Development of genomic resources and whole-genome prediction in the Pacific Whiteleg shrimp *Litopenaeus vannamei*.

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Traditional breeding programs

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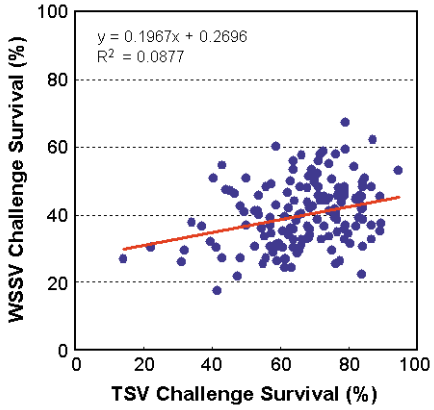
Some limitations with traditional shrimp breeding programs

- Hard to measure traits or can't measure early
  - Disease resistance – labour intensive / accuracy
  - Carcass quality – mature animals



- Some traits low or variable heritability
  - Disease resistance – WSSV (h^2= 0.01-0.05)
  - Fertility - no of eggs (h^2= 0.09)

- Negative or low genetic correlations.
  - Disease & growth - WSSV (up to $_gc$ = -0.40)
  - Low between diseases - TSV and WSSV survival ($_pc$ = 0.09)
  - Increased difficulty in performing multi-trait selection

Traditional breeding programs

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- **Limitations continued..**
  - Selection candidates not always directly evaluated
    - sacrificed in testing – eg., disease & carcass composition
  - Genetic change = (**accuracy of selection***selection intensity*genetic standard deviation)/generation interval

Solution

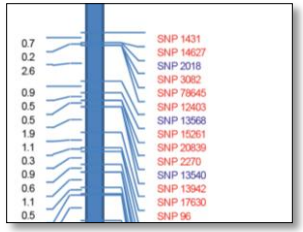
Directly evaluate genes or parts of the genome responsible for favourable traits

1. QTL mapping / GWAS and MAS
2. Genomic Selection

Program Objectives

Years

0	1)	Generate a large genomic sequence and SNP resource for *L. vannamei*
1	2)	Develop trait recording program and pedigree / genetic parameter evaluation
2	3)	Create dense genetic maps for genome structure and trait association studies
3	4)	Perform GWAS / QTL investigations
4+	5)	Evaluate genomic selection options based on dataset and farm resources



1. Illumina Infinium SNP array

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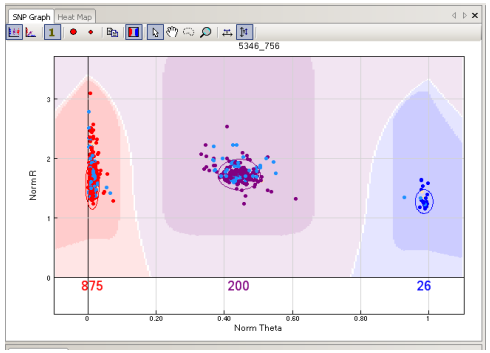
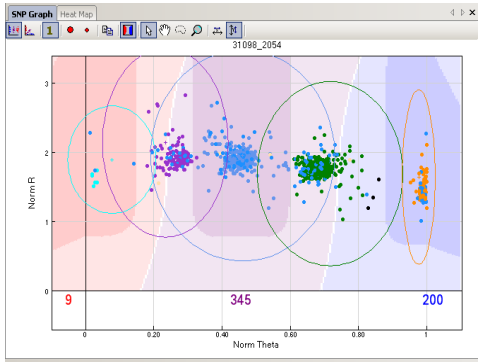
❑ SNP validation of **8,967** SNPs across 1,327 *L. vannamei* (GenomeStudio)

SNP Exclusion Reason	# SNPs (Percentage)
Probe didn't bind	262 (2.91)
Ambiguous Clusters (poor probe)	1,391 (15.51)
Het Excess	163 (1.82)
MAF < 0.01	276 (3.10)
Mendelian Inheritance Errors	425 (4.77)
MSV (genome duplication)	43 (0.5)
Total Useable	***6,407 (71.55)***
Total SNPs	8,967

Good SNP Cluster



MSV



❑ Average MAF = 31.21%

❑ Average call rate = 98.16 %

2. *L. vannamei* animal resources

- Indonesian based shrimp breeding company
  - Nucleus breeding centre
  - Broodstock multiplication
  - Hatchery PL production
  - SPF (9 pathogens)

- Diverse foundation stock (5 sources)
- Family based selection began 2008 (288+ FS families / year)
  - Growth
  - Reproduction
  - Survivability
  - Low salinity tolerance
  - WSSV, IMNV, TSV resistance

~300-1000 family average records

3. Genome resource development

Genetic Linkage Maps

- 13 mapping reference families

  → 688 informative meiosis

- 4,390 SNPs successfully mapped

  → LOD 2 & 3 framework = 2,898 SNPs

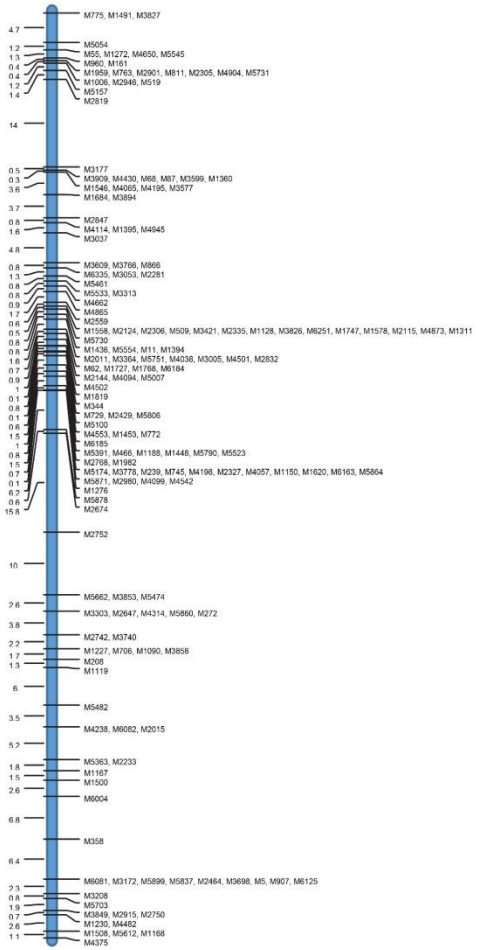
- 45 linkage groups → 97.89% coverage
- Sex average total length → 4559 cM
- Average inter-locus distance (no 0's)

  → 2.67cM

QTL analysis – sex

- 3 major QTL - 63%, 23%, 39% effect

Linkage group 6



4. GWAS analysis

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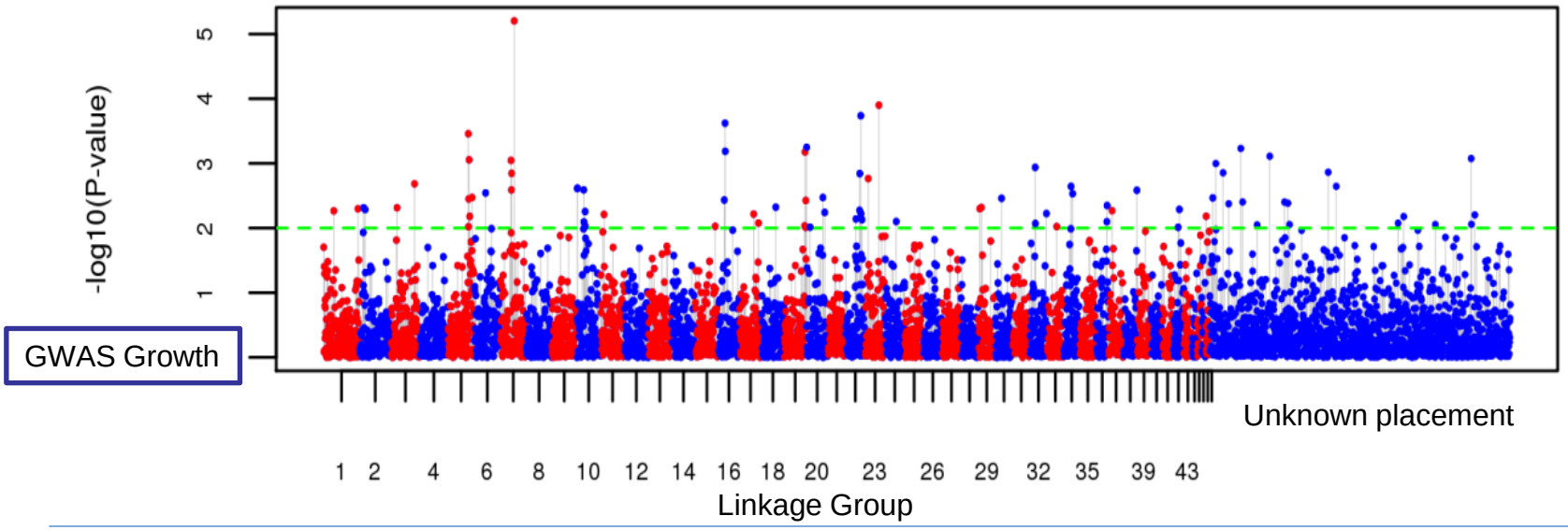
Genes of major effect - power analysis

- >90% power for moderate-high heritability (>0.15, ie., growth)
- 30-50 % power for low heritability (0.02-0.08, ie., disease resistance)

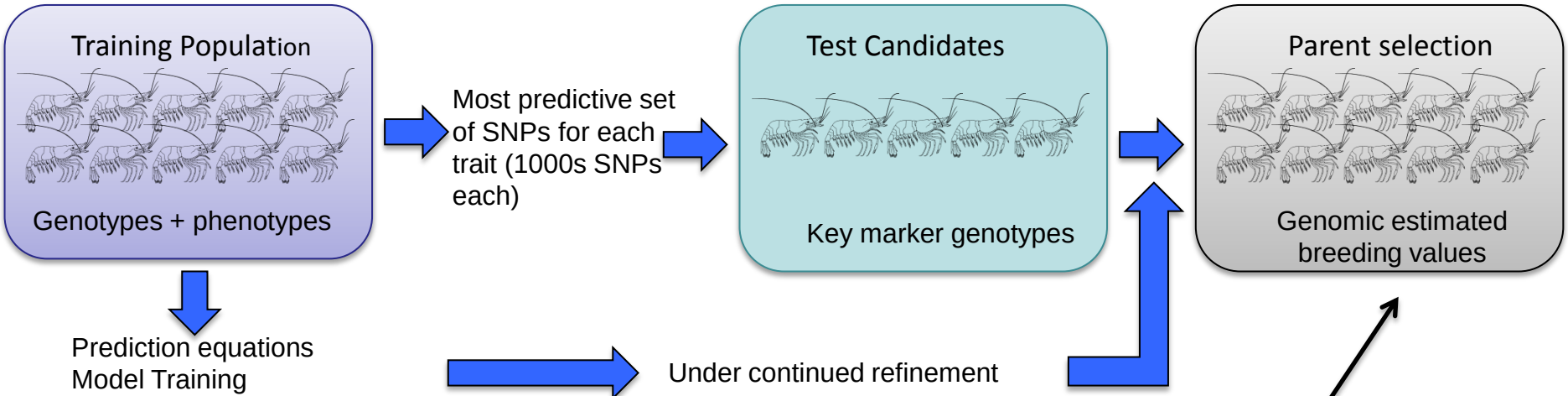
GWAS results

- No significant SNPs following FDR correction across all traits
- No genes of major effect for moderate-high heritable traits
- Insufficient power to detect genes of small effects



5. Genomic selection

- ***Most traits are complex involving many genes of small effect***
  - *Need to simultaneously search for all genes of small effects*



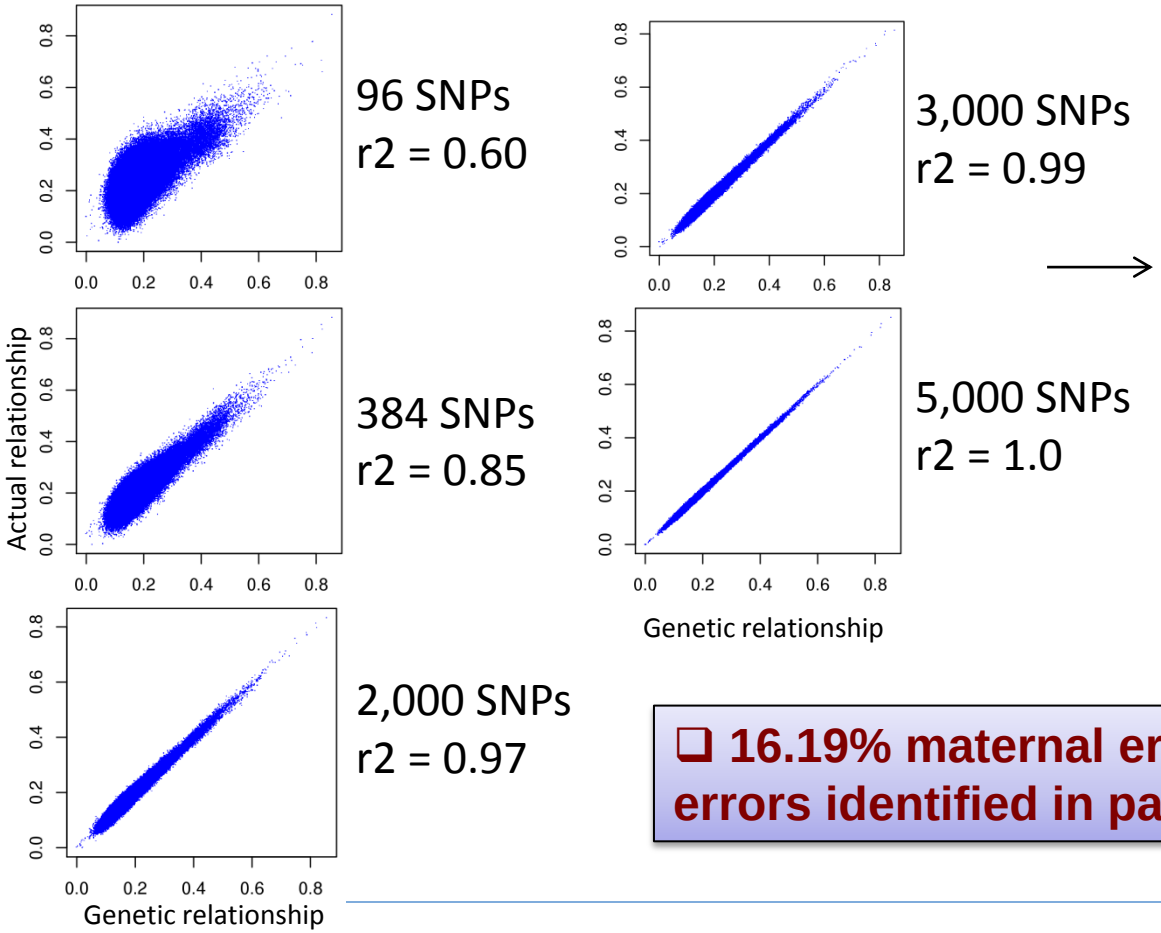
ID #	SNP1	SNP2	SNP3	SNP4	SNPn	GEBV
1M	8	3	4	8	6	29
2M	6	1	3	0	1	11
3M	0	1	0	1	-3	-1
4F	6	3	2	5	1	17
5F	1	2	--1	2	-4	2

Meuwissen et al, (2001)

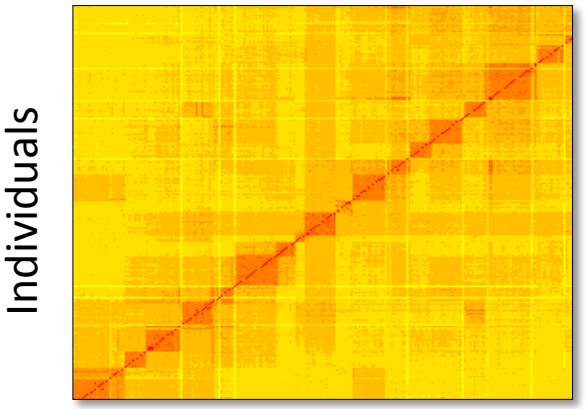
5. Genomic Selection application

What's required?

Accurate pedigrees – Genomic relationship matrix



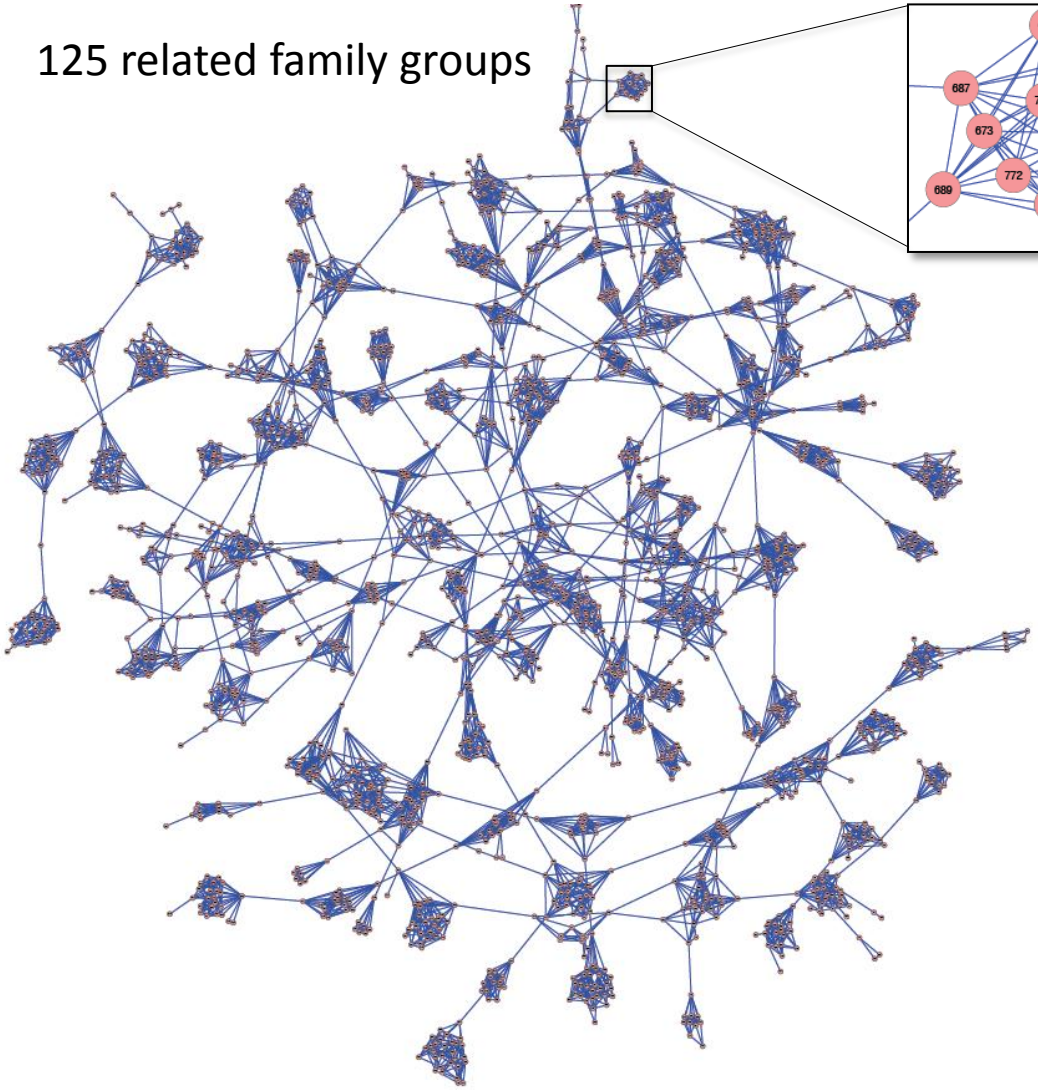
Pair wise IBS Heat map



Red blocks = family groups

❑ **16.19% maternal errors and 21.17% paternal errors identified in paper pedigree**

5. Genomic Selection application



NETVIEW analysis

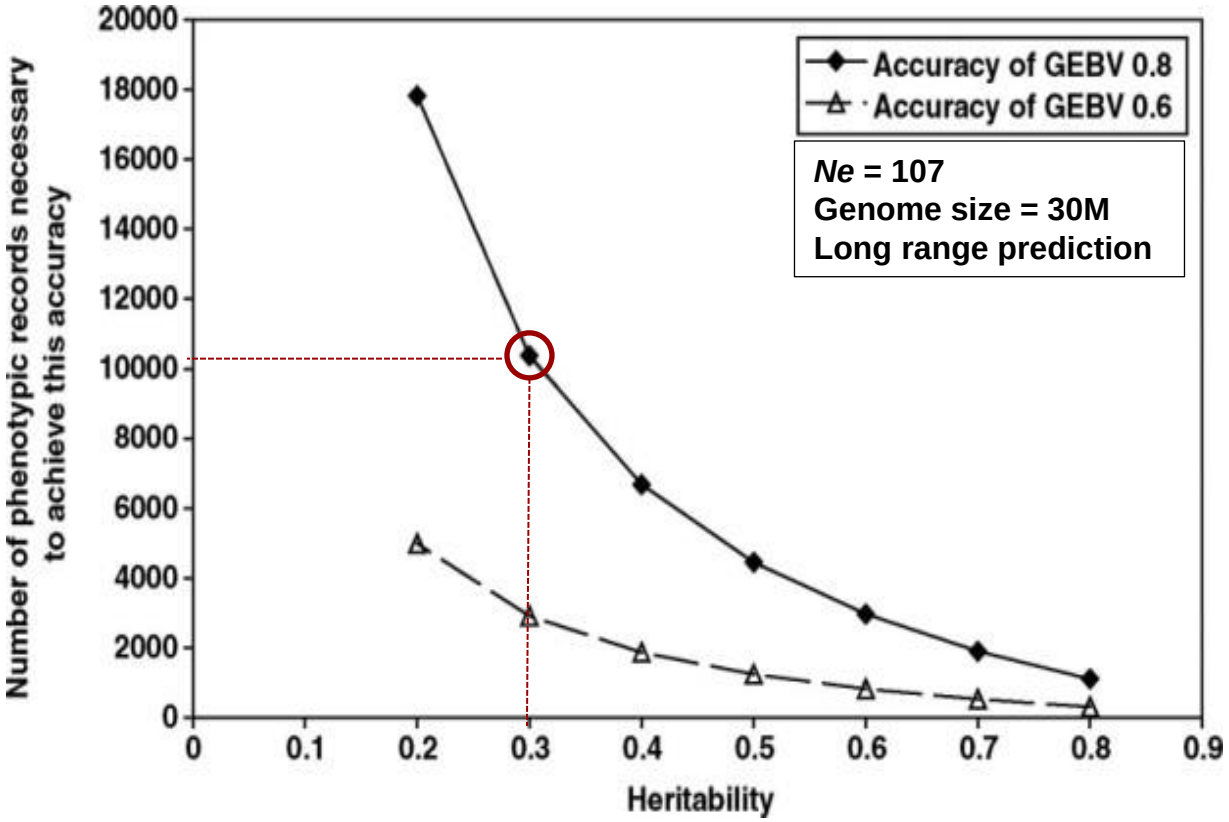
- Uses genome-wide SNPs
- Groups individuals into clusters without prior ancestry info
- Reveals fine-scale individual relatedness
- Reveals common ancestors and family connections
- Useful in correcting / inferring pedigree information

Neuditschko et al, *PLOS one* (2012), Steinig et al, *Mol Ecol Res* (2015)

5. Genomic Selection application

What's required?

Unrelated Animal Numbers[1]



Random SNP Numbers[1,2]

Unrelated individuals

10*Ne*L = 32,500

8*Ne*L = 26,000

6*Ne*L = 19,500

2*Ne*L = 6,500

1. Hayes & Goddard *Genome* (2010); 2. Sonesson et al, *GSE* (2010)

5. Genomic Selection application

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What's available ?

Large numbers of accurate phenotypic records required

- ❑ Commercial farm data only
  - Limited number of family average phenotypic records (~300-900)
  - Both parents not always genotyped
  - Needed to infer family parental allele frequencies
    - Matching family average phenotype & genotype
    - Used pooled genotyping approach on nauplii (300+) to reconstruct family parent allele frequencies (ie, 0, 0.25, 0.5, 0.75, 1)
    - Pooled genotyping approach had 99.49% accuracy at reconstructing parental allele frequencies / genotypes

Although limited data – what's the GS results for growth and TSV resistance!

5. Genomic Selection application

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Accuracy of genomic prediction (MIRROR) for Growth – moderate-high heritability

% in training	Number of families		gBLUP results for Test set			rrBLUP results for Test set		
	Training	Test	Accuracy	Bias	MSE	Accuracy	Bias	MSE
75	312	104	**0.667**	1.251	0.072	**0.693**	1.256	0.053
67	277	139	**0.597**	0.965	0.075	**0.632**	1.039	0.056
50	208	208	**0.618**	1.428	0.077	**0.647**	1.478	0.051

Accuracy of genomic prediction (MIRROR) for TSV resistance – low heritability

% in training	Number of families		gBLUP results for Test set			rrBLUP results for Test set		
	Training	Test	Accuracy	Bias	MSE	Accuracy	Bias	MSE
75	237	79	**-0.069**	-8.759	0.943	**-0.026**	-0.492	0.947
67	211	105	**0.135**	16964	0.897	**0.045**	16.352	0.896
50	158	158	**-0.038**	-24678	0.863	**0.152**	90.081	0.862

Final Comments

- Comprehensive SNP and map resources developed
- Family average phenotype and parent average allele frequency used (single record and genotype)
- Genomic relationships used to replace pedigree matrix @ $r^2 = 0.97$
  - ~3000 SNPs required
- Current SNP density appears to be sufficient for GS
  - more required for GWAS analysis (small effect genes)
- No indication of genes of major effect
- Genomic Selection a real option in this commercial farm
  - More phenotypic/genotypic data required for low heritable traits


Questions
ISGA 2018 in JCU Cairns!