# PWD/Ph and PWK/Ph Inbred Mouse Strains of *Mus m. musculus* Subspecies – a Valuable Resource of Phenotypic Variations and Genomic Polymorphisms

( functional genomics / microsatellite polymorphism / *Igf2r* / mouse genome / QTL / *Mus m. musculus* / hybrid sterility / genomic imprinting )

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**Abstract. PWD/Ph and PWK/Ph (abbreviated PW*) are highly inbred mouse strains (F66 and F70) derived from wild mice of *Mus musculus musculus* subspecies. When compared with laboratory inbred strains, they display a plethora of differences in many complex phenotypes such as body weight, fat distribution pattern, blood levels of intermediary metabolites, sensitivity to type-1 diabetes or behaviour patterns. The PWD/Ph genes can rescue the lethal effect of lack of the Igf2 receptor. The male-limited hybrid sterility of (PWD/Ph x laboratory strain)$F_1$ hybrids is a specific phenotype controlled by three or four unlinked loci. These complex phenotypic traits can be genetically dissected by QTL analysis using microsatellite markers of known genetic location. The PW* strains are particularly useful for such genome-wide scans since 70–80% of randomly chosen microsatellite markers are polymorphic in (PW* x laboratory strain) crosses compared to 35–45% in crosses between two laboratory strains. The list of polymorphic microsatellite loci is included in this report. The high degree of sequence polymorphism allows easier distinction between paternal and maternal mRNA transcripts in PW* hybrids, which makes the PW* strains a useful tool also in molecular studies of genomic imprinting. The high frequency of phenotypic differences together with the high degree of sequence polymorphism and the relatively easy breeding of PW* strains make them a valuable mammalian model organism for the functional genomics of the traits of biomedical importance.**

The inbred strains of mice represent a favourite model of genetically defined mammals particularly suitable for ambitious goals of functional genomics. A significant difference in any phenotypic trait between two inbred strains is genetic in nature and can be meaningfully analyzed with the power of the presently available genetic and physical mapping tools. For decades the genetic analysis was restricted to phenotypes controlled by single genes. At present, traits such as susceptibility to diseases, control of embryonic development or body size are amenable to complex genetic dissection for the first time. Genetics and genomics thus quickly meet physiology to answer the questions about the nature of normal and pathological body functions. To assign the complex, disease-related phenotypes to DNA sequences, functional (physiological) genomics needs variants of both, the studied phenotypes and the homologous DNA sequences. However, in contrast to human genetic resources, DNA polymorphism between laboratory inbred strains is very restricted. This is because a rather restricted number of unrelated founders were used to create more than 400 laboratory mouse strains available today (Festing, 1996). Their genome appears to be mostly but not entirely of *Mus m. domesticus* origin (Moriwaki, 1994). For this reason, the creation of inbred strains from wild mice of other (sub)species and their use in interspecies crosses proved to be particularly rewarding. In the last 20 years, inbred strains were established from several mouse species, namely *Mus spretus, Mus mollosinus* and *Mus castaneus* (Bonhomme and Guenet, 1996), that are reproductively isolated in nature but still can breed in captivity. Most often used are the strains of *Mus spretus*, a species separated from *Mus m. domesticus* (laboratory mice) for 3 million years. The long separate evolution of genomes of both species generated a high frequency of DNA sequence variants that can now be used as markers in the whole genome scans. However, the use of *Mus spretus* in genetic analysis has some drawbacks, since interbreeding with classical laboratory inbred strains can be very inefficient and it can be difficult to generate reciprocal $F_1$ hybrids. *Mus m. musculus* ancestors have separated from ancestors of *Mus m. domesticus* about 1 million years ago, yet the degree of sequence polymorphism is almost as high as in the *spretus – domesticus* pair (Montagutelli et al., 1991). They show many phenotypic differences when compared with *Mus m. domesticus*, and it is much easier to produce $F_1$ hybrids with classical laboratory strains. Twenty five years ago, we established the first wild-derived inbred strains of

Supported by the grants 204/98/K015 and 302/99/0354 from the Grant Agency of the Czech Republic and by the grant A505209/1997 from the Grant Agency of the Academy of Sciences of the Czech Republic. J.F. is a recipient of an International Research Scholar's award from Howard Hughes Medical Institute.

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Abbreviations: PW* – PWD/PH and/or PWK/Ph mouse inbred strains, QTL – quantitative trait loci, RFLP – restriction fragment length polymorphism, RLGS – restriction landmark genomic scanning, SSLPs – simple sequence length polymorphisms.

*Mus m. musculus* origin, PWB/Ph, PWD/Ph and PWK/Ph (Pavljukova and Forejt, 1981, and unpublished). Here we want to briefly point to their value as a unique resource for functional genomics studies of traits of biomedical importance. We also summarize hitherto known polymorphisms at the microsatellite (SSLPs) loci that can be used for genetic and physical mapping of the mouse genome. We believe that these mice will serve the community of mouse geneticists as excellent model organisms in the evolving era of functional genomics.

## PWD/Ph, PWK/Ph and PWB/Ph inbred strains were generated from wild mice of *Mus m. musculus* subspecies

In 1972, each of the three strains started from a single pair of wild mice of *Mus m. musculus* origin trapped in the central part of the Czech Republic. The PWB/Ph inbred strain originated from mice trapped in the Prague Zoo, PWD from mice in the locality "Kunratice" near Prague and PWK/Ph from founders in the locality "Lhotka". The founders were pre-selected by progeny testing with C57BL/10 mice. Only wild mice that produced sterile sons with C57BL/10 were used for brother x sister mating that initiated the inbred strains. At the time of writing this manuscript (October 1999), the PWD/Ph strain was at the F66 generation of brother x sister mating and PWK/Ph was at F70. The PWB strain went through a breeding crisis in 1998. Therefore, the few remaining males were outcrossed to PDW/Ph females, and $F_1$ hybrid females were backcrossed four times to PWB/Ph fathers. The $BC_4$ progeny was used to start an inbreeding programme that should save over 95% of the original PWB/Ph genome in the newly developed strain. The PWD/Ph strain was transferred to SPF status in 1999, the other strains will be clean from specific pathogens (SPF) in the near future.

## PW* inbred strains facilitate studies of monoallelically expressed genes

The molecular analysis of monoallelic expression of imprinted genes takes advantage of the high degree of sequence polymorphism between laboratory and PW* mouse strains, allowing to distinguish between mRNA transcripts of maternal and paternal origin in the reciprocal $F_1$ hybrids. Several methods were used to distinguish monoallelic expression. In the case of mouse *U2af1-rs1* gene, the RT PCR products of C3H and PWK alleles revealed restriction fragment length polymorphism (RFLP) after digestion with *Msp*I restriction endonuclease (Hatada et al., 1995; Nabetani et al., 1997). The PWK inbred mice were used in the restriction landmark genomic scanning (RLGS) assay for detection of parent-of-origin specific CpG methylation patterns known to accompany parent-of-origin monoallelic expression (Shibata et al., 1995). The RNase protection assay was successfully used to distinguish PWD and C3H transcripts of *H19* and *Igf2* genes in cell hybrids between lymphocytes from reciprocal (PWD x C3H)$F_1$ hybrids and embryonal carcinoma cell lines (Forejt et al. 1999).

The $T^{hp}$ deletion is acting as late embryonic lethal when inherited from the female parent, but is viable when of paternal origin, and so does the null mutation of the *Igf2r* gene located in the region deleted by $T^{hp}$. In crosses with PWD, the lethality of maternally transmitted $T^{hp}$ deletion disappeared (Forejt and Gregorova, 1992). The genetic control of the rescue phenomenon is being dissected by genome scanning of $BC_1$ mice from (C3H x PWD) x C3H $BC_1$ backcross (Gregorova, Divina, Dimitrov and Forejt, 1999, in preparation). The monoallelic expression of the *Pax5* gene, independent of parental origin, was described in mature B cells using the PWD inbred mouse strain (Nutt et al., 1999). These examples demonstrate that interspecies PW* hybrids with laboratory mice are a favourite model for investigation of the monoallelically expressed genes. It also indicates that *Mus m. musculus* and *Mus m. domesticus* subspecies did not diverge their control mechanisms of genomic imprinting in such a way that would disturb the monoallelic expression of the imprinted genes. Admittedly, this conclusion requires additional scrutiny, since in hybrids of two closely related species of *Peromyscus* the imprinting seems to be disturbed (Vrana et al., 1998).

## The genetic mechanism of hybrid sterility phenomenon can be dissected in interspecies crosses with PWD mice

In accordance with the Haldane rule, the male $F_1$ hybrids from interspecies crosses are often sterile. The rule holds true for $F_1$ hybrids of laboratory strains such as BALB/c and wild mice of *Mus spretus*, *Mus hortulanus, Mus macedonicus, Mus spicilegus* or *Mus abbotti* origin (Matsuda and Chapman, 1994). Wild mice of *Mus m. musculus* origin can give rise to fertile or sterile male hybrids (Table 3 in Forejt, 1981), indicating that the genes controlling this form of reproductive isolation have not been fixed yet. The PWB/Ph, PWD/Ph and PWK/Ph mouse inbred strains were initiated from wild mice that had been previously proved to yield sterile male progeny with C57BL/10 inbred mice. So far, one gene responsible for hybrid sterility, *Hst1*, was located on mouse chromosome 17, 8.4 cM distally from the centromere (Forejt et al., 1991). The high resolution genetic mapping was possible because C57BL/10 and C3H mice differ only at one locus, *Hst1*, in respect to hybrid sterility. The allelic difference at this single gene causes that (C57BL/10 x PWD) male hybrids are completelly sterile, while (C3H x PWD)$F_1$ hybrids are fertile (Fig. 1). Physical mapping within a YAC contig identified a 580 kb interval with several candidate genes showing testicular expression and zero recombination with *Hst1* (Gregorova et al., 1996; Trachtulec et al., 1997). The genomic scan of (C7BL/10 xPWD) x C57BL/10 $BC_1$ progeny indicates that, contrary to hybrid sterility seen in *Drosophila* inter-

species crosses, the hybrid sterility of *Mus m. musculus* x laboratory strain hybrids can be controlled by only three or four major genes (Brennerova, Gregorova, Dimitrov, Divina and Forejt, in preparation).

The fact that hybrid males cannot make any progeny makes the genetic studies of phenotypes other than sterility more complicated. One way to avoid the problem is to use hybrid females since they are always fertile. In the case of PW* strains, the hybrids with C3H or CBA inbred strains are fertile in both sexes and can be used for further crosses.

## List of polymorphic microsatellite (SSLP) loci used for genome scanning: a useful tool for QTL analysis

Since the PW* strains are not on the list of 8 strains for which the strain distribution pattern of microsatellite loci was established (Dietrich et al., 1996), we have combined the available data from several sources on polymorphisms associated with PWK/Ph, PWB/Ph or PWB/Ph strains (Table 1). The PWK/Ph data are based on allelic comparisons in EtBr-stained polyacrylamide gels; our data are from Genetic Analyser ABI310 using fluorescently labelled primers obtained from Research Genetics (http://www.resgen.com/). C3H and C57BL/6 data are from the Research Genetics WEB page. In our experience as well as of others (Montagutelli et al., 1991), 70–80% of randomly chosen microsatellite markers are polymorphic in (PW* x laboratory strain) crosses compared to 35–45% in crosses between two laboratory strains.

## Mapping of simple mutations and complex traits in crosses with PW* strains

The high degree of sequence polymorphism was used for mapping of simple mutations, including a T cell receptor mutation (Cazenave et al., 1990; Jouvin-Marche et al., 1992) or pigment mutation (Holcombe et al., 1991). PWK crosses were used to dissect the genetic control of the complex phenotypes such as the mouse model for human type I von Willebrand disease (Nichols et al., 1994), the bleeding time (O'Brien et al., 1994) or sensitivity to insulin-dependent diabetes (Melanitou et al., 1998). At present we are using the PWD strain for QTL mapping of Tme-modifiers (Forejt and Gregorova, 1992), for mapping genes governing the hybrid sterility of (PWD xC57BL/10) male hybrids (Forejt, 1996) and for body weight and fat distribution pattern (Brennerova, Gregorova, Divina, Dimitrov, Kopecký and Forejt, in preparation). The potential of PW* strains for QTL mapping is much higher than the above mentioned examples. We have obtained preliminary data showing that the PWD/Ph strain differs from C57BL/10 in blood levels of 20% of tested intermediary metabolites. We can see dramatic differences in behaviour when PWD or PWK mice are compared to any "classical" laboratory inbred strains.

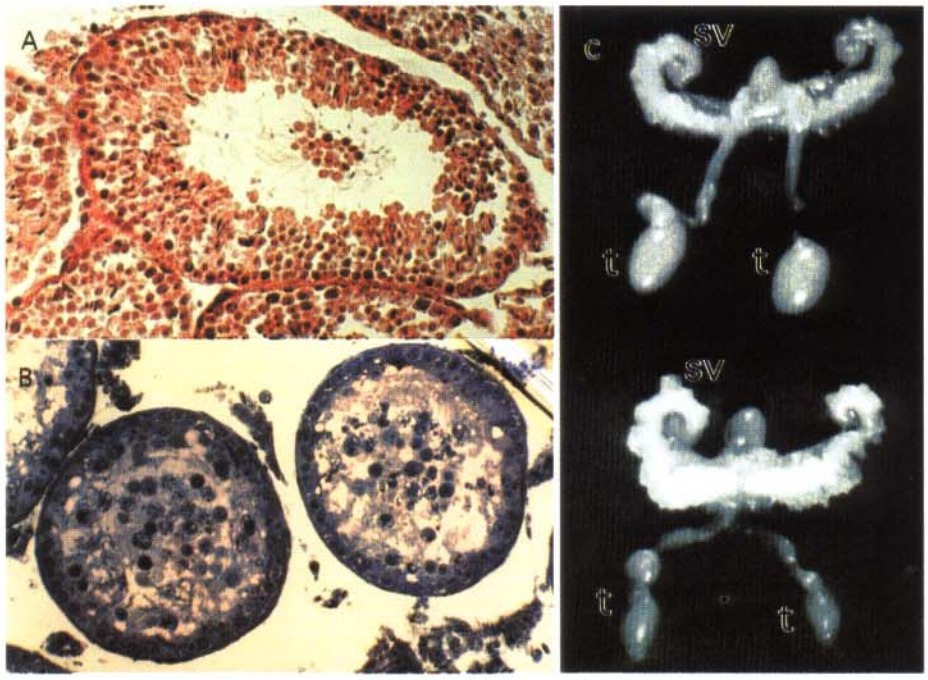


*Fig. 1.* Spermatogenic breakdown in $F_1$ hybrids of PWD/Ph strain and laboratory inbred strains. (A) histological crossection of seminiferous tubules of a fertile $F_1$ hybrid (PWD x C3H). (B) Sterile $F_1$ hybrids (PWD x C57BL/10) reveal only one layer of cells in seminiferous tubules. A more detailed EM analysis indicates the presence of spermatogonia, Sertoli cells and early leptotene and zygotene primary spermatocytes (data not shown). The lumen of the spermatogenic tubules is filled with degenerated apoptotic primary spermatocytes. (C) The early arrest of spermatogenesis in (PWD x C57BL/10) hybrids causes a dramatic reduction of testis (t) size. The upper seminal vesicles (sv) and testes (t) were dissected from a (PWD x C3H) hybrid male (compare with histology in 1a), the lower dissection is from a (PWD x C57BL/10) sterile male. The size of seminal vesicles reflects the testosterone level but does not differ between fertile and sterile males.