Large interspecies genomic replacements and super-Mendelian inheritance to connect genotype and phenotype over non-genetically hybridizable phylogenetic distances

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ABSTRACT

Quantitative trait analysis identifies causative genetic modifications that drive evolution, but its usefulness is limited to closely related species that can interbreed. Other approaches must be developed to test the sufficiency of genetic change to cause phenotypic change over larger evolutionary distances. Here, we outline two genome engineering approaches in mouse that can be used to investigate the complex genetics that direct limb patterning and development of the the Lesser Egyptian Jerboa, *Jaculus jaculus*, a bipedal rodent with elongated feet, three toes, and fused foot bones. The relatively recent common ancestor with the laboratory mouse (55 mya) places the jerboa's extreme phenotype in a genomic context similar enough to the mouse to test the sufficiency of evolved jerboa sequences to alter the developing mouse limb. We are building the tools to precisely replace a large piece of the mouse genome (30kb) with its jerboa homologue using hybrid mouse-jerboa BACs. This approach aims to preserve the genomic architecture of the jerboa locus of interest including de novo enhancers that are difficult to identify computationally. Our replacement is likely to yield a "jerbouse," a mouse with jerboa-like phenotypes, allowing us to identify sequences within the replaced region that are responsible for the phenotype. We also present evidence for a CRISPR/Cas9-based system that, when mobilized in the female germline, can induce copying of an allele to the homologous chromosome, biasing inheritance towards a specific allele. Such a system could be used to combine alleles of interest much faster than possible by Mendelian inheritance, allowing for the efficient assemblage of complex genotypes. These approaches can be applied to test the genotype-phenotype relationship of a variety of morphologies and species to understand the mechanisms that produced organismal diversity.

LARGE INTERPSPECIES GENOMIC REPLACEMENTS

The Jerboa is a bipedal desert dwelling rodent with elongated hindlimbs, three toes, and fused metatarsals (Fig 1). In order to understand the regulation of genes responsible for these phenotypes we propose to engineer large genomic replacements in mouse cells.

We will construct hybrid BACs[1] with 31kb of jerboa sequence flanked by 50+kb of mouse homology. **When the jerboa sequence is incorporated into mouse cells by homologous recombination, we hope that the resulting animals will have a hybrid "jerbouse" phenotype** (Fig 1). Specific components of the phenotype can then be retraced to specific DNA sequences within the replacement.

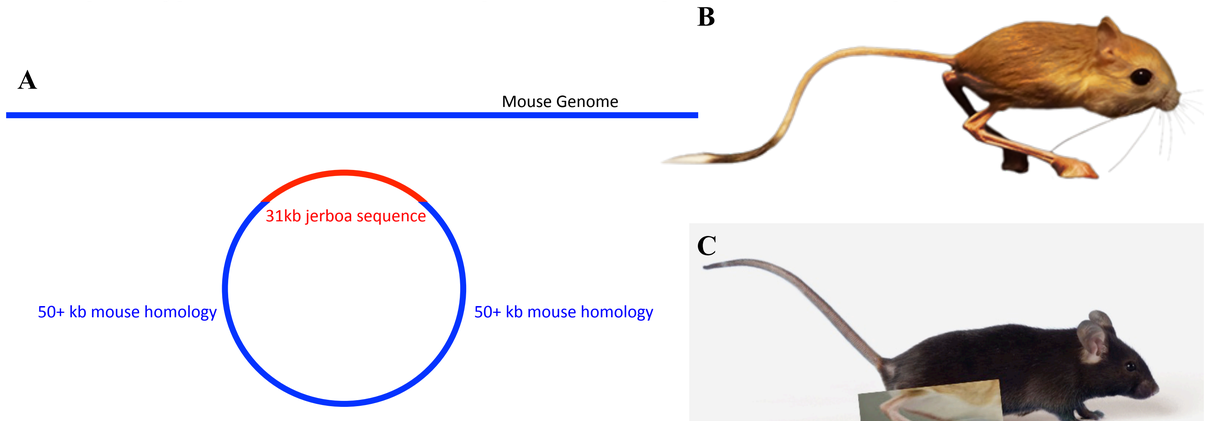


Figure 1: Engineering large genomic replacements. A) Hybrid BAC for homologous recombination. B) The lesser Egyptian jerboa, *Jaculus jaculus*. C) Jerbouse.

SUPER-MENDELIAN INHERITANCE[2]

Combining many alleles for a complex genetic model is prohibitive. In order to engineer an allele that would be inherited at greater-than-Mendelian rates, we inserted a fluorescent red marker and a gRNA targeting *Tyrosinase* into the gRNA's own cut site on one chromosome (Fig 2a). This "CopyCat" allele is inherited normally unless combined with a source of Cas9. In the presence of Cas9, the gRNA targets the opposite chromosome, inducing a double stranded break (DSB) (Fig 2b).

DSBs in the genome can be repaired by non-homologous end-joining (NHEJ), which frequently leaves a mutation, or by homology directed repair (HDR), where the break is repaired by copying information from a homologous template. If the break is repaired by HDR using the opposite chromosome as a template, the mouse will become homozygous for our allele, increasing the rate of inheritance.

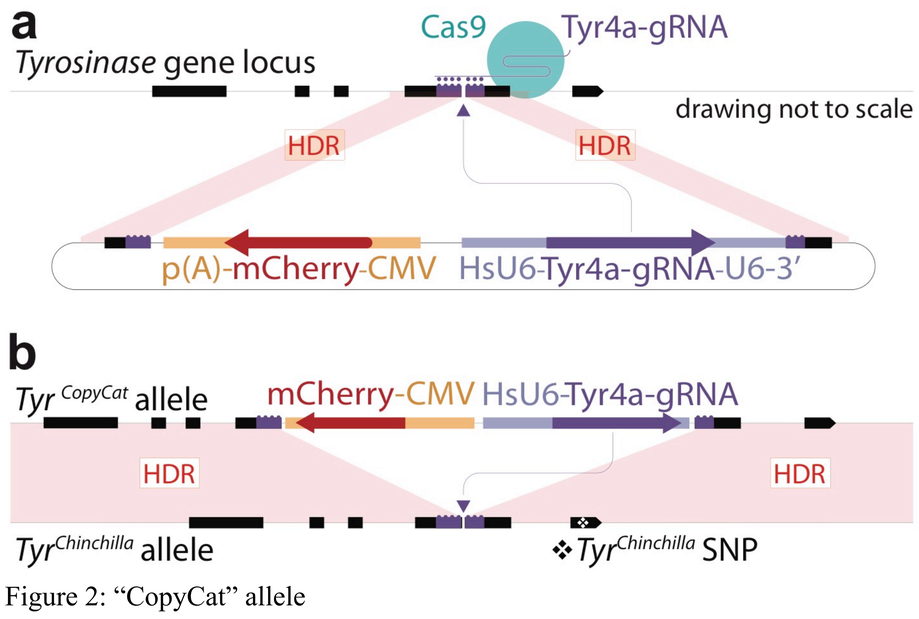
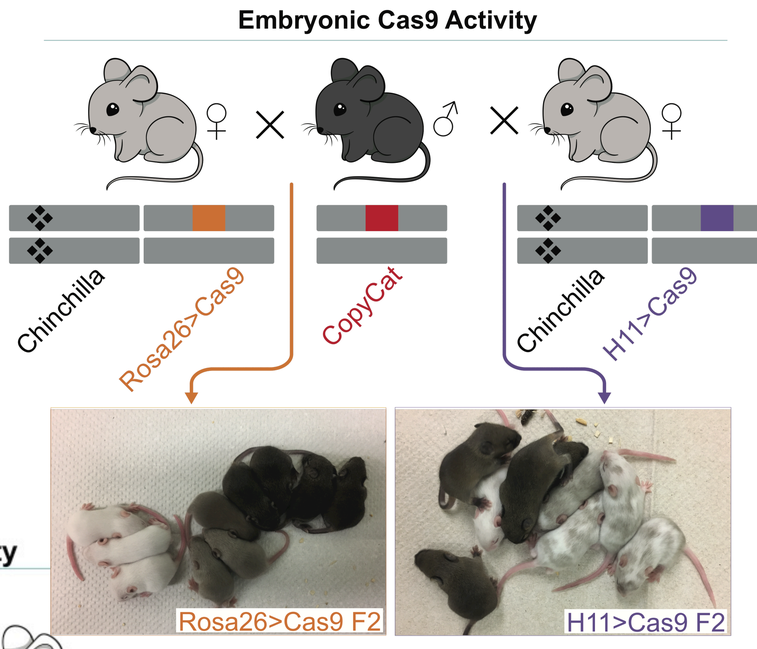


Figure 2: "CopyCat" allele

To track the targeted chromosome we used a closely linked SNP, *Tyrosinase*^{Chinchilla}.

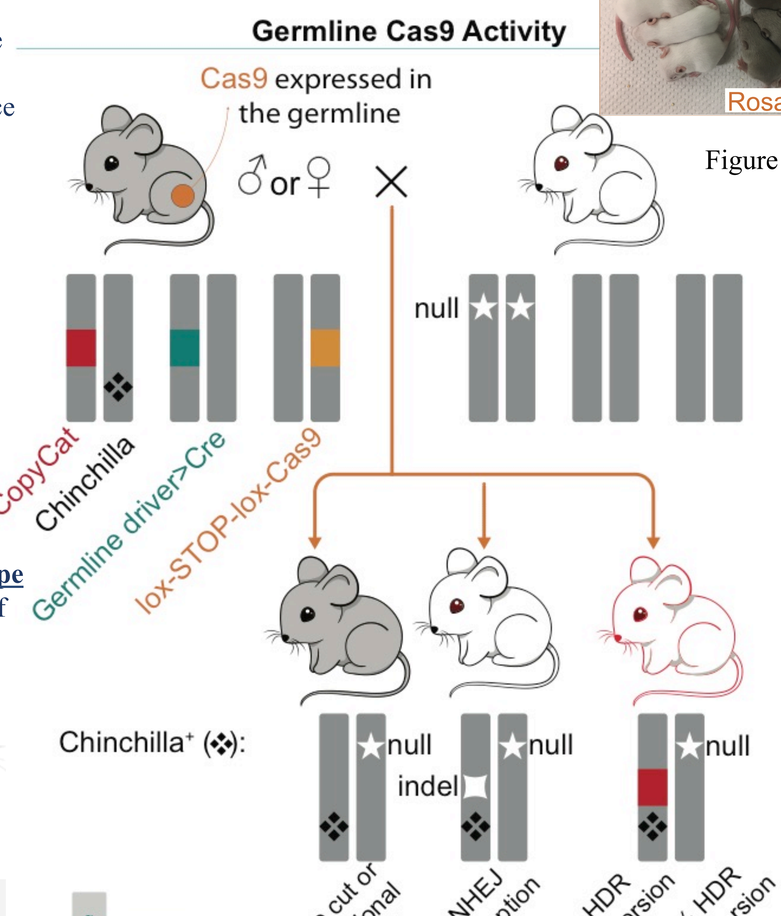
First, we crossed CopyCat males to females carrying *Tyrosinase*^{Chinchilla} as well as Cas9 encoded at the Rosa26 or H11 locus to assess embryonic Cas9 activity (Fig 3).



Embryonic activity of Cas9 in Rosa26-Cas9 mice efficiently generated null mutants (Fig 3, white mice). H11 was less efficient at producing null mutations (Fig 3, mosaic mice).

Embryonic Cas9 activity never induced repair by HDR. Mutants were efficiently generated by NHEJ, but inheritance of the CopyCat allele was never affected.

Figure 3: Embryonic Cas9 activity produces NHEJ mutations



Cre	Cas9	F3	no cut or functional repair	NHEJ disruption	HDR conversion	% HDR conversion
Vasa-Cre	Rosa26 ♀	1	-	15	-	-
		2	1	2	-	-
		3	-	3	1	25%
		4	4	5	3	25%
		5	6	6	1	8%
	Rosa26 ♂	1	-	25	-	-
		2	-	17	-	-
	H11 ♀	1	4	1	13	72%
		2	10	7	4	19%
		3	4	-	5	56%
		4	8	4	4	25%
		5	8	3	10	48%
	H11 ♂	1	-	15	-	-
		2	-	5	-	-
		3	-	2	-	-
		4	-	3	-	-
Stra8	R26 ♂	1	-	19	-	-
		2	-	3	-	-
	H11 ♂	1	3	21	-	-

Figure 4: Cas9 activity in the female germline induced gene conversion

We restricted Cas9 activity to the germline by using Cre under the control of a germline promoter (Vasa or Stra8) combined with a lox-STOP-lox (LSL) Cas9 (Fig 4).

Cas9 activity in the male germline only produced mutations by NHEJ.

Cas9 activity in the female germline induced gene conversion up to 72% of the time, **combining closely linked alleles (CopyCat and *Tyr*^{ch}) at rates impossible through normal inheritance**.

We hypothesize that chromosomes must be aligned in order to facilitate gene conversion by HDR from one template chromosome to the other.

The only time chromosomes are aligned in mammals is during Prophase I of meiosis. We suspect that Cas9 activity in the male germline failed to induce HDR because our Cre-based breeding system resulted in Cas9 expression before Prophase I in males.

In summary, embryonic Cas9 activity can produce highly efficient DSBs which are never resolved by HDR, whereas germline Cas9 activity can influence inheritance rates by inducing gene conversion. Timing of DSB formation seems to be critical to bias repair towards HDR (Fig 5).

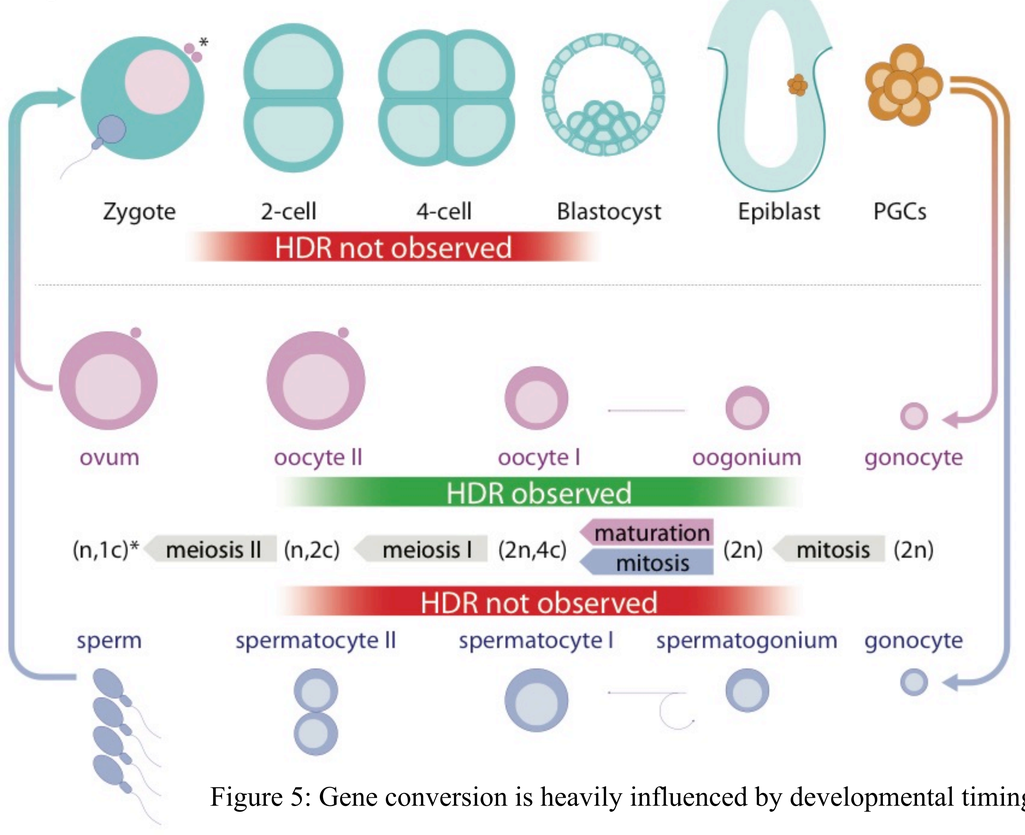


Figure 5: Gene conversion is heavily influenced by developmental timing

REFERENCES

1. Macdonald, L. E. *et al.* Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 5147–5152 (2014).

2.Grunwald, H. A. *et al.* Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline. *Nature* **566**, 105–109 (2019).