Loss of Function Mutations in Essential Genes cause Embryonic Lethality in Pigs

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Data

Breed	80K	WGS	RNA-seq	Phenotypes (TNB, NBA, MUM)
Duroc	11.255	119	25	23,132
Landrace	28,085	167	34	9,920
Large White	23,117	89	3	33,595
Total	**62,457**	**375**	**62**	**66,647**

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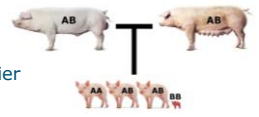
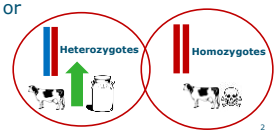
Lethal recessives

- Harmful genetic mutations causing early (embryonic) lethality in homozygous state
- Produced from carrier-by-carrier matings
- Produce a 1:2 phenotypic and genotypic ratio.



- Maintained by **genetic drift** or **heterozygote advantage** (overdominance)



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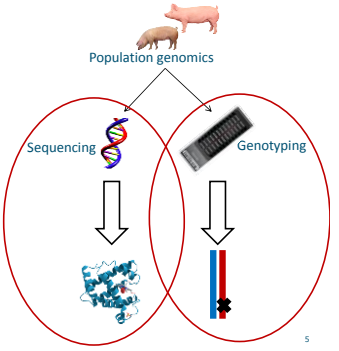
Methodology

- Infer from missing homozygosity.
- Direct prediction from whole genome sequence data.
  - LoF mutations
  - Missence mutations
  - Structural variation



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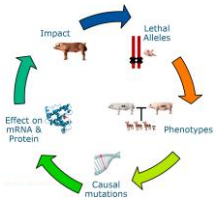
Objective

- 1. Map recessive lethal haplotypes in pig populations
- 2. Confirm lethality from carrier-by-carrier (CxC) matings
- 3. Identify causal mutation and downstream effect on protein
- 4. Investigate impact on population



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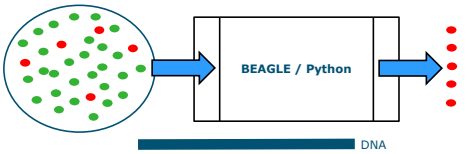
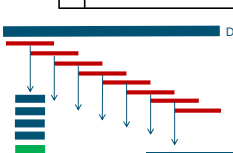
Methodology (missing homozygosity)





Fritz et al. 2013

$$E(k) = \sum_{i=1}^{ns} p_{ik} \sum_{j=1}^{nd} r_{jk} n_{ij}$$

Haplotype	Homozygous Observed	Homozygous Expected
	12	10
	14	13
	0	12

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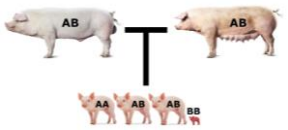
Candidate lethal recessives

Abbr.	SSC	Carrier. Freq (%)	Expected (trio)	Observed	# Genotyped progeny	# Heterozygote progeny
DU1	12	**9.6**	26.1	0	28	18 (64.3%)
LA1	3	**13.4**	126.0	0	208	120 (57.7%)
LA2	13	**8.4**	49.5	2	73	53 (72.6%)
LA3	6	**4.7**	15.5	0	24	11 (45.8%)
LW1	18	**10.8**	14.4	0	227	167 (73.6%)

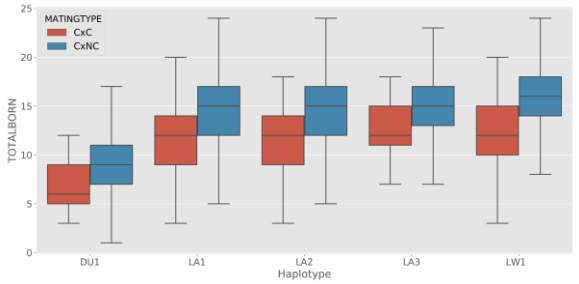
Relative high carrier frequencies

Candidate lethal haplo

1:2 genotype ratio



CxC matings produce significantly smaller litters



Reduction totalborn in range 15.1 – 21.6%

Search for the causal variant

Assessment on **co-segregation** and **functional impact** (LoF and missense)

1. Located **within 5 Mb** of the haplotype boundaries.
2. The mutation is **carried in heterozygote state** by the haplotype carriers
3. **No homozygous individuals** are observed.
4. **Absent** non-haplotype-carrier animals.

Hap.	#WGS carriers	#RNA-seq carriers
LA1	21	4
LA2	17	3
LA3	7	0
LA4	9	0
DU1	9	3
LW1	15	1

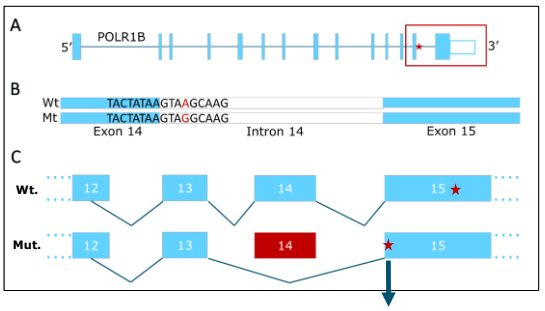
Candidate mutations

Candidate mutations

Hap.	Type	SSC	Position	Ref	Alt	Gene	Gene name
DU1	Splice-donor	12	38922102	G	A	*TADA2A*	Transcriptional adapter-Ada2/Gcn5/Ada3
LA1	Splice-region	3	43952776	T	G	*POLR1B*	RNA polymerase I subunit B
LA2	Frameshift	13	195977037	C	-	*URB1*	Ribosome Biogenesis 1 Homolog
LA3	Non-synonymous	6	54880241	T	C	*PNKP*	polynucleotide kinase 3'-phosphatase
LW1	Deletion	18	39817373-40029300	-	-	*BBS9*	Bardet-Biedl syndrome 9

- **Analysis yields strong candidate mutations for each haplotype!**
- Mutations of different types all inducing a **loss-of-function**: Splice, frameshift, deletion, missense.

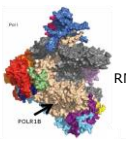
A splice region mutation in *POLR1B* inducing embryonic lethality for LA1



Premature stop codon → truncated protein

Candidate genes involved in essential cellular housekeeping functions

EL.	Type	Gene name	Gene ontology
DU1	Splice-donor	*TADA2A*	Regulation of **transciption**, DNA binding
LA1	Splice-region	*POLR1B*	RNA polymerase I **transcription**, regulation of **gene expression**
LA2	Frameshift	*URB1*	**mRNA** binding, maturation of **rRNA**.
LA3	Non-synonymous	*PNKP*	**DNA repair**
LW1	Deletion	*BBS9*	**Cilium assembly,** fat cell differentiation



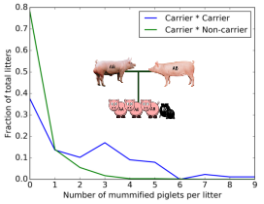
RNA polymerase I

Population impact of lethal recessives

Population	Hap.	#CxC	#CxNC	TNB (CxC)	TNB (CxNC)	Reduction	% Affected litters	Piglet loss	% Death
Landrace	LA1	297	2,350	11.51	14.18	18.8%	1.796	792.99	0.338
	LA2	127	1,527	12.00	14.26	15.9%	0.706	287.02	0.112
	LA3	30	872	11.96	14.09	15.1%	0.212	63.90	0.032
	LA4	29	950	11.48	14.05	18.3%	0.212	74.53	0.039
	SUM	**483**	**5,699**	-	-	-	**2.926**	**1218.44**	**0.521**
Duroc	DU1	21	293	7.33	9.35	21.6%	0.922	42.42	0.199
Large White	LW1	169	5,231	12.54	15.9	21.1%	1.210	567.84	0.256

- 2.9% of Landrace litters affected by recessive lethals identified in this study!
- Responsible for the death of 0.52% of the total population of embryos
- Reduction in TNB never reaches (expected) 25%.

Homozygotes for Large White *BBS9* deletion die during mid-late gestation resulting in mummification



Litter	Liveborn	Stillborn	Mummified
CC1	10	1	4
CC2	12	0	3
CC3	17	3	2
CC4	10	3	5

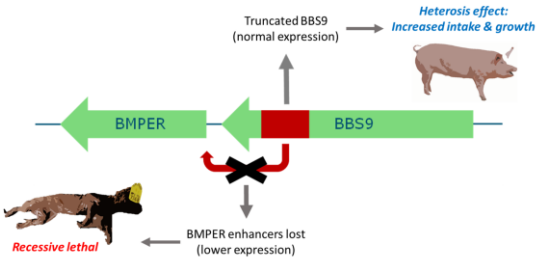
Derks et al. BMC Genomics (2017)

Balancing selection for *BBS9* deletion in Large White

EBVs	TSI	Growth rate	Daily feed intake
Carrier (n=412)	112.13	19.618	-10.095
Non-carrier (n=3030)	109.24	17.450	-4.968
Ratios	1.027	1.124	2.032

- Carriers have on average **~3% higher total selection index** (TSI)
- Carriers have **~12% higher growth rate** breeding value (EBV) and higher feed intake EBVs
  - *BBS9* null-mutants associated with **obesity** in human and mouse.

Derks et al. PLOS Genetics (2018)



Derks et al. PLOS Genetics (2018)

Conclusions

- Combining population genomic (genotype, WGS) and transcriptomic (RNA-seq) data is powerful to detect embryonic lethal mutations.
- Different types of **loss of function** mutations in **essential genes** cause embryonic lethality in commercial breeds.
- Recessive lethals driven by **genetic drift** or **over-dominance** (heterozygote advantage)
- Lethal recessives have significant impact on purebred fertility
  - Mutations breed-specific, no impact on crossbreds (heterosis?).

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