UNIVERSITY OF GEORGIA
College of Agricultural & Environmental Sciences

SNP effects and weights from ssGBLUP using BLUPF90 family (postGSf90)

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BLUPF90 Team – 02/2022

Equivalence between GBLUP and SNP-BLUP

GBLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W}+\mathbf{G}^{-1}\boldsymbol{\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{G}\sigma_u^2$$

SNP-BLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\mathbf{I}\boldsymbol{\lambda_2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix}$$

↓

SNP effects

$$\mathbf{u} = \mathbf{Za}$$

GEBV = w_1**PA** + w_2**DGV** + w_3**PP**

Are GBLUP and SNP-BLUP equivalent?

In SNP-BLUP: $\mathbf{u} = \mathbf{Za}$

$$\mathrm{Var}(\mathbf{u}) = \mathrm{Var}(\mathbf{Za})$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{Z}\,\mathrm{Var}(\mathbf{a})\,\mathbf{Z}'$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{Z}\,\mathbf{Z}'\sigma_a^2$$

$$\sigma_a^2 = \frac{\sigma_u^2}{2\sum_{i=1}^{SNP} p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{ZZ}'\frac{\sigma_u^2}{2\sum_{i=1}^{SNP} p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \frac{\mathbf{ZZ}'}{2\sum_{i=1}^{SNP} p_i(1-p_i)}\sigma_u^2$$

$$\mathbf{G} = \frac{\mathbf{ZZ}'}{2\sum p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{G}\sigma_u^2$$

GBLUP and SNP-BLUP are equivalent

If we can get **u** from SNP-BLUP, we can get **a** from GBLUP!

genes

MDPI

Review

Single-Step Genomic Evaluations from Theory to Practice: Using SNP Chips and Sequence Data in BLUPF90

Daniela Lourenco [1,*], Andres Legarra [2], Shogo Tsuruta [1], Yutaka Masuda [1], Ignacio Aguilar [3] and Ignacy Misztal [1]

https://www.mdpi.com/2073-4425/11/7/790

Pages 11-12

SNP effect in ssGBLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W+H^{-1}\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

$$\hat{\mathbf{a}} = \sigma_a^2 \, \sigma_u^{-2} \, \mathbf{D} \, \mathbf{Z'G^{-1}} \hat{\mathbf{u}}$$

Matrix of SNP weights

Matrix of SNP content

Genomic relationship matrix

- What can we do with SNP effects?

1) Predictions for animals not included in the evaluation

$$\mathbf{DGV} = \mathbf{Z}\hat{\mathbf{a}}$$

Indirect predictions

Indirect Genomic Predictions

SNP effect in ssGBLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W+H^{-1}\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

$$\hat{\mathbf{a}} = \sigma_a^2 \, \sigma_u^{-2} \, \mathbf{D} \, \mathbf{Z'G^{-1}} \hat{\mathbf{u}}$$

Matrix of SNP weights

Matrix of SNP content

Genomic relationship matrix

2) Compute weights for SNP

a) Quadratic SNP weights (or variance) (Falconer & Mackay, 1996)

$$d_i = \hat{a}_i^2 2p_i(1-p_i)$$ Default

b) Nonlinear A SNP weights (or variance) (VanRaden, 2008)

$$d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a})}-2}$$ Recommended

Weights or variances for SNP in ssGBLUP

- What can we do with weights or variance for SNP?

1) Single-step Genome-Wide Association Studies - ssGWAS

   proportion of σ_u^2 explained by SNP

2) Weighted single-step GBLUP - WssGBLUP

   different σ_a^2 for each SNP when constructing **G**

Weighted single-step GBLUP - WssGBLUP

Weights for SNP in ssGBLUP

- ssGBLUP
  - Same weights for SNP

$$G = \frac{ZZ'}{2\sum p_i(1-p_i)}$$

- WssGBLUP
  - Different weights for SNP

$$G = \frac{ZDZ'}{2\sum p_i(1-p_i)}$$

- Weights may increase accuracy of GEBV
- If SNPs explain high %variance

SNP weighting in ssGBLUP: WssGBLUP

- Wang et al. (2012):

"Iterative method needs convergence"

1) Set $\mathbf{D}_t = \mathbf{I}$ and $\mathbf{G}_t = \frac{\mathbf{ZDZ'}}{2\sum p_i(1-p_i)}$

2) Compute GEBV using ssGBLUP approach

Diagonal matrix of weights

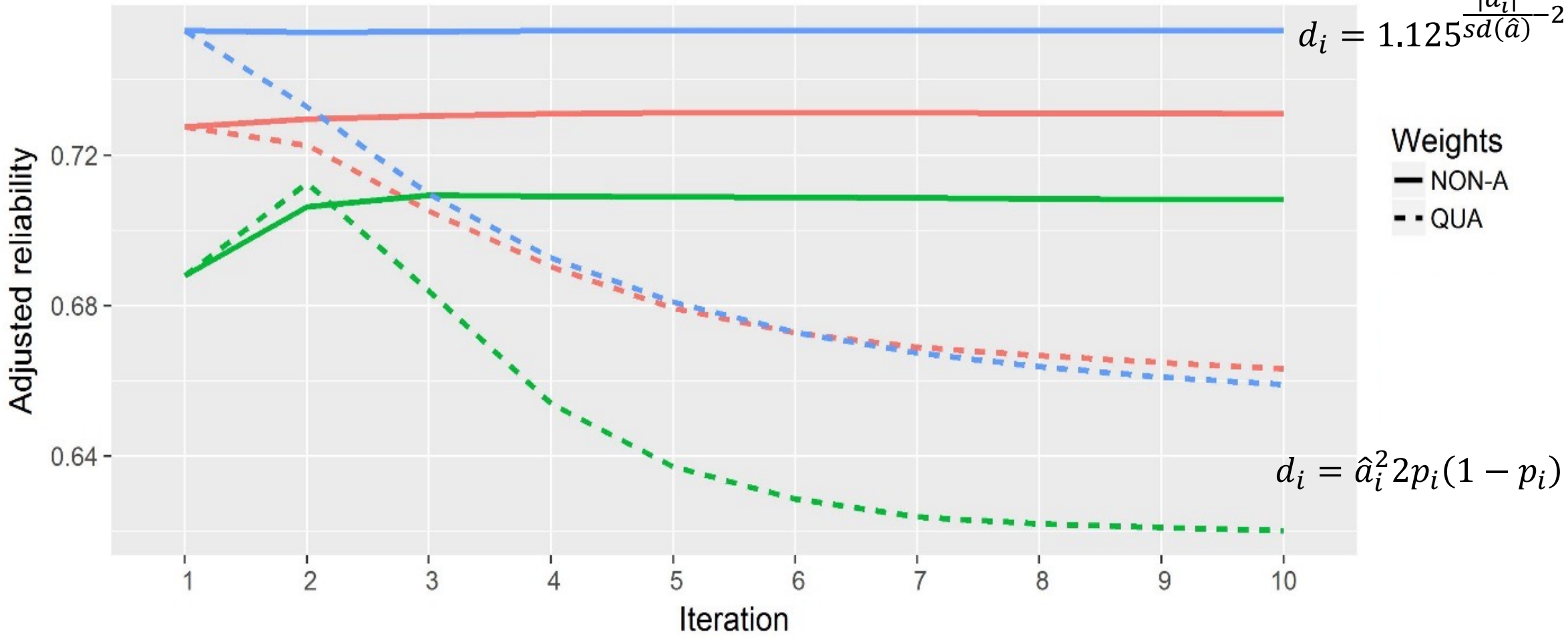
3) Compute SNP effects as $\hat{\boldsymbol{a}} = \lambda \mathbf{D}\,\mathbf{Z'G^{-1}\hat{u}}$

4) Calculate SNP weight

5) Normalize $\mathbf{D}_{(t+1)}$ so tr($\mathbf{D}$) = number of SNP

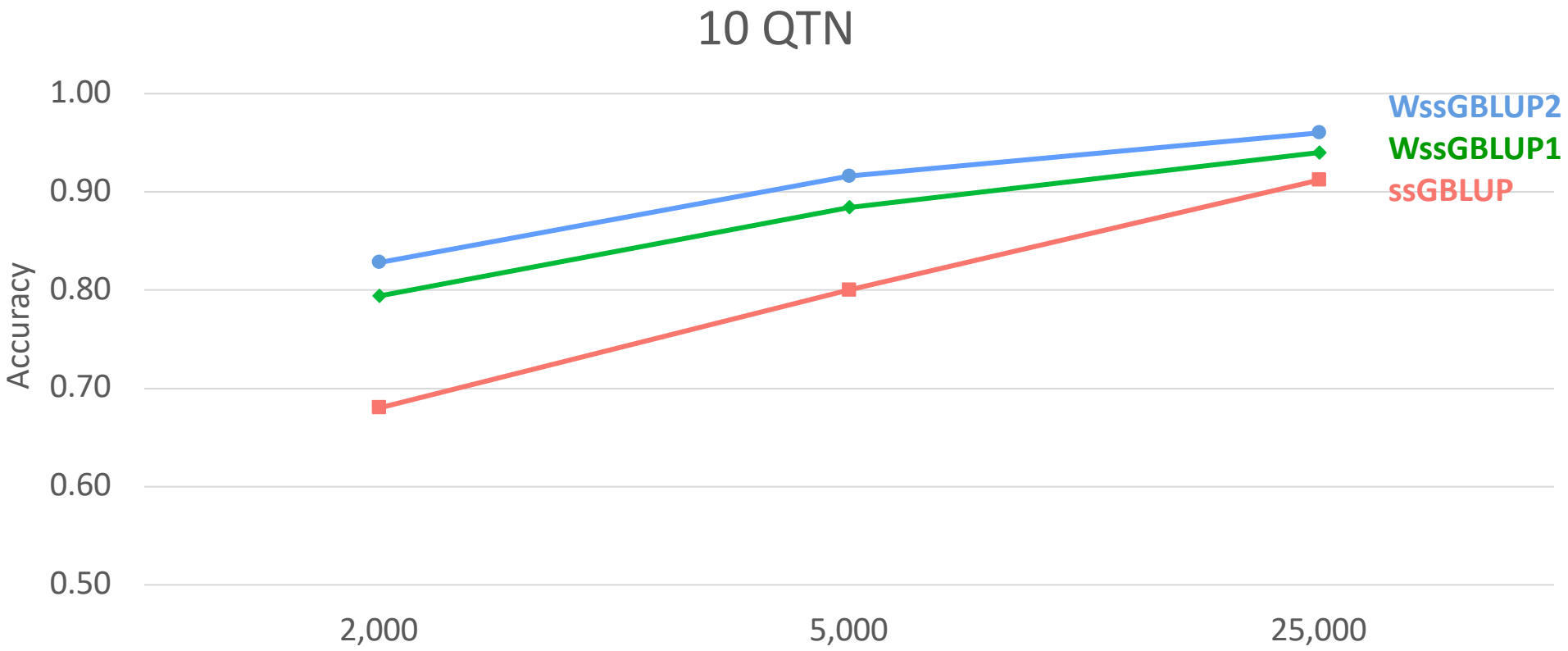
6) $\mathbf{G}_{(t+1)} = \frac{\mathbf{ZD}_{(t+1)}\mathbf{Z'}}{2\sum p_i(1-p_i)}$

Convergence for nonlinear A and quadratic weight



Fragomeni et al., 2019

WssGBLUP for large populations



Lourenco et al. (2017)

How to compute SNP effect and weight in BLUP90?

- After renumf90 and preGSf90 to save clean files:

  - blupf90 to estimate GEBV
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION saveGInverse`
    - `OPTION saveA22Inverse`

  - postGSf90 to backsolve GEBV to SNP effect
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION readGInverse`
    - `OPTION readA22Inverse`
    - `OPTION which_weight nonlinearA`

OPTION which_weight nonlinearA

`OPTION which_weight` nonlinearA $d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a})}-2}$

This option assumes the default constant (CT) is 1.125. To change the constant value to reflect a distribution closer to normal, use a CT value closer to 1:

`OPTION which_weight` nonlinearA 1.05

By default, the maximum change in SNP variance is limited to 5, which is calculated as $CT^{(5-2)}$ and returns a value of 1.4238 with CT=1.125. If this limit is to be changed to 10, the following option can be used, where the value provided (x) is the result of the expression $CT^{(x-2)}$. As an example, if CT is 1.05 and x is 10, the value provided to the option should be 1.4775:

`OPTION SNP_variance_limit` 1.4775

How to run WssGBLUP in BLUPF90

- After renumf90 and preGSf90 to save clean files:
  - Blupf90 to estimate GEBV
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION saveGInverse`
    - `OPTION saveA22Inverse`
    - `OPTION weightedG w.txt    #vector of weights`
  - postGSf90 to backsolve GEBV to SNP effect
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION readGInverse`
    - `OPTION readA22Inverse`
    - `OPTION which_weight nonlinearA`
    - `OPTION weightedG w.txt    #vector of weights`
    - `OPTION windows_variance 1   #to get variance explained`

How to run WssGBLUP for 3 iterations in BLUPF90

```
awk 'BEGIN { for (i==1;i<45000;i++) print 1}' > w.txt   # number of lines = number of SNP

for j in {1..3}
    do
     echo blup.par | blupf90 | tee blup.log1_$j
     cp solutions solutions1_$j
     echo post.par | postGSf90 | tee post.log1_$j
     cp snp_sol snp_sol1_$j
     cp w.txt w.txt_$j
     awk '{ if ($1==1) print $7}' snp_sol > w.txt
     mkdir plot1_$j
     cp chrsnp plot1_$j/chrsnp
     cp chrsnpvar plot1_$j/chrsnpvar
     rm chrsnp chrsnpvar snp_sol solutions
   done

rm Gi A22i
```

How to run WssGBLUP for 3 iterations and multi-trait models in BLUPF90

- Although the model can be multi-trait, there is only one **G**
  - Only one set of weights can be used
- To estimate correct weights for each trait in a multi-trait model:
  - Add an option in postGSf90

```
OPTION postgs_trt_eff x1 x2
```

  - x1 is the trait you are interested (number of the trait)
  - x2 is the effect (number of effect in this case)

- Run once for each trait or effect of interest using weights for the specific trait or effect

Output from postGSf90

```
snp_sol
```

http://nce.ads.uga.edu/wiki/doku.php?id=readme.pregsf90

contains solutions of SNP and weights

- 1: trait
- 2: effect
- 3: SNP
- 4: Chromosome
- 5: Position
- 6: SNP solution
- 7: weight

if `OPTION windows_variance` is used

- 8: variance explained by *n* adjacents SNP.

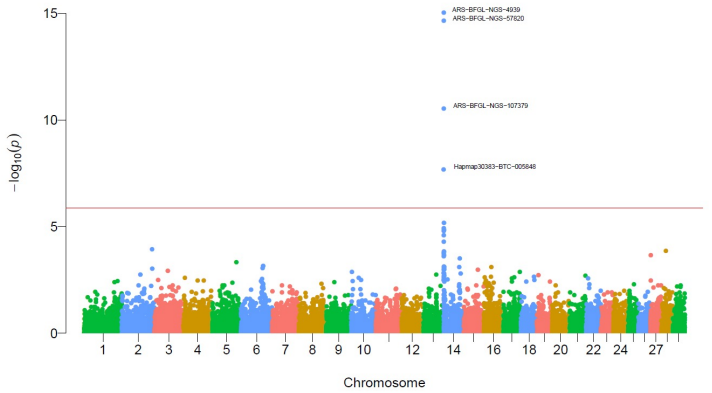
Single-step GWAS

Genome-Wide Association Studies

Current standard for GWAS

- Run single marker regression with **G** to compensate for relationships
  - $\mathbf{y} = \mathbf{Xb} + \mathbf{z}_i\mathbf{a}_i + \mathbf{u} + \mathbf{e}$
    - **z:** gene content {0,1,2}
    - **a:** SNP effect

- Estimate SNP effects
- Get p-values as $pval_i = 2\left(1 - \Phi\left(\left|\frac{\hat{a}_i}{sd(\hat{a}_i)}\right|\right)\right)$
- Apply Bonferroni to correct for multiple testing

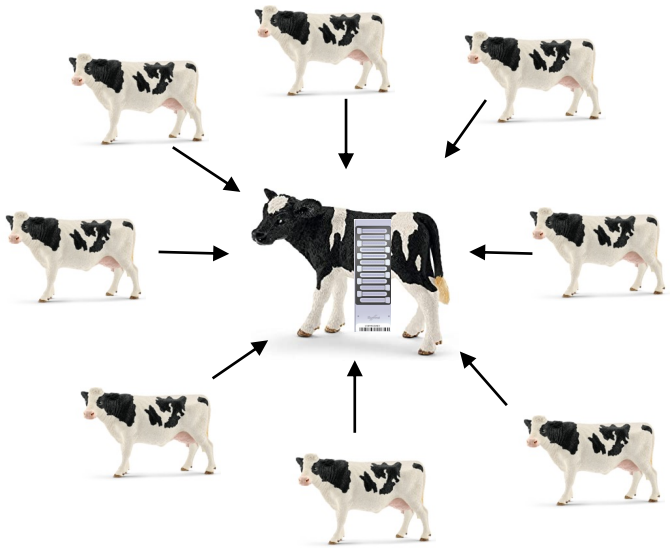


- **Assumption: Genotyped individuals have phenotypes**

GWAS in livestock populations

- Most animals are non-genotyped
- Animals may not have phenotypes
- Some traits are sex-limited
  - milk, fat, protein

- Single marker regression
  - Only genotyped animals with phenotypes
  - Deregressed EBV



- Need a method that fits the livestock data

Single-step GWAS

SNP effects

GEBVs

$$\hat{\mathbf{a}} = \lambda \mathbf{Z}'\mathbf{G}^{-1}\hat{\mathbf{u}}$$

VanRaden 2008
Stranden and Garrick 2009
Wang et al. 2012

a) Quadratic SNP weights (Falconer & Mackay, 1996)

$$d_i = \hat{a}_i^2 2p_i(1-p_i)$$

b) Nonlinear A SNP weights (VanRaden, 2008)

$$d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a})}-2}$$

How to run ssGWAS in BLUPF90

- After renumf90 and preGSf90 to save clean files:
  - Blupf90 to estimate GEBV
    - OPTION SNP_file snp.dat_clean
    - OPTION map_file mrkmap.txt_clean
    - OPTION saveGInverse
    - OPTION saveA22Inverse
    - OPTION weightedG w.txt #vector of weights
  - postGSf90 to backsolve GEBV to SNP effect
    - OPTION SNP_file snp.dat_clean
    - OPTION map_file mrkmap.txt_clean
    - OPTION readGInverse
    - OPTION readA22Inverse
    - OPTION which_weight nonlinearA
    - OPTION weightedG w.txt #vector of weights
    - OPTION windows_variance 1

postGSf90 options

```
OPTION SNP_moving_average n
```

Solutions for SNP effects will be by moving average of *n* adjacents SNPs.

```
OPTION windows_variance n
```

Calculates the variance explained by *n* adjacents SNPs.

When this option is used, the sum of variance explained by *n* adjacent SNPs (column 8 of snp_sol or column 3 of chrsnpvar) is not 100%.
This is because moving variance is used. If windows size is 20, the proportion of variance assigned to SNP 1 is calculated from SNP 1 to 20, for SNP 2 it goes from 2 to 21, for SNP 3 it goes from 3 to 22, and so forth.
A file called windows_variance has variance that sums to 100% in column 9.

```
OPTION windows_variance_mbp n
```

Calculates the variance explained by *n* Mb window of adjacents SNPs.

```
OPTION which_weight x
```

Generates a weight variable w to be used in the creation of a weighted genomic relationship matrix G=ZDZ'

- 1: w = y^2 * (2(p(1-p)))
- 2: w = y^2
- 3: experimental with the degree of brief
- 4: w = C**(abs(y)/sqrt(var(y's))-2) from VanRaden et al. (2009)
- nonlinearA: same as 4

postGSf90 options

```
OPTION Manhattan_plot
```

Plot using GNUPLOT the Manhattan plot (SNP effects) for each trait and correlated effect.

```
OPTION Manhattan_plot_R
```

Plot using R the Manhattan plot (SNP effects) for each trait and correlated effect.
`pdf` images are created: *manplot_St1e2.pdf*, but other formats can be specified.
Note: *t1e2* corresponds to trait 1, effect 2.

```
OPTION Manhattan_plot_R_format <format>
```

Control the format type to create images in R
`format` values accepted:

- pdf (default)
- png
- tif

```
OPTION plotsnp <n>
```

Control the values of SNP effects to use in Manhattan plots

- 1: plot regular SNP effects: abs(val)
- 2: plot standardized SNP effects: abs(val/sd) (default)

Output from postGSf90

http://nce.ads.uga.edu/wiki/doku.php?id=readme.pregsf90

```
chrsnp
```

contains data to create plot by GNUPLOT

- 1: trait
- 2: effect
- 3: values of SNP effects to use in Manhattan plots
- 4: SNP
- 5: Chromosome
- 6: Position

```
chrsnpvar
```

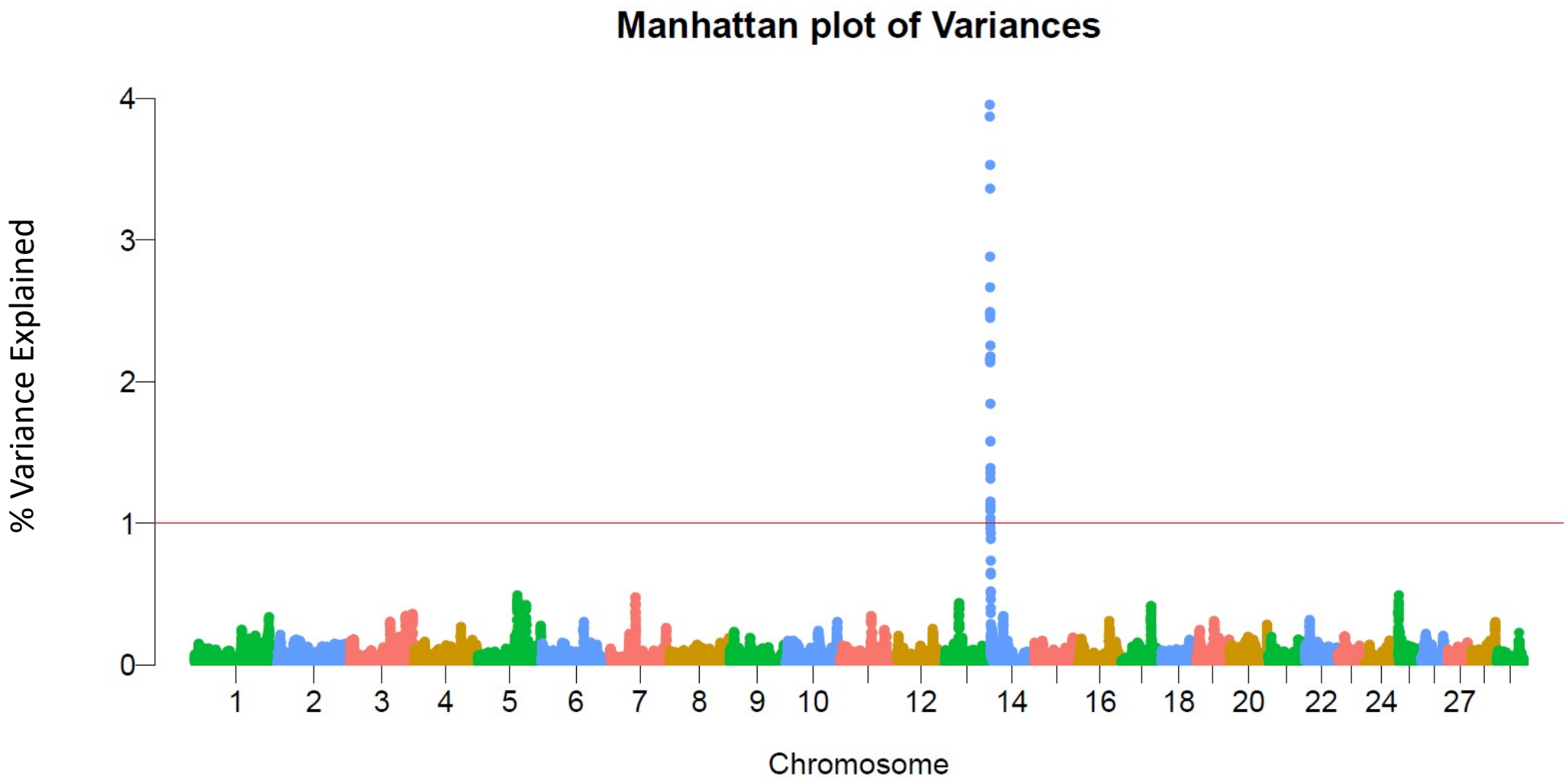
contains data to create plot by GNUPLOT

- 1: trait
- 2: effect
- 3: variance explained by *n* adjacents SNP
- 4: SNP
- 5: Chromosome
- 6: Position

Single-step GWAS

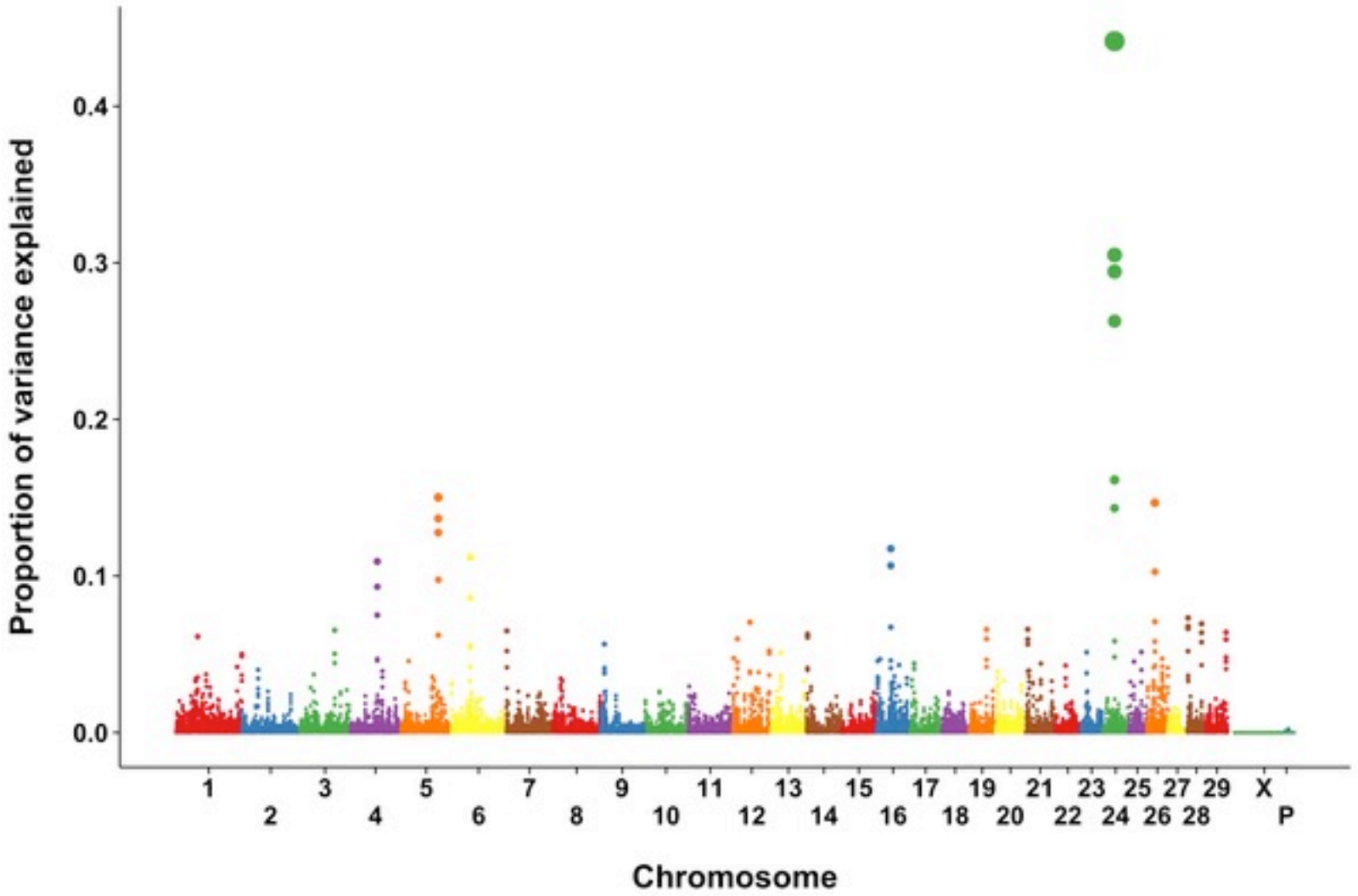
Fat – US Holsteins

No P-value!!!



Single-step GWAS

Figure 2. Proportion of SNP variance explained by 5-SNP moving windows for rectal temperature from a single-step GBLUP analysis



No P-value!!!

PLOS ONE

Dikmen S, Cole JB, Null DJ, Hansen PJ (2013) Genome-Wide Association Mapping for Identification of Quantitative Trait Loci for Rectal Temperature during Heat Stress in Holstein Cattle. PLOS ONE 8(7): e69202. https://doi.org/10.1371/journal.pone.0069202
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069202

Can we have p-values in ssGWAS?

Gualdrón Duarte *et al. BMC Bioinformatics* 2014, **15**:246
http://www.biomedcentral.com/1471-2105/15/246

BMC Bioinformatics

METHODOLOGY ARTICLE Open Access

Rapid screening for phenotype-genotype associations by linear transformations of genomic evaluations

Jose L Gualdrón Duarte[1], Rodolfo JC Cantet[1], Ronald O Bates[2], Catherine W Ernst[2], Nancy E Raney[2] and Juan P Steibel[2,3*]

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/age.12378

Meta-analysis of genome-wide association from genomic prediction models

Y. L. Bernal Rubio*†, J. L. Gualdrón Duarte*, R. O. Bates*, C. W. Ernst*, D. Nonneman‡, G. A. Rohrer‡, A. King‡, S. D. Shackelford‡, T. L. Wheeler‡, R. J. C. Cantet†§ and J. P. Steibel*¶

Genome-Wide Association Analyses Based on Broadly Different Specifications for Prior Distributions, Genomic Windows, and Estimation Methods

Chunyu Chen,[1] Juan P. Steibel, and Robert J. Tempelman
Department of Animal Science, Michigan State University, East Lansing, Michigan 48824
ORCID ID: 0000-0002-7833-6730 (R.J.T.)

J. Dairy Sci. 101:3140–3154
https://doi.org/10.3168/jds.2017-13364

Genome-wide association analyses based on a multiple-trait approach for modeling feed efficiency

Y. Lu,* M. J. Vandehaar,* D. M. Spurlock,† K. A. Weigel,‡ L. E. Armentano,‡ E. E. Connor,§ M. Coffey,# R. F. Veerkamp,‖ Y. de Haas,‖ C. R. Staples,¶ Z. Wang,** M. D. Hanigan,†† and R. J. Tempelman*[1]

P-values in ssGWAS

1) Factorize and Invert LHS of ssGBLUP with YAMS (Masuda et al., 2014)

blupf90

2) Solve the MME for $\begin{bmatrix}\hat{\beta}\\ \hat{u}\end{bmatrix}$ using the sparse Cholesky factor

Ignacio Aguilar

3) Extract from LHS^{-1} coefficients for genotyped animals ($\mathbf{C}^{u_2u_2}$)

4) Obtain individual prediction error variance of SNP effects:

$$Var(\hat{a}_i) = \frac{1}{2\sum p_i q_i}\mathbf{z}_i'\mathbf{G}^{-1}(\mathbf{G}\sigma_u^2 - \mathbf{C}^{u_2u_2})\mathbf{G}^{-1}\mathbf{z}_i\frac{1}{2\sum p_i q_i}$$

(Gualdron-Duarte et al., 2014)

postGSf90

Yutaka Masuda

Andres Legarra

5) Backsolve GEBV to SNP effects ($\hat{a}$): $\hat{a} = \frac{1}{2\sum p_i q_i}\mathbf{Z}'\mathbf{G}^{-1}\hat{u}$

6) p-value$_i$ $= 2\left(1 - \Phi\left(\left|\frac{\hat{a}_i}{sd(\hat{a}_i)}\right|\right)\right)$

OPTION in blupf90 and postGSf90

- Single option for both programs

  OPTION snp_p_value

- Output

Aguilar *et al. Genet Sel Evol* (2019) 51:28
https://doi.org/10.1186/s12711-019-0469-3

GSE Genetics Selection Evolution

SHORT COMMUNICATION Open Access

Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle

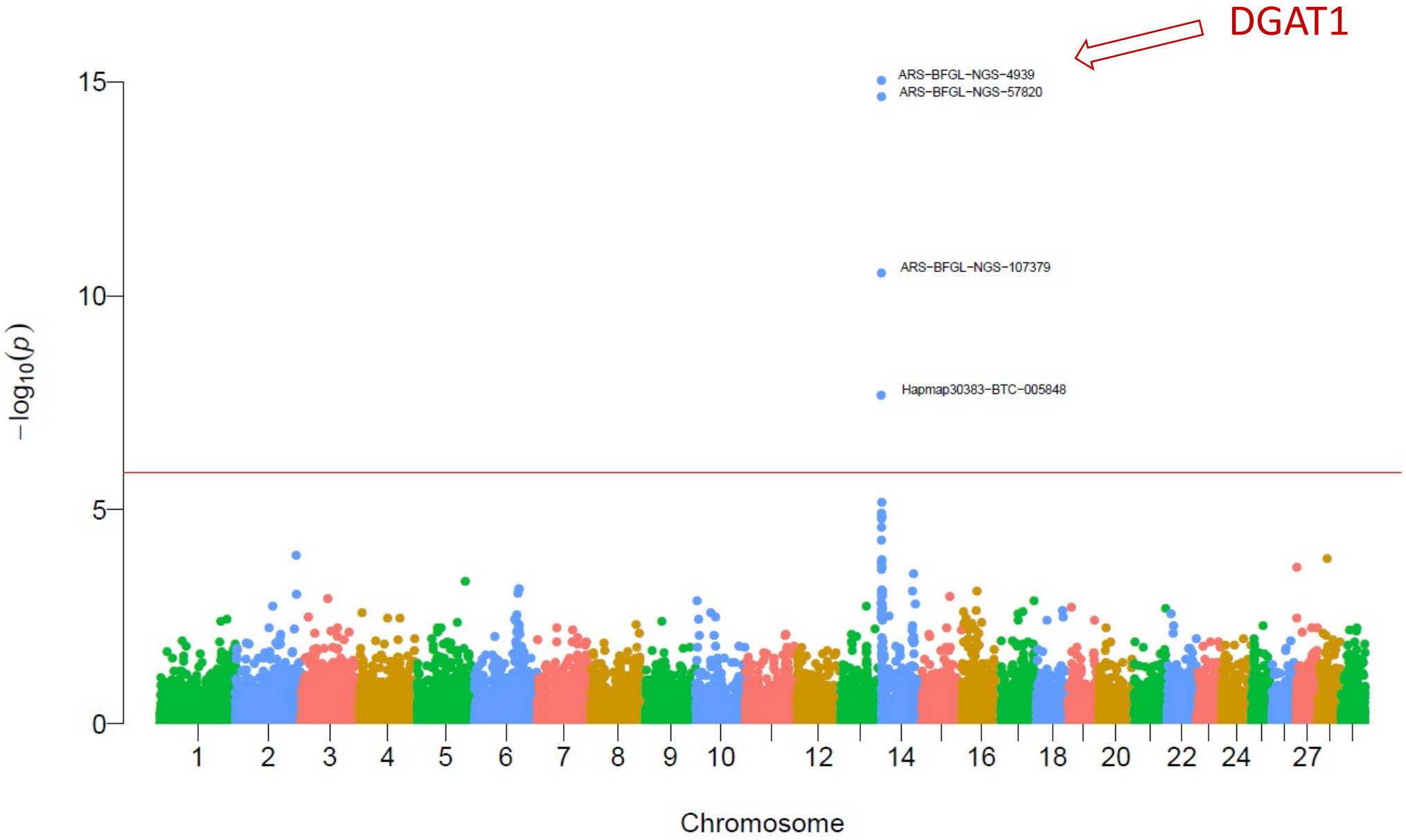
Ignacio Aguilar[1], Andres Legarra[2*], Fernando Cardoso[3,4], Yutaka Masuda[5], Daniela Lourenco[5] and Ignacy Misztal[5]

trait	effect	-log10(p-value)	SNP	CHR	POS
1	5	0.6467097526	1	1	120183
1	5	0.3510786763	2	1	135098
1	5	0.3606678137	3	1	158820
1	5	0.2585950992	4	1	183040
1	5	0.6969161959	5	1	208728
1	5	1.7646253513	6	1	267940
1	5	1.0802326921	7	1	278952
1	5	0.6819748588	8	1	290690
1	5	1.0131137254	9	1	309487
1	5	0.0038533074	10	1	393248

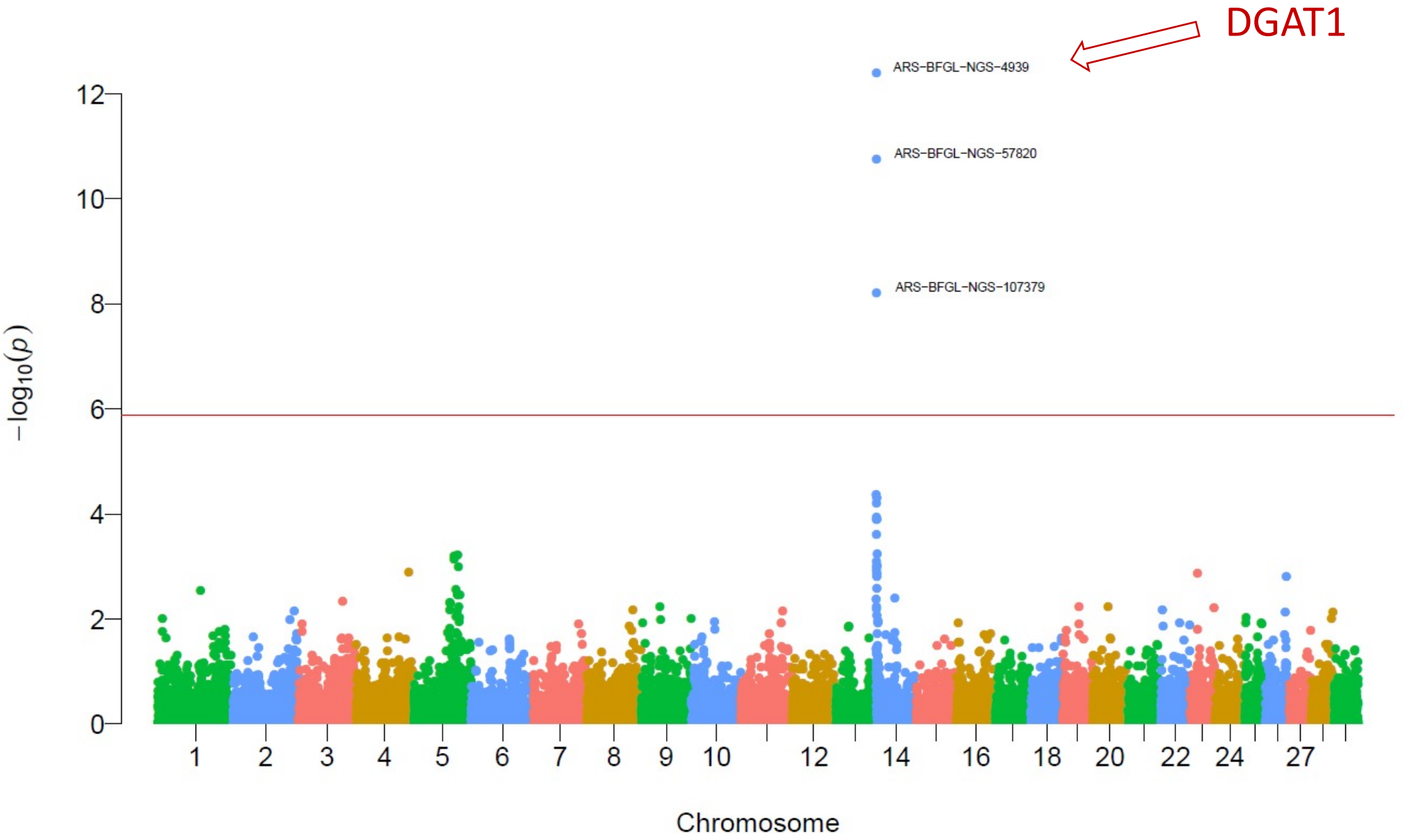
P-values in ssGWAS for US Holsteins

- US HOL 2009 data: milk, fat, protein
- Single-trait models
  - 10k genotyped bulls
  - 752k records for 100k daughters
  - 303k animals in ped

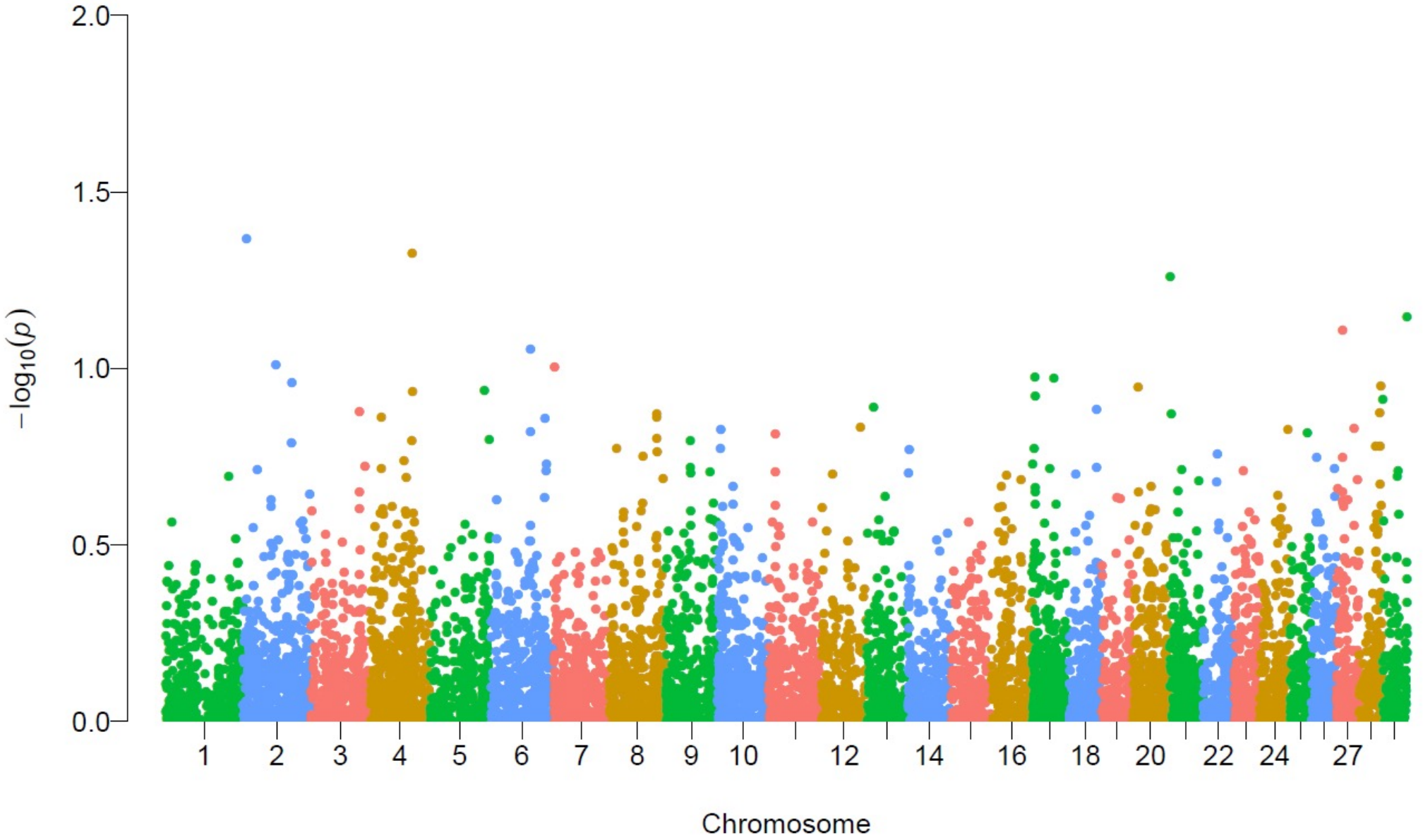
P-values in ssGWAS - Milk



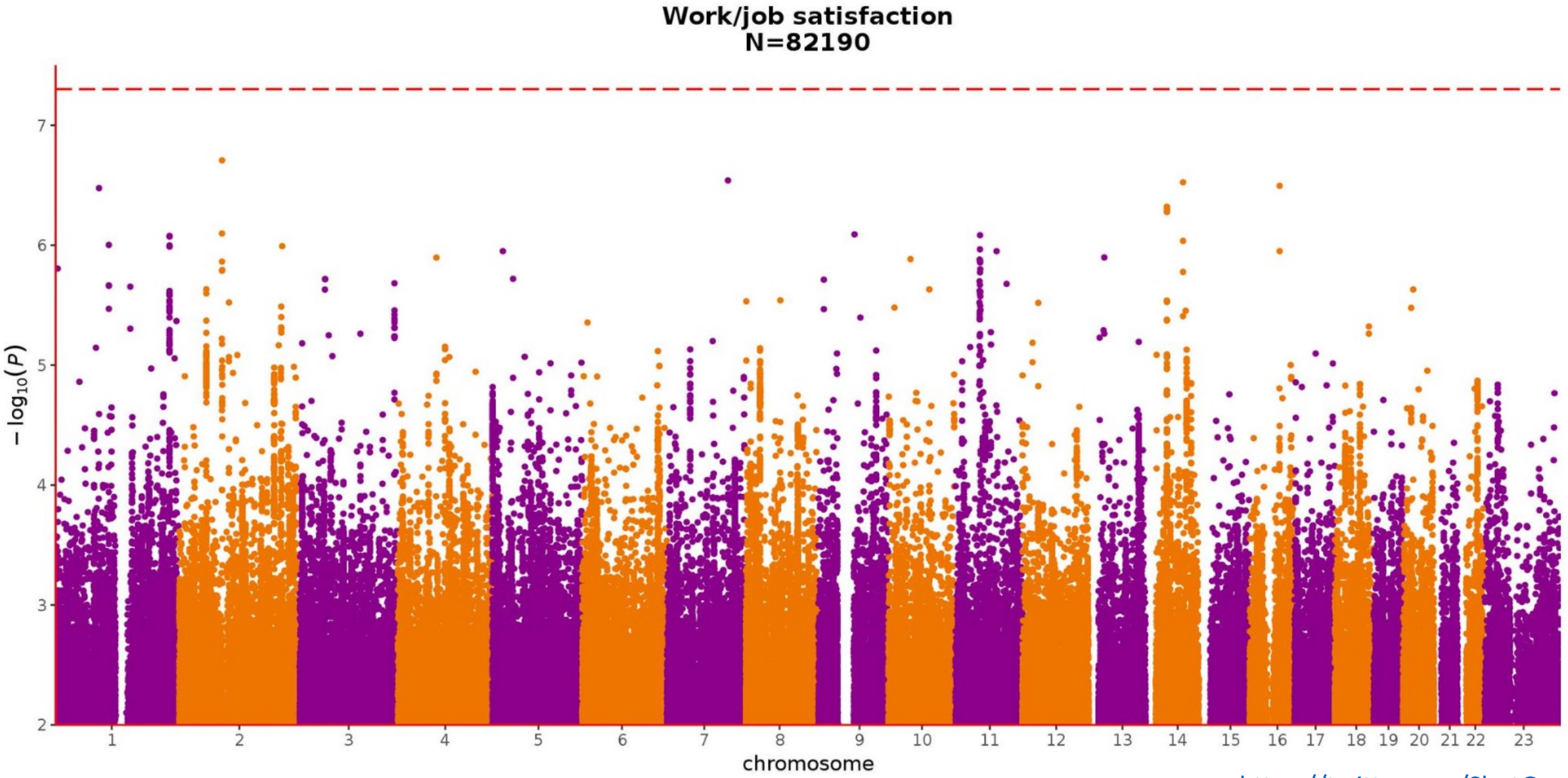
P-values in ssGWAS - Fat



P-values in ssGWAS - Protein



Non-significant hits



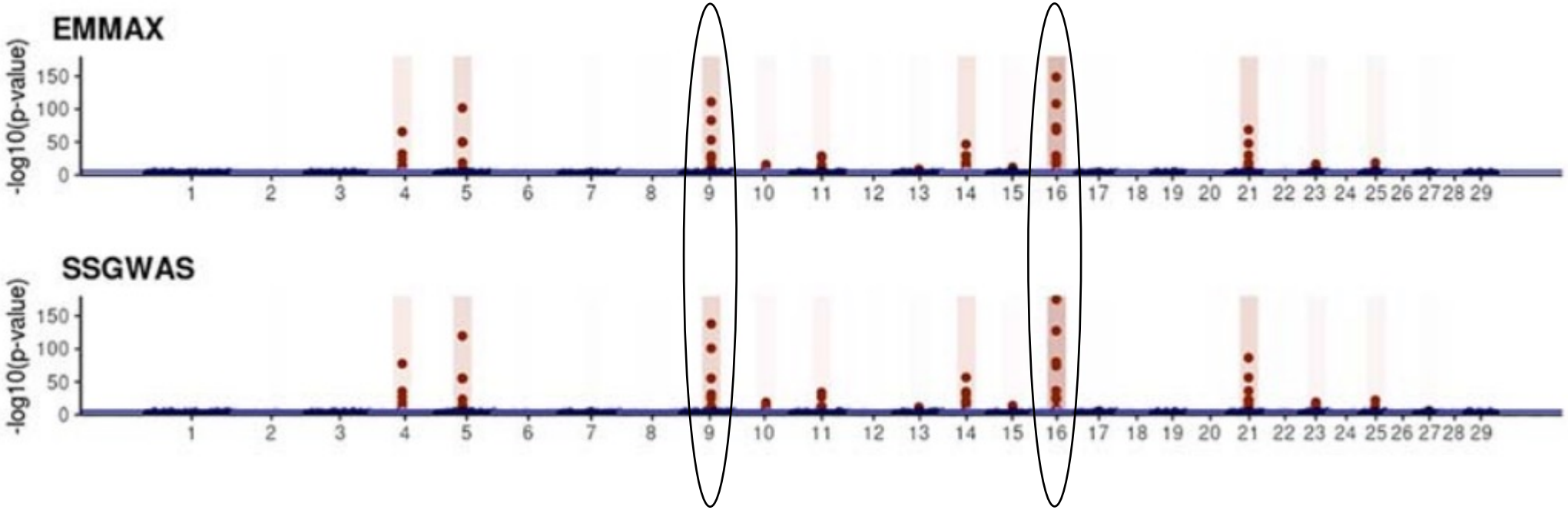
https://twitter.com/SbotGwa

ssGWAS vs. EMMAX

- Simulated population (1 QTN per CHR)

14k genotyped sires
Deregressed EBV
(10 daughters)

14k genotyped sires
500k Pedigree
250k phenotypes



Association	EMMAX (Khang et al., 2010)	ssGWAS (Aguilar et al., 2019)
True Positive	55.2[a] (3.7)	61.6[a] (8.7)
False Positive	0.0	0.0

Mancin et al. (2021)

How to run ssGWAS with p-values in BLUPF90

- Should not use iterations!
- After renumf90 and preGSf90 to save clean files:

  - Blupf90 to estimate GEBV
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION saveGInverse`
    - `OPTION saveA22Inverse`
    - `OPTION snp_p_value`

  - postGSf90 to backsolve GEBV to SNP effect
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION readGInverse`
    - `OPTION readA22Inverse`
    - `OPTION which_weight nonlinearA`
    - `OPTION windows_variance 1`
    - `OPTION snp_p_value`

Do not run iterations for p-values!

Output from postGSf90

```
chrsnp_pval
```

contains solutions of SNP and weights

- 1: trait
- 2: effect
- 3: -log10(p-value)
- 4: SNP
- 5: Chromosome
- 6: Position in bp

nce.ads.uga.edu/wiki

start

BLUPF90 Family of Programs

Now with support for genomic selection

Ignacy Misztal and collaborators, University of Georgia

BLUPF90 family of programs is a collection of software in Fortran 90/95 for mixed model computations in animal breeding. The goal of the software is to be as simple as with a matrix package and as efficient as in a programming language. For general description, see a paper from the CCB'99 workshop or see a paper on BGF90 at 7th WCGALP.

For variance component estimation, the family offers choices for simple and complicated models; see paper "Reliable computing in estimation of variance components" . From 2009 the programs are successively modified for genomic selection using a single-step approach (or ssGBLUP) by Ignacio Aguilar and Shogo Tsuruta.

For support, join blupf90 group at yahoo.com.

Troubleshooting

If the software crashes with segmentation fault, please change settings in your operating system. See FAQ:Segmentation fault for details. Also, The FAQ pages provide useful suggestions and solutions.

Headline

- History
- Modules
- Condition of use
- Distribution / Download
- Documentation / Manual / Tutorial
- Application program details
- Support
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- Tricks / Tips
- To Do
- Courses
- Sample data
- Undocumented options

Single-step GBLUP

```
DATAFILE
data3.txt
TRAITS
 4
FIELDS_PASSED TO OUTPUT
2
WEIGHT(S)

RESIDUAL_VARIANCE
0.60
EFFECT
3 cross alpha
EFFECT
1  cross alpha
RANDOM
animal
FILE
ped3.txt
FILE_POS
1 2 3 0 0
SNP_FILE
snp3.2k
PED_DEPTH
0
INBREEDING
pedigree
(CO)VARIANCES
0.40
OPTION map_file mrkmap.txt
OPTION use_yams
```

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

Computed using Henderson-Quaas' algorithm with inbreeding

Computed using VanRaden's formula, which considers inbreeding

Computed using Colleau's algorithm, which considers inbreeding