Natural history of a copy-number variant in mouse

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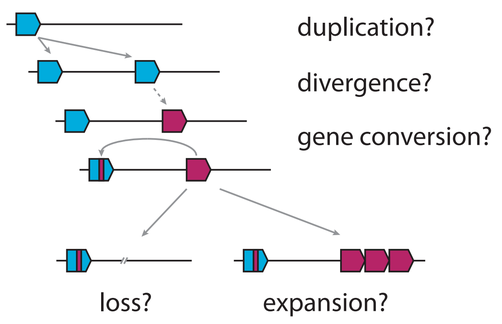
Background

Departures from expected Mendelian segregation (transmission ratio distortion, TRD) on mouse chr2 have been reported in several independent crosses. In all cases, TRD is in favor of alleles with a copy-number gain at chr2: 77.9 Mbp. We have recently shown that this due, in part, to female meiotic drive[1]. High-copy alleles have been subject to rapid selective sweeps in both laboratory and wild populations (see poster **P-14**).

In order to understand and correctly interpret extant patterns of sequence variation around this locus, we aimed to reconstruct its evolutionary history using whole-genome sequence from samples across the phylogeny of *Mus*. Specifically, we sought to account for the following events:



msBWT: new tool for sequence analysis

The multi-string Burrows-Wheeler transform (`msBWT`) is a compressed representation of unaligned reads with an associated index which allows efficient search[2]. Queries for sequences of length k take $O(k)$ time, regardless of the size of the dataset.

```
                    ???:CTGGCCTGTCCACAGTGTC
------CATGAGCCTCATTTATCATGCCTTCTGGCCTGTCCACAGTGTCTAACATA----------------------
----------AGCCTCATTTATCATGCCTTCTGGCCTGTCCACAGTGTCTAACATAGTAC----------------
----------GCCTCATTTATCATGCCTTCTGGCCTGTCCACAGTGTCTAACATAGTACT----------------
--------------CATTTATCATGCCTTCTGGCCTGTCCACAGTGTCTAACATAGTACTAGAT-----------
--------------------TATCATGCCTTCTGGCCTGTCCACAGTGTCTAATATAGTACTAGATACAC-------
--------------------TATCATGCCTTCTGGCCTGTCCACAGTGTCTAATATAGTACTAGATACAC-------
--------------------TATCATGCCTTCTGGCCTGTCCACAGTGTCTAATATAGTACTAGATACAC-------
----------------------CATGCCTTCTGGCCTGTCCACAGTGTCTAATATAGTACTAGATACACAAT----
```

The `msBWT` enables targeted *de novo* assembly and variant discovery, even in repetitive sequence.

Acknowledgements

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References

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Conclusions

Using whole-genome sequence from three mouse species, we have shown that the multiple copies of *R2d* present in several classical and wild-derived laboratory strains represent a segmental duplication ancestral to the divergence of *Mus musculus*. The resulting duplicates in the *R2d1* and *R2d2* loci have undergone multiple independent gene conversion events. Evidence that copies of *R2d* in the distal locus are unstable is twofold. First, multiple independent copy-number losses have occurred within the *M. m. musculus* and *M. m. domesticus* lineages. Second, although expansion alleles at *R2d2* arose recently on a single haplotype, copy number at *R2d2* is highly polymorphic in wild *M. m. domesticus*.

Evidence for a CNV

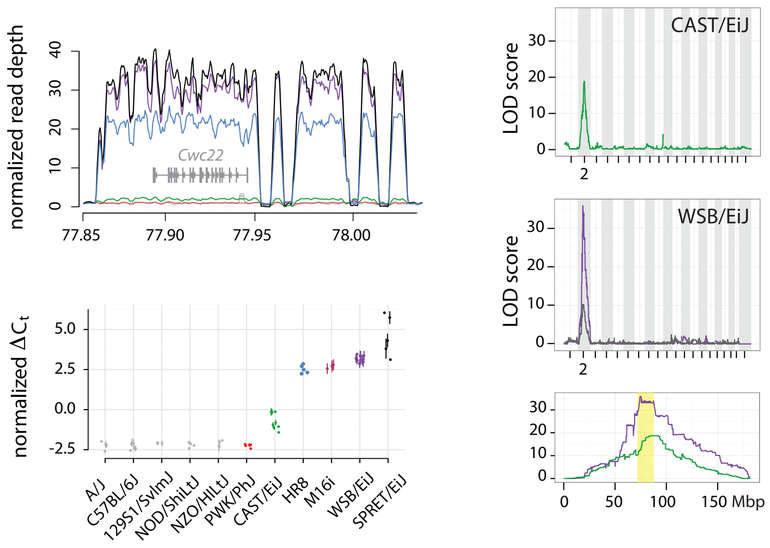

Figure 1. Copy-number gain over 150 kbp at chr2: 77.9 Mbp in four inbred strains, revealed by sequencing[3] and confirmed by qPCR. The extra copies are not in tandem: they map ~ 6 Mbp away[1].

Structure of the locus

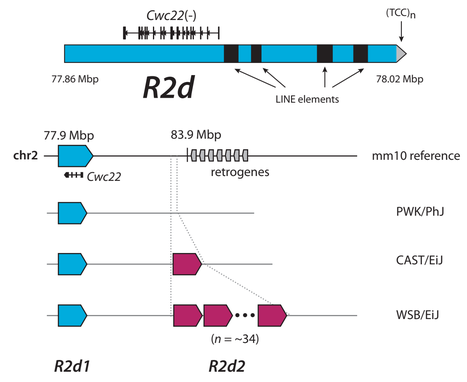

Figure 2. A single copy of the 150 kbp duplicated sequence (named "responder to (meiotic) drive", *R2d*) is located chr2: 77.9 Mbp (locus *R2d1*) in the reference genome. Additional copies are located at *R2d2* in other strains.

The *R2d* unit contains a single protein-coding gene, *Cwc22*. It encodes a spliceosome protein which is essential for development in mouse. Four recent LINE insertions are present in the reference genome, and the unit terminates in a $(\mathrm{TCC})_n$ microsatellite.

Phylogenetic context

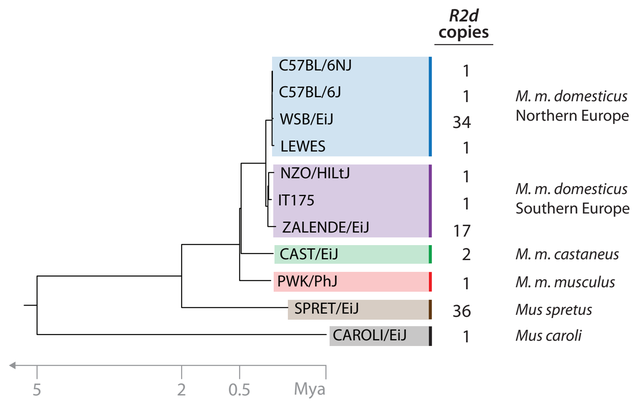

Figure 3. Mitochondrial phylogeny of subgenus *Mus* for samples used in this study (dates from [4]), with copy number in *R2d1* + *R2d2*.

We assembled *R2d* sequence(s) in 2 classical and 8 wild-derived inbred strains, plus 1 wild-caught mouse (IT175). The samples span all 3 subspecies of *M. musculus* as well as outgroups *M. spretus* and *M. caroli*.

Ancestral duplication of *R2d*

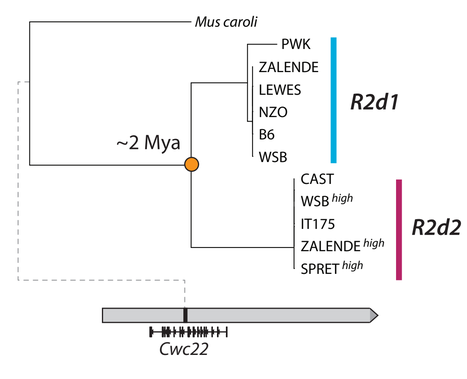

Figure 4. Maximum-likelihood phylogeny built from 1557 bp of non-coding sequence in *R2d*, assembled from Illumina sequence reads using the `msBWT` toolkit[2].

R2d was duplicated ~ 2 million years ago (orange node); since then, the copies in *R2d1* and *R2d2* have diverged by $\sim 2.5\%$. Sequence diversity within *R2d2* is very low.

R2d2 expansion alleles of recent European origin

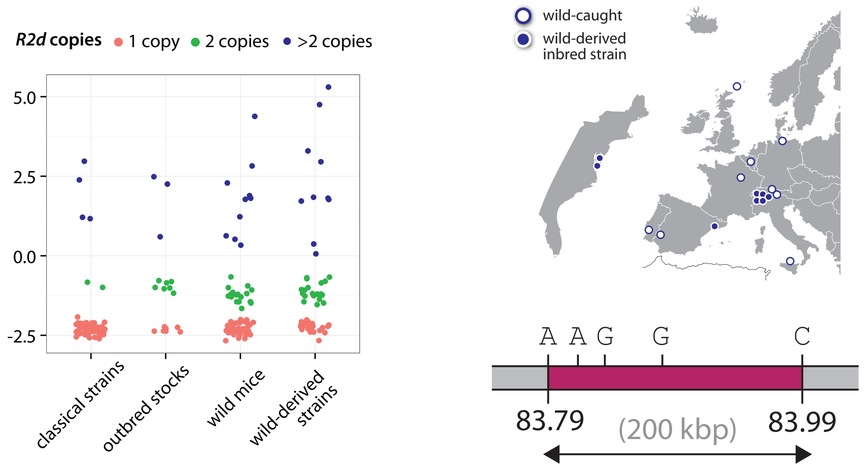

Figure 5. *R2d* copy number varies widely in laboratory and wild mice of *M. m. domesticus* origin (left). Expansion alleles – those with copy number > 2 – are widespread in Europe.

We surveyed *R2d* copy number by qPCR in a large panel of laboratory and wild mice[5]. We found that expansion alleles (copy number > 2) are common in mice of *M. m. domesticus* origin (MAF = 0.21 in the wild) and are associated exclusively with a single 200 kbp haplotype.

This haplotype is present both in populations with Robertsonian karyotypes and those with standard karyotypes, suggesting that it arose before the separation of the chromosomal races ($\sim 5 - 10$ kya[6]).

Inter-locus gene conversion between *R2d1* and *R2d2*

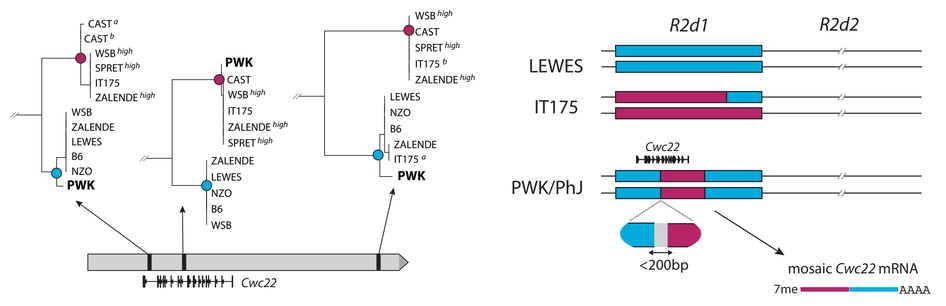

Figure 6. Changes in tree topology along *R2d* (left) are a signature of inter-locus gene conversion[7]. Resulting mosaic *R2d1* alleles are shown at right.

The presence of *R2d2*-like sequence within *R2d1* in extant mouse lineages with a single *R2d* copy is evidence of inter-locus gene conversion prior to the loss of copies at *R2d2*. PWK/PhJ shows evidence of a conversion event between exons of *Cwc22*, such that transcripts are a chimera of two sequences separated by 2 million years of evolution.