INTRODUCTION. In humans, less than 25% of disease mutations encoding enzymes are dominant; of these, many arise due to loss-of-heterozygosity or dominant interfering effects. In true haploinsufficiency, the phenotype is usually manifested immediately in heterozygous progeny. For example, in mice heterozygous for BLM or p27, an increase in genetic instability is observed in heterozygous progeny without a concomitant loss of the remaining wild-type allele. We describe an unusual type of progressive haploinsufficiency involving the essential protein component of telomerase, the telomerase reverse transcriptase, \textit{mTert}. Animals heterozygous for \textit{mTert} show a gradual telomere shortening with each generation, and an accelerated loss of telomere function when bred to nullizygosity. Despite an inability to maintain average telomere lengths, \textit{mTert+/-} animals are nonetheless proficient at retaining minimal telomere DNA at all chromosome ends.

METHOD. We previously reported the generation and characterization of mice deficient in \textit{mTert}, in a mixed C57BL/6-129J background, for up to two successive generations of \textit{mTert-/-} progeny [1]. Similar to mice lacking the telomerase RNA, \textit{mTert-/-} mice lack telomerase activity and undergo telomere attrition [1-3]. Here, we carried out successive breeding of \textit{mTert-/-} mice in this mixed genetic background until the 8\textsuperscript{th} generation. Concurrently, mixed genetic background \textit{mTert+/-} mice were successively crossed with wild-type C57BL/6 mice, for up to 10 generations. After 6 successive backcrosses to wild-type mice, \textit{mTert+/-} mice were bred together to generate \textit{mTert-/-} animals in a C57BL/6 background, and these mice were bred successively for up to four generations. Telomerase
activity, *mTert* mRNA levels, and quantitative telomere length measurements were performed as previously described [1].

RESULTS. It was previously reported that loss of the telomerase RNA leads to telomere shortening and eventual sterility in C57BL/6-129J mice after approximately 6 generations (G6) [3, 4]. Similarly, C57BL/6-129J *mTert*-/- progeny became sterile at G8, whereas *mTert*+/− progeny in a C57BL/6 background showed a surprising decline in fertility after only 1 generation (Figure 1). We therefore examined the distribution of individual telomere ends in metaphase chromosomes of *mTert*+/− mice after successive breeding to C57BL/6 WT mice, and *mTert*-/− mice in both a mixed and C57BL/6 background. We observed a loss of telomere signal that was accelerated by up to 4 generations in *mTert*-/− C57BL/6 mice (Figure 2). This accelerated telomere erosion could be rationalized by the observation that *mTert*+/− animals exhibited gradual telomere erosion when crossed to WT C57BL/6 mice, eventually reaching an apparently stable short, average length (Figure 2, right panel). Despite average telomere lengths comparable to infertile *mTert*-/− mice, *mTert*+/− mice were fertile and retained minimal telomere DNA at all chromosome ends (Figure 2). In addition, *mTert* exhibited a dominant ability to rescue telomere-free ends in crosses between *mTert*+/− and *mTert*-/− mice (not shown).

![FIGURE 1. Fertility upon successive generations (G1-G8) of *mTert*-/− mice derived from C57BL/6-129J mice with initially long telomeres (green) compared with *mTert*-/− mice derived after 6 successive crosses of *mTert*+/− mice with C57BL/6 wild-type mice (blue).](image-url)
DISCUSSION. \( mTert \) exhibited unique dosage effects \textit{in vivo}; a haploinsufficiency for overall telomere length maintenance, and a haplosufficiency for healing critically shortened chromosome ends. Several human tissues exhibit progressive telomere attrition despite a low level of telomerase activity. These low levels of telomerase may serve a critical role in maintaining minimal telomere DNA at chromosome ends. In humans, an autosomal dominant disease, dyskeratosis congenita, is linked to mutations in the telomerase RNA \([5, 6]\). These patients suffer from bone marrow failure, anemia, and myeloid cell proliferation disorders that worsen with each generation (termed genetic anticipation) \([5, 6]\). The \( mTert^{+/-} \) mice may thus provide a genetic framework for the role of limiting telomerase in human cell proliferation, including the contribution of progressive telomere loss to genetic instability and anticipation.

ACKNOWLEDGMENT. This work was supported by the National Institutes of Health Grant AG16629 (to L.H.). This work has been submitted for publication elsewhere, and should therefore be regarded as personal communication.
REFERENCES.


