XXXVI SIMPOSIUM ANAPORC. GIRONA 22 Y 23 OCTUBRE 2015

PFTS Y GENÉTICA: ¿TENEMOS EVIDENCIAS DE SU RELACIÓN?

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Dpto. Producción Animal
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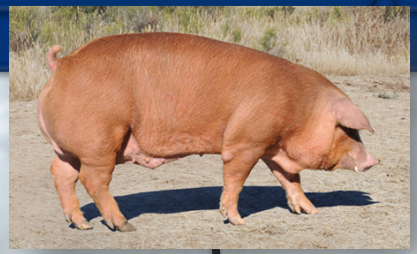

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AAATAATAGAAAACAAACTAAAATGAAAAT
TATTACTTAACAAATAGTTTTTAAGAATTAT
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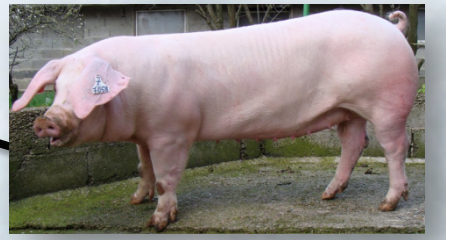
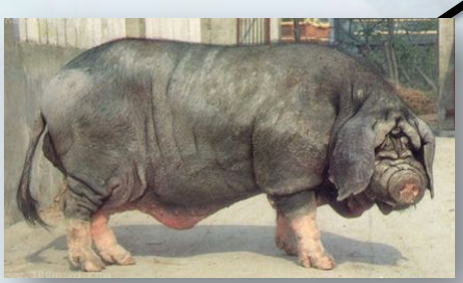
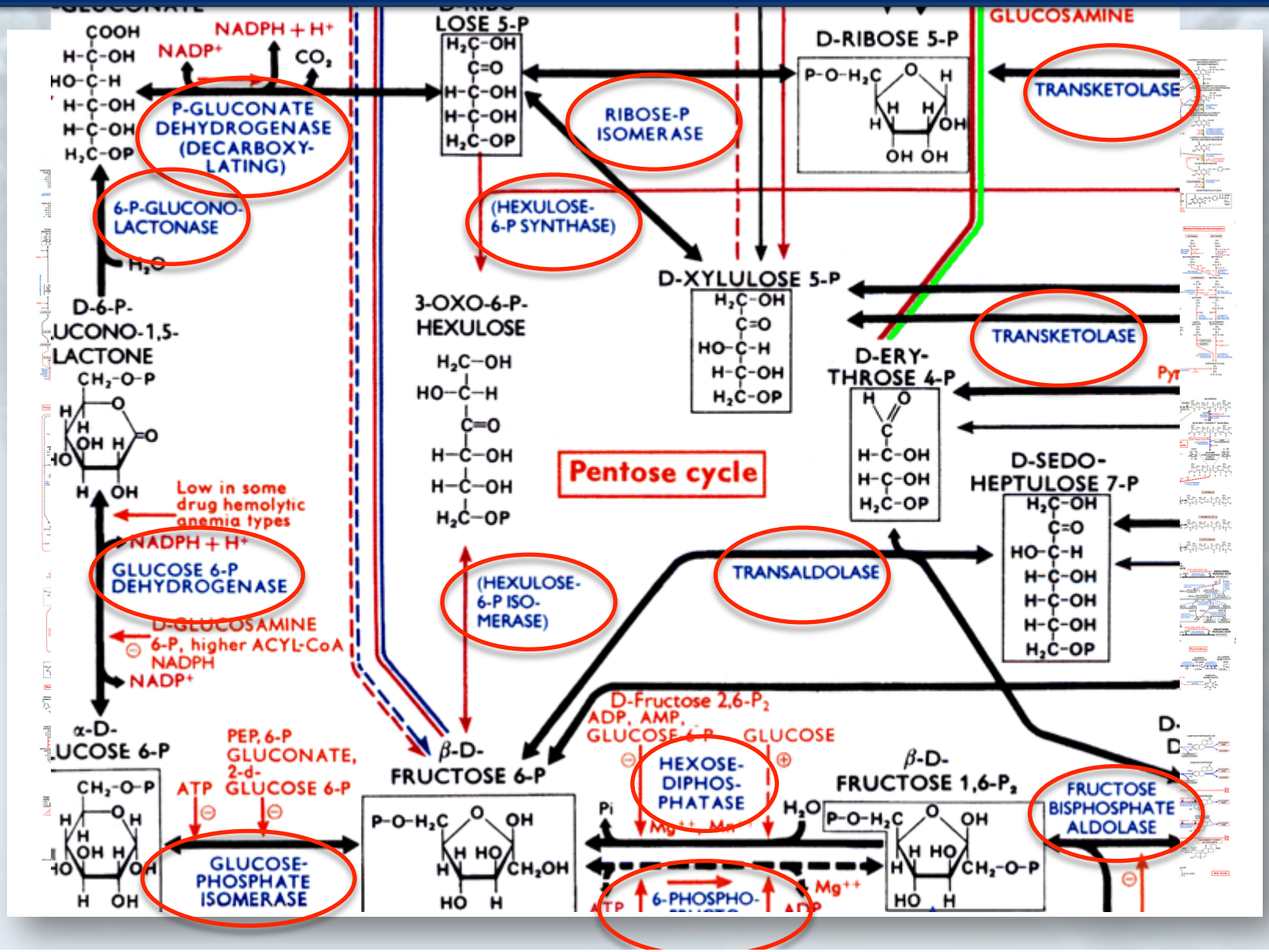





NADPH + H+
NADP+
CO2
P-GLUCONATE DEHYDROGENASE (DECARBOXY-LATING)
6-P-GLUCONO-LACTONASE
D-6-P-GLUCONO-1,5-LACTONE
Low in some drug hemolytic anemia types
NADPH + H+
GLUCOSE 6-P DEHYDROGENASE
D-GLUCOSAMINE 6-P, higher ACYL-CoA
NADPH
NADP+
α-D-GLUCOSE 6-P
PEP, 6-P GLUCONATE, 2-d-GLUCOSE 6-P
ATP
GLUCOSE-PHOSPHATE ISOMERASE
RIBOSE-P ISOMERASE
(HEXULOSE-6-P SYNTHASE)
3-OXO-6-P-HEXULOSE
(HEXULOSE-6-P ISO-MERASE)
β-D-FRUCTOSE 6-P
D-RIBOSE 5-P
GLUCOSAMINE
TRANSKETOLASE
D-XYLULOSE 5-P
TRANSKETOLASE
D-ERY-THROSE 4-P
Pentose cycle
D-SEDO-HEPTULOSE 7-P
TRANSALDOLASE
D-Fructose 2,6-P2
ADP, AMP, GLUCOSE 6-P
GLUCOSE
HEXOSE-DIPHOS-PHATASE
Pi
H2O
Mg++
β-D-FRUCTOSE 1,6-P2
FRUCTOSE BISPHOSPHATE ALDOLASE
6-PHOSPHO-

PFTS

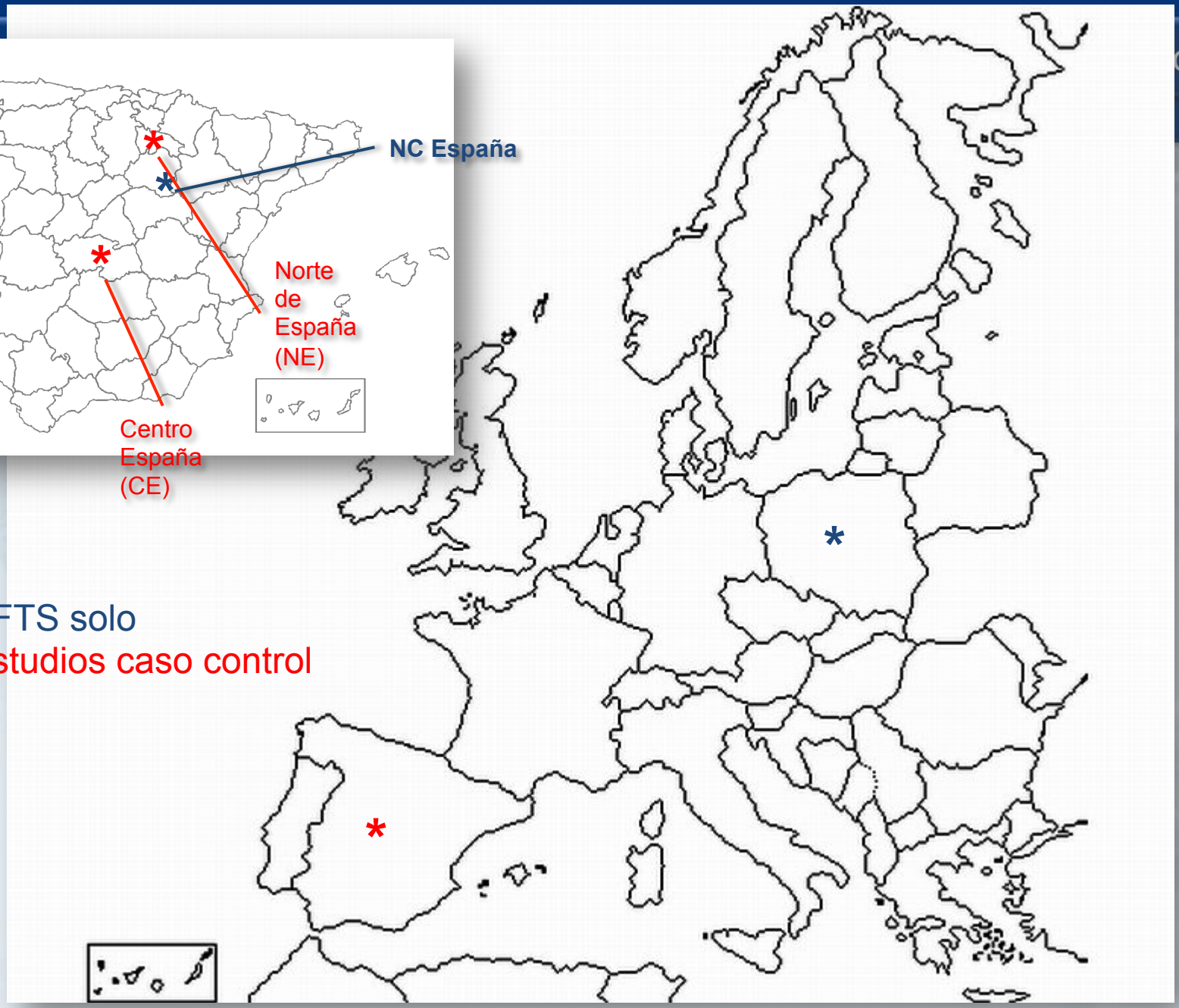
- La cuestión es:

¿Hay alguna influencia genética en la aparición de PFTS?

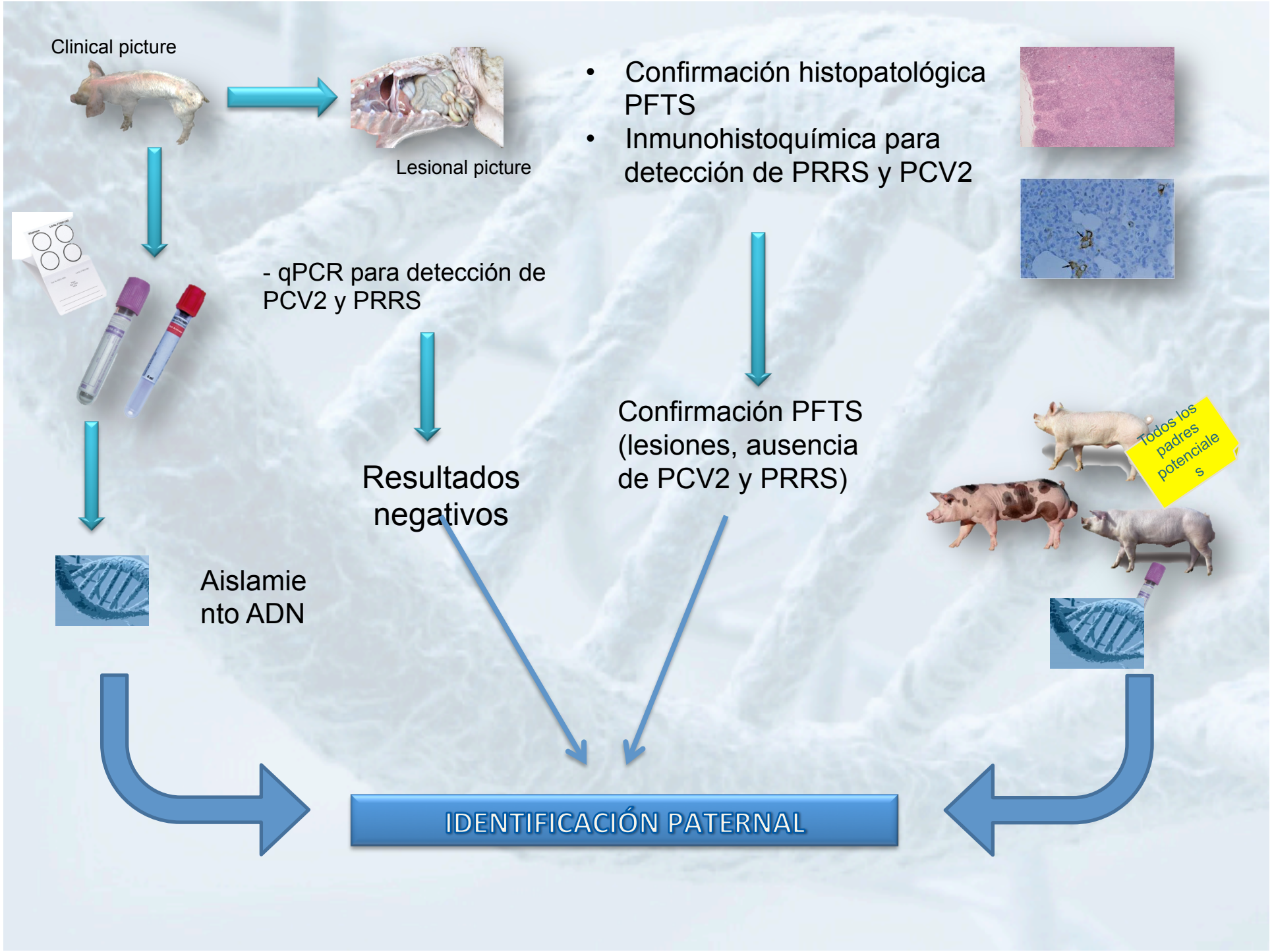
PFTS

- Pruebas de paternidad
- Estudios de asociación
  - Microsatélites
  - GWAS con SNPs
- Sexo

ones

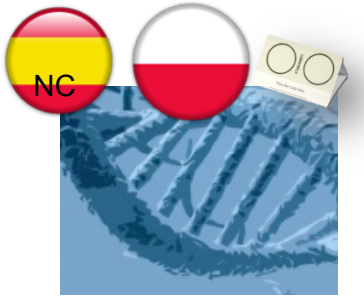


* PFTS solo
* Estudios caso control

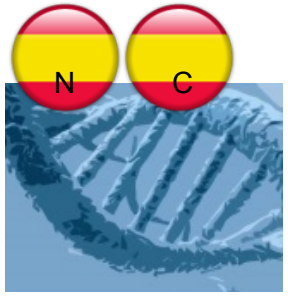

Clinical picture
Lesional picture
• Confirmación histopatológica PFTS
• Inmunohistoquímica para detección de PRRS y PCV2
- qPCR para detección de PCV2 y PRRS
Resultados negativos
Confirmación PFTS (lesiones, ausencia de PCV2 y PRRS)
Todos los padres potenciales
Aislamiento ADN
IDENTIFICACIÓN PATERNAL

IDENTIFICACIÓN PATERNAL

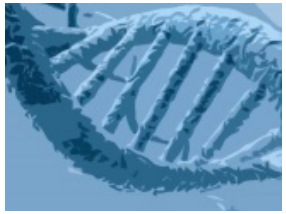
MUESTRA



DNA
PFTS



DNA PFTS
+
DNA control



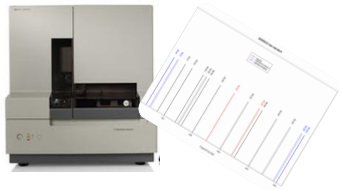
DNA verracos (todos los potenciales padres)

MOLECULAR

Análisis de fragmentos

Marker	Chro.	Marker	Chro.
S0026	16	Swr1941	13
S0155	1	Sw936	15
S0005	5	Sw122	6
Sw2410	8	Sw857	14
Sw830	10	S0097	4
S0355	15	Sw72	3
Sw24	17	S0226	2
Sw632	7	S0090	12
S0218	x	Sw1067	6
S0228	6	S0101	7
Sw240	2	Sw1828	1
IGF1	5	S0143	12
Sw2406	6	S0068	13
Sw2008	11	S0178	8
Sw911	9	S0002	3

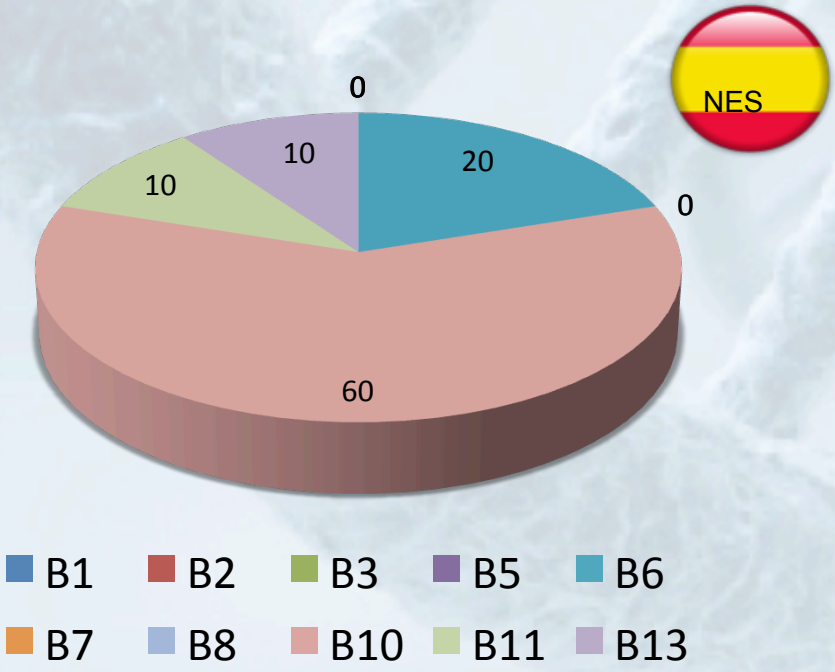
4 PCRs multiplex



Electroforesis en secuenciador

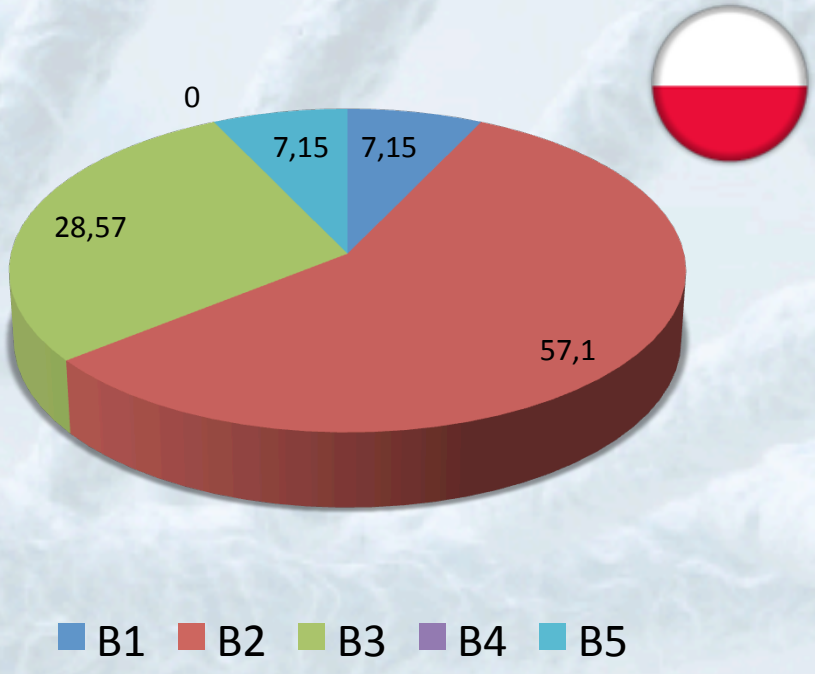
NC. PFTS

N=10



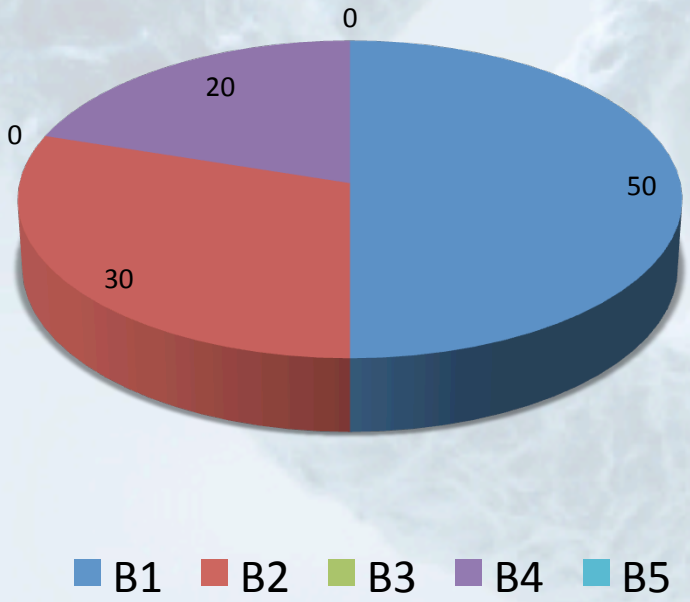
Polonia. PFTS

N=14



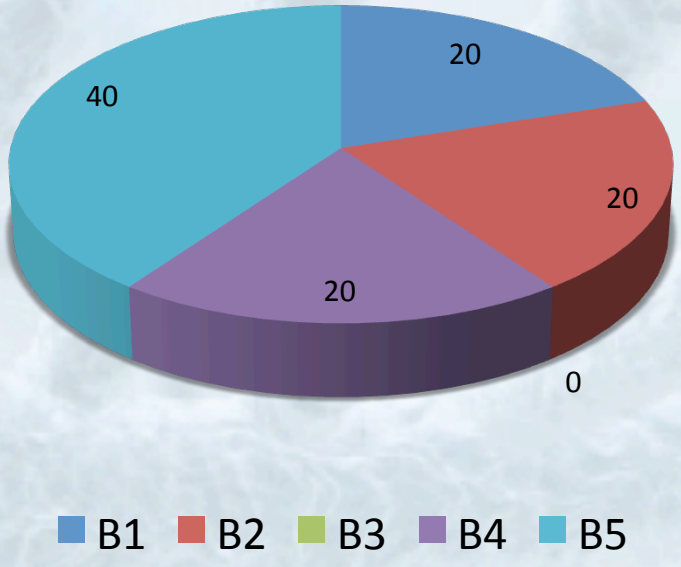
C. PFTS

N=10



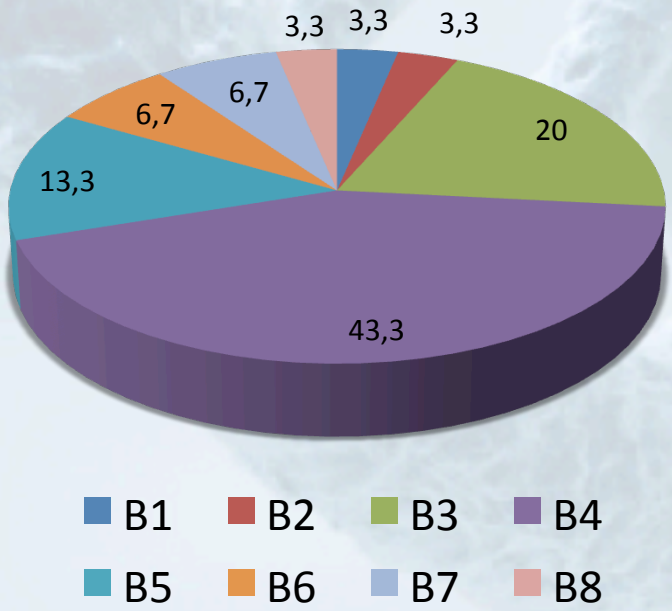
C. Control

N=10



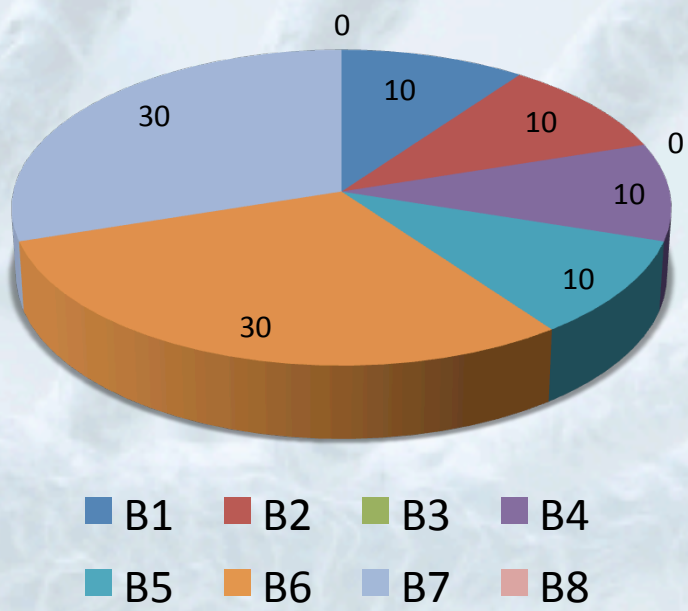
N. PFTS

N= 30



N. Control

N= 10



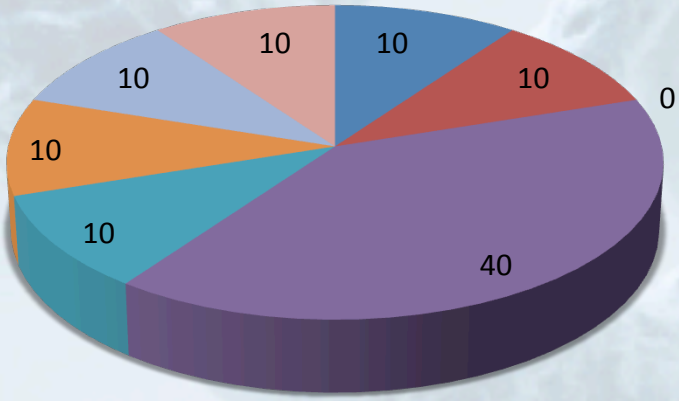
Test exacto de Fisher, p<0,03

N. PFTS
Junio, 2013

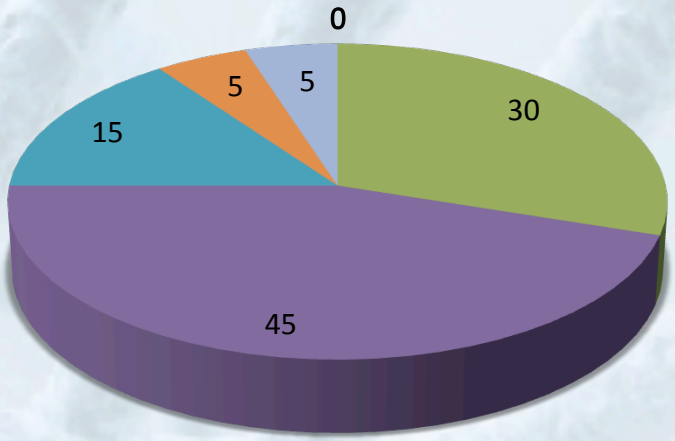
N= 10

N. PFTS
Febrero, 2014

N= 20



B1 B2 B3 B4
B5 B6 B7 B8



B1 B2 B3 B4
B5 B6 B7 B8

Hechos

Polonia

- 250 Cerdas– genética A.
- Verracos- genética B
- Autoreposición. Manejo en bandas 3 semanas.
- Vacunaciones cerdas– Parvo , Mal rojo, *E.coli* , *C. perfringens*.
- Lechones– vacunados frente a PCV ; destete a 4 semanas.
- Problemas aparecen 7-10 días post-destete.

España NC (Burgos)

- Comienza tras una introducción de verracos
- Cerdas genética A, Verracos genética C
- Problemas aparecen 7-10 días post-destete.
- ADG mayor de 900 g/día
- Afecta a los lechones más grandes al destete

Hechos

Caso	Inicio	Cerdas/genética	verracos	Clínica	producción
Polonia	Comienza tras una introducción de verracos	250 Genética A	Genética B	7-10 días post-destete.	Ciclo cerrado
España NC (Burgos)	Comienza tras una introducción de verracos	Genética A	Genética C	7-10 días post-destete.	
España C (Toledo)	Comienza tras una introducción de verracos	250 Genética A	Genética A y Genética D	7-10 días post-destete.	Ciclo cerrado
España N (Logroño)	Comienza tras una introducción de verracos	1200 Genética D	Genética D		Ciclo cerrado

España NE (Burgos)

- Se eliminan los verracos implicados y desaparece la presentación clínica de la enfermedad

(Comunicación personal E. Marco

España N (Logroño)

- Se eliminan los verracos implicados (V3, 4 y 5):

	Prevalencia PFTS	Mortalidad transición
Antes	10%	4
Después	0,5%	1,4%

(Comunicación personal V. Magaña)

Polonia

- Se eliminan los verracos implicados y desaparece la presentación clínica de la enfermedad

(Comunicación personal G. Swierczynski)

España C (Toledo)

- No se han eliminado los verracos
- Se han probado distintas estrategias sanitarias, de manejo y nutricionales
- Prevalencia PFTS: 10-12%
- Mortalidad en lechoneras: 15% con picos del 20% an algunos lotes (2% previo a la emergencia de PFTS).

(Comunicación personal P. González-Contreras)

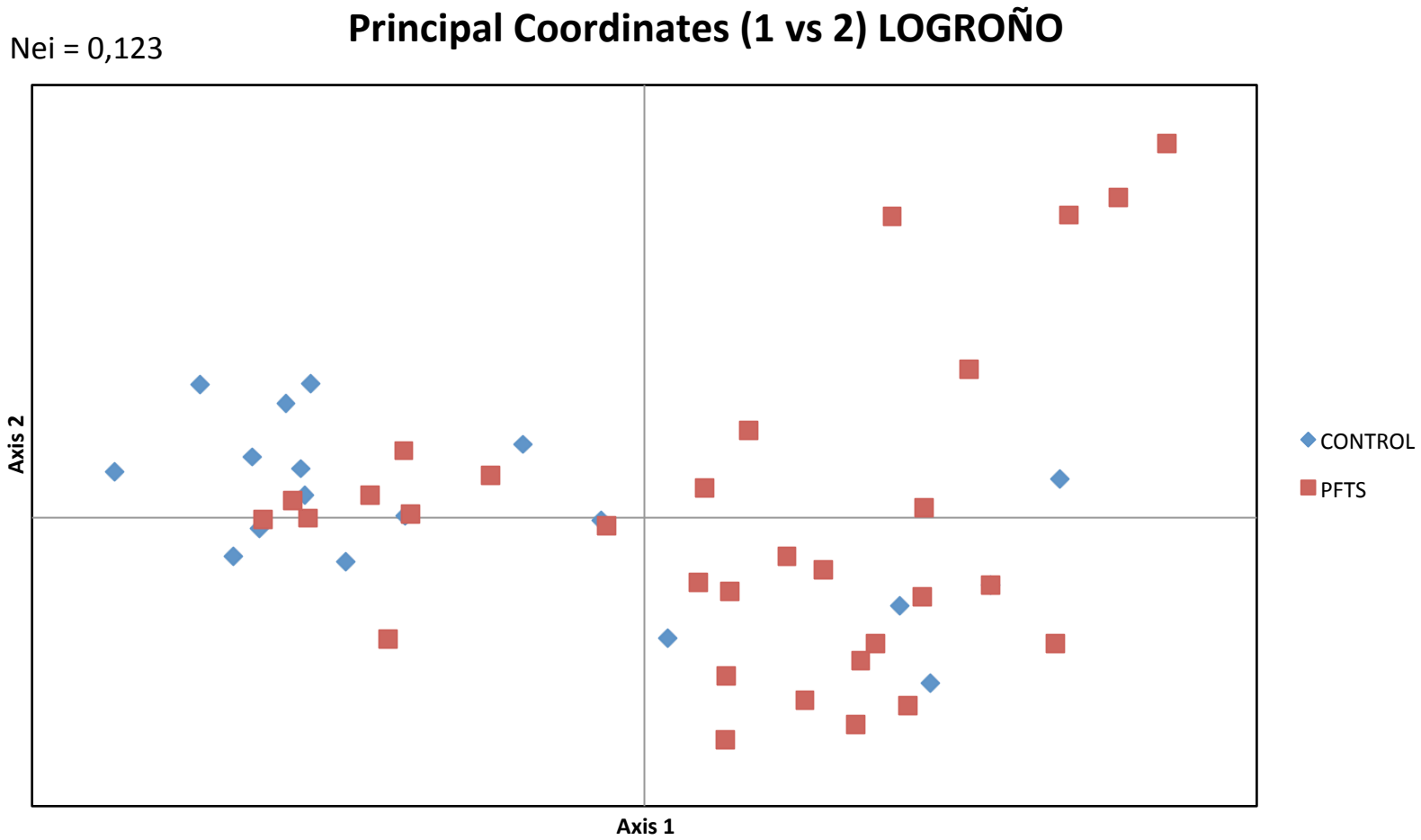
Ya hay documentados casos donde cambiar el origen de los lechones produce una desaparición de la enfermedad (Frienship et al., 2011)

No es achacable a una línea genética en concreto (en este estudio hay 4 líneas en 4 combinaciones diferentes)

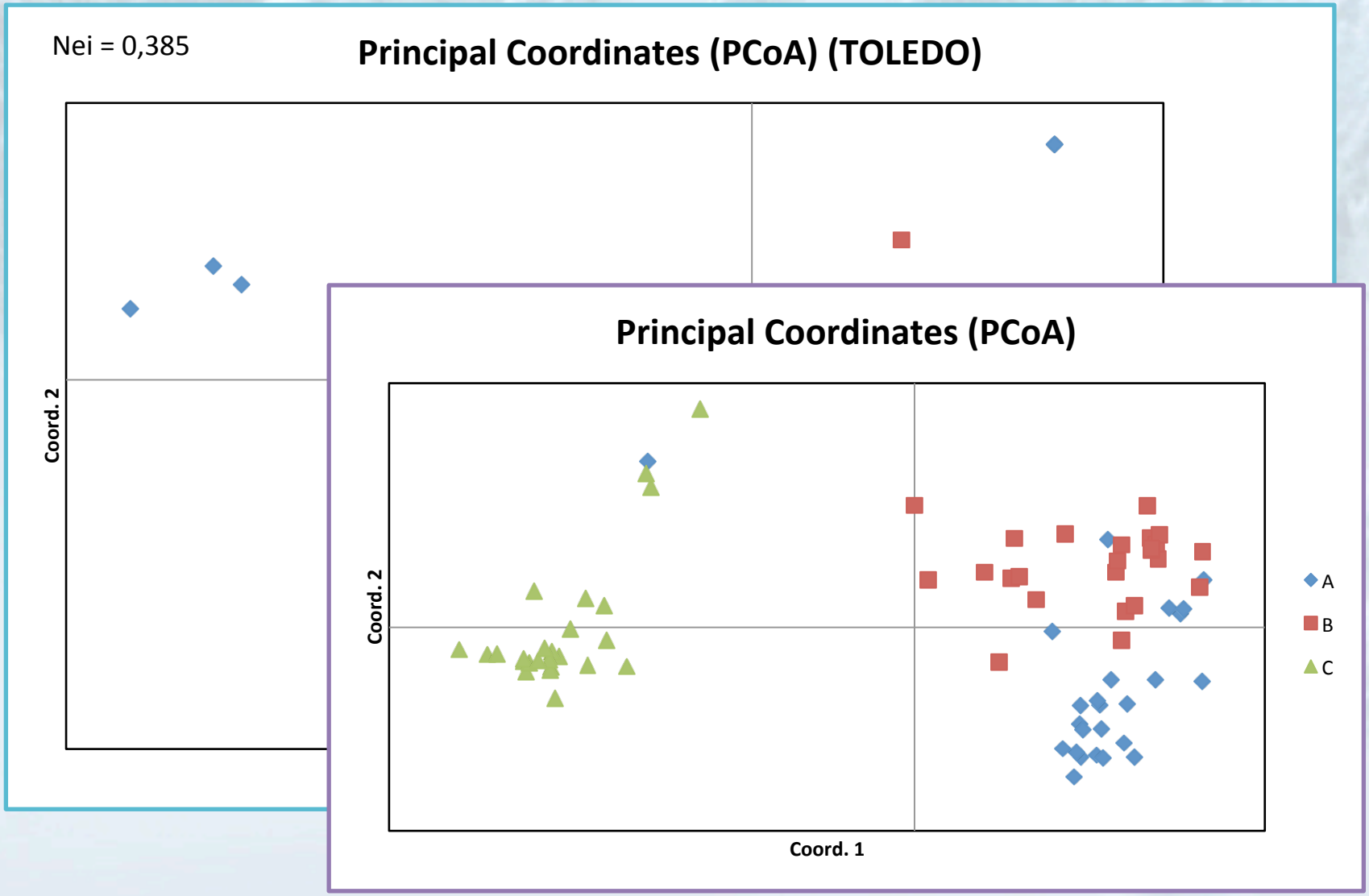
Cambiar los verracos por verracos de la misma línea genética puede hacer que desaparezca la enfermedad: parece una cuestión de susceptibilidad individual y de combinación de individuos

- Microsatélites: ¿Es posible diferenciar dos subpoblaciones entre sanos y enfermos?

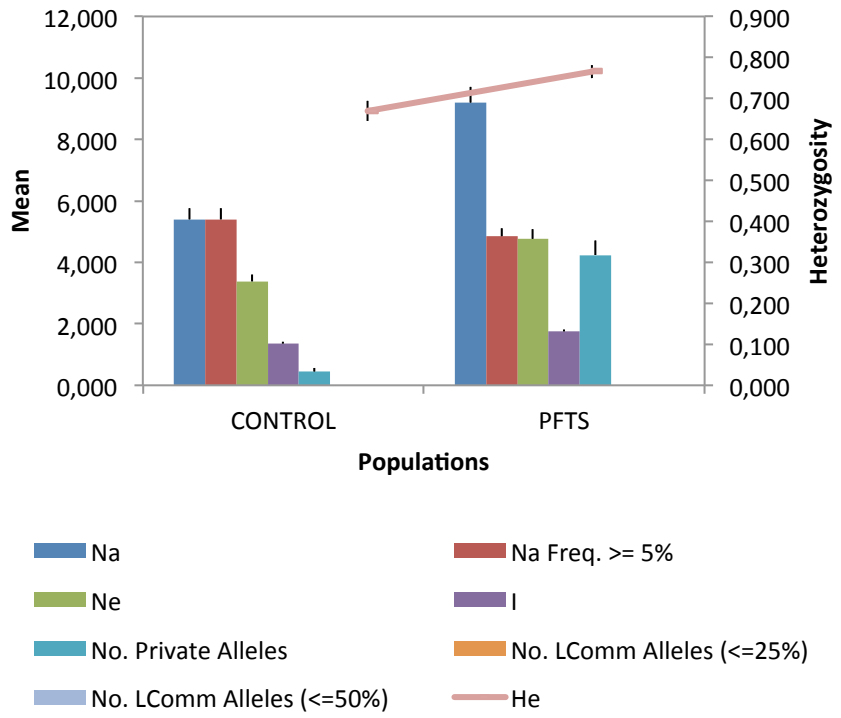
Nei = 0,123


Principal Coordinates (1 vs 2) LOGROÑO
Axis 2
Axis 1
CONTROL
PFTS

Nei = 0,385

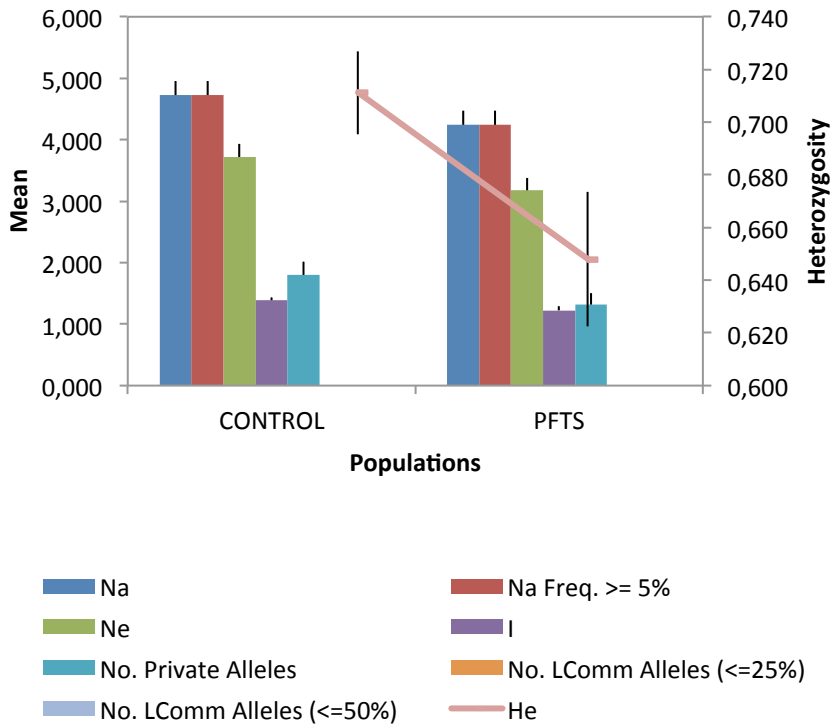

Principal Coordinates (PCoA) (TOLEDO)
Coord. 2
Principal Coordinates (PCoA)
Coord. 2
Coord. 1
A
B
C

Allelic Patterns across Populations. LOGROÑO

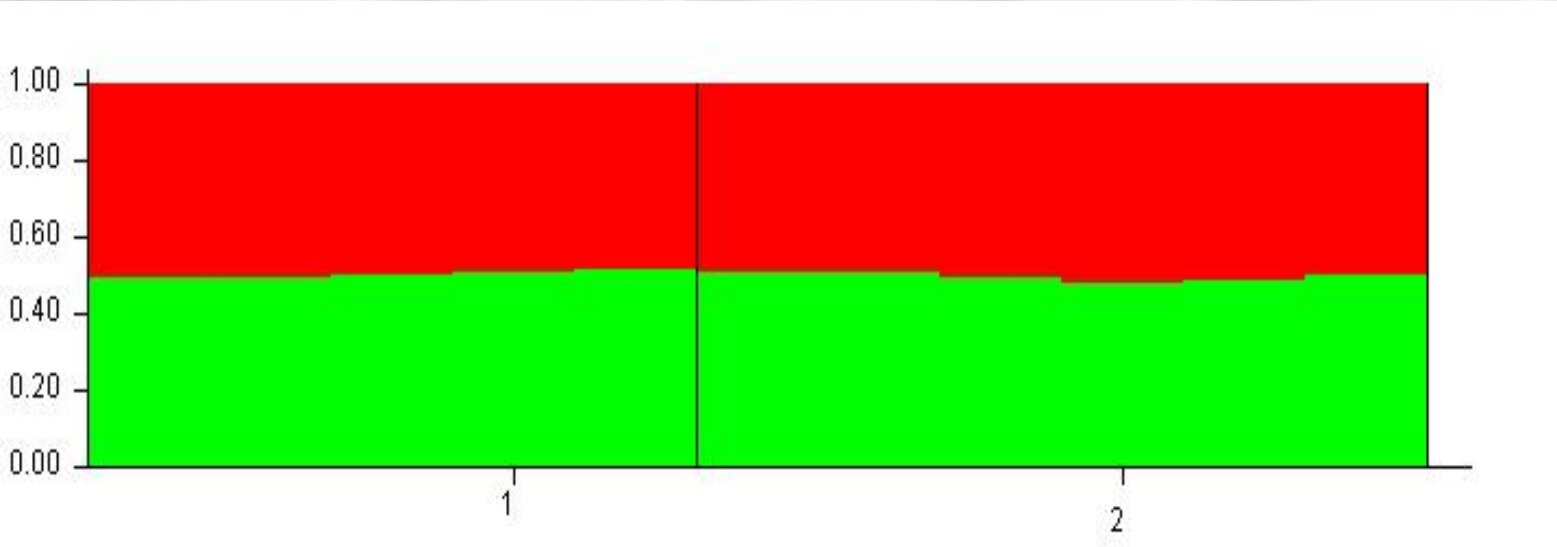


Population	CONTROL	PFTS
Na	5,400	9,200
Na Freq. >= 5%	5,400	4,840
Ne	3,383	4,755
I	1,353	1,751
No. Private Alleles	0,440	4,240
He	0,669	0,766

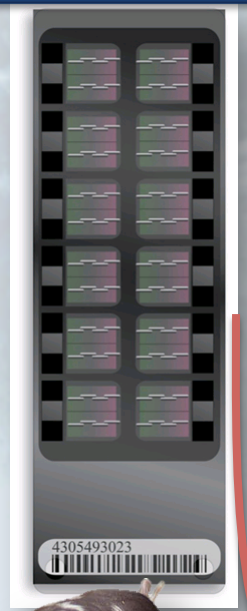
Allelic Patterns across Populations. TOLEDO

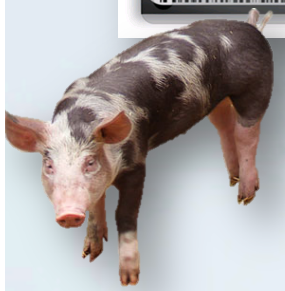


Population	CONTROL	PFTS
Na	4,720	4,240
Na Freq. >= 5%	4,720	4,240
Ne	3,720	3,172
I	1,387	1,226
No. Private Alleles	1,800	1,320
He	0,711	0,648


1.00
0.80
0.60
0.40
0.20
0.00
1
2

- 60K SNPs: estudio de asociación (GWAS; Genome Wide Association Study)
- Se busca la asociación entre el polimorfismo de un panel de SNPs y un carácter cuantitativo.
- Requiere mucha computación (hay que comparar el rendimiento de n animales con 60.000 puntos del ADN).
- Se han usado los 48 animales de LOGROÑO (38 sanos, 10 enfermos)
- Illumina Porcine 60K SNPs Beadchip (Geneseek, USA)





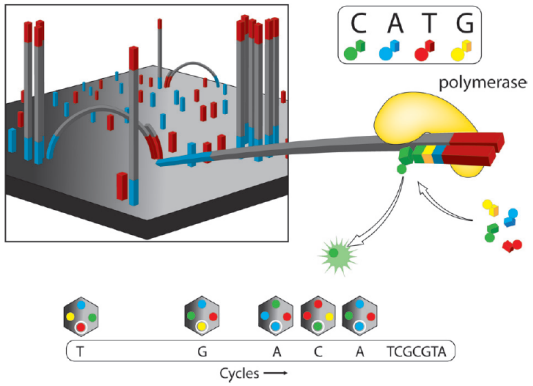
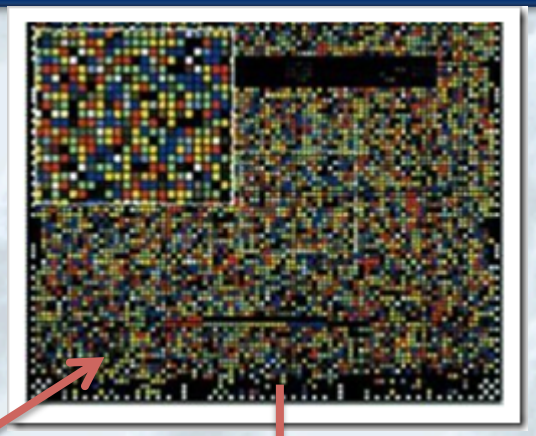
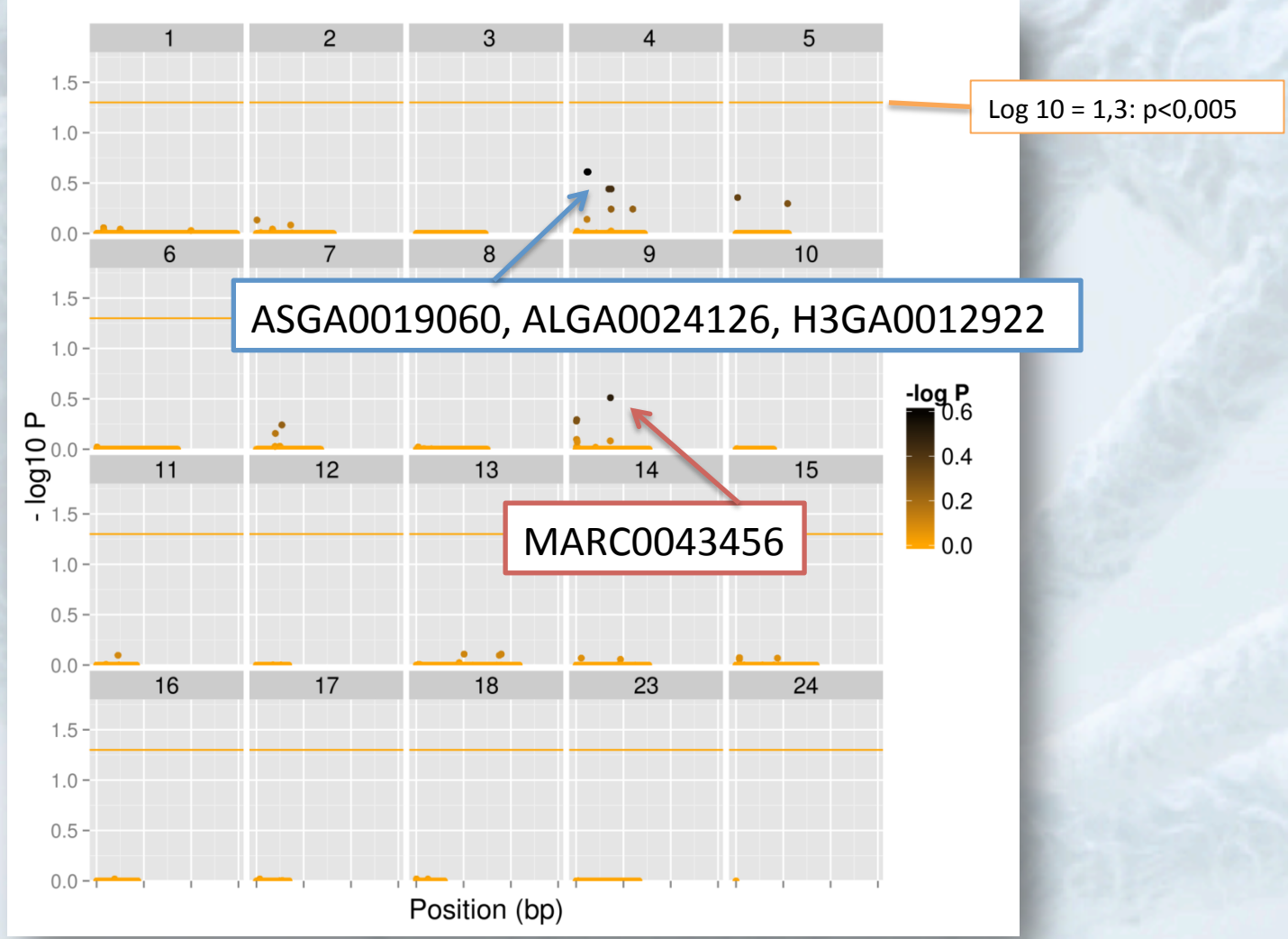
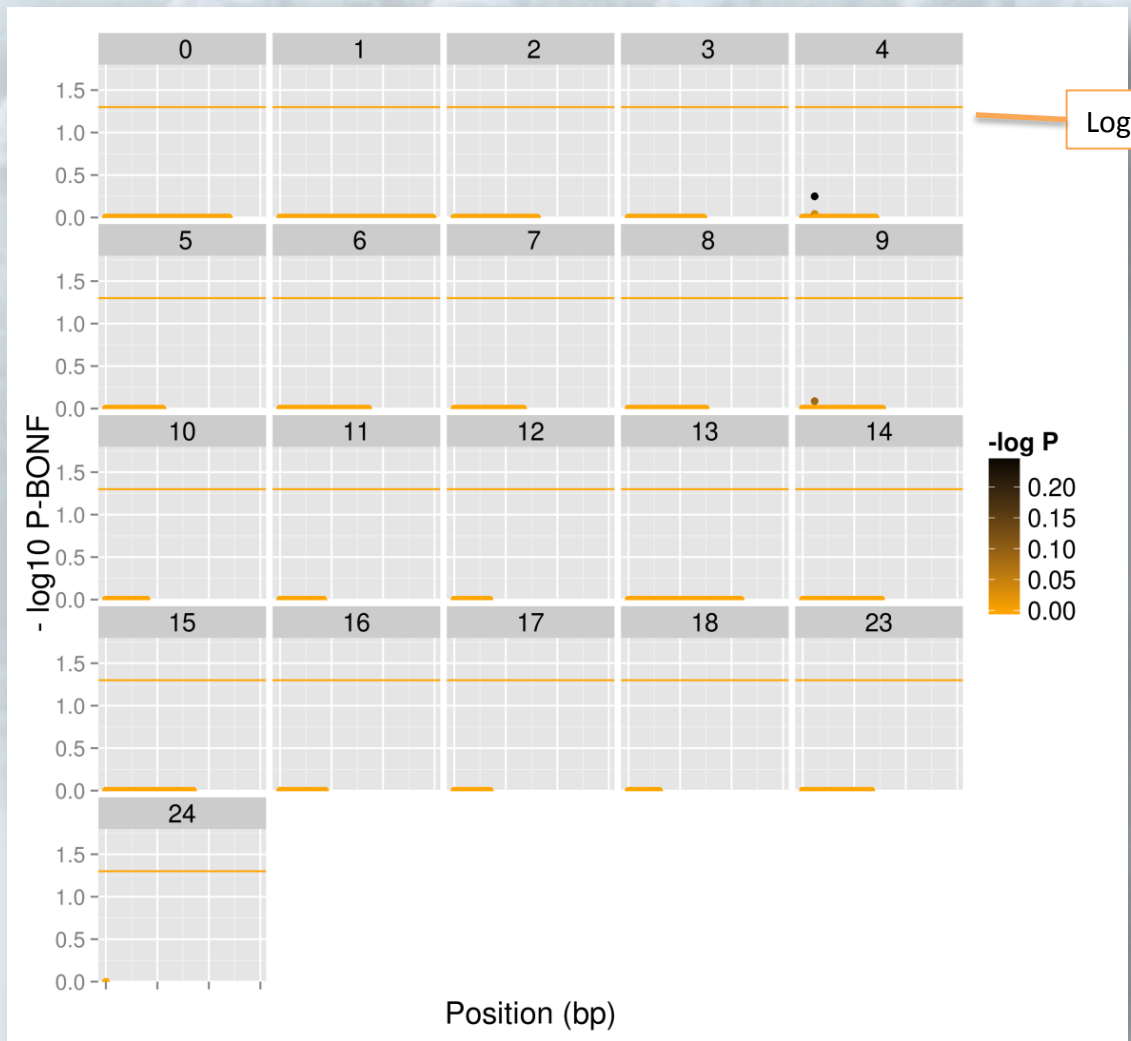


Figure 2. Illumina. *Solid phase amplification* (upper left). The initial step is the coupling of oligonucleotides (priming) and the extension of single-strand molecules. The next step is bridge amplification of the immobilized template with the adjacent primer to form clusters. *Four fluorescent color reversible termination* (upper right). This platform uses a terminator and fluorescent label that differs for each base type. After the addition of a new base, a picture is taken and fluorescence removed leaving the hydroxyl group free for a new addition (bottom). As an example, the bottom part of the figure shows 4 color images that represent the sequence data from one template. Modified from Metzker (2010).

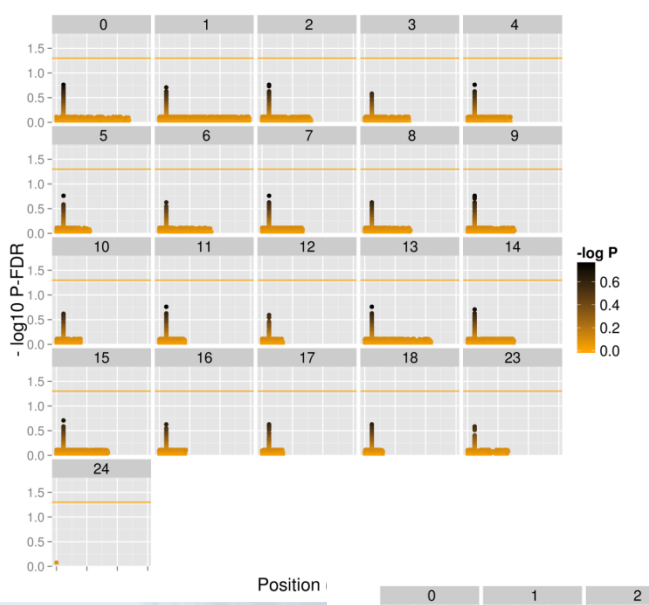


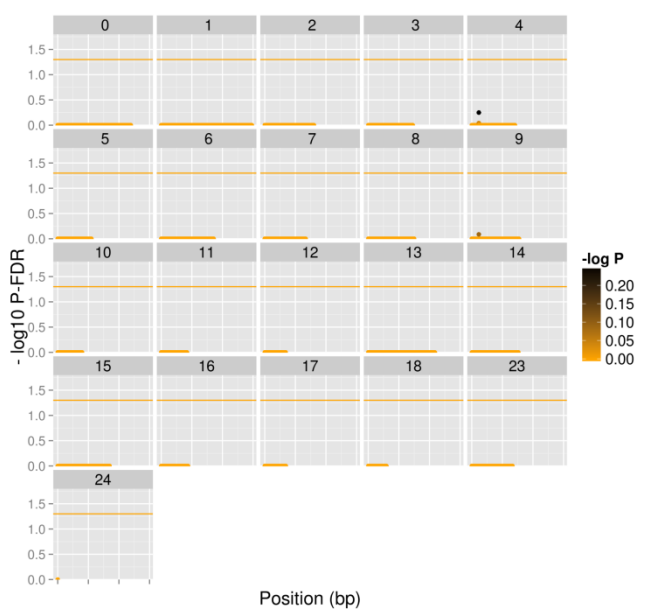
CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR
0	ALGA0100552	0	A	0,3833	0,25	G	1.172	0,279	1.865
0	ALGA0100636	0	A	0,3333	0,45	G	0,8835	0,3473	0,6111
0	ALGA0100804	0	T	0,3167	0,25	G	0,3175	0,5731	1,39
0	ALGA0100881	0	G	0,14	0,2222	A	0,6599	0,4166	0,5698
0	ALGA0100978	0	T	0,4167	0,5	G	0,4233	0,5153	0,7143
0	ALGA0100987	0	A	0,1724	0,2222	G	0,2268	0,6339	0,7292
0	ALGA0101032	0	A	0,45	0,4	G	0,1524	0,6963	1.227
0	ALGA0101170	0	G	0,3667	0,35	A	0,01803	0,8932	1.075
0	ALGA0101209	0	A	0,08333	0,15	G	0,7407	0,3894	0,5152
0	ALGA0101247	0	A	0,3333	0,45	G	0,8835	0,3473	0,6111
0	ALGA0101301	0	G	0,4	0,2	T	2.637	0,1044	2.667
0	ALGA0101384	0	A	0,25	0,55	G	6.154	0,01311	0,2727
0	ALGA0101441	0	C	0,15	0,1667	A	0,02955	0,8635	0,8824
0	ALGA0101549	0	A	0,4667	0,35	G	0,8296	0,3624	1.625
0	ALGA0101653	0	A	0,3833	0,15	G	3.723	0,05368	3.523
0	ALGA0101716	0	A	0,3667	0,35	G	0,01803	0,8932	1.075
0	ALGA0101775	0	A	0,2833	0,35	G	0,3175	0,5731	0,7342
0	ALGA0101810	0	C	0,4167	0,35	T	0,2778	0,5982	1.327
0	ALGA0102027	0	A	0,3167	0,3	G	0,01939	0,8892	1.081

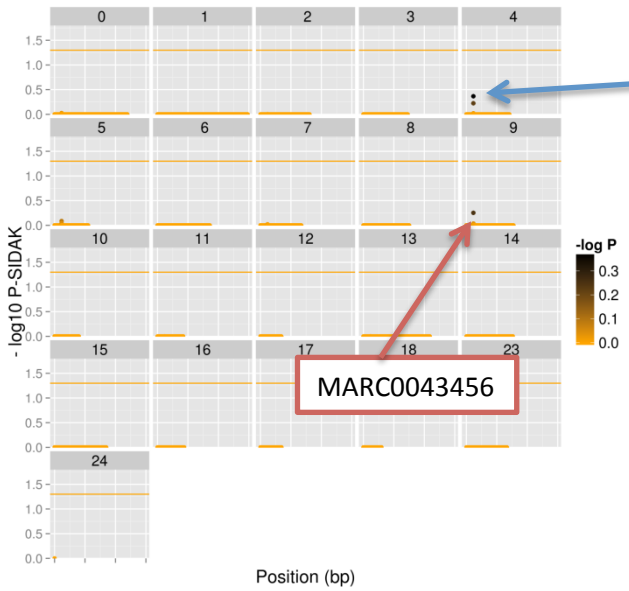

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
23
24
1.5
1.0
0.5
0.0
- log10 P
Position (bp)
-log P
0.6
0.4
0.2
0.0
Log 10 = 1,3: p<0,005
ASGA0019060, ALGA0024126, H3GA0012922
MARC0043456



Log 10 = 1,3: p<0,005







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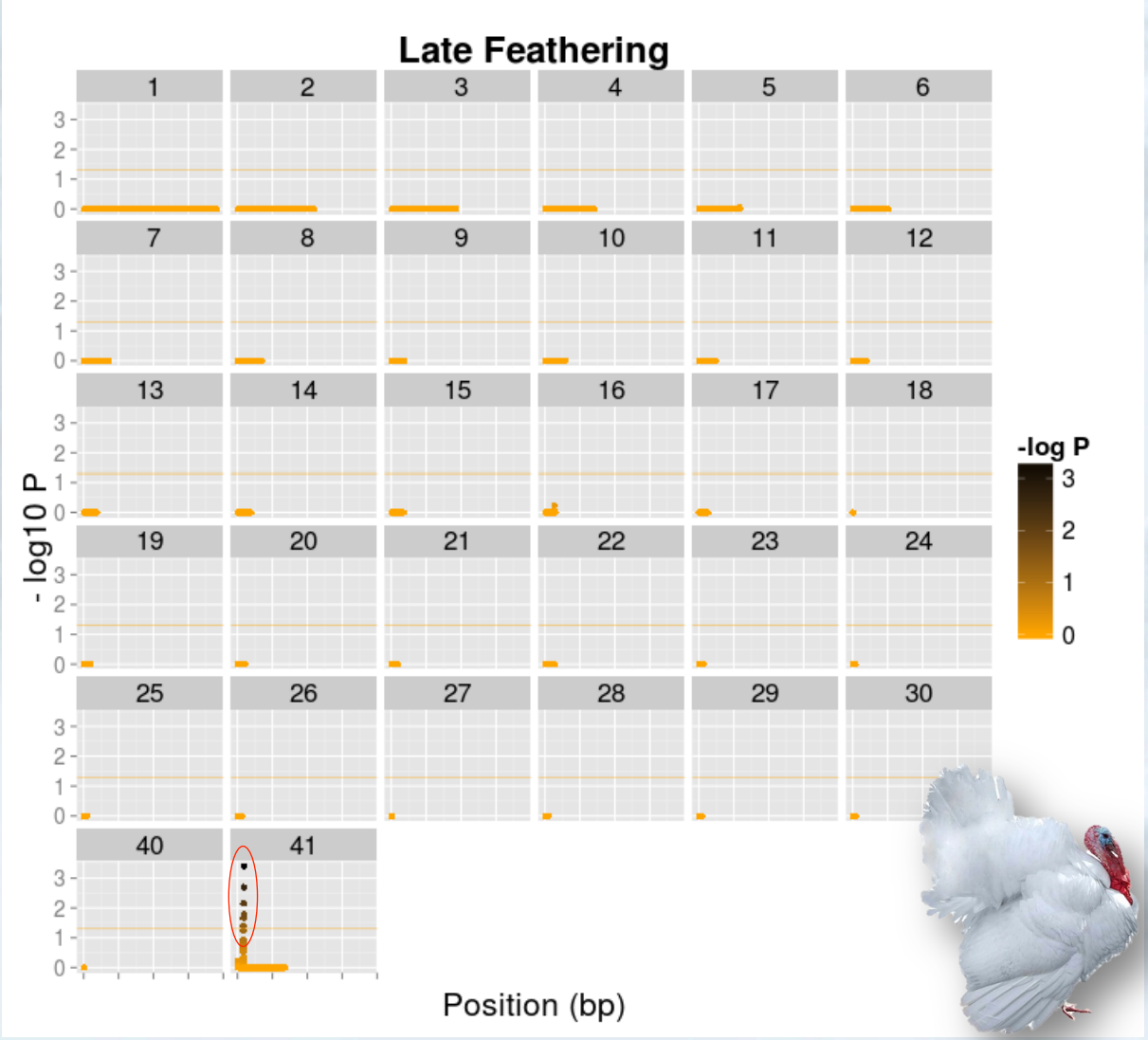


WGS: whole genome sequencing; 2M SNPs

Conclusiones

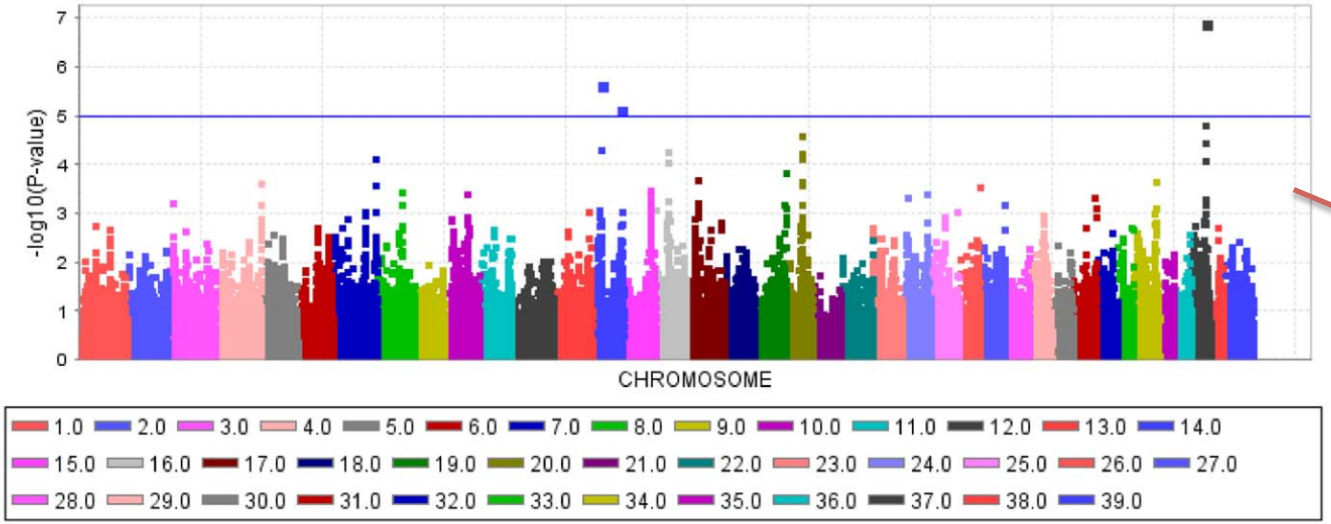


Figure 1. Manhattan plot of $-\log_{10}$P-values of the genome-wide association study for the canine hip dysplasia score in Bernese mountain dogs using a general model analysis. On the X-axis, the SNPs are given by dog chromosome number. The $-\log_{10}$P-values for each SNP genotype effect are plotted against the SNP position on each chromosome. Chromosomes are differentiated by colors. The color keys are given below the plot. The blue line indicates the threshold of the $-\log_{10}$P-values for genome-wide significance after correcting for multiple testing.
doi:10.1371/journal.pone.0049782.g001

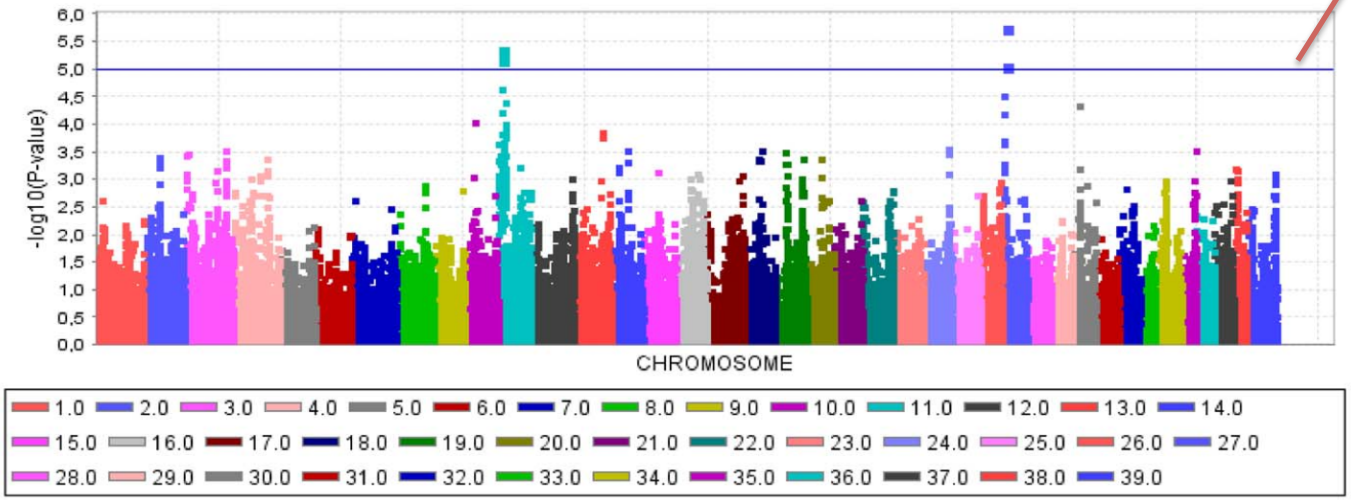
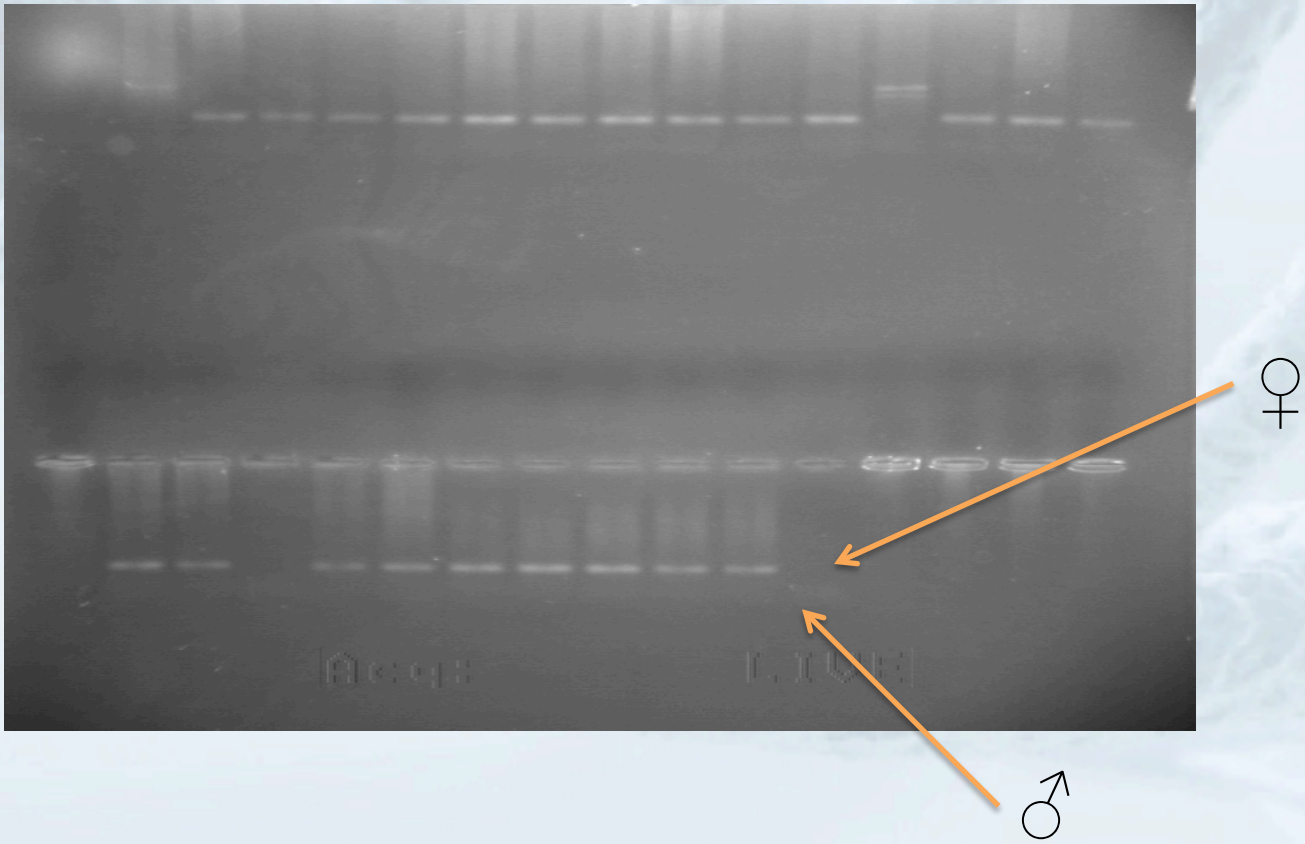


Figure 2. Manhattan plot of $-\log_{10}$P-values of the genome-wide association study for the canine elbow dysplasia score in Bernese mountain dogs using a general model analysis. On the X-axis, the SNPs are given by dog chromosome number. The $-\log_{10}$P-values for each SNP genotype effect are plotted against the SNP position on each chromosome. Chromosomes are differentiated by colors. The color keys are given below the plot. The blue line indicates the threshold of the $-\log_{10}$P-values for genome-wide significance after correcting for multiple testing.
doi:10.1371/journal.pone.0049782.g002

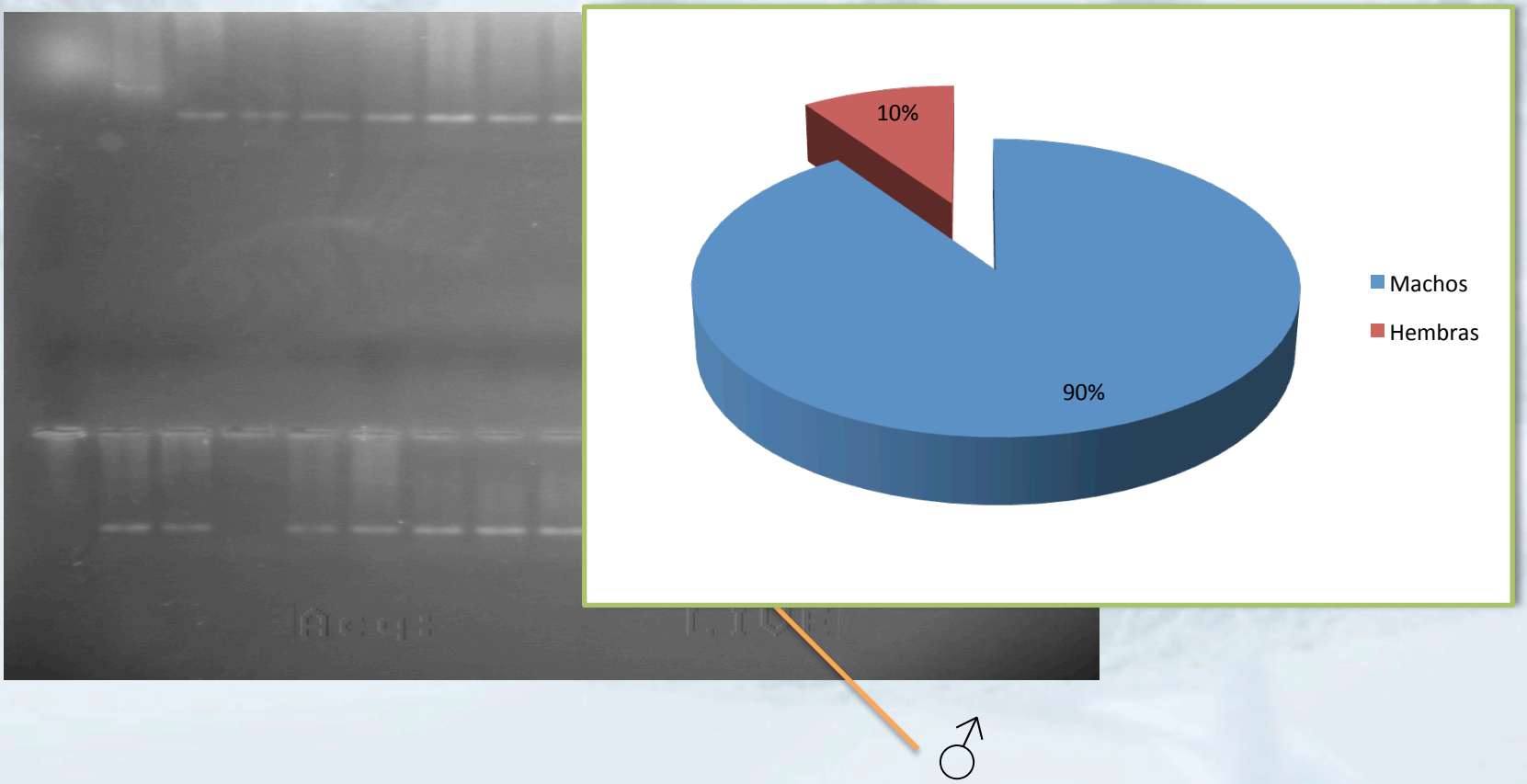
- Debilidades del GWAS usando 60K SNPs:

1. «Solo» tipamos 60.000 puntos del ADN frente a los más de 2.000.000 de un WGS
2. Se tipan muy pocos marcadores en el cromosoma X
3. Prácticamente no se tipa ningún marcador en el cromosoma Y…… ¿Qué sexo tenían los animales tipados para GWAS?

- Determinación del sexo mediante tipado del marcador SYRB



- Determinación del sexo mediante tipado del marcador SYRB



- Los test de paternidad nos permiten identificar de forma fiable qué animales son los padres de ciertos lechones
- En al menos 4 casos se han identificado uno o dos verracos como los productores de la mayoría de lechones con PFTS.
- Parece ser una predisposición individual, no racial ni de línea genética
- En estudios de asociación no se ha encontrado ningún SNP del panel de 60K que esté directamente relacionado con esta enfermedad
- Es necesario seguir investigando

Introdu

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Paper

Evidence that periweaning failure-to-thrive syndrome (PFTS) has a genetic predisposition

G. Ramis, E. Marco, V. Magaña, P. González-Contreras, G. Swierczynski, J. M. Abellaneda, A. Sáez-Acosta, A. Mrowiec, F. J. Pallarés

Genetic susceptibility or resistance to diseases is currently drawing increasing attention. This work describes two different breeding herds showing signs of periweaning failure-to-thrive syndrome (PFTS), an emergent swine disease. The disease was diagnosed based on clinical picture and confirmed by histopathology. The possibility of main infectious pathogens was ruled out by immunohistochemistry and PCR. In a simple approach, sires of the affected piglets have been determined using microsatellite paternity analysis, including a healthy group in each case. In each of the two farms, a single boar was found to have sired 45–50 per cent sick animals. Removal of this sire from two farms resulted in a significant decrease in the prevalence of the disease among the offspring, in accordance with other two cases diagnosed, although without including a control group. Since the analysed animals belonged to three different genetic lines, these findings point to the existence of individual genetic susceptibility to this syndrome.

Introduction

Many of the common diseases have a strong genetic component to their occurrence. Extensive work has been done on genetic predisposition to certain diseases in human beings. However, greater focus is currently being laid on genetic susceptibility to animal disease, with research on porcine diseases receiving a lot of funding. One of the leading projects related to genetic predisposition to porcine diseases is the Reproductive and Respiratory Syndrome Host Genetics Consortium (PHGC) project, in which different (public and private) research institutions from the USA investigate the genetic basis of host resistance or susceptibility to Porcine Reproductive and Respiratory Syndrome virus (PRRSV).

Periweaning failure-to-thrive syndrome (PFTS), formerly

between main infectious agents (such as PRRSV, porcine circovirus type 2 (PCV-2), Influenza A Virus, transmissible gastroenteritis virus, Rotavirus A, porcine cytomegalovirus, porcine enteric calicivirus, porcine haemagglutinating encephalomyelitis virus, *Clostridium perfringens*, pathogenic *Escherichia coli*, *Brachyspira hyodysenteriae*, *B. pilosicoli*, *Bordetella* species, *Streptococcus* species, *Haemophilus parasuis*, *Pasteurella multocida* and coccidian) and the PFTS has been demonstrated by Huang and others (2012) by immunohistochemistry or PCR.

Although the PFTS syndrome shows a non-specific clinical picture, some frequent histological lesions (such as superficial lymphocytic fundic gastritis, atrophic enteritis, superficial colitis, lymphocytic and neutrophilic rhinitis, mild non-

- **Muchas gracias por vuestra atención!!!**
- **Moltes gràcies per la vostra atenció!!!**