

Genetic diversity, linkage disequilibrium and genomic selection of the local Ashanti Dwarf Pig (ADP) of Ghana

The ALBORADA Trust



Richard Osei-Amponsah
CAPREx Postdoctoral Fellow
Department of Animal Science
University of Ghana, Legon.



presentation outline

- background and objective of project
- data collection
- studies carried out and preliminary results
- future studies/way forward
- acknowledgements

background and objective of project

background

- Ghana is yet to attain self sufficiency in protein production
- non-ruminant production has been identified as a means to bridging this protein deficiency gap
- **local breeds** are important for genetic diversity and food security and their characterisation is the first strategic priority area of the GPA on AnGR

(FAO, 2007; MOFA, 2012)

background

- the Ashanti Black Forest Dwarf Pig (Ashanti Dwarf Pig) (**ADP**) is an adapted breed with relatively good adaptive traits



problem statement

- origin and phylogeny of the ADP is unknown
- unique favourable genes not identified
- indiscriminate crossbreeding with exotic breeds
- lack of a sustainable breeding scheme
- low human and institutional capacity in animal genetics

genomics and animal breeding

- genomic selection predicts **breeding values** on the basis of a large number of marker haplotypes across the entire genome.
- underlying assumption is that haplotypes at some loci are in linkage disequilibrium (**LD**) with QTL alleles that affect **traits of economic importance**.

genomics and animal breeding

- significant associations have been demonstrated for candidate genes for:
 - litter size** (ESR, PRLR, RBP4),
 - growth** (MC4R)
 - meat quality** (PRKAG3, RYR1, H-FAPB),
 - disease resistance** (FUT1, SLA/MHC, NRAMP, SLC11A1, LIF, IRF1)
 - coat colour** (KIT, MCIR)

(Meuwissen *et al.*, 2001; Bidanel and Rothschild, 2002; Chen *et al.*, 2007)

genomics and animal breeding

- genome sequencing and development of chips that are able to genotype thousands of single nucleotide polymorphisms (**SNP**) across the genome is a breakthrough for animal breeding.
- **SNP** genotyping increases the accuracy of selection at young ages, shorten generation interval and increase overall profitability of animal production.

(Ibañez-Escríche and Gonzalez-Reício, 2011, Fernández *et al.*, 2012)

objective of project

- to utilise genomics to determine the origin and characterise unique genetic attributes of the ADP
- to select and maintain beneficial alleles conferring traits of economic importance

hypothesis

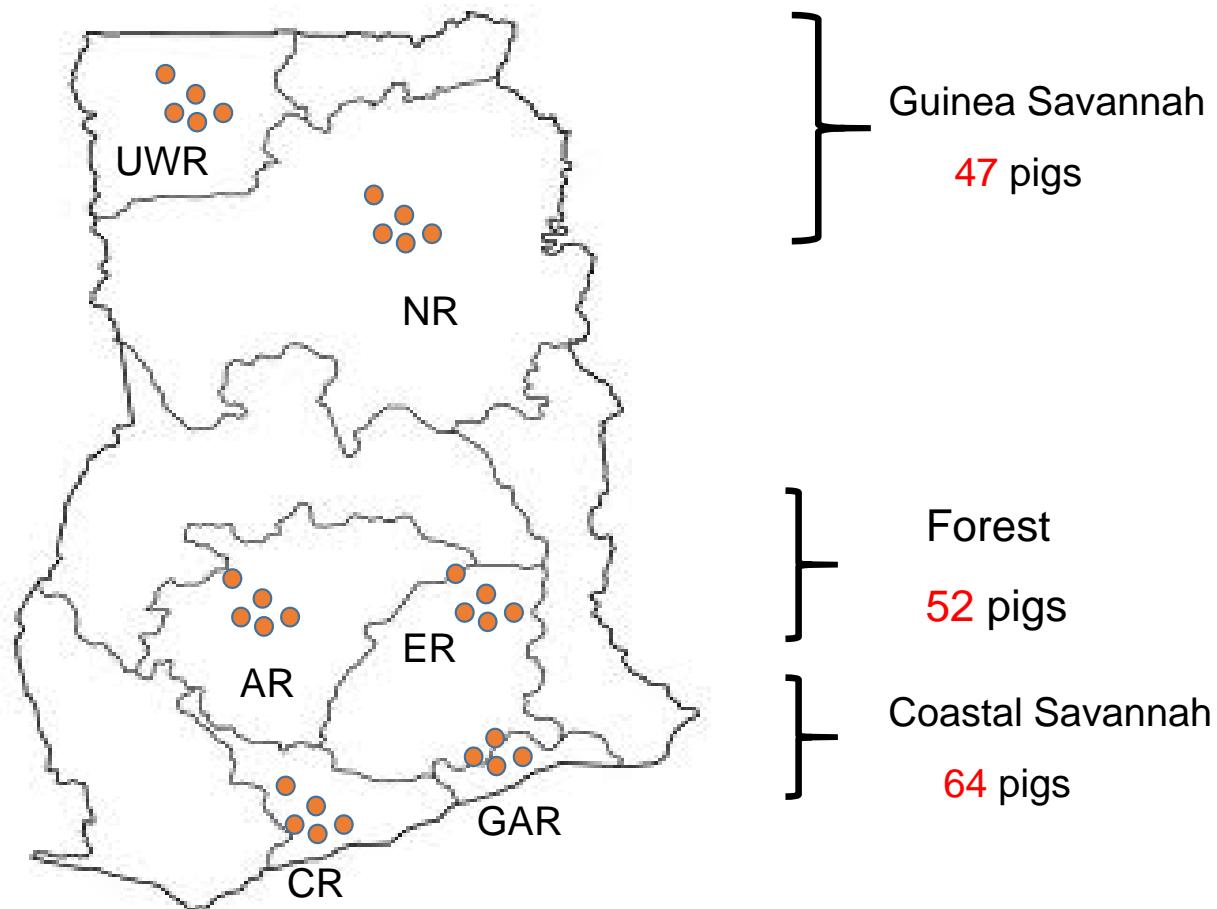
- application of genomic selection tools should enable genetic characterisation and identification of unique and beneficial alleles of the ADP.

specific objectives

- i. determine the origin and genetic diversity of the ADP
- ii. identify QTL of growth, carcass, reproductive and disease resistance traits in the ADP
- iii. develop a genomic selection scheme for ADP
- iv. propose a breeding programme for the ADP
- v. build capacity in genomic characterisation of pigs

data collection

data collection



data collection



data collection



ADP pig breeding station, Babile



ear tissue sampling



ear tissue sampling



ear tissue sampling



DNA Extraction

- DNA extracted from ear tissues using QIAGEN blood and tissue kit following the manufacturer's protocol at the Biotechnology laboratory of CACS, UG
- DNA samples were stored at -80°C and subsequently transported to Cambridge for analyses



studies carried out and preliminary results

study 1

origin and phylogenetic status of local ADP by mitochondrial DNA analysis



origin and phylogenetic status of the ADP

- evolutionary relationships, levels of variability and geographic sub-structuring within and between groups of animals can be derived by comparing their DNA sequences
- correlations between phylogenetic signals and geographic provenance allows pigs to be important proxies of human dispersal

(Avise *et al.*, 1987, Kim *et al.*, 2002, Larson *et al.*, 2010)

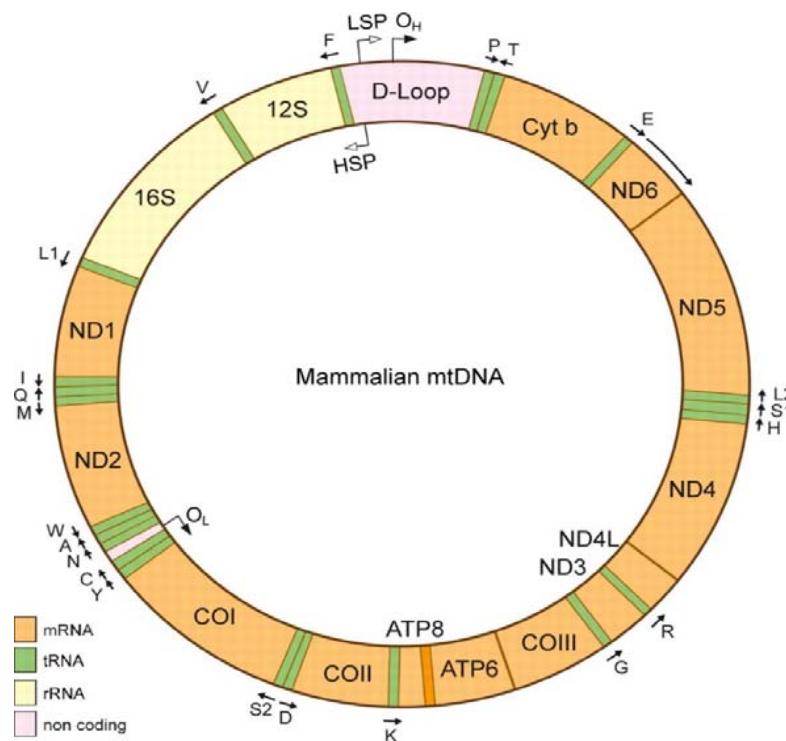
origin and phylogenetic status of the ADP

- comparative genomics of distantly related species makes it possible to decipher the major steps in evolution including genome rearrangements and mutation pressure
- mitochondrial DNA (**mtDNA**) is routinely used to produce phylogenetic trees at several taxonomic levels, from within species to among orders

(Primose and Twyman, 2003, Larson *et al.*, 2005; Haile *et al.*, 2010)

mitochondrial DNA

- size of 16,613bp, consists only of structural genes without any non-coding bases except the D-loop region,



mitochondrial DNA

- MtDNA is maternally inherited, haploid, non-recombining and its evolutionary rate of base substitution is much faster than that of nuclear DNA
- the D-Loop region is non-coding and known to be more variable in sequence than other regions and mutations in this interval have been investigated in a number of evolution studies

(Giles *et al.*, 1980; Cann *et al.*, 1984; Huang *et al.*, 1999; Avise, 2000 Gongora *et al.*, 2004; Yu *et al.*, 2013)

mitochondrial DNA

- has also been used to describe variation in putative wild ancestor and modern domestic livestock populations including pigs
- a significant differentiation between the European and Chinese domestic pigs has been revealed by mtDNA analyses

(Ursing and Arnason, 1998; Giuffra *et al.*, 2000; Okimura *et al.*, 2001, Watanabe *et al.*, 2001, Kim *et al.*, 2002; Bruford *et al.*, 2003; Mccann *et al.*, 2014).

analysis of mtDNA D-loop

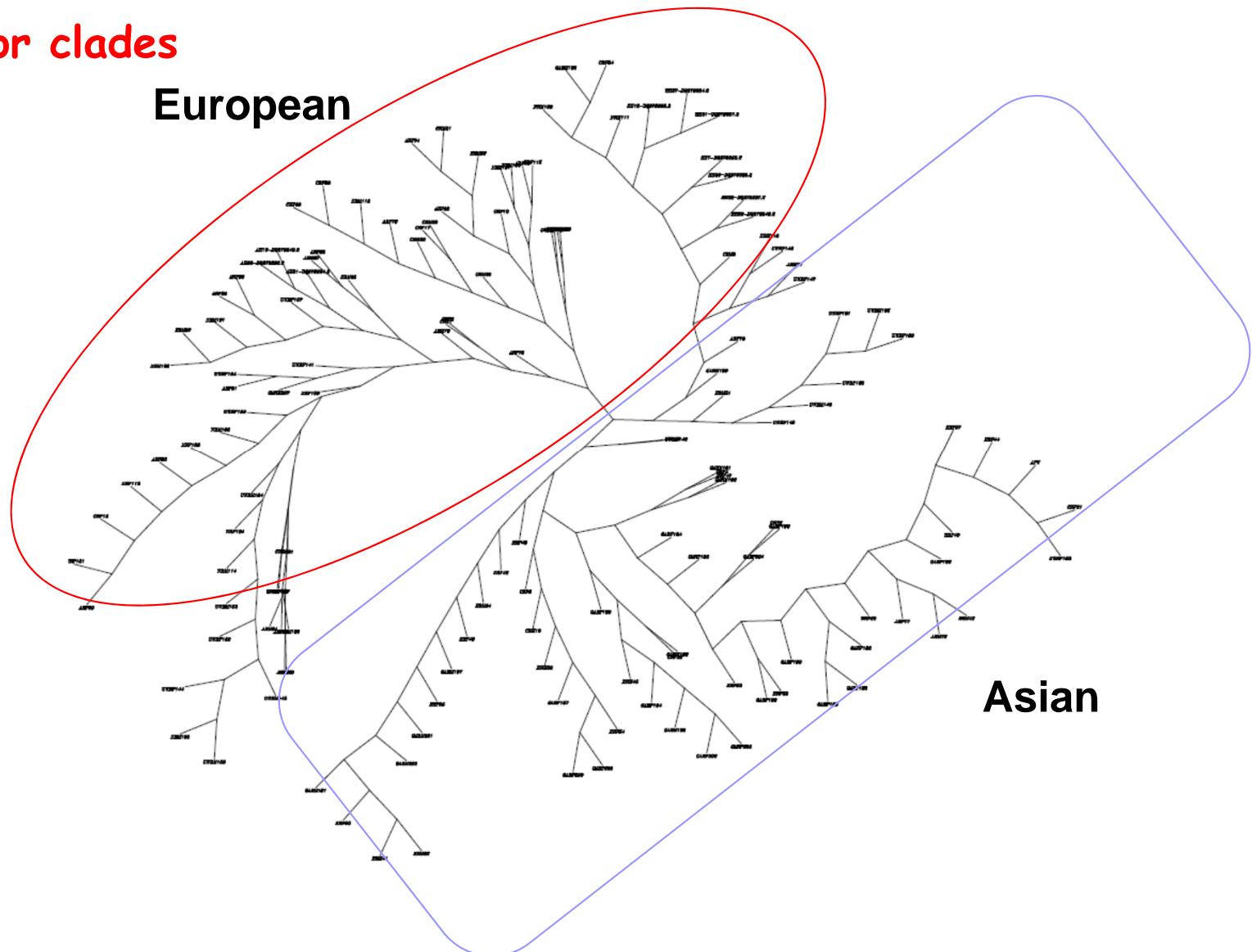
- a 620bp fragment of the D-loop region of mtDNA from 142 animals was amplified by touchdown PCR and purified
- amplicons were sequenced and traces edited using Chromas version 2.2
- sequences were viewed using the MultAlin program and within the ClustalW2 program
- BLAST (in NCBI) was used to view and obtain the consensus sequence of D-loop region for each individual relative to pig mtDNA sequence

phylogenetic analysis

- genetic distances between whole control region sequences were calculated using Kimura's two-parameter substitution model and with the same pattern among lineages and uniform rates among sites, as implemented in the **MEGA** software
- phylogenetic tree was produced using **MRBAYES** with bootstrap = 1,000,000 replicates
- the frequency of each haplotype in each population and the frequency of Asian and European haplotypes were calculated

(Kimura *et al.*, 2001; Edgar, 2004; Tamura *et al.*, 2011)

two major clades



results

- 2 major clades – European and Asian wild boars
- 43 haplotypes:

11 unique ADP haplotypes

5 haplotypes unique to Asian wild boars

6 haplotypes unique to European wild boars

3 haplotypes – Ghanaian + European

2 haplotypes – Ghanaian + Asian

11 ADP unique haplotypes

- ADP haplotypes:
 - AR(1)
 - GAR (2) + 1(ER)
 - AR(1)
 - UWR(3)
 - GAR(14) + ER(5) + CR(1)
 - AR(1)
 - ER (1)
 - UWR (1)
 - UWR (1)
 - UWR(1) + NR(1)
 - CR(2)
-
- ```
graph TD; A[ADP haplotypes] --- B[AR(1)]; A --- C[GAR (2) + 1(ER)]; A --- D[AR(1)]; A --- E[UWR(3)]; A --- F[GAR(14) + ER(5) + CR(1)]; A --- G[AR(1)]; A --- H[ER (1)]; A --- I[UWR (1)]; A --- J[UWR (1)]; A --- K[UWR(1) + NR(1)]; A --- L[CR(2)]; B --- EuropeanClade[European Clade]; C --- EuropeanClade; D --- EuropeanClade; E --- EuropeanClade; F --- EuropeanClade; G --- EuropeanClade; H --- AsianClade[Asian Clade]; I --- AsianClade; J --- AsianClade; K --- AsianClade; L --- AsianClade;
```

## within haplotype genetic distance

|    | 1     | 2     | 4     | 7     | 12    | 15    |
|----|-------|-------|-------|-------|-------|-------|
| 1  |       | 0.004 | 0.004 | 0.008 | 0.008 | 0.008 |
| 2  | 0.008 |       | 0.004 | 0.008 | 0.008 | 0.008 |
| 4  | 0.009 | 0.011 |       | 0.007 | 0.008 | 0.008 |
| 7  | 0.025 | 0.028 | 0.024 |       | 0.003 | 0.000 |
| 12 | 0.027 | 0.030 | 0.027 | 0.005 |       | 0.003 |
| 15 | 0.026 | 0.028 | 0.023 | 0.001 | 0.004 |       |

Haplotypes 1, 2 and 4 fell in the Asian clade

Haplotypes 7, 12 and 15 fell in the European clade

## within breed genetic distance

|               | ADP   | Asian | European | Pacific | Sus outgroups | AFW   |
|---------------|-------|-------|----------|---------|---------------|-------|
| ADP           |       | 0.005 | 0.003    | 0.005   | 0.007         | 0.013 |
| Asian         | 0.027 |       | 0.005    | 0.004   | 0.006         | 0.014 |
| European      | 0.018 | 0.027 |          | 0.005   | 0.007         | 0.014 |
| Pacific       | 0.026 | 0.022 | 0.025    |         | 0.006         | 0.014 |
| Sus outgroups | 0.043 | 0.038 | 0.042    | 0.033   |               | 0.012 |
| AFW           | 0.095 | 0.099 | 0.096    | 0.095   | 0.090         |       |

(Blench, 1999; Ramirez *et al.*, 2009)

## conclusions

local pigs of Ghana

- display multiple origins based on mtDNA sequence analysis
- segregate into two major clades – one clustering with the European wild boars and the other with the Asian wild boars
- are more likely to be of European than Asian origin

## study 2



MC1R gene polymorphism and coat colour variation in the ADP and its crosses with modern commercial breeds

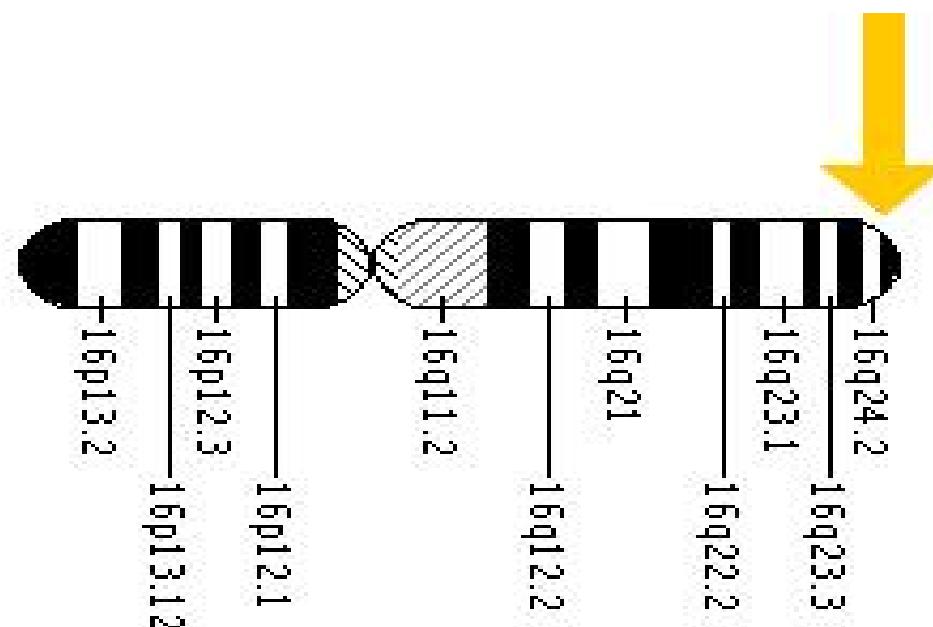
## **genetics of coat colour**

- pigmentation is due to the presence or absence of melanins
- two types of melanins are synthesized:
  - eumelanins (black/brown pigments)
  - pheomelanins (yellow/red pigments)
- variability in several genes have been shown to affect pigmentation but 2 main ones are involved in pig colour – **MC1R** and **KIT**

(Kijas *et al.*, 1998, 2001)

## MC1R gene

- a single coding exon of about 950bp
- located on the long arm of chromosome 16 at position 24.3



## **MC1R gene – coat colour genetics**

- melanocortin receptor 1 (MC1R) expressed in melanocytes is a G-protein-coupled receptor
- MC1R signalling determines whether a melanocyte produces a black eumelanin or red/yellow pheomelanin
- loss-of-function mutations are associated with recessive red coat colour
- dominant black colouring is linked with mutations causing activation of MC1R signalling

(Kijas *et al.*, 1998, 2001; Dun *et al.*, 2007; Li *et al.*, 2010; Margeta *et al.*, 2013)

## Mutations in MC1R gene

- 5 allele groups have been reported

| allele         | symbol            | variants |        |        |
|----------------|-------------------|----------|--------|--------|
| wild type      | $E^+$             | MC1R*1   | MC1R*5 |        |
| dominant black | $ED^1$ and $ED^2$ | MC1R*2   | MC1R*7 | MC1R*3 |
| black spotted  | $E^P$             | MC1R*6   |        |        |
| recessive      | $e$               | MC1R*4   |        |        |

(Kijas *et al.*, 1998, 2001; Dun *et al.*, 2007; Li *et al.*, 2010; Margeta *et al.*, 2013)

## **black spotted allele ( $E^P$ )**

- a consequence of two C nucleotide insertions, at the position 67 nucleotide leading to the frame shift and premature stop codon
- the insertion of CC occurs in a GC-rich rich region and within a stretch of six Cs that is expanded to a mononucleotide repeat of eight Cs

## coat colour variation in ADP



## **genetic analysis of the MC1R gene polymorphisms**

- An 823bp fragment covering the region containing the allelic variant was amplified by PCR and the products sequenced from 33 Ghanaian local pigs, 1 large white and 1 duroc
- sequences edited using Chromas and blasted in NCBI
- MC1R sequences of local and exotic pigs compared using **MULTIALIN** software

# results

| Animal ID | CC insertion | Phenotype | Black spotting |
|-----------|--------------|-----------|----------------|
| 85        | ++           | white     | +              |
| 111       | ++           | white     | +              |
| 80        | ++           | white     | +              |
| 10        | ++           | white     | +              |
| 108       | ++           | white     | -              |
| 110       | ++           | white     | -              |
| LW        | ++           | white     | -              |

# results

| <i>Animal ID</i> | <i>CC insertion</i> | <i>Coat colour</i> | Black spotting |
|------------------|---------------------|--------------------|----------------|
| 114              | --                  | black              | n/a            |
| 27               | --                  | black              | n/a            |
| 164              | --                  | black              | n/a            |
| 5                | --                  | black              | n/a            |
| 167              | --                  | white              | -              |
| 162              | --                  | white              | +              |
| 90               | --                  | white              | +              |

## results

- pigs with a CC insertion at nt67 had white coat colour
- pigs lacking the CC insertion were both black and white
- The nt67insCC is associated with the white colour
- absence of the CC insertion however does not guarantee black coat colour

(Kijas *et al.*, 1998, 2001; Dun *et al.*, 2007; Yang *et al.*, 2010; Margeta *et al.*, 2013)

## conclusions

- Ghanaian local pigs have diverse coat colour inheritance
- ADP lacks the nt67insCC
- occurrence of spotted piglets may be due to epistatic interactions
- further studies recommended
- coat colour alone cannot be used to adequately characterise local pigs

## study 3

whole-genome SNP analysis  
of growth, reproduction,  
meat quality and disease  
resistance traits in the ADP  
of Ghana



## snp genotyping

- the genome sequencing and development of chips that are able to genotype thousands of SNPs across the genome is a breakthrough for animal breeding.
- hundreds of thousands of porcine SNPs have been discovered using NGS technologies and these SNPs, as well as others from different public sources have been used to design a high-density SNP genotyping assay – **porcine Illumina 60k beadchip**

(Ramos *et al.*, 2009).

## Illumina Porcine SNP60k BeadChip

- identify SNP associated with **reproduction traits** in the Finnish Landrace
- study porcine colonization of the Americas detailing a **genome wide overview** of local populations
- study linkage disequilibrium (**LD**) pattern and **selection signatures** at the genome level
- estimated **breeding values** for meat quality, production and reproduction traits

(Uimari *et al.*, 2011, Burgos-Paz *et al.*, 2012, Ai *et al.*, 2013, Beker *et al.*, 2013)

## SNP Genotyping

- genotyping was done at the genome analysis facility of the Mammalian Molecular Genetics Research Group, Cambridge University following the Illumina protocol
- 72 Ghanaian local pigs were genotyped with the **Illumina's porcine SNP60 beadchip** (Ramos et al., 2009)
- the use of a large number of markers permits a more rigorous analysis of genetic relationships

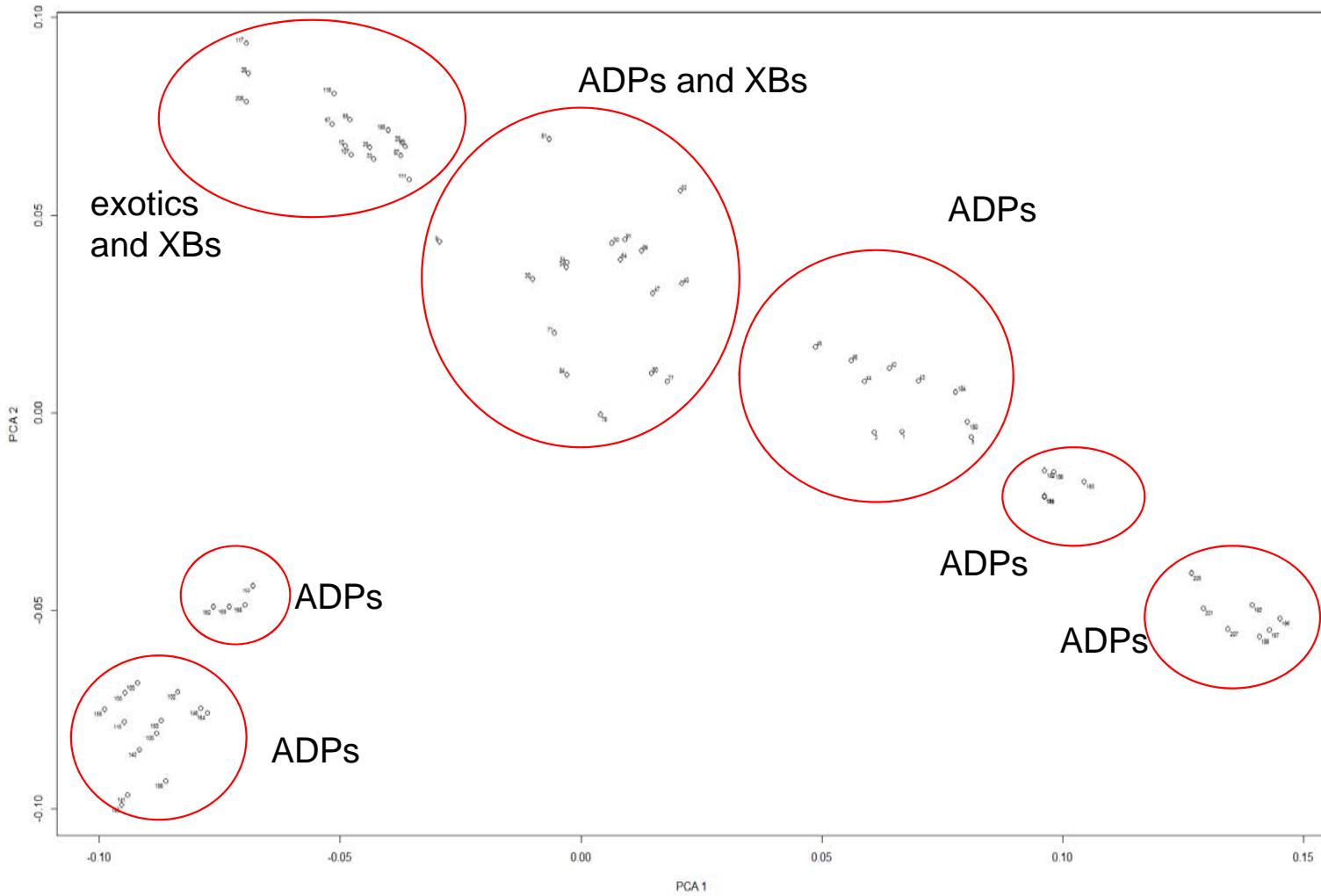
# Genome analysis facility



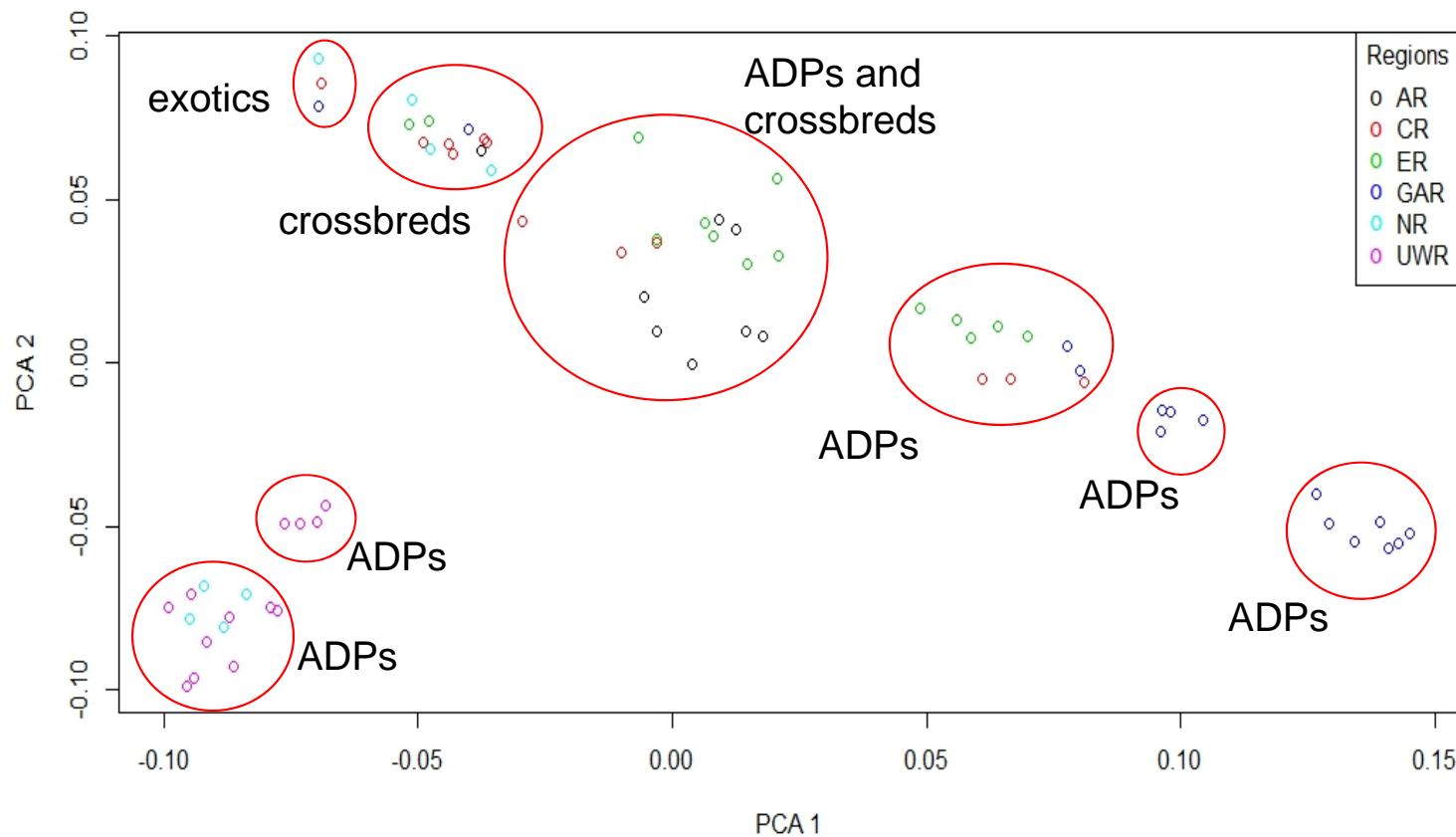
## analyses of genomic data

- data were visualised and analysed with the **PLINK** software
- the following criteria was used to filter animals and SNP before analysis:
  - monomorphic SNPs were removed
  - call rate > 0.98
  - minor allele frequency (**MAF**) > 0.05
- relatedness among the pigs was examined using a PCA to determine identity by descent (IBD)
- polymorphisms in the genes for selected traits of economic importance being studied

## PCA analysis of SNP data

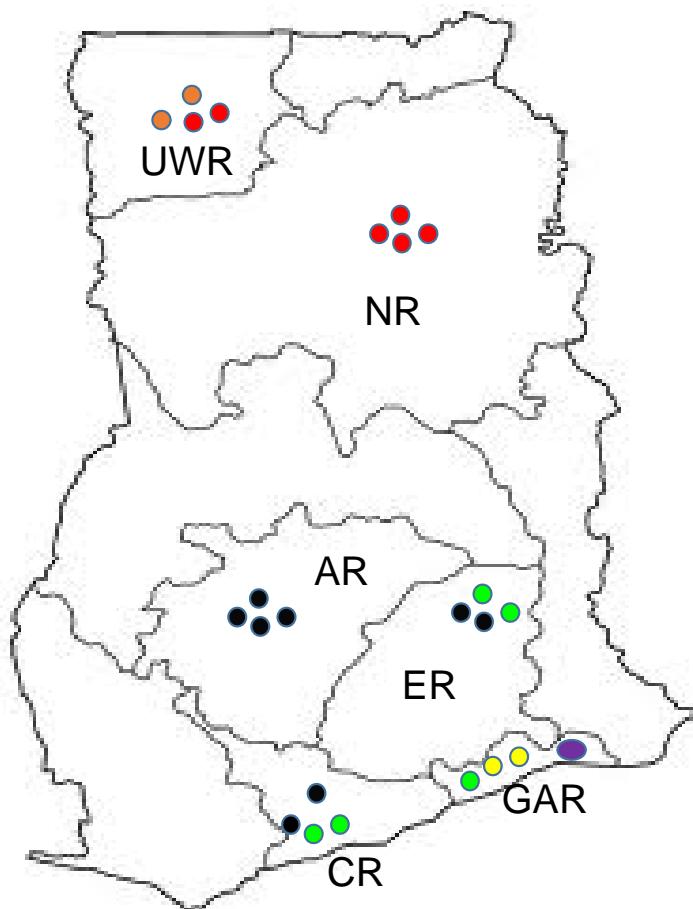


## PCA analysis of SNP data



## conclusions

- 6 genotypic groups of the ADP were found
  - 2 are unique to the GAR
  - 1 unique to UWR
  - 1 unique to UWR and NR
  - 1 unique to CR, ER and GAR
  - 1 unique to AR, ER and CR



## conclusions

- ADPs from the UWR and NR display the **farthest** origin from the others
- ADPs from AR, ER and CR are closest to exotic pigs
- one of the two ADP groups from the GAR displays white patches in coat colour

## findings

- there appear to be diverse genetic origins of the ADP but it is has more European than Asian origin
- there are five distinct genetic groupings of the ADP
- phenotypic coat colour data is insufficient to distinguish these diverse genetic groups

## **publications in preparation**

- origin and phylogenetic status of local ADP – using evidence from mtDNA analysis, MC1R and Y-chromosome haplotypes
- identification of unique genomic signatures associated with disease resistance, growth rate and meat quality traits in local swine genetic resources of Ghana

**way forward**

## **way forward**

- whole-genome SNP analysis of growth, reproduction, meat quality and disease resistance traits in the ADP of Ghana
- study on the genetic diversity and linkage disequilibrium of African local pigs
- use of these data to develop a breeding programme to introgress desirable production and health traits into crossbred pigs

## way forward

- grant application – to finish study
- PAG - January 2015
- joint PhD students supervision
- workshops on genomic selection for various stakeholders

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appreciation



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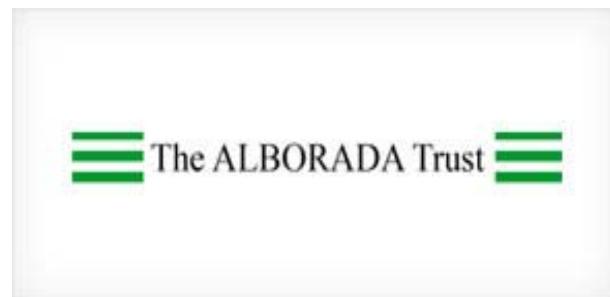
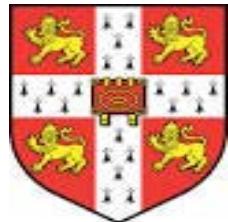
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My family



ISAAC NEWTON TRUST





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