Hybrid sterility is one of the postzygotic reproductive barriers that can prevent gene flow between diverging populations. Identification of sterility genes and their function is consequently demanded for understanding of processes that can ultimately lead to speciation. The sterility locus Hst1 (Hybrid sterility-1) on chromosome 17 was originally described in hybrid progeny delivered in intersubspecific crosses between wild Mus musculus musculus, sampled at localities in Prague, and two classical laboratory strains C57BL/10 (producing sterile hybrid males) and C3H (producing fertile hybrid males), derived from M. m. domesticus; Hst1 was defined by the presence of polymorphism in the domesticus genome. We confirmed that Hst1-related sterility is polymorphic in natural allopatric populations and noted additional polymorphic genes affecting hybrid sterility in crosses between wild M. m. musculus from Studenec and C57BL/10. To determine the genetic basis underlying male fertility, we derived two wild-origin musculus strains, STUS and STUF, producing alternative phenotypes in crosses with C57BL/10 mice (sterile and fertile males, respectively). From the cross of musculus (STUS × STUF)F1 females and C57BL/B10 we obtained both fertile and sterile hybrid males. Infertile hybrid males suffered from significantly reduced testes and epididymis size with no sperm or very low number of sperm in epididymis. Histology of testes showed that sperm development was arrested at the pachytene/zygotene stages of meiosis. Alternatively, spermiogenic defect was observed in the seminiferous tubules of some hybrid males. QTL analysis of the male progeny revealed significant male sterility loci on chromosomes 17 and X. The data suggest that genetic incompatibilities on chromosome 17 associated with male sterility have evolved independently and are polymorphic both within the M. m. domesticus and M. m. musculus genomes. The new wild-derived inbred strains STUS and STUF represent an effective tool for elucidating the genetic basis of hybrid sterility and the geographical variation of hybrid sterility within natural populations of house mouse.