Royal Veterinary College
University of London

Autozygosity by difference – a method for locating autosomal recessive mutations

Geoff Pollott

Background

- Mutations occur regularly in all species
- Autosomal recessive conditions arise in most breeds from time to time and may cause genetic diseases
- Genetic tests are often required to identify carriers or affected animals
- The DNA around the mutation in an affected animal has specific characteristics
- Genome-wide SNP data may be used to locate this area by looking for characteristic patterns

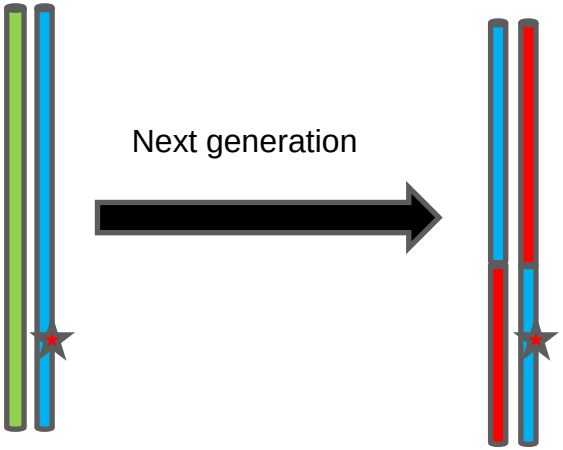
RVC

Development of autosomal recessive offspring

Mutation occurs on one of the autosomes

RVC

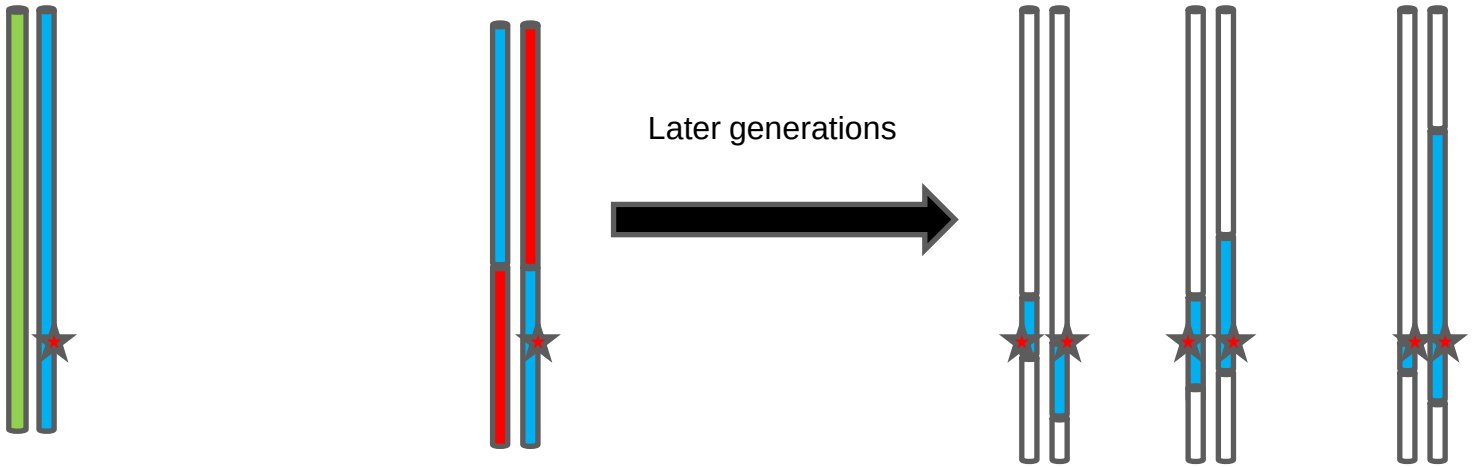
Development of autosomal recessive offspring



All offspring inheriting the mutation will have some of the original ‘blue’ DNA around it

RVC

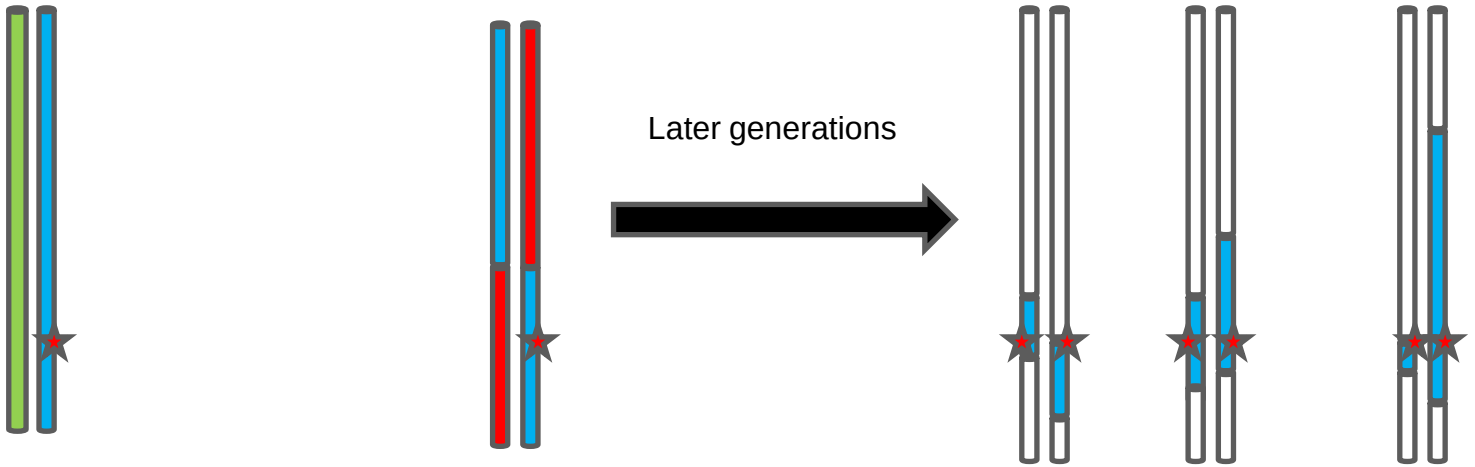
Development of autosomal recessive offspring



Affected animals will appear with two copies of the mutation plus a variable amount of homozygous ‘blue’ DNA from the original mutated chromosome

RVC

Development of autosomal recessive offspring



Sections of overlapping ‘blue’ chromosome will be autozygous. Throughout the ‘population’ of affected animals, DNA closest to the site of the mutation will be more homozygous than DNA further away

RVC

Basis of autozygosity by difference method (ABD)

1) The chromosome containing the mutation will contain relatively more homozygous SNPs :
    a) than other autosomes
    b) in the cases compared to controls

2) The region containing the mutation will comprise longer runs of homozygosity in cases compared to controls

3) Both cases and controls may have runs of homozygosity that are a breed characteristic and individuals will have differing levels of homozygosity

4) SNPs closest to the mutation should all be homozygous for the same genotype

RVC

Autozygosity by difference method

1) Summarise genotypes by SNP in all cases and define most common case homozygous genotype

2) In ALL animals, score all homozygous SNP = 1 when they are the same as the most common case homozygous genotype

3) Score all other SNP = 0

4) Summarise the SNP score by chromosome within animal

RVC

Autozygosity by difference method - 2

Use GLM to fit model:

Chr score = Chr + Animal within Status + Chr by Status

Takes account of: level of inbreeding for each animal
different chromosome SNP #

Significant interaction indicates chromosome containing mutation

RVC

Autozygosity by difference method - 3

Use SNP genotype data from identified chromosome

Identify runs of homozygosity (ROH)

Length of ROH, weighted by the animal's level of homozygosity, is the autozygosity score for each constituent SNP

Calculate mean autozygosity score by SNP for cases and controls

Calculate the difference between case and control mean autozygosity score at each SNP

Mutation should be at SNP with greatest difference or in a run of autozygosity with the maximum difference

RVC

Autozygosity by difference method - 4

Look for SNPs within the run of homozygosity which are all homozygous for the same SNP genotype (monomorphic)

The mutation should be close to or within this area

RVC

Example – Perinatal calf mortality in a rare breed

Apparently normal calves at birth died within the first 10 days of life

Previous study identified the condition to be due to an autosomal recessive condition and identified likely animals of origin (Pollott et al., BSAS meeting, 2011)

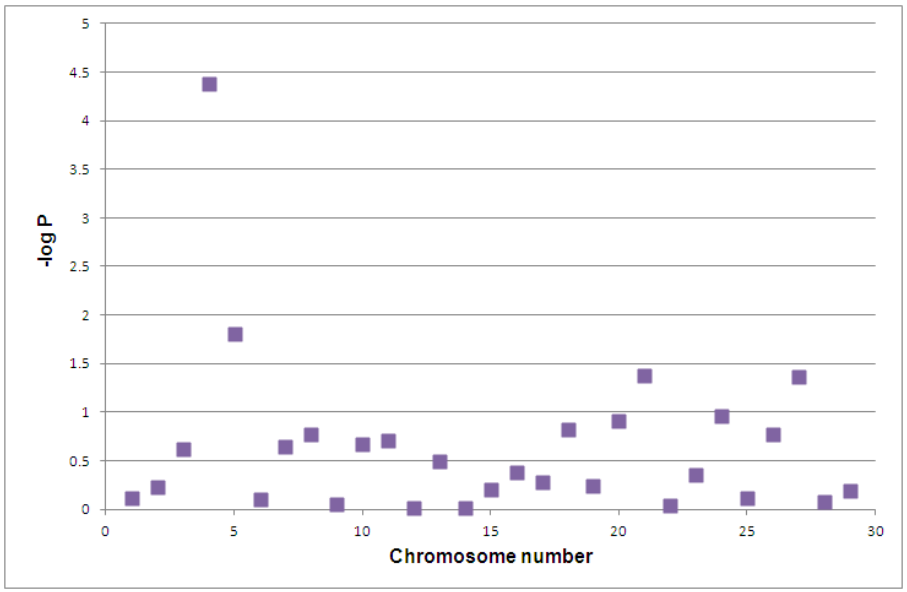
Used ABD method to try to locate the area containing the mutation

RVC

Which chromosome?

ANOVA of homozygous SNP by chromosome and disease status – Significant interaction (P = 0.003)

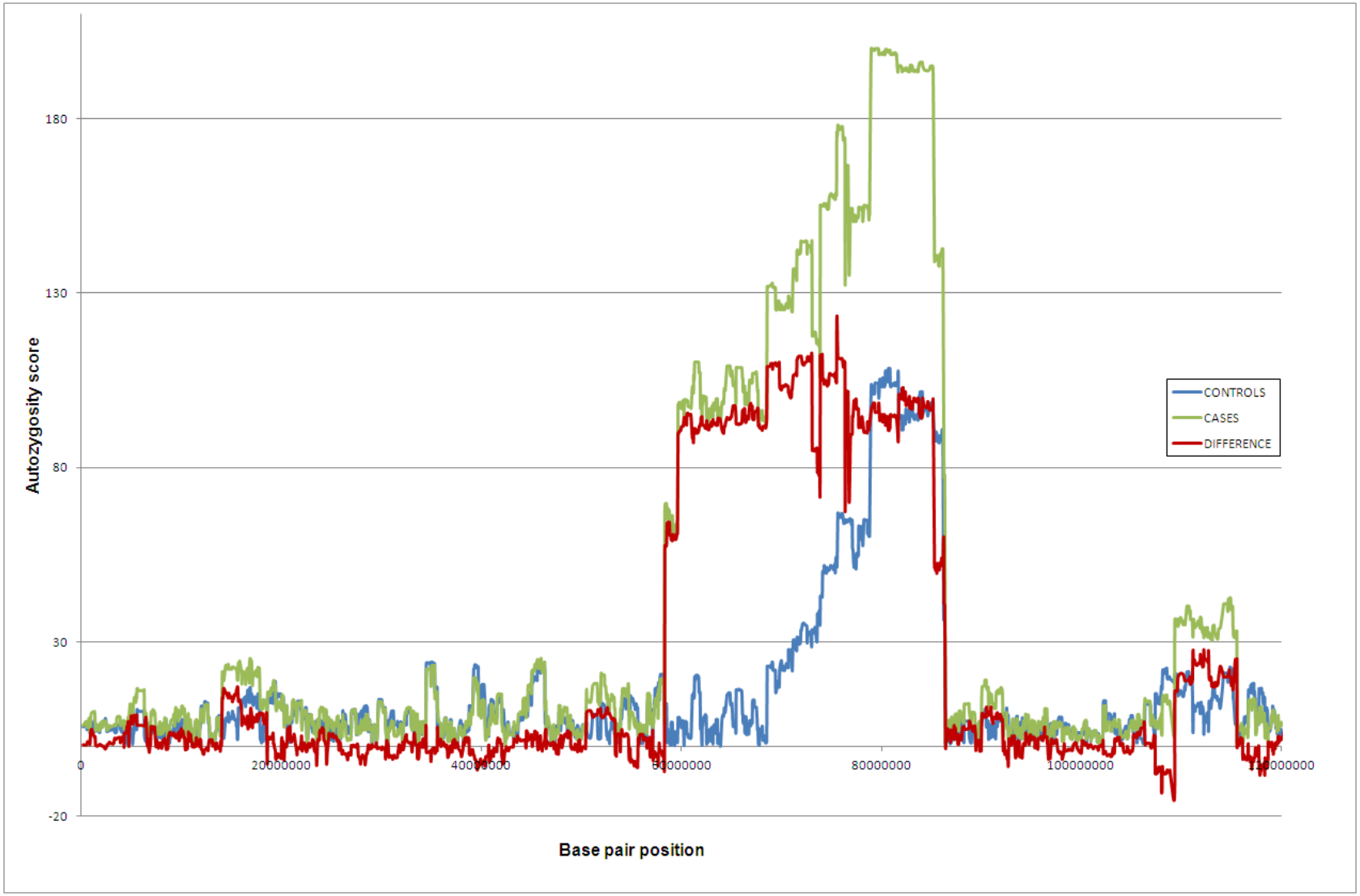
Comparison of # homozygous SNP between cases and controls tested with paired t-test within chromosome



Highly significant interaction on Chromosome 4

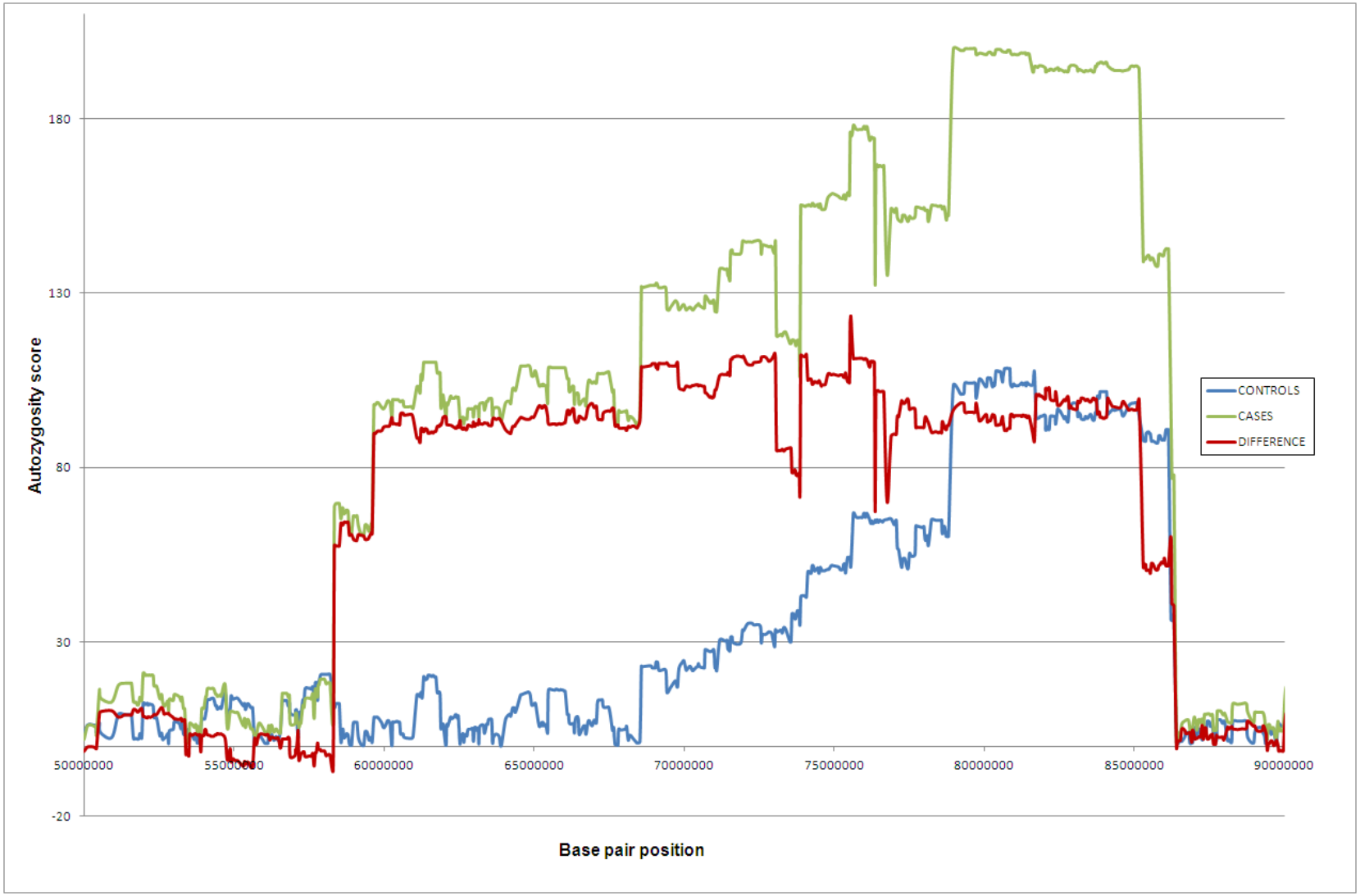
RVC

Chromosome 4 autozygosity score



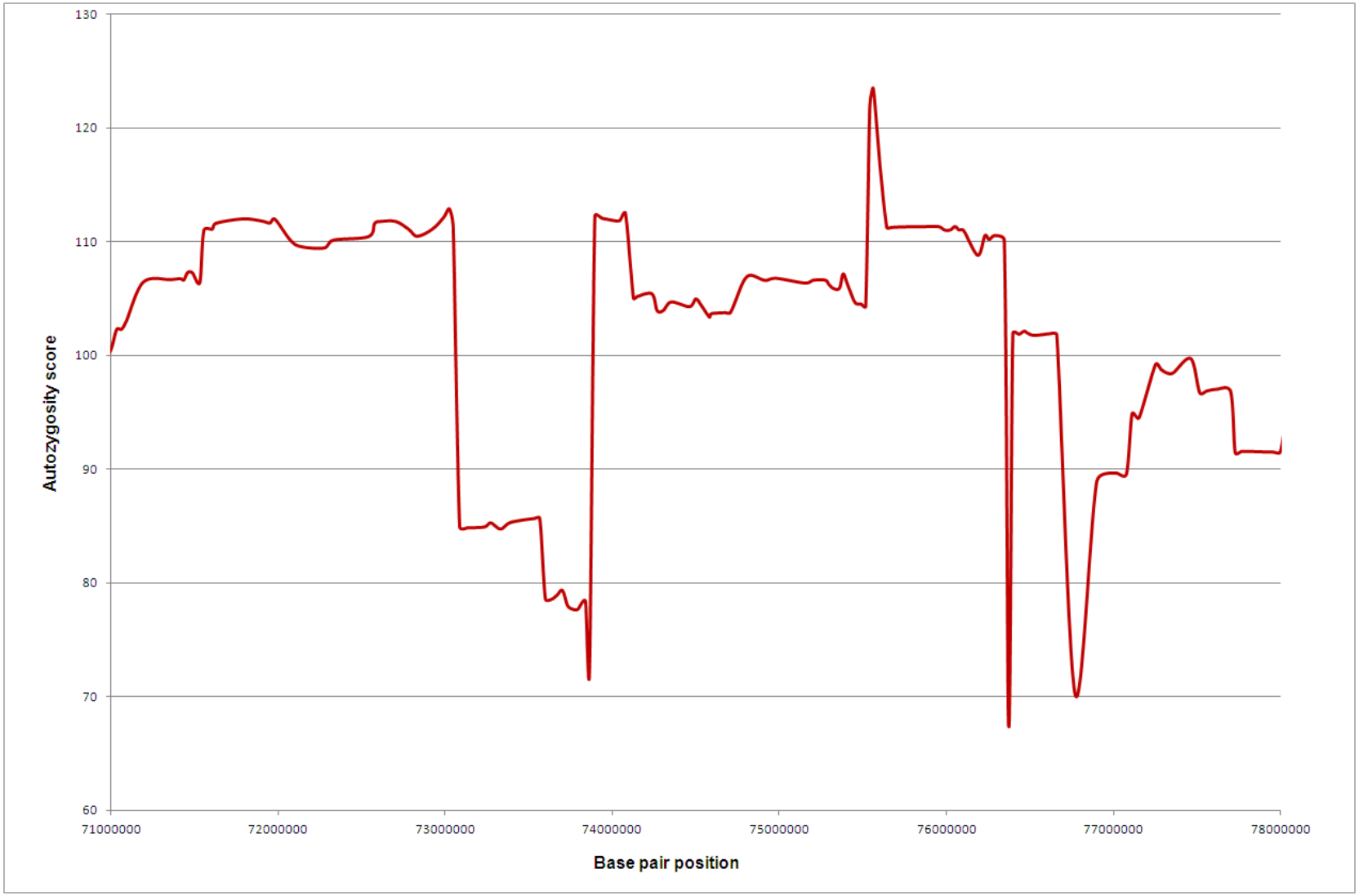
RVC

Chromosome 4 autozygosity score



RVC

Chromosome 4 autozygosity score

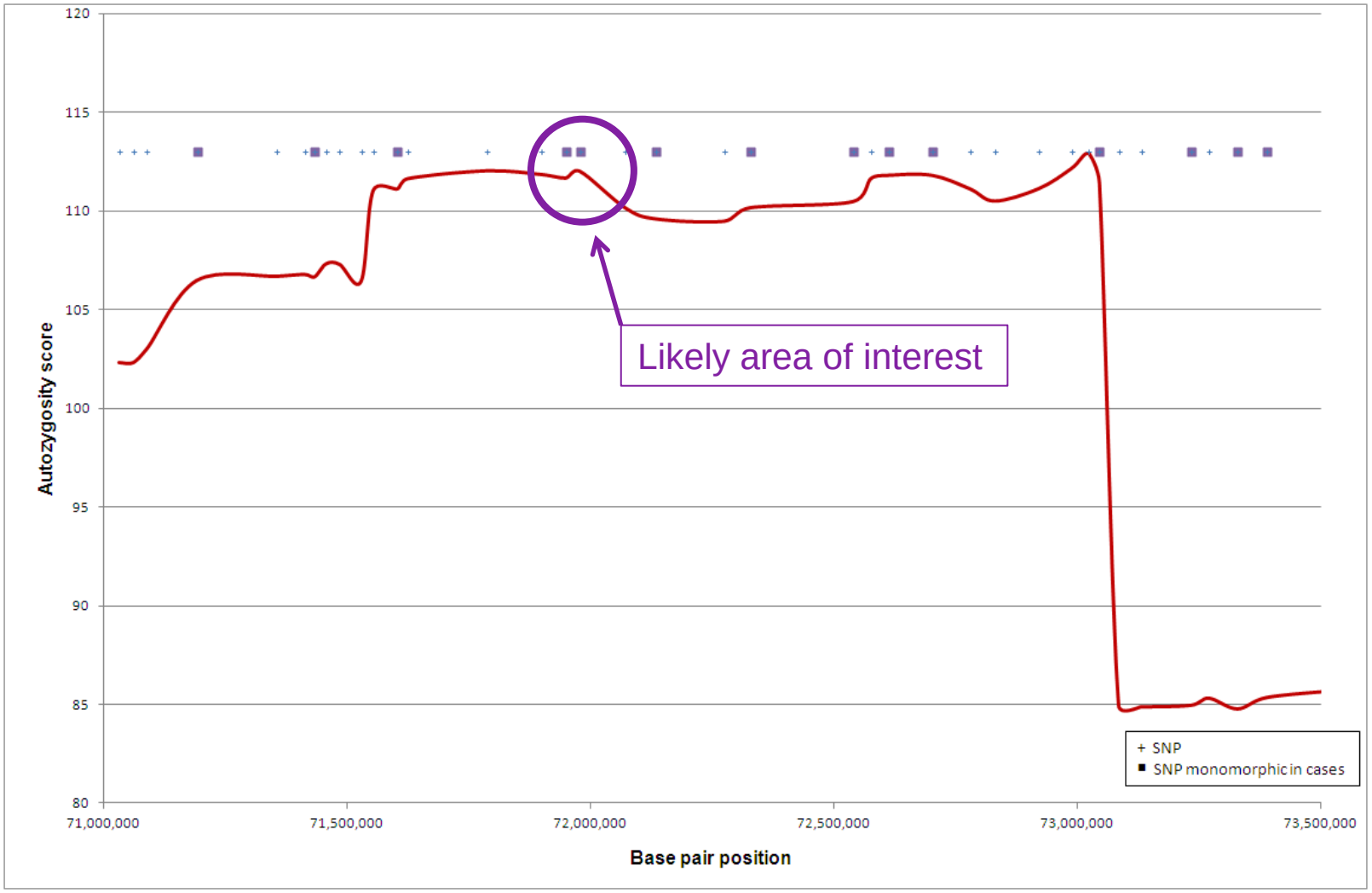


RVC

Chromosome 4 autozygosity score plus monomorphic SNP

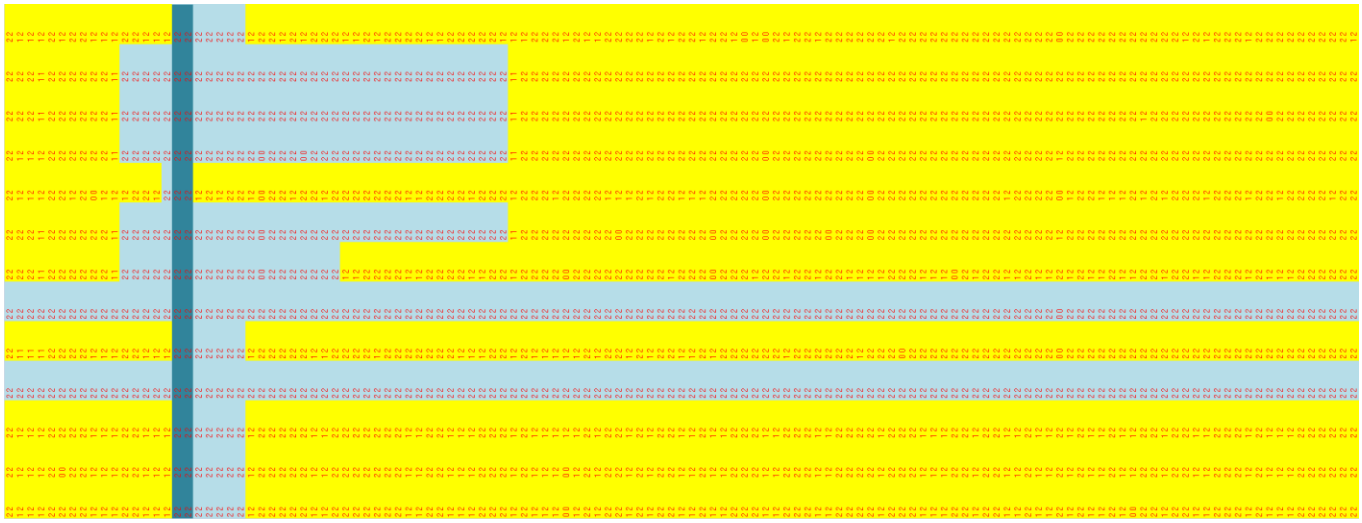
RVC

Chromosome 4 autozygosity score plus monomorphic SNP



RVC

Heatmap of likely area containing mutation



Two momomorphic SNP in dark blue

Runs of homozygosity in light blue

RVC

Concluding remarks

Issues in rare breeds

Phenotyping

Drop one methods?

Significance levels

RVC

Acknowledgements

The breed society members for their time, data and cooperation.

Biosciences-KTN for a SPARK award to cover the cost of genotyping.

Thank you for your attention

RVC