the differences in the rate of senescence in litter size at birth between the three female genotypes over the whole maternal lifespan (with the same color code as in A, B, and C).

whole dataset and for all genotypes separately (slope of -0.19 ± 0.08 , slope of -0.21 ± 0.08 , slope of -0.27 ± 0.06 for XX, X*X, and X*Y females, respectively; *SI Appendix, Table S3*). However, the decline in neonatal survival was not statistically different between the three genotypes (Table 1c and Fig. 4).

When investigating possible effects of selective disappearance by adding maternal lifespan to the previous selected models, we did not detect any association between maternal lifespan and litter size at birth on the whole dataset or within both XX and X*Y females (*SI Appendix, Table S1*). For X*X females, we detected a negative relationship between litter size at birth and maternal lifespan (*SI Appendix, Table S4*). On the contrary, we detected a positive relationship between maternal lifespan and litter size at weaning within the whole dataset (nonsignificant trend) and within XX females (*SI Appendix, Table S4*). In addition, we found a positive relationship between neonatal survival and maternal lifespan within the whole dataset and within each genotype (*SI Appendix, Table S4*). Finally, we did not detect any effect of paternal age on litter size at birth, at weaning or on neonatal survival (*SI Appendix, Tables S1, S2, and S5*).

Discussion

Irrespective of their genotype, African pygmy mouse females show evidence of reproductive senescence in litter size at birth and neonatal survival. This logically extends to a marked senescence in litter size at weaning, which thus plead for an unequivocal decline in reproductive fitness with increasing age in females from this species (31, 32). While support of reproductive senescence in

mammalian females is increasingly documented (2), most evidence so far has been published on slow species [i.e., species displaying a covariation of long biological times (33)] such as large herbivores, primates, or hibernating mammals (2). This study focused on a particularly fast species [e.g., pygmy mouse females reach sexual maturity at 4 to 6 wk and display a low survival rate in natural conditions with only a few individuals living up to 1 y of age (34)] highlighting the pervasive nature of the reproductive senescence process along the full slow-fast continuum of life history strategies in mammals (see also ref. 35). More surprisingly, senescence in litter size at birth starts straight from the age at first reproduction. Although predicted by the evolutionary theories of aging (26), this particularly early onset of reproductive senescence is far to be common in nature (36), especially for litter size (e.g., refs. 37–40).

We found that the rates of reproductive senescence in litter size at birth and weaning are much more pronounced in X*Y females compared to other female genotypes. For instance, from the age at first reproduction by almost 8 mo of age, the litter size at weaning decreases faster (of almost 1 offspring every 100 d) for X*Y females than for XX or X*X females. This result highlights that sex chromosomes display extended and largely unappreciated effects on the aging phenotype and contribute to the among-individual variability in reproductive senescence patterns in mammalian females with unconventional sex-determining systems. However, whether the steep senescence in litter size exhibited by X*Y females is caused by the presence of a Y chromosome (i.e., toxic Y hypothesis) or the absence of another copy of the X

Table 3. Parameter estimates of the models testing for an interaction between mother's age and the female genotype on litter size at birth (a), at weaning (b), and on neonatal survival (c)

(a)	Litter size at birth	Estimates	SE	t	P
	Intercept	4.604	0.155	29.667	<0.001
	Mother age	-0.004	0.001	-6.722	<0.001
	Genotype (XX)	-1.429	0.248	-5.76	<0.001
	Genotype (X*X)	-0.958	0.236	-4.052	<0.001
	Maternal age x Genotype (XX)	0.002	0.001	2.129	0.0335
	Maternal age x Genotype (X*X)	0.002	0.001	2.516	0.012
(b)	Litter size at weaning	Estimates	SE	t	P
	Intercept	4.111	0.275	14.965	<0.001
	Mother age	-0.009	0.002	-5.676	<0.001
	Genotype (XX)	-2.026	0.455	-4.457	<0.001
	Genotype (X*X)	-1.659	0.450	-3.689	0.0002
	Maternal age x Genotype (XX)	0.007	0.002	2.822	0.0049
	Maternal age x Genotype (X*X)	0.007	0.002	2.859	0.0044
(c)	Neonatal survival	Estimates	SE	z-value	P
	Intercept	0.555	0.091	6.099	<0.001
	Mother age	-0.243	0.060	-4.056	<0.001
	Genotype (XX)	-0.199	0.141	-1.413	0.158
	Genotype (X*X)	-0.001	0.134	-0.011	0.991
	Maternal age x Genotype (XX)	0.025	0.105	0.239	0.811
	Maternal age x Genotype (X*X)	0.041	0.096	0.425	0.671

These two models include the mother identity as a random effect (N = 432 females and 1,476 observations for a, N = 431 females and 1,463 observations for b and c). X*Y females are used as the reference group.

chromosome (i.e., unguarded X hypothesis)—two nonmutually exclusive pathways—remains to be determined.

Using a population genetic modeling approach, Connallon and colleagues (41) have recently suggested that the unguarded X was unlikely to explain the sex differences in lifespan and actuarial senescence patterns generally observed in mammals, especially in species carrying a short X chromosome. But in the context of the African pygmy mouse and the evolution of a third sex chromosome, the unguarded X hypothesis needs to be reevaluated since the X* chromosome does not recombine anymore over a large region (20, 23) and may have accumulated deleterious mutations that fasten the aging process and contribute to the age-specific decline in reproductive performance.

The Y chromosome could contribute to the high rate of senescence in litter size through multiple mechanisms. For instance, the large content in TEs on the Y chromosome can be derepressed with increasing age, which progressively increase the risk of deleterious mutations on autosomes [(15, 24), but see ref. 42] and more generally genomic instability—a key hallmark of aging (43). This genomic instability could even be triggered by a progressive and selective loss of Y chromosome in somatic cells, as recently documented in Wistar rats, *Rattus norvegicus* (44). Moreover, the epistatic effects of the female-specific X* chromosome and the Y chromosome are at the origin of a complex X*Y phenotype that may suffer from acute life-history trade-offs between reproductive success early in life and senescence, as proposed by evolutionary theories of aging (26, 45, 46). X*Y females differ from the XX and X*X females in gene expression profiles (20), hormone concentrations [e.g., lower baseline concentration in corticosterone (47)] as well as in several life history and behavioral traits (21, 48, 49). This is notably confirmed by the present study where unlike

most sex-reversed mammals that are usually sterile, X*Y females of the African pygmy mouse are fully fertile and outperform X*X and XX females in terms of litter size (see also ref. 21). More strikingly, X*Y females display a higher ovulation rate (producing almost one and a half-times as many ova per cycle) and reproduce earlier than the other females (21). Therefore, X*Y females might allocate more resources to the production and the raising of pups at the expense of somatic maintenance, which could be responsible for their fast senescence in litter size (39), as predicted by the disposable soma theory (50). Finally, it has been shown that X*Y females experienced complex meiotic configurations (quadrivalents involving the sex chromosomes with synapsis and cross-over deficiencies), albeit they manage to successfully complete meiotic divisions (23), but these hurdles may intensify with age, as commonly observed, and increase the production of aneuploid gametes (51, 52). This mechanism, in addition to a more rapid depletion of the oocyte pool in X*Y females, could explain the particularly marked senescence in litter size.

Our study also adds to the increasing evidence that maternal age negatively impacts neonatal survival in the living world (53), which in the case of mammals might be explained by a decline in maternal care (e.g., declines in milk quantity or quality, in antibodies transfer) (25). This decline in neonatal survival was similar between the three female genotypes. Therefore, while X*Y females display distinct maternal strategy and are more likely solitary mothers compared to XX and X*X females that commonly share parental care with males (20), they do not pay extra cost of raising their offspring alone, at least in terms of lower neonatal survival at advanced maternal age. Contrary to maternal age, paternal age did not influence neonatal survival despite evidence that, at least in humans, advanced paternal age is positively associated with an increased risk of genetic mutations

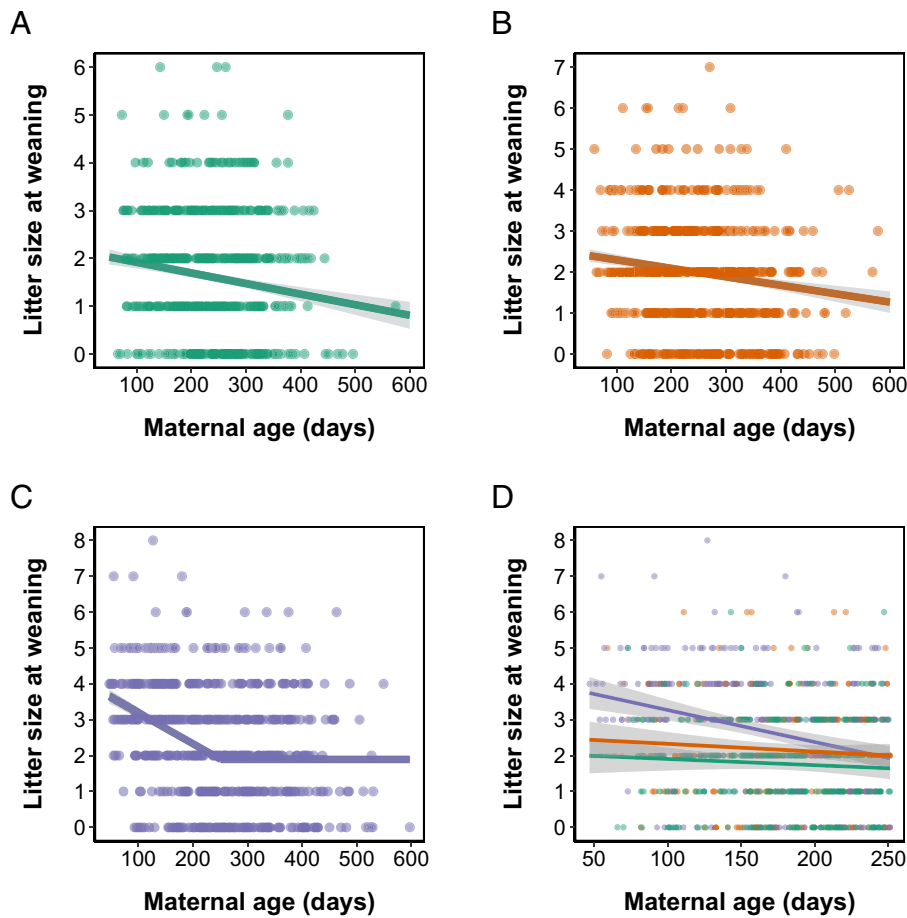


Fig. 3. Relationship between litter size at weaning and maternal age for XX (A), X*X (B), and X*Y (C) females. The threshold value for X*Y females is 251 d. The figure (D) represents the differences in the rate of senescence in litter size at weaning between the three female genotypes when maternal age varies between 42 and 251 d of age (with the same color code as in A, B, and C).

and epigenetic alterations, leading to negative health outcomes in offspring (54) and a lower reproductive success (55). Our analyses reveal no such detrimental effect of increased paternal age on reproductive traits but we cannot exclude long-term adverse consequences like neurodevelopmental disorders as described previously (e.g., ref. 56).

Conclusion and Perspectives

Our results suggest that X*Y females might have evolved a faster pace of life (see ref. 57) characterized by their earlier onset of reproduction and their higher aggressiveness (as previously documented) (21, 49) but also their higher reproductive senescence than XX and X*X females. This faster pace of life might—despite their pronounced reproductive senescence—be beneficial for X*Y females as they produce significantly more pups at weaning during their lifetime than XX and X*X females (see also ref. 21).

Regarding the particularly steep senescence in litter size observed in heterogametic (X*Y) females, multiple mechanisms encompassing genomic instability, resource-based allocation trade-offs, and constraints on meiosis might be involved. A logical and salient objective—at the crossroad of biogerontology and evolutionary biology—would now be to decipher the relative contribution of the mechanism underpinning both unguarded X and toxic Y hypotheses on the evolution of aging. In that context, our finding that heterogametic females suffer from a pronounced reproductive aging opens timely research opportunities. Very interestingly, *Mus triton* a closely related species of *M. minutoides* sharing similar life history traits (e.g., litter size), has been described with another atypical sex determination system where males and females are X0 (loss of the Y in males and of the second X in females) (19, 58). Taken together, these two species of African pygmy mice present all kinds of sex chromosome combinations (i.e., males with or without Y, females with or without Y and with one or two Xs) that would allow us to discriminate between the two main hypotheses. No such reproductive data are yet available for wild or captive populations of *M. triton*, but this offers very tantalizing prospects and

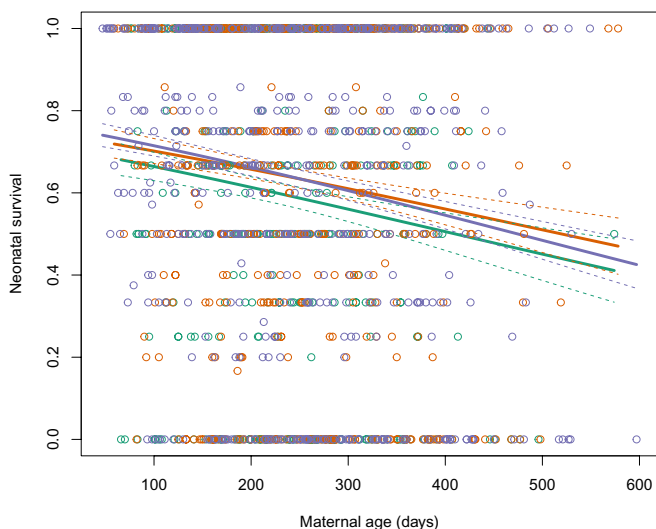


Fig. 4. Relationship between neonatal survival and maternal age for each genotype. X*Y females are in blue, XX females are in green, and X*X females are in orange.

confirms that nonconventional sex chromosome systems in mammals, although unexpectedly poorly studied, represent unique natural models for the study of fundamental processes such as aging.

Materials and Methods

Laboratory Colony and Reproductive Success Measurements. The breeding colony of *M. minutoides* is housed in the animal breeding facility of the University of Montpellier (CECEMA), it was established in 2010 from wild-caught animals trapped in Caledon Nature Reserve (South Africa). Mice are provided with food and water ad libitum, light regime is set to 15:9 h (light:dark) in a room held at 23 °C. The genotype of females is systematically assessed by karyotyping and/or PCR amplifications of the Y-linked *Sry* gene and an X-specific region that gives different sized amplicons in X and X* chromosomes (for further details in genotyping protocols, see refs. 20 and 59). Pairs of known-age individuals are isolated in environmentally enriched terrariums and maintained together for over 6 mo (in the absence of incident). Occurrence of birth and breeding performance are monitored every 2 to 3 d. For each litter, the number of offspring at birth and at weaning (4 to 5 wk) is noted. Data were collected from 2011 to 2023. Our dataset contained information on the number of pups at birth for 1,476 litters corresponding to 432 females (131, 140, and 161 females for XX, X*X, and X*Y genotypes, respectively) with a mean number of litters per females of 3.42 (4.50, 3.47, and 3.37 for XX, X*X, and X*Y genotypes, respectively). Within this dataset, the information regarding the litter size at weaning was absent for 13 litters (7, 5, and 1 litters for XX, X*X, and X*Y genotypes, respectively), for which pups were experimentally culled straight after birth. Information on the number of pups at weaning was thus available for 1,463 litters corresponding to 431 females (130, 140, and 161 females for XX, X*X, and X*Y genotypes, respectively). The litter size at birth ranges from 1 to 8 and the litter size at weaning ranges from 0 to 8.

Statistical Analyses. For all analyses, data on litter size at birth and weaning were analyzed using linear mixed effect models (LMM), with a normal error distribution (e.g., refs. 37 and 38). Results were qualitatively similar when generalized linear mixed effect models (GLMM) using a Poisson distribution were used instead (SI Appendix, Table S6). However, in a few cases, models using a Poisson distribution failed to converge and we thus report results obtained with LMM only. Neonatal survival analyses were performed with a GLMM using a binomial error by including both litter size at weaning and the number of dead offspring between birth and weaning as the dependent variable (using the R function *cbind*). Analyses were conducted using R version 4.2.2. Parameter estimates are given as \pm one SE. Analyses were performed with the package *lme4* (60).

Mean differences in litter size at birth, weaning, and neonatal survival. We compared the mean litter size (at birth and at weaning) and neonatal survival over the life course according to the female genotypes by including the female genotypes as an explanatory factor (XX, X*X, or X*Y) and mother's identity as a random effect.

Reproductive senescence in litter size at birth and weaning. To identify the best model describing the age-specific trajectory in female's litter size at birth and weaning across all females independently of their genotypes and for each genotype separately (XX, X*X, and X*Y), we fitted a set of linear mixed effect models (see below). All models included mother's identity as a random effect.

For both litter size at birth and weaning and following classical approaches in the study of reproductive senescence patterns (e.g., refs. 61 and 62), we compared models that included no effect of maternal age (equivalent to a constant litter size through life), a linear effect of maternal age, a quadratic effect of maternal age and three threshold models (i.e., piecewise linear functions) corresponding to three different forms of age-specific reproductive patterns (SI Appendix, Fig. S1). The first threshold model corresponds to a constant litter size up to a threshold maternal age after which litter size decreases with increasing maternal age, the second threshold model corresponds to an increase and/or a decrease in reproductive performance to a threshold age after which it remains constant and the third threshold model includes an increase and/or a decrease in reproductive performance to a threshold age after which it increases or decreases with maternal age (see ref. 61 for a similar approach). We then used the Akaike Information Criterion (AIC) (63) to select the best model among the five tested models. We retained the model with the lowest AIC, and when the difference of AICs between competing models was less than two, we retained the model with the lowest

number of parameters to satisfy parsimony rules (63). For both litter size at birth and litter size at weaning, this procedure was repeated four times (i.e., for all females pooled together and for XX females, X*X females, X*Y females, separately). AIC values of each tested model are reported in SI Appendix, Tables S1 and S2 for litter size at birth and weaning, respectively.

Based on the results of our model selection procedure, we then tested whether the female genotypes influence the decline in age-specific decline litter size at birth and weaning by running LMMs with litter size as the dependent variable, mother's identity as a random effect and an interaction between maternal age and the female genotypes as explanatory variables.

Reproductive senescence in neonatal survival. We analyzed the age-specific changes in neonatal survival throughout mother's life course by fitting a GLMM with the mother's identity as a random effect and the maternal age as the covariable. We then investigated whether the age-specific patterns of senescence in neonatal survival change between the three genotypes by including an interaction between maternal age and the three-level factor female genotypes. Threshold models led to convergence issues with binomial distribution and the occurrence of reproductive senescence were investigated through linear models. For neonatal survival, explanatory variables are scaled to facilitate model's convergence.

Confounding effects of selected disappearance and father's age. For the three reproductive traits considered in this study (litter size at birth, weaning, and neonatal survival), we performed on a subset of the initial dataset, two complementary analyses in order to both assess and control for possible effects of i) selective disappearance and ii) paternal age on reproductive senescence patterns. We investigated these two effects separately in order to maximize the sample size for each analysis (see below).

- i) The possible selective disappearance of females with low reproductive performance could mask senescence or lead to an underestimation of this process at the population level (64). A classical procedure to control for selective disappearance is to include the individual's lifespan as a covariate in the different model considered (64, 65). Data on natural lifespan are available for a subset of females in the colony (72 females, 16.67% of whole the dataset) and on this restricted sample size, we compared the previous selected models with a similar model including the maternal lifespan (ranging from 55 to 609 d) as covariate. Models were then compared through an AIC selection procedure (see above). The AIC values of these models are reported in SI Appendix, Tables S1, S2, and S3 for litter size at birth, at weaning, and neonatal survival, respectively.
- ii) There is increasing evidence that mating with old males can potentially modulate female's reproductive success through a reduced litter size or a lower neonatal survival (see ref. 25). The age of the father was known for 98.04 % percent of the litters (1,447 out of 1,476 litters at birth). Therefore, to control for a possible confounding effect of paternal age, we compared (for each reproductive trait and for each set of data), the selected models with a similar model including paternal age as an additional covariate. In our dataset, the positive correlation between maternal and paternal age ($r = 0.48, P < 0.001, N = 1,447$, SI Appendix, Fig. S2) was below the threshold value of 0.7 above which multicollinearity issues can occur (66). Both variables were thus concomitantly added in these models. Models were compared with an AIC selection procedure (see above) and the AIC values of these models are reported in SI Appendix, Tables S1, S2, and S5 for litter size at birth, at weaning and neonatal survival, respectively.

Data, Materials, and Software Availability. The data that support the findings of this study as well as the R script of the statistical analyses are openly available at Zenodo: <https://zenodo.org/records/13940837> (67).

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