

# Eagle: multi-locus association mapping on a genome-wide scale made routine

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## Abstract

**Motivation:** We present Eagle, a new method for multi-locus association mapping. The motivation for developing Eagle was to make multi-locus association mapping "easy" and the method-of-choice. Eagle's strengths are that it a. is considerably more powerful than single-locus association mapping b. does not suffer from multiple testing issues c. gives results that are immediately interpretable and d. has a computational footprint comparable to single-locus association mapping.

**Results:** By conducting a large simulation study, we will show that Eagle finds true and avoids false SNP-trait associations better than competing single- and multi-locus methods. We also analyse data from a published mouse study. Eagle found over 100% more validated findings than the state-of-the-art single-locus method.

**Availability and Implementation:** Eagle has been implemented as an R package, with a web-based Graphical User Interface (GUI) for users less familiar with R. It is freely available via the CRAN website at <https://cran.r-project.org>.

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# 1 Introduction

Over the past decade, genome-wide association studies (GWASs) have changed considerably in both their analysis and design. Early studies followed a case-control design. Association mapping methods were no more complicated than contingency table tests or simple linear regression. These designs though had a tendency to yield spurious findings if there was unrecognised population stratification (Cardon and Palmer, 2003). This prompted a shift towards family-based designs and score tests, such as the transmission/disequilibrium test (TDT) and its variants (Spielman and Ewens, 1996). Today, instead of by design, it is through statistical modelling that we account for the effects of population stratification (Price *et al.*, 2010). This has meant that data can be collected from general populations, even if these populations are highly structured. Analysis via sophisticated association mapping methods, such as linear mixed model based approaches, is now almost routine (Yu *et al.*, 2006; Zhao *et al.*, 2007).

What has not changed is that it remains common practice to analyse genome-wide association study (GWAS) data on a locus-by-locus basis. This is despite there being several significant problems with analysing data in this way. First, for each SNP, a hypothesis test is performed. The null hypothesis is that there is no association between the SNP and trait. The alternative is that the SNP is in association with the trait. It is straight forward to guard against wrongly rejecting the null hypothesis (or making a type 1 error) if only a single hypothesis test is being performed. However, the analysis of GWAS data with locus-by-locus methods necessitates conducting a large number of correlated hypothesis tests, simultaneously. This leads to an increased risk of type 1 errors. To deal with this challenge, many different solutions have been offered (Storey and Tibshirani, 2003; Li and Ji, 2005; de Bakker *et al.*, 2005). Second, the aim of association mapping is to identify regions of the genome that house genes that are influencing a trait. The identification of these regions from these analyses is not always straightforward. GWAS results are reported, typically, via Manhattan plots that plot the  $-\log_{10}$  of the  $p$  value for each locus against the map position of the locus. The  $p$  value is obtained from the hypothesis test. The location of peaks in this plot identify genomic regions of interest. Inferring

the exact number of regions though can be difficult if the peaks are not well separated. Third, many of the traits whose genetic secrets we are trying to discover are complex. There will be multiple SNPs in linkage disequilibrium with genes that are influencing the trait. Yet, a locus-by-locus mapping approach only assesses the evidence for association between a single marker locus and trait.

It is somewhat surprising then that multi-locus association mapping methods haven't attracted more attention. Methods based on regularisation techniques, such as ridge regression (Shen *et al.*, 2013) and lasso (Rakitsch *et al.*, 2013), measure all locus-trait associations simultaneously. These techniques though are computationally demanding. Also, the strength of association is not measured by a  $p$  value but by the size of the regression coefficient for the SNP in the model. Further processing is required before the results can be interpreted (Cho *et al.*, 2010; Rakitsch *et al.*, 2013). More recently, associations have started to be mapped with random forests (Szymczak *et al.*, 2016). Similar to regularisation techniques though, it is not clear how to infer genomic regions of interest from their findings. A multi-locus method that does show promise is the multiple-locus linear mixed model method (Segura *et al.*, 2012). The best multi-locus model is built with forward and backward stepwise selection. Results are immediately interpretable in that the SNP closest to the genes underlying the trait are identified but computation does become challenging for large datasets.

In this paper, we present our new multi-locus method for genome-wide association mapping, which we are calling Eagle. Eagle combines the strength of regularisation techniques (being able to fit all SNP-trait associations jointly), with forward selection giving easy-to-interpret threshold-free results. We are able to achieve a computational performance similar to the fastest single-locus linear mixed model implementations through a dimension reduction step. Our aim was to make multi-locus association mapping on a genome-wide scale routine. To this end, we have implemented Eagle within an R package of the same name. Our package accepts marker data of different formats, can handle data larger than a computer's memory capacity, and makes heavy use of parallel computing for computation when available.

## 89 2 Methods

### 90 2.1 Mouse Data

91 The data were obtained from a large genome-wide association study that was  
92 performed in outbred mice (Nicod *et al.*, 2016). Phenotypic and genotypic  
93 data were available on 1,887 adult mice. The phenotypic data included raw  
94 and adjusted (for fixed effects) measurements from 200 behavioural, tissue, and  
95 physiological traits. Of these traits, 45 yielded SNP-trait associations that could  
96 be corroborated through other independent published work. It was these 45  
97 traits that were the focus of our real data analyses. As in the original study  
98 (Nicod *et al.*, 2016), our analyses were based on the adjusted traits. Genotypic  
99 data were available on 359,559 (353,697 autosomal) SNPs in the form of marker  
100 dosages (expected allele counts that ranged from zero to one). All missing  
101 data had been imputed. We converted the dosages into discrete genotypes by  
102 clustering around 0, 0.5, and 1, corresponding to SNP genotypes AA, AB, and  
103 BB, respectively. We focused our analyses on the autosomal SNPs.

### 104 2.2 Eagle Approach for Multi-locus Association Mapping

105 Eagle is a method for multi-locus association mapping on a genome-wide scale.  
106 It is based on linear mixed models. It differs from most other single- and multi-  
107 locus association mapping methods in that Eagle treats association mapping as  
108 a model selection problem (Ball, 2001; Broman and Speed, 2002; Yi *et al.*, 2005).  
109 The "best" model is found via forward selection. It makes use of a modified form  
110 of the Bayesian information criterion, BIC, for model selection. A "best" model  
111 is built iteratively. At each iteration, a hypothesis test is performed. Only  
112 a small number of iterations are needed in building the "best" model. Con-  
113 sequently, Eagle does not suffer from multiple testing issues. In contrast, for  
114 single-locus methods, multiple testing is an issue because each SNP is assessed  
115 separately, culminating in the need for a large number of hypothesis tests to be  
116 performed. Eagle reports as its findings only those SNPs that are in strongest  
117 linkage disequilibrium with the genes influencing a trait. The methodologi-  
118 cal foundation for Eagle comes from a whole-genome linkage analysis method

that was developed for mapping quantitative trait loci in experimental crosses (Verbyla *et al.*, 2007).

Let  $S = \{S_1, S_2, \dots, S_s\}$  be a set of  $s$  ordinal numbers where  $S_k$  is the  $S_k$ th ordered SNP that was selected in the  $k$ th iteration of the model building process. Suppose three iterations ( $s = 3$ ) have been performed and say the 500023rd, 15th, and 420th SNP were selected. Then  $S = \{500023, 15, 420\}$ . Let  $\mathbf{y}^{(n \times 1)}$  be a vector containing  $n$  measurements of the quantitative trait. Let  $\mathbf{M}^{(n_g \times L)} = [\mathbf{m}_1 \mathbf{m}_2 \dots \mathbf{m}_L]$  be a matrix containing the genotype data which have been collected from  $L$  loci that span the genome on  $n_g$  groups/lines/strains. Here,  $n \geq n_g$  meaning that a single or several trait measurements may be taken of the same group/line/strain. It is common for the columns of  $\mathbf{M}$  to be in map order but this is not a requirement. The vector  $\mathbf{m}_j^{(n_g \times 1)}$  contains the genotypes for the  $j$ th SNP. The genotypes are coded as -1, 0, and 1 corresponding to SNP genotypes AA, AB, and BB, respectively.

The specifics of the Eagle method are as follows. Eagle builds the "best" model iteratively, via forward selection. Suppose  $s$  iterations of our model building process have already been performed. This means  $s$  SNP-trait associations have been identified. It also means that  $s$  separate genomic regions of interest have been found. To perform the  $s + 1$ th iteration, we first fit the current model to the data. The (current) model is of the form

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Z}\mathbf{u}_g + \mathbf{e} \quad (1)$$

where  $\mathbf{X}^{(n \times p)}$  and  $\mathbf{Z}^{(n \times n_g)}$  are known design matrices with  $\mathbf{X}$  being of full rank and  $\mathbf{Z}$  containing zeros and ones that assign the appropriate genetic effect to each measurement. The vector  $\boldsymbol{\tau}^{(p \times 1)}$  has  $p$  fixed effects parameters including the intercept. The vector  $\mathbf{u}_g^{(n_g \times 1)}$  contains the genetic effects. The vector of residuals is  $\mathbf{e}^{(n \times 1)}$  whose distribution is assumed to follow  $N(\mathbf{0}, \sigma_e^2 \mathbf{I}^{(n \times n)})$ . So far, this model differs little from standard linear mixed models for association mapping (Yu *et al.*, 2006; Zhao *et al.*, 2007) However, it is how we specify  $\mathbf{u}_g$  that distinguishes our model from the others.

147 The genetic effects  $\mathbf{u}_g$  are modelled as

$$\mathbf{u}_g = \sum_{k=1}^s \mathbf{m}_{S_k} a_{S_k} + \mathbf{M}_{-S} \mathbf{a}_{-S} \quad (2)$$

148 where  $\mathbf{m}_{S_k}^{(n_g \times 1)}$  is the vector of genotypes for the  $k$ th selected SNP,  $a_{S_k}$  is  
 149 the additive effect of the  $k$ th selected SNP,  $\mathbf{M}_{-S}^{(b \times L-s)}$  is the matrix of SNP  
 150 genotypes with the data for the SNP in  $S$  removed, and  $\mathbf{a}_{-S}^{(L-s \times 1)}$  is a random  
 151 effect whose distribution is  $\mathbf{a}_{-S} \sim N(\mathbf{0}, \sigma_a^2 \mathbf{I}^{(L-s \times L-s)})$ . The terms in the  
 152 summation on the left hand side are fixed effects. They account for the additive  
 153 effects of those SNPs that have been found to be in association with the trait.  
 154 The other term is a random effect. It accounts for the joint effect of the yet-to-  
 155 be-identified SNP that are in association with the trait. This is a simple genetic  
 156 model but it is effective for discovering SNP-trait associations.

157 Second, we estimate the parameters of (1) and (2) via **restricted** maximum  
 158 likelihood (REML). For complex models, REML can be computationally de-  
 159 manding. However, our model only contains a single random effect ( $\mathbf{a}_{-S}$ ).  
 160 Here, highly efficient single-dimension optimisation via spectral decomposition  
 161 is possible (Kang *et al.*, 2008).

162 Third, we identify the  $(s+1)$ th SNP that is in strongest association with the  
 163 trait, based on the maximum score statistic  $t_j^2 = \frac{\tilde{a}_j^2}{\text{var}(\tilde{a}_j)}$  where  $\tilde{a}_j$  is the best  
 164 linear unbiased predictor (BLUP), and  $\text{var}(\tilde{a}_j)$  is its variance. This statistic is  
 165 not only appealing intuitively, where we identify a SNP based on its (random)  
 166 effect size and accuracy, but is justifiable, theoretically (Verbyla *et al.*, 2012).

167 Fourth, we determine the importance of the  $(s+1)$ th selected SNP via a  
 168 model selection strategy (Verbyla *et al.*, 2007). We begin by reforming (2)  
 169 where  $S$  now contains the  $s+1$  selected SNP. We then fit this new model to the  
 170 data via maximum likelihood and calculate its extended Bayesian information  
 171 criteria (extBIC) (Chen and Chen, 2008). The extBIC is a model selection  
 172 measure that takes into account the number of unknown parameters and the  
 173 complexity of the model space. It is well suited to the model selection problem  
 174 in genome-wide association studies (Chen and Chen, 2008). It is different to the  
 175 model selection measure used in (Verbyla *et al.*, 2007). If this new model has a

larger extBIC than the current model, then the  $s + 1$ th selected SNP is added to the current model and the above process is repeated. If this new model has a smaller extBIC than the current model, then the model building process is complete. The set of SNP in strongest association with the trait is the  $s$  SNPs previously identified.

### 2.2.1 Reducing the dimension of the model:

In practice, estimating the parameters of (2) can be demanding, computationally. The vector  $\mathbf{a}_{-S}$  has  $L - s$  random effects where in modern genome-wide association studies,  $L$ , the number of SNPs, can be extremely large. An alternative model is given by Verbyla (Verbyla *et al.*, 2012, 2014). They show how to reformulate (2) to be a model with a random effect with only  $n$  elements

$$\mathbf{u}_g = \sum_{k=1}^s \mathbf{m}_{S_k} a_{S_k} + (\mathbf{M}_{-S} \mathbf{M}_{-S}^T)^{1/2} \mathbf{a}_{-S}^* \quad (3)$$

where  $\mathbf{a}^* \sim N(\mathbf{0}, \sigma_a^2 \mathbf{I}^{(n_g \times n_g)})$ , and  $(\mathbf{M}_{-S} \mathbf{M}_{-S}^T)^{1/2}$  can be calculated via singular value decomposition (Golub and Van Loan, 2012). Although it may not be obvious, the two models are equivalent, having identical variance structures. Yet, the computational cost of model (3) compared to model (2) is much less, due to the random term in model (3) having only  $n$  instead of  $L - s$  effects needing estimating.

Verbyla (Verbyla *et al.*, 2012, 2014) go on to show how to recover  $\tilde{\mathbf{a}}$  from estimates from model (3) with

$$\tilde{\mathbf{a}} = \left[ \mathbf{M}_{-S}^T (\mathbf{M}_{-S} \mathbf{M}_{-S}^T)^{-1/2} \right] \tilde{\mathbf{a}}^* \quad (4)$$

where its variance matrix is

$$\text{var}(\tilde{\mathbf{a}}) = \mathbf{M}_{-S}^T (\mathbf{M}_{-S} \mathbf{M}_{-S}^T)^{-1/2} \text{var}(\tilde{\mathbf{a}}^*) (\mathbf{M}_{-S} \mathbf{M}_{-S}^T)^{-1/2} \mathbf{M}_{-S} \quad (5)$$

These values are needed in order to calculate the score statistic  $t_j^2$  for identifying the SNP in strongest association with the trait. Fortunately, when calculating  $t_j^2$ , only the diagonal elements of the variance matrix are needed which simplifies

199 the calculation of (5).

## 200 **2.3 Comparison Methods**

### 201 **2.3.1 Multi-locus methods:**

202 We compare the computational and statistical performance of Eagle against five  
203 multi-locus methods. They are bigRR (Shen *et al.*, 2013), LMM-Lasso (Rakitsch  
204 *et al.*, 2013), glmnet (Friedman *et al.*, 2010), MLMM (Segura *et al.*, 2012), and  
205 r2VIM (Szymczak *et al.*, 2016). All but glmnet have been purposely designed  
206 for genome-wide association mapping. BigRR, LMM-Lasso, and glmnet are  
207 regression-based regularisation methods. BigRR is based on generalised ridge  
208 regression, LMM-Lasso is based on lasso, and glmnet is based on elastic net.  
209 Regularisation methods make parameter estimation possible in models where  
210 the number of predictors is far greater than the number of samples. They allow  
211 the strength of association between all the SNPs and trait to be measured within  
212 a single model, simultaneously. A limitation of these methods though is that  
213 the statistical significance of the SNP effects cannot be easily determined. Due  
214 to the adaptive nature of the estimation procedures, to do this analytically is  
215 challenging and is an area of active research (Lockhart *et al.*, 2014). Instead,  
216 we calculate significance empirically via stability selection (see below).

217 MLMM is closest in philosophy to Eagle. It too is based on building the  
218 best model via **stepwise** selection, within a linear mixed model framework, and  
219 uses the extBIC as one of its model selection criterion. However, there are  
220 differences between the two methods. MLMM does not make use of dimension  
221 reduction. Also, how SNP are selected to enter the model differs between the  
222 two methods. Eagle identifies a SNP of interest from its score statistic (see  
223 Section 2.2 for details). This score statistic was originally developed for outlier  
224 detection in linear (mixed) models but it is being used by Eagle to identify  
225 SNP with unusually large random effects. MLMM instead uses the statistical  
226 significance of a SNP, when treated as a fixed effect in the model. This involves  
227 fitting a separate linear mixed model for each candidate SNP, a potentially  
228 computationally expensive exercise. However, MLMM does this in a clever and  
229 efficient way via the Gram-Schmidt process. Both are R packages but there



is a significant difference in computational performance (see Results). Note, even though a hypothesis test is being performed for each SNP by MLMM, it does not suffer from multiple testing issues. Neither the null nor the alternative hypothesis is being accepted or rejected. Only the hypothesis yielding the most significant association is of interest.

R2VIM differs to the other four methods in that it is a non-parametric model-free approach. It implements random forests but where multiple parallel runs are performed. Each run leads to different random forests being created. A relative importance score is calculated, within a run, for each SNP. This is done by dividing a SNP’s importance score by the minimum importance score observed across all the SNPs within a run. Only those SNPs with relative importance scores above a certain threshold across all the runs are deemed to be significant. Unfortunately, the relationship between threshold value and false positive rate is unknown. The threshold could be found empirically via permutation but the computational cost is high, restricting the size of data that can be analysed.

### 2.3.2 Single-locus methods:

We also compare the performance of Eagle against two single-locus methods, GEMMA (Zhou and Stephens, 2012) and FaST-LMM (Lippert *et al.*, 2011). Both are based on linear mixed models. The models have a single fixed effect for the SNP, other fixed effects, a single random effect to account for familial relatedness (or polygenic background), and an error. The significance of the SNP effect in the model is a measure of the strength of association. They are of the same computational complexity (Zhou and Stephens, 2012), and produce exact results. Both perform a single spectral decomposition of the relationship (or similarity) matrix  $K$ , use an eigenvector matrix to rotate the data, and reformulate the (residual) log likelihood for easier computation. They do differ in their estimation procedure. GEMMA implements Newton-Raphson. FaST-LMM implements Brent’s algorithm. Newton-Raphson is more complicated but has better convergence properties than Brent’s algorithm. Both methods are state-of-the-art and have been implemented in highly efficient computer programs.

## 2.4 Generation of Simulation Data

The data are generated via data perturbation (Zhao *et al.*, 2007). Data perturbation amalgamates real with simulated data to generate replicates. It is a way of introducing greater realism into a simulation study. Here, the genotype data are real, the quantitative trait data are simulated. The SNP genotypes are drawn, according to the specifications of a particular simulation scenario, from data collected from the 1000 Genome Project, version 3 (Consortium *et al.*, 2010). Six different scenarios are considered. These scenarios differ in their sample size and number of SNPs (see Results for details). Here, across scenarios, the SNP data differs. Across replicates within a scenario, the SNP data are the same. For each scenario, 100 replicates are generated.

To generate the trait data  $\mathbf{y}$ , first,  $q$ , the number of SNPs that are to be assigned a quantitative value is drawn from a Poisson distribution with mean 30. Second,  $q$  SNP are selected randomly. Third, we assume an additive model for the SNPs. The SNP genotypes AA, AB, and BB are assigned the values -1, 0, and 1, respectively. Fourth, the SNP effects are summed across the  $q$  selected loci, for each individual, to generate a  $\mathbf{g}^{(n \times 1)}$  vector of genetic values where  $n$  is the number of individuals. Fifth,  $\mathbf{e}^{(n \times 1)}$ , a vector of residuals, is drawn from a normal distribution where  $e_i \sim N(0, \sigma_e^2)$  and  $\sigma_e^2$  is the residual variance that has been set to yield a trait with heritability 0.5. Sixth, the trait data are formed as  $\mathbf{y} = \mathbf{g} + \mathbf{e}$ . In forming  $\mathbf{y}$ , we have purposely not included any other environmental variables such as age, sex, or experimental design effects. This is because not all the methods were implemented to handle the inclusion of additional fixed effects. A two-stage modelling approach is often adopted to deal with this situation, but we chose not to introduce this complexity into the analyses.

## 2.5 Stability Selection

Stability selection (Meinshausen and Bühlmann, 2010) is a subsampling strategy with a range of applications. It is used here to estimate, empirically, the statistical significance of the results from LMM-Lasso, glmnet, and bigRR analyses of the simulated data. These three regularisation methods give the effect sizes

292 of the SNPs, but not their significance as their results. Stability selection was  
293 chosen over permutation and other sampling procedures because of its reduced  
294 computational cost.

295 The stability selection procedure for LMM-Lasso and glmnet is as follows.  
296 For a particular scenario, we begin by finding, via a binary search, the value of  
297 the regularisation parameter that yields 20 to 30 non-zero SNP effects. We know  
298 that 20 to 30 SNP-trait associations is a reasonable number of findings to expect  
299 from the analysis of a replicate in the simulation study. The regularisation  
300 parameter though could have been tuned to give any reasonable number of  
301 non-zero SNP effects. This tuning was done for each of the six scenarios but  
302 only for a single replicate, selected at random, from within a scenario. It is not  
303 necessary to tune the regularisation parameter on every replicate when replicates  
304 are generated under the same (trait, sample size, and number of SNP) conditions  
305 within a scenario.

306 Once a suitable value for the regularisation parameter had been found, for  
307 the replicate whose SNP results are to be assigned statistical significance, we  
308 subsample repeatedly, 100 data sets of size  $n/2$ . A larger number of data sets  
309 and/or larger sized data sets could have been chosen here but we found these  
310 changes to have little impact on the final significance estimates. The subsamples  
311 are drawn without replacement. Also, the matching of trait to genotype is  
312 preserved in the subsamples. A subsample differs to the replicate in size only.  
313 The subsamples are analysed with LMM-Lasso (glmnet) with its regularisation  
314 parameter fixed to the tuned value found previously. From the analysis of a  
315 subsample, a binary vector, of length the number of SNP, is recorded as the  
316 result where a one (zero) means the SNP had a non-zero (zero) effect size.  
317 Calculating a SNP's statistical significance is now a simple task. We calculate  
318 the vector sum of the binary vectors over all 100 subsamples. This vector sum  
319 will contain elements in the range of 0 to 100. By dividing each element in  
320 this vector sum by the number of subsamples upon which the sum is calculated  
321 (which is 100), we obtain empirical probabilities. These probabilities measure  
322 the strength of evidence for the SNPs to be in association with the trait.

323 For bigRR, stability selection is implemented in a different way. Unlike  
324 LMM-Lasso and glmnet, bigRR yields non-zero SNP effects for all the SNPs.

325 Also, there is no need to tune the regularisation parameter for bigRR as an  
326 optimal value is found as part of its analysis procedure. We still draw 100  
327 subsamples of size  $n/2$ , without replacement, and each subsample is analysed  
328 with bigRR. However, from each analysis, we order the SNPs according to the  
329 absolute size of their SNP effect estimates from bigRR. A binary vector, of  
330 length the number of SNPs is then formed where a one (zero) means the SNP  
331 is (not) in the top 20 ordered SNPs. Calculating the significance of the SNPs  
332 then proceeds as described above.

## 333 2.6 Implementation

334 Eagle has been implemented as an R package of the same name. Much of  
335 the computation though is performed outside of R via C++ functions that  
336 utilise Eigen C++ routines. Eagle has been purpose built to rely heavily on  
337 calls to BLAS and LAPACK, mathematical libraries common to most computer  
338 systems. By making use of multi-threaded BLAS and LAPACK libraries, many  
339 of the calculations in Eagle are parallelised. We have gone to great lengths to  
340 make Eagle easy-to-use. Tutorials, videos, How-To guides, and a link to our  
341 server for demonstrating Eagle on some test data are available on the Eagle  
342 website. Eagle is available for download from the CRAN website.

## 343 3 Results

### 344 3.1 Association Mapping Methods

345 We compared Eagle, in terms of computational and statistical performance,  
346 against seven other association mapping methods. We chose methods that al-  
347 most all had been purpose built for genome-wide analysis, that could handle  
348 data from quantitative traits, and where the methods had been implemented in  
349 freely available computer programs or packages. Two of the methods are based  
350 on single-locus (or locus-by-locus) models and five are based on multi-locus  
351 models. Of the many ways of performing single-locus association mapping, we  
352 chose GEMMA and FaST-LMM because of their popularity and computational  
353 speed. For multi-locus association mapping, we chose bigRR, glmnet, LMM-

Lasso, MLM, and r2VIM. Each takes a different approach to multi-locus association mapping. A summary of the key attributes of the different computer programs/packages is given in Supplementary Table 1 (see Methods for further details).

## 3.2 Simulation Study

A large simulation study was performed where we sought to answer two questions. First, how well does Eagle find true associations (power) and avoid false associations (type 1 errors)? Second, how does Eagle compare, in terms of run time and memory usage, to competing implementations? Data were generated under six different scenarios; a study of size 150 individuals and 5,000 single SNPs (150 x 5K), 350 individuals and 400,000 SNPs (350 x 400K), 1,500 individuals and 50,000 SNPs (1500 x 50K), 2,000 individuals and 500,000 SNPs (2000 x 500K), 4,000 individuals and 1,500,000 SNPs (4000 x 1.5M), and 10,000 individuals and 1,500,000 SNPs (10000 x 1.5M). These scenarios reflect, at least in some cases, the sizes of study being performed in animals, plants, and humans.

For each scenario, 100 replicates were generated. A single replicate consisted of SNP and quantitative trait data (see Section 2.4). Extra realism was introduced into the simulation study through the drawing of the SNP genotypes from the 1000 Genome Project, phase 3 (Consortium *et al.*, 2010). The quantitative trait data were generated by selecting, randomly, a set of SNPs and assigning these loci additive allelic effects. Random errors were then drawn from a normal distribution with variance set to give a heritability of 50% for the trait. For each individual, a quantitative trait value was obtained by summing its random error and additive allelic effects. The number of randomly selected SNPs follows a Poisson distribution with mean 30. The size of the allelic effects across the selected loci are equal, because the SNP genotypes AA, AB, and BB are assigned the values -1, 0, and 1, respectively (Section 2.4).

Analyses by the eight programs/packages of a replicate proceeded as follows. They were all run at their default settings. Eagle and MLM were the easiest of the programs/packages to implement. The only parameters requiring speci-

385 fication were the amount of available memory and number of CPUs for Eagle  
386 and the number of chunks for MLMM. **MLMM breaks its matrices into blocks**  
387 **or chunks, reducing its memory footprint but at the cost of increased compu-**  
388 **tation.** Their results were also immediately interpretable. Their findings were  
389 the set of SNPs in strongest association with the trait. Each SNP in this set  
390 identified a separate genomic region of interest, whose position was given by the  
391 map location of the SNP.

392 BigRR, LMM-Lasso, and glmnet required more effort to implement. They  
393 are based on regularisation methods and as such, all the SNPs were fitted si-  
394 multaneously in a regression framework. The difficulty was in calculating the  
395 significance of the SNP effects. To do this analytically is challenging. We instead  
396 opted for stability selection (see Methods), an empirical approach for calculating  
397 significance.

398 R2VIM is different from the rest in that it is a nonparametric approach for  
399 association mapping. It is based on random forests. Three important param-  
400 eters needed to be set. These were the number of trees, the number of variables  
401 for building a tree, and the minimum size of a terminal node. Ideally, these pa-  
402 rameters would be "tuned" on a replicate-by-replicate basis (Boulesteix *et al.*,  
403 2012). However, this was not practical here. We instead used the same settings  
404 as in (Szymczak *et al.*, 2016) where the number of trees was set to 1000, the  
405 number of variables was set to 20% of the number of SNPs, and the minimum  
406 size of a node was set to 10% of the sample size. A relative importance measure  
407 was calculated for each SNP measuring its strength of association with the trait.

408 FaST-LMM and GEMMA implement single-locus association mapping. FaST-  
409 LMM was run in two ways. One way was where a subset of the SNP data were  
410 used in calculating the similarity (or relationship) matrix. Here, FaST-LMM is  
411 highly efficient, computationally. The other was where calculation of the sim-  
412 ilarity matrix was based on all the SNP data. The  $p$  values of the SNP were  
413 reported as their results.

414 The results from all but Eagle and MLMM required post-processing be-  
415 fore the findings were interpretable. The SNPs were placed in map order, a  
416 significance threshold was set, peak regions containing SNPs with significance  
417 measures above the threshold were identified, and the SNP with the largest

significance measure in each of the peak regions was recorded as a finding.

### 3.3 Power and False Positive Rates

Here, we answer the question of how well Eagle finds true SNP-trait associations and avoids false SNP-trait associations. We do this by estimating the power and false positive rates of Eagle and the other methods for the six scenarios. Since, for a replicate, we knew which SNPs were assigned additive effects, we knew the SNPs that were in true association with the trait. We will refer to these SNPs as being true SNPs. By knowing the true SNPs, we were able to assess the validity of a method's findings. A finding was counted as true if it was positioned within 40 kilobase pairs of the location of a true SNP. **This was the average (confidence interval) distance used by Nicod *et al.* (2016) for determining if their findings were close to candidate genes and whose mouse data we analyse below.**

When a replicate was analysed, we obtained an estimate of the power of the method by taking the number of findings that were found to be true and dividing by the number of true SNPs. We also obtained an estimate of a method's false positive rate. It is the number of findings that were found to be false divided by the number of true SNPs. Both these estimates varied with replicate. The power (false positive rate) of a method, for a scenario, was found by taking the median of the power (false positive rate) estimates over the 100 replicates.

The power and false positive rates of Eagle and the other multi-locus methods across the scenarios 150 x 5K, 350 x 500K, 1500 x 50K, and 2000 x 500K are shown in Supplementary Figure 1. We restricted our attention to these scenarios because not all multi-locus methods could cope with the size of data in the other scenarios. Each plot contains single points and power curves. The single points are the power and false positive rates for Eagle and MLM. These two methods treat association mapping as a model selection problem. There are no significance thresholds to be set. The power curves are for those methods that treat association mapping as a variable selection problem. Here, the significance of the findings are assessed against a significance threshold. The power curves in the plot show how power changes with the false positive rate as the significance threshold is adjusted. The power and false positive rate of Eagle and the two

single-locus methods, GEMMA and FaST-LMM, are shown in Figure 1.

In answer to the question of how well Eagle finds true SNP-trait associations and avoids false SNP-trait associations, it does extremely well. Of the multi-locus methods, Eagle had the highest power while keeping its false positive rate low (Supplementary Figure 1). MLMM also performed well. However, it was when Eagle was compared against single-locus methods that the difference in power was most noticeable. Eagle had much higher power than single-locus methods for finding SNP in true association with a trait while avoiding false associations (Figure 1).

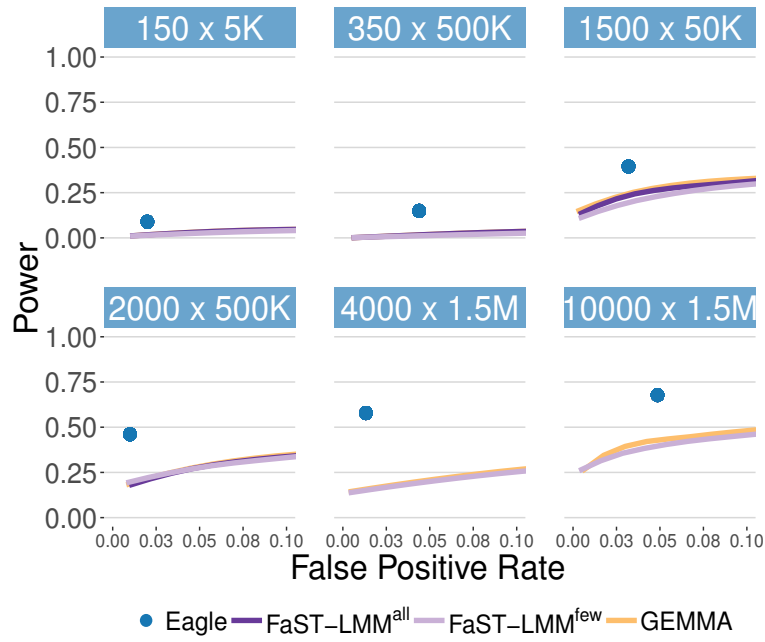
### 3.4 Memory Usage and Run Times

Memory usage and run (or elapse) times were recorded for Eagle and the other computer programs/packages across the simulation scenarios. Analyses were performed on a high-end desktop computer with dual 8-core Xeon processors and 128 gigabytes of RAM. Not all data generated under the six scenarios could be analysed by all implementations. Memory usage for many of the computer programs/packages was the limiting factor (see Supplementary Figure 2). The single-locus program GEMMA was by far the most memory efficient. Not surprisingly, the multi-locus programs were memory intensive. Most required in excess of the 128 gigabytes of available RAM for the analysis of data generated under 4000 x 1.5M and 10000 x 1.5M. Even FaST-LMM, when all the SNP data were being used to calculate the similarity matrix, ran out of memory for the larger scenarios. Of the multi-locus programs/packages, only Eagle, with its ability to handle data larger than the memory capacity of the computer, was capable of producing findings for data from our largest scenario, 10000 x 1.5M.

The median run times for Eagle and the other computer programs/packages across the six scenarios are shown in Figure 2. The x- and y-axes are on a log scale. A unit change on the x- or y-axis is equivalent to a change in the order of magnitude. In answer to our question of how does Eagle compare in terms of run time to competing implementations, Eagle was significantly faster, sometimes by orders of magnitude, than the other multi-locus implementations and is comparable to the single-locus implementations. For a simulation study



Figure 1: Power verse false positive rates for Eagle and the single-locus methods GEMMA and FaST-LMM. FaST-LMM was run where all the SNP data are used to estimate the relationship matrix (FaST-LMM<sup>all</sup>) and where genotype data from every five-hundredth SNP are used to estimate the relationship matrix (FaST-LMM<sup>few</sup>). Eagle has substantially higher power than the single-locus methods.



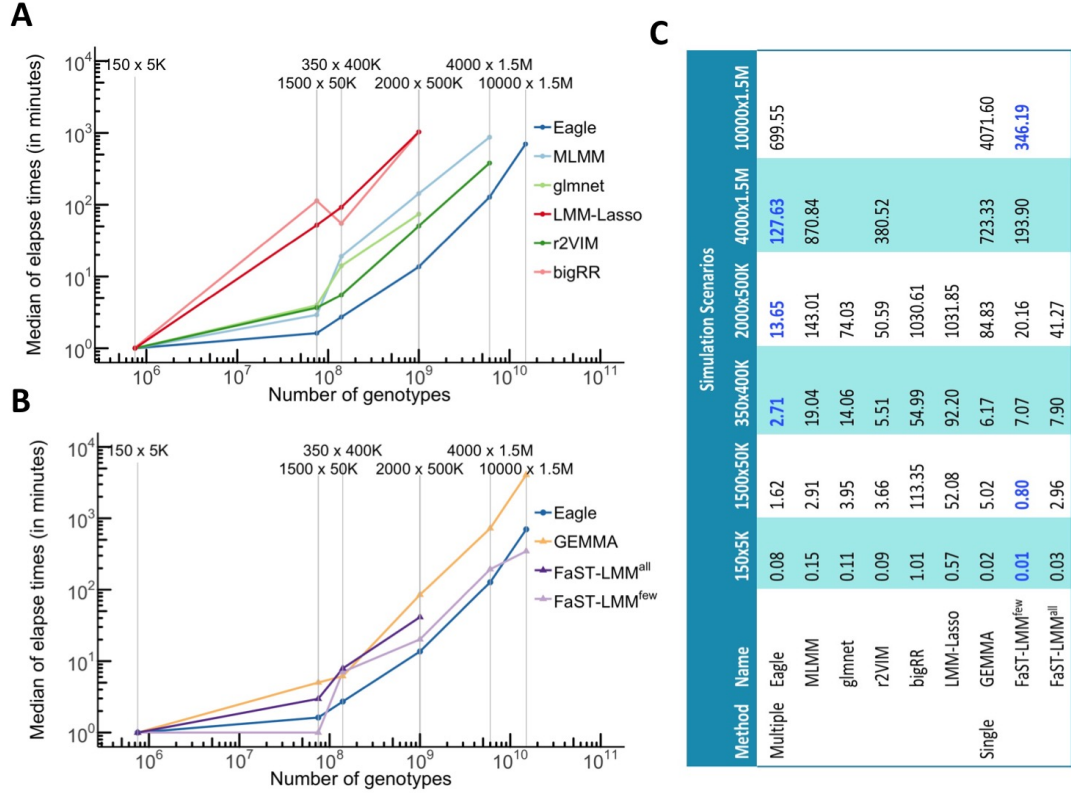
480 with 150 individuals and 5000 SNPs, Eagle produced results in seconds. For the  
481 larger simulation scenarios of 1500 x 50K and 350 x 400K, analyses with Eagle  
482 took under two minutes. Even for data from a couple of thousand individuals  
483 and half a million SNPs (2000 x 500K), the median run time of Eagle was under  
484 14 minutes. For our scenarios where there were thousands of individuals and  
485 1.5 million SNPs, Eagle took just over two hours for the analysis of data from  
486 4000 x 1.5M and 12 hours for the analysis of data from 10000 x 1.5M. Towards  
487 the final stages of writing this paper, we gained access to a high-end server with  
488 14-core Xeon processors and 256 gigabytes of RAM. We reran Eagle on data  
489 from the largest scenario 10000 x 1.5M to measure the impact on run time. The  
490 median run time dropped by more than 70% from 12 hours to 3.31 hours.

### 491 3.5 Mouse Data Analysis

492 We were interested in comparing results from Eagle with those from single-  
493 locus association mapping for a real data set. We chose to focus on data from  
494 a large outbred mouse study (Nicod *et al.*, 2016). This study was unusual in  
495 that it collected and analysed SNP dosages (continuous values from zero to  
496 one of expected allele counts) instead of the more common SNP genotypes.  
497 Analyses based on dosages rather than discrete genotypes have been shown  
498 to have greater power for the detection of genes that are influencing a trait  
499 (Zheng *et al.*, 2011). By converting the dosages into genotypes and analysing  
500 the data with the single-locus program FaST-LMM, we obtained a subset of  
501 those findings reported in the original study. We then analysed the data with  
502 Eagle. Due to Eagle's increased power, we found SNP-trait associations not  
503 found with FaST-LMM. However, we were able to confirm the validity of these  
504 new findings as they matched what was found in the original study. Having the  
505 ability to confirm new findings in a real study was one of the primary motivators  
506 for choosing these data for analysis.

507 We repeated the single-locus analyses as first performed (Nicod *et al.*, 2016)  
508 but with some exceptions. We focused on autosomal SNPs, our analyses were based  
509 on SNP genotypes rather than SNP dosages, we sought to control the false  
510 positive rate not false discovery rate of the methods, and significance thresholds

Figure 2: Median run times, in minutes, for the analysis of simulation study data from the six scenarios. Eagle is compared against five other multi-locus programs/packages (A) and two single-locus programs (B). The x- and y-axes are on a log scale for improved aesthetics. Eagle has the lowest run-times of the multi-locus programs/packages, sometimes by orders of magnitude. Eagle can even produce results faster than single-locus programs. The median run times for the programs/packages across the scenarios are given in the table (C). The entries in a blue font correspond to the lowest run-time for a scenario. FaST-LMM<sup>all</sup> is where calculation of the similarity matrix is based on all the SNP data. FaST-LMM<sup>few</sup> is where calculation of the similarity matrix is based on a subset of the SNP data.



511 were found empirically via permutation (Doerge and Churchill, 1996).

512 We ran Eagle in three ways. Eagle chooses the best model via the extended  
513 Bayesian information criteria (extBIC) (Chen and Chen, 2008). The conser-  
514 vativeness of the extBIC can be adjusted by a single regularisation parameter  
515  $\gamma$  that ranges from zero to one. In the simulation study, this parameter was  
516 set to one, its most conservative and default setting. The mouse data were also  
517 analysed under this setting (Eagle<sup>default</sup>). An alternate (Chen and Chen, 2008),  
518 less conservative way of setting  $\gamma$  is to let  $\gamma = 1 - \frac{1}{(2\kappa)}$  with  $\kappa = \frac{\log(L)}{\log(n_g)}$  where  $L$   
519 is the number of loci that span the genome, and  $n_g$  is the number of individu-  
520 als/groups/lines/strains in the study (Eagle<sup>alt</sup>). However, our preferred way is  
521 to set the  $\gamma$  parameter for each trait via permutation (Eagle<sup>optimal</sup>). We used  
522 100 permutations to set  $\gamma$  to give a false positive rate of 5%. This only took six  
523 times as long as a single analysis of the data. This is because the marker data  
524 need only be read once, and only the trait data changes across permutations  
525 leading to other computational efficiencies. This permutation method has been  
526 implemented within the Eagle package.

527 The genome wide results from the analyses of the mouse data are shown  
528 in Figure 3, with the Manhattan plots of the single-locus analysis shown in  
529 Supplementary Figure 3. The mouse study recorded measurements on 200 traits.  
530 Of these, in the original study, 45 were able to have their findings corroborated  
531 by previously published work. We focused our analyses here on these same 45  
532 traits. Overall, FaST-LMM<sup>Bonf</sup>, FaST-LMM<sup>perm</sup>, Eagle<sup>default</sup>, Eagle<sup>alt</sup>, and  
533 Eagle<sup>optimal</sup> found 47, 68, 37, 67, 106, SNP-trait findings, respectively, across 39  
534 traits. No associations were found by FaST-LMM and Eagle for the other six  
535 traits. Eagle<sup>alt</sup> and Eagle<sup>optimal</sup> also found SNP-trait associations not found  
536 in the original study. This is despite their analyses being based on the SNP  
537 genotype data and the original study being based on SNP dosage data. Eagle<sup>alt</sup>  
538 found two and Eagle<sup>optimal</sup> found seven new findings (Supplementary Table 2).  
539 These new findings all involved SNPs whose association had been confirmed for  
540 other related traits in the original study.

541 In the simulation study, Eagle outperforms single-locus association mapping.  
542 Here, Eagle<sup>default</sup>, where  $\gamma = 1$ , finds less associations than FaST-LMM. Why  
543 the discrepancy in performance? The answer lies in the conservativeness of

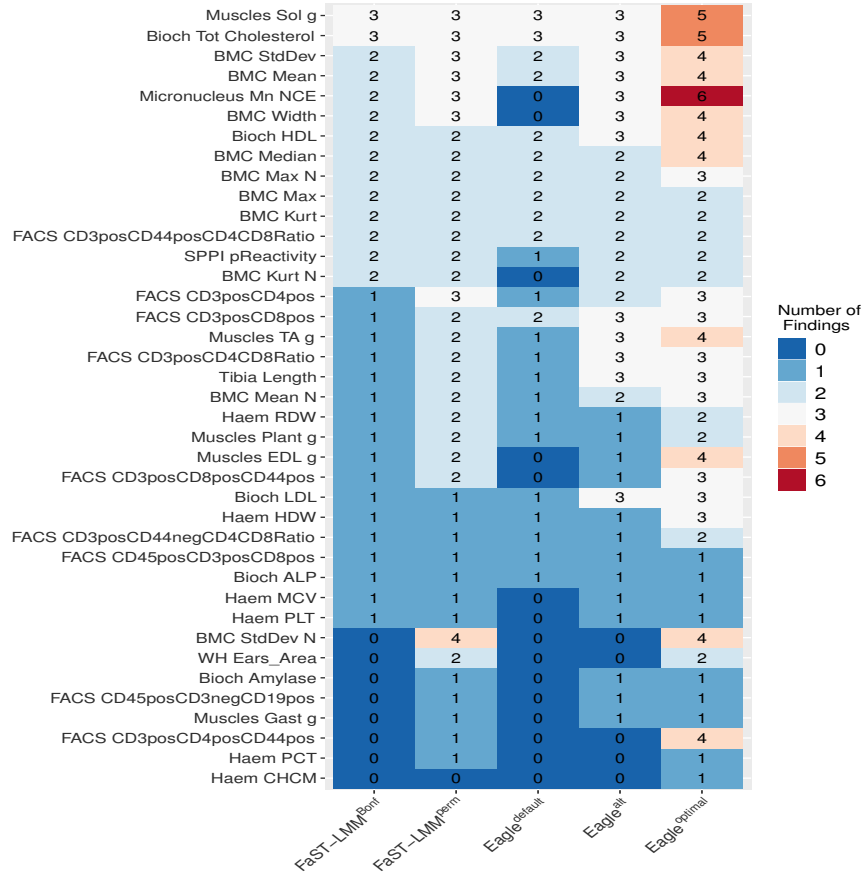
544 Eagle. With the added genetic complexity implicit within the mouse data,  
545 Eagle is more conservative when  $\gamma$  is set to one than in the simulation study.  
546 However, the relative results of the simulation study remain true. For similar  
547 false positive rates, Eagle is superior to single-locus association mapping. As  
548 a case in point, here FaST-LMM<sup>perm</sup> found 68 SNP-trait associations with a  
549 false positive rate of 5%. Eagle, with the same false positive rate (Eagle<sup>optimal</sup>)  
550 found 106 SNP-trait associations, more than a 50% increase in findings.

## 551 4 Discussion/Conclusion

552 Eagle is a new linear mixed model based method (and R package) for multi-locus  
553 association mapping. It advances the state of association mapping in several  
554 ways. First, its computational footprint is much smaller than other multi-locus  
555 implementations. Eagle makes multi-locus analysis practical, even when the  
556 datasets are large. Second, the results from Eagle are immediately interpretable.  
557 They are the set of SNPs in strongest association with the trait where each  
558 SNP identifies a separate genomic region of interest. Third, it treats association  
559 mapping as a model selection problem, avoiding multiple testing issues. As we  
560 saw in the simulation study, Eagle has considerably higher power than single-  
561 locus methods but is comparable in run time. Also, when analysing the mouse  
562 data, Eagle found more than double the SNP-trait associations than with single-  
563 locus association mapping, the method of choice. **Furthermore, because we**  
564 **converted the SNP dosages of the original study into genotypes and focusing**  
565 **our analyses on these data, the validity of the extra findings can be confirmed**  
566 **against the original findings.** These extra findings were all true.

567 Eagle outperformed the other multi-locus methods in our simulation study.  
568 However, we are cognisant of the fact that we made several implementation  
569 choices that impact our conclusions. For instance, we chose to calculate the  
570 significance of the SNP effects from bigRR, LMM-Lasso, and glmnet via stability  
571 selection. Permutation and its variants (Browning, 2008; Pahl and Schafer,  
572 2010) are also equally valid empirical approaches. Stability selection though has  
573 the advantage of being based on repeated sampling of only a proportion (50%  
574 in our case) of the data. Also, when analysing the (sub)samples, it was not

Figure 3: Genome-wide association mapping results from analyses of the mouse data for the single-locus method FaST-LMM and the multi-locus method Eagle. Genome-wide significance thresholds for FaST-LMM were calculated, via the Bonferroni correction (FaST-LMM<sup>Bonf</sup>) and permutation (FaST-LMM<sup>perm</sup>), at the 5% significance level. Eagle was run under three settings; its default setting (Eagle<sup>default</sup>), an alternate less conservative setting based on the number of SNPs and sample size (Eagle<sup>alt</sup>), and where the model selection had been optimised for a false positive rate of 5% (Eagle<sup>optimal</sup>). The number of SNP-trait associations found are reported in the cells.



necessary to calculate the entire solution path for a method. Instead, analyses are performed for a fixed value of the regularisation parameter, greatly reducing the amount of computation required. For r2VIM, an R package implementing random forests, we had to decide on the minimum size of a terminal node, the number of trees, and number of potential variables. The setting of these parameters greatly affects performance. We acknowledge that in the hands of an expert, r2VIM could be fine-tuned for a better balance of computational and statistical performance. However, we would like to think that the parameter settings we used are sensible since they match the values in the original r2VIM publication (Szymczak *et al.*, 2016).

Eagle’s computational speed does come at a cost. It is a weakness shared by all of the methods considered here, although in different ways. Eagle cannot handle extra random effects which are sometimes needed when more advanced study designs are employed. One solution is to adopt a two-stage analysis procedure. In the first stage, a single linear mixed model is fitted to the data. Much of the modelling complexity, including the extra random effects, is captured in this first-stage model. In the second stage, Eagle is run not on the original trait data but adjusted trait data which are obtained from the first stage analysis. Even though this is a well accepted practice, it is approximate (Gogel *et al.*, 2018). A better solution is to fit a single model to the data. Although not specifically designed for association mapping, WGAIM (Verbyla *et al.*, 2007), upon which Eagle is based, and RWGAIM (Verbyla *et al.*, 2012) are two R packages where this is possible. The difficulty is that for large datasets and/or complex models, run time and memory usage can become limiting factors for analysis.

Over the coming years, computationally, the demand placed upon association mapping methods is going to increase. High-throughput array-based technologies continue to decrease the cost of genotyping, permitting ever larger GWASs to be performed. Whole-genome sequencing is also now a reality. Already sequence across entire genomes are being collected for GWASs (Gudbjartsson *et al.*, 2015; Long *et al.*, 2017) culminating in data on millions of SNPs. It is because of this growing demand that we have purposely structured the Eagle package for continued development. We are already experimenting with a GPU-based version of Eagle. Early results suggest that for small to moderate

608 sized datasets (<10,000 samples), there is little improvement in performance  
609 over CPU-based computation. However, for larger study sizes, we are seeing  
610 up to a 40% decrease in run times. We also have plans for Eagle to run on  
611 computer clusters. Structuring Eagle for larger-than-memory calculations was  
612 a preemptive step in this direction. GWASs have changed significantly in the  
613 past decade but the size and complexity of GWASs is expected to change even  
614 more in the coming decade.

## 615 Data Availability

616 The input files for Eagle containing the mouse GWAS data are available for  
617 download from <https://doi.org/10.25919/5bc08287717dd>. The original data  
618 were obtained from the Heterogeneous Stock Mice website [http://wp.cs.ucl.ac.uk/outbredmice/heterogeneous-](http://wp.cs.ucl.ac.uk/outbredmice/heterogeneous-stock-mice/)  
619 [stock-mice/](http://wp.cs.ucl.ac.uk/outbredmice/heterogeneous-stock-mice/).

## 620 Acknowledgements

621 We would like to thank the IM&T Scientific Computing group at the Common-  
622 wealth Scientific and Industrial Research Organisation (CSIRO) for providing  
623 access and support to the CSIRO Accelerator Cluster upon which all compu-  
624 tation was performed. We also gratefully acknowledge the comments of two  
625 anonymous reviewers whose suggestions have helped "polish" this paper.

## 626 Funding

627 This research received no specific grant from any funding agency in the public,  
628 commercial, or not-for-profit sectors.

629 *Conflict of interest:* The authors declare that they have no competing financial  
630 interests.



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