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Mixed linear model approach adapted for genome-wide association studies

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Mixed linear model (MLM) methods have proven useful in controlling for population structure and relatedness within genome-wide association studies. However, MLM-based methods can be computationally challenging for large datasets. We report a compression approach, called 'compressed MLM', that decreases the effective sample size of such datasets by clustering individuals into groups. We also present a complementary approach, 'population parameters previously determined' (P3D), that eliminates the need to re-compute variance components. We applied these two methods both independently and combined in selected genetic association datasets from human, dog and maize. The joint implementation of these two methods markedly reduced computing time and either maintained or improved statistical power. We used simulations to demonstrate the usefulness in controlling for substructure in genetic association datasets for a range of species and genetic architectures. We have made these methods available within an implementation of the software program TASSEL.

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Although genome-wide association studies (GWAS) have the potential to pinpoint genetic polymorphisms underlying human diseases and agriculturally important traits, false discoveries are a major concern[1] and can be partially attributed to spurious associations caused by population structure and unequal relatedness among individuals in a given cohort. Population stratification was initially addressed using general linear model (GLM)-based methods such as structured association[2], genomic control[3] and family-based tests of association[4]. The introduction of MLM approaches has more recently been demonstrated as an improved method to simultaneously account for population structure and unequal relatedness among individuals[5].

In the MLM-based methods, population structure[2,6] is fit as a fixed effect, whereas kinship among individuals is incorporated as the variance-covariance structure of the random effect for the individuals. Regardless of the applied statistical method, GWAS require large sample sizes to achieve sufficient statistical power[7], especially in order to detect the small effect polymorphisms that underlie most complex traits[8]. For the MLM approach, datasets with these large sample sizes create a heavy computational burden because the computing time for solving a MLM increases with the cube of the number of individuals fit as a random effect. The earliest effort to reduce the size of the random effect in an MLM can be traced back to the sire model approach used in animal breeding[9–12], which replaces an individual's genetic effect with that of its sire. Consequently, the sire-model approach requires pedigrees, which are not always available, and which in particular are often not available in plant studies. Even with available pedigrees, the use of a marker-based kinship is preferred because of its higher accuracy[13,14]. The computing time is further increased because iteration is needed to estimate population parameters, such as variance components[15], for each tested marker. Even though a number of studies have sought to improve the speed of the iteration process, including development of the recent efficient mixed-model association (EMMA) algorithm[16], solving an MLM for a large number of individuals and markers remains computationally intensive. To address this issue, a residual approach was proposed based on a two-step strategy[17]. The first step optimized a reduced MLM with the genetic marker effect excluded. In the second step, the residual from the reduced MLM was fit as the dependent variable to test each marker in a GLM. Because the random genetic effect was not fit in the second step, iteration was not required when testing markers. This residual approach can be performed much faster than the one-step MLM with full optimization for all unknown parameters, but it has a statistical power equivalent to that of the full optimization approach only for traits with low heritability. We propose here methods to reduce the size of the random genetic effect in the absence of pedigree information and eliminate iterations to re-estimate the population parameters for each marker without compromising statistical power. We show that the joint use of these two methods greatly reduces computing time and maintains or even increases statistical power.

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The total computing time for a GWAS with a standard MLM is mpn^3, where m is the total number of markers, p is the number of iterations to solve the MLM and n is the total number of individuals assessed. Conducting a GWAS with a large sample size becomes computationally intensive because each iteration takes an amount of time that is proportional to the cube of the number of individuals in the random effect[15,18]. An approach for reducing this computational burden is to reduce the size of the random effect. We achieve this by substituting n individuals with a smaller number of groups, s ($s \leq n$), clustered based on the kinship among individuals. Consequently, the kinship between pairs of groups replaces the kinship between pairs of individuals for the random effect of an MLM. If $c = n/s$ is the average number of individuals per group, referred to hereafter as the compression level, this approach will reduce computing time by a factor of c^3. We named this approach compression, referring to how the random effect in a MLM is compressed from individuals to groups. An MLM that uses compression is called a compressed MLM.

Because in this method individuals are clustered into groups based on kinship estimates, we consider the compressed MLM to be an extension of the pedigree-based sire model[9–12] with notable advancements. The groups used in the compressed MLM can be clustered from kinship calculated from either markers or pedigrees. In addition, the number of groups in the compressed MLM can vary from n to 1, whereas the number of sires is fixed in the traditional method for a particular pedigree. This flexibility in the number of groups allows the accuracy of the group mean and number of groups to be optimized, which is a method similar to choosing the numbers of sires and progeny per sire to maximize genetic improvement in a breeding program[19–21]. The ability to optimize the number of groups could lead to increased statistical power in GWAS.

Compressed MLM crosses the boundary between GLM and MLM because GLM and MLM can both be considered extreme cases of compressed MLM (**Fig. 1**). MLM is equivalent to compressed MLM when each individual is treated as a single group (that is, $s = n$), whereas GLM is equivalent to compressed MLM when all individuals are in one group ($s = 1$). The latter causes the random effect to have a single level, thereby preventing the separate estimation of the random effect and residual variance components. In addition, the random effect and the overall mean are linearly dependent and thus cannot be estimated separately.

To further reduce computing time, we developed the P3D algorithm, a two-step approach that does not require iteration to estimate population parameters such as genetic variance and residual variance for each marker. The first step in the algorithm is to optimize the reduced MLM with the marker effect excluded. If compression is incorporated in the model, the population parameters also include the clustering algorithm and compression level. Taken from a similar approach that was applied to marker-assisted breeding[22], the second step of the algorithm continues to fit the random genetic effect in the MLM with the previously determined population parameters fixed as empirical Bayesian priors[23]. Subsequently, the non-population parameters, including the marker effect and the random genetic effect, are estimated for each marker.

P3D is similar to the two-step residual approach[17], but it also has notable differences. The residual approach fits the residuals from the reduced MLM as the dependent variable in the second step, whereas the original phenotype is fit as the dependent variable in the second step of P3D. In addition, the residual approach does not fit the random genetic effect and uses a GLM when testing markers, whereas P3D fits the random genetic effect with previously determined population parameters fixed in an MLM framework.

To evaluate compression and P3D relative to the standard MLM with full optimization of all unknown parameters for each marker, we conducted a series of association studies between observed or simulated phenotypes and observed markers in human, dog and maize. For the observed phenotypes, we evaluated the fit of compressed MLMs at different compression levels and with different clustering algorithms. Under the assumption that there is no association between the observed phenotypes and the observed genetic markers, we investigated the distribution of false positives by using the compressed MLM. The simulated phenotypes were used to evaluate statistical power by considering the potential true associations between the observed phenotypes and the observed markers. The simulated phenotypes were generated from the observed SNPs by adding the genetic effects. The SNPs with assigned genetic effects are called quantitative trait nucleotides (QTNs). Total number of QTNs, heritability and dominance and epistatic effects were varied to validate the robustness of P3D for phenotypes with different genetic architectures. We used the distribution of the F statistics from the association tests between the simulated phenotypes and the non-QTN markers to determine an empirical threshold[5] at a significance level of 5% ($P < 0.05$). We then calculated the statistical power as the proportion of QTNs with F values greater than the thresholds.

RESULTS

Compression

We examined the fit of the compressed MLM on human height with eight hierarchical clustering algorithms[24,25]: unweighted pair group method with arithmetic average (UPGMA); unweighted pair-group centroid; complete linkage; Lance-Williams flexible-beta method; McQuitty's similarity analysis (weighted pair-group method using

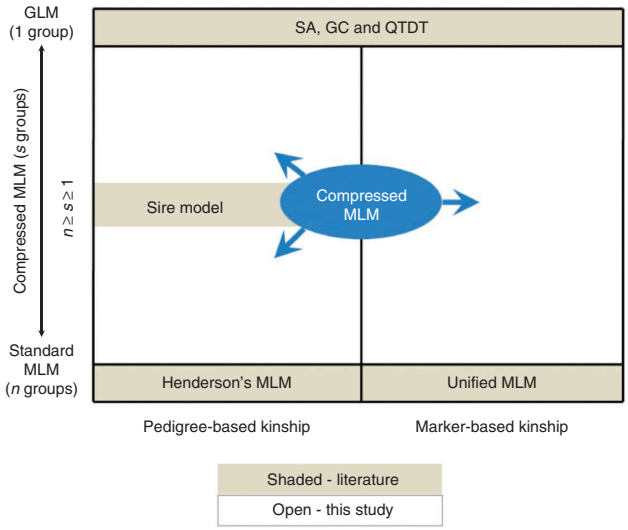

Figure 1 The forms of MLM classified by the random effect size and types of kinship. The GLM and standard MLM are the two extremes of the compressed MLM with the number of groups determined as 1 and n (number of individuals), respectively. The sire model is a special case of the compressed MLM, with the groups determined as the sires derived from pedigrees. Kinship used in Henderson's MLM[15] was calculated from the pedigrees. It was extended to marker-based kinship in the unified MLM[5]. The GLM approach appears in many formats in various GWAS, including structure association (SA)[2], genomic control (GC)[3] and the quantitative transmission disequilibrium test (QTDT)[4]. The compressed MLM can be flexibly applied to the entire area by varying the number of groups (s), including the area investigated previously (shaded area) and the area proposed in this study (open area).

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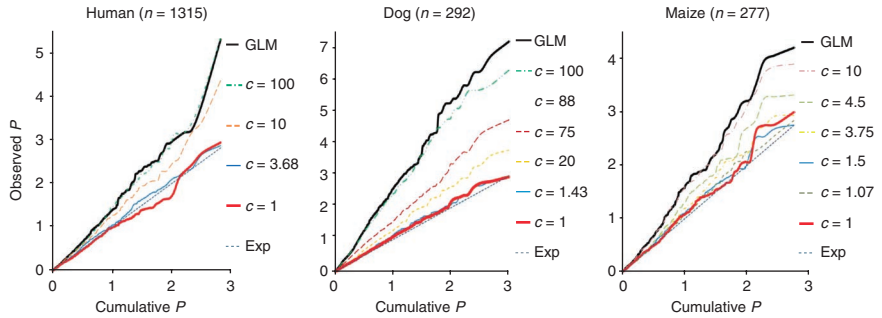

Figure 2 Quantile-quantile plots of type I error (false positive) rates of association tests using the compressed MLM under different compression levels. The observed phenotypes are height in humans, hip dysplasia (Norberg angle) in dogs and flowering time (days to pollination) in maize. The distributions of *P* values are shown by plotting the observed *P* values against the cumulative *P* values in the negative $\log_{10}$ scale. Under the assumption that this set of genetic markers are unlinked to the polymorphism controlling the phenotypes, the *P* values of the association tests have a uniform distribution, indicated by the expected diagonal line (Exp)[5]. A statistical approach that has a distribution closer to the diagonal line indicates a better control for type I errors. The GLM that is equivalent to the compressed MLM at the maximum compression level had the most type I errors. For all the species, at least one compression level was found at which the compressed MLM performed better than the standard MLM, which is equivalent to the compressed MLM with compression level of 1.

arithmetic averages); weighted pair-group centroid median; single linkage (nearest neighbor); and Ward's method. The fit of each model varied considerably with the use of different combinations of clustering algorithms and compression levels. For each clustering algorithm, at least one compression level had a better fit with the data than the standard MLM, with the exception of the unweighted and weighted pair-group centroid median algorithms in the human dataset (**Supplementary Fig. 1**). The variation in model fit among clustering algorithms suggests that additional research is needed to better understand the relationship between clustering algorithms and compression levels; however, this is beyond the scope of our study. Because UPGMA produced models that were generally equivalent to or better than other clustering algorithms, we chose to use that in the rest of the work presented here, including the examination of model fit for different phenotypes within the same population (**Supplementary Fig. 2**).

Under the assumption that there is no association between the observed phenotypes and the tested markers, the *P* values from the association tests should follow a uniform [0,1] distribution. This distribution is shown in the quantile-quantile plot in **Figure 1**. Notably, compressed MLM controlled the false positive rate better than the standard MLM when the compression levels were within the range of 1.5 to 10 (**Fig. 2**). At these same compression levels, the compressed MLM had a better model fit than the standard MLM when marker effects were excluded (the top panel in **Fig. 3**).

To deal with the risk that reducing the number of false positives might affect the ability to detect true positives (that is, statistical power), especially in the case of assuming that no association is violated, we examined the performance of the compressed MLM by simulation studies. After QTN effects were added to the observed phenotypes, tests of association between these simulated phenotypes and markers showed that the statistical power (that is, the ability

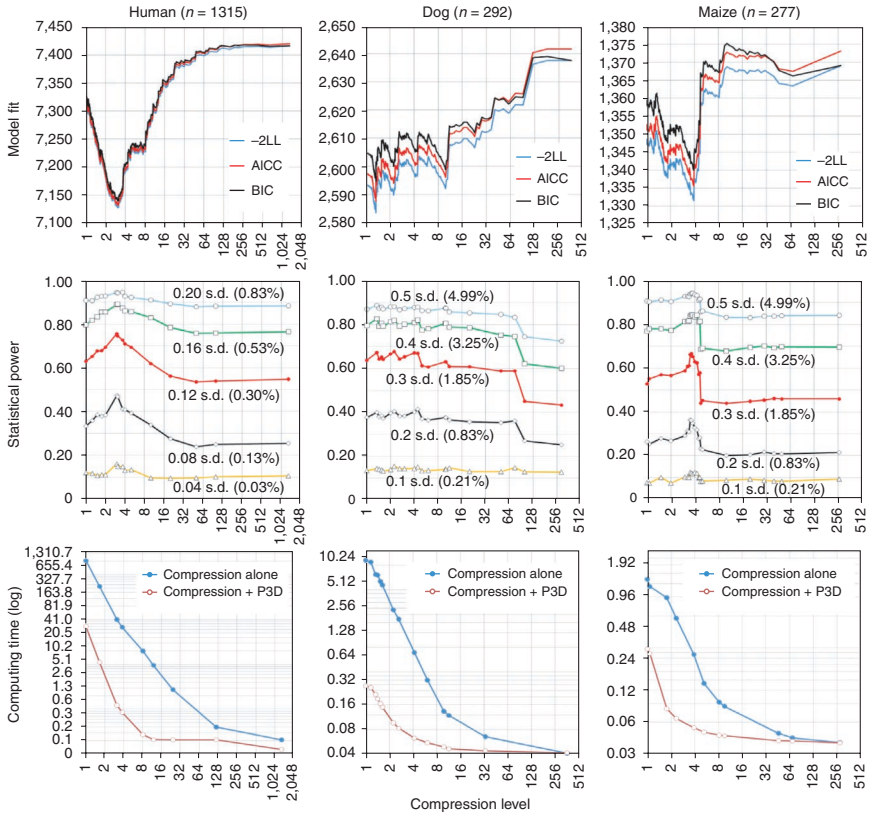

Figure 3 The performance of the compressed MLM under different compression levels (horizontal axis). The two extremes of the compression level at 1 and *n* (the number of individuals) correspond to the standard MLM and the GLM, respectively. Performances were examined based on model fit, statistical power and computing time (s). The observed phenotypes are height in humans, hip dysplasia (Norberg angle) in dogs and flowering time (days to pollination) in maize. Individuals in each of the datasets were clustered into groups according to kinship by using the UPGMA algorithm implemented by proc cluster in SAS[26]. Model fit was evaluated using negative log likelihood (−2LL), adjusted Akaike information criterion (AICC) and Bayesian information content (BIC). Smaller values of −2LL, AICC and BIC indicate better fit. The statistical power was evaluated for QTNs with different size effect. The size of QTN effect is expressed in the unit of phenotypic standard deviation (s.d.). The average computing time was calculated from the observed CPU time for association tests on 647 markers in human datasets; 1,000 markers in dog datasets; and 553 markers in maize datasets. The computations were performed by proc mixed in SAS[26] on a computer from Dell (Optiplex 755) with two physical CPUs (E6850 @ 3.00 GHz) and 3.25 GB RAM operated under Windows XP.

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to detect the simulated QTN) and model fit followed the same trend. The compression level that best fit a model without markers also provided the highest power to detect QTN (middle, **Fig. 3**). Compared to the standard MLM, equivalent power was achieved using compressed MLM with as much as 5- to 10-fold compression. The compression level with the best-fitting model increased statistical power by 34%, 42% and 20% for human, dog and maize for a QTN that explained 0.12, 0.30 and 0.30 units of the phenotypic standard deviation, respectively.

P3D

We compared *P* values obtained from using P3D to *P* values from using full optimization for testing the association between observed phenotypes and markers in human, dog and maize. The coefficient of determination (r^2; Pearson's correlation coefficient squared) between corresponding *P* values obtained from the two approaches were all greater than 0.96. Therefore, we concluded that the association tests obtained from the P3D and full optimization methods were approximately the same.

To evaluate the performance of P3D using phenotypes with different genetic architectures, we performed association tests on simulated phenotypes. Different numbers of QTNs with various levels of heritability, dominance and epistatic effects were simulated. Similarly, strong correlations ($r^2 > 0.97$) between the corresponding *P* values from the P3D and full optimization approaches were observed for both QTN and non-QTN SNPs (top two panels in **Fig. 4** and **Supplementary Figs. 3** and **4**).

For a simulated phenotype with a heritability of 50% and that is controlled by 20 QTNs randomly sampled from the SNPs in the

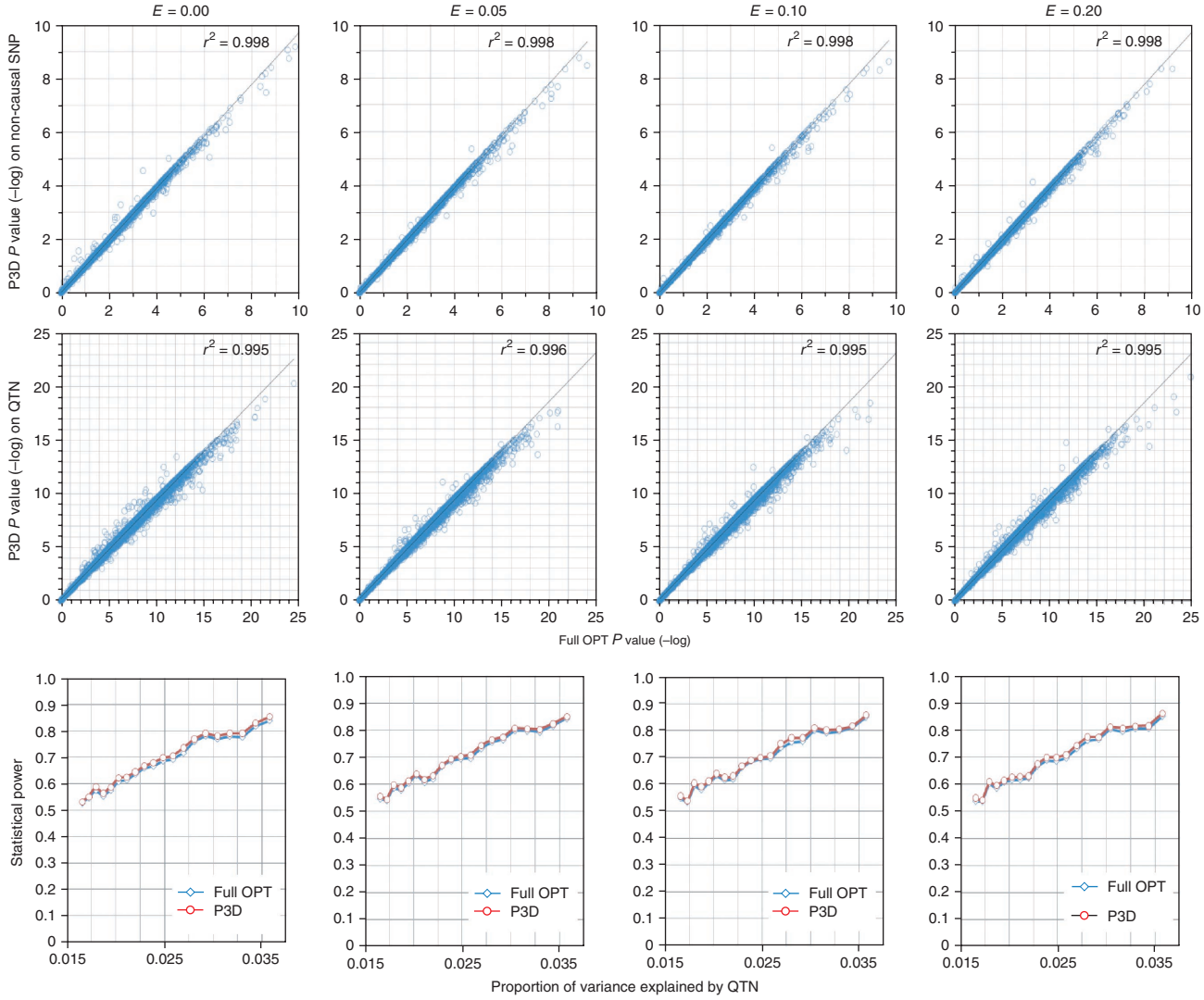

Figure 4 The *P* values and statistical power of association tests obtained by using the one-step MLM with the full optimization (full OPT) for all unknown parameters compared to P3D on a maize phenotype simulated with different epistatic effects (*E*). The phenotype was controlled by 20 QTNs, which were randomly assigned to the SNPs from the maize dataset[5]. Heritability was defined as the proportion of additive genetic variance over the total variance (the sum of additive genetic variance, epistatic variance and residual variance) and was set at 0.5. Because all maize used here belonged to inbred lines, no dominance effect was included. The experiment was repeated 1,000 times. For each replicate, the number of non-causal SNPs that were randomly sampled was the same as the number of causal QTNs. The top two panels display the *P* values using the full OPT (*x* axis) and P3D (*y* axis). Each dot represents a test on a non-causal SNP (top) and a causal QTN (middle). The *P* values from P3D are highly correlated with the ones from the full OPT for the non-causal SNPs and causal QTNs ($r^2 > 99\%$). The empirical statistical power for detecting the causal QTNs is displayed (bottom) as a function of the proportion of the total variation explained (*x* axis). The P3D approach and the full OPT had approximately the same statistical power for detecting the causal QTNs.

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human dataset, we used four compression levels. At each compression level, association tests were performed using both the P3D and full optimization approach. Strong correlations between the corresponding P values from P3D and the full optimization were also observed ($r^2 > 0.99$) for both QTN and non-QTN SNPs across the different compression levels (top two panels in **Supplementary Fig. 5**).

We used the distribution of the F statistics for the non-QTN SNPs to derive the empirical threshold for evaluating F values at each compression level. We calculated the empirical statistical power as the proportion of QTNs with F values greater than the threshold corresponding to a significance level of 5% ($P < 0.05$). The empirical statistical power of the P3D and full optimization approaches were approximately the same in all tested scenarios (bottom panels in **Fig. 4** and **Supplementary Figs. 3–5**).

DISCUSSION

Compression decreases computing time in proportion to the inverse of the cube of the compression level. For instance, a compression level of 2 will reduce the computing time by about 87%. The standard MLM with each individual as a single group has a compression level of 1 and requires the most computing time. The GLM, equivalent to the highest compression level with all individuals assigned to a single group, requires the least computing time. In our analyses, both model fit and statistical power improved as the compression level increased from one. After reaching the optimum compression level, further compression reduced model fit and statistical power, which eventually became the same as the power with the GLM at the maximum compression.

The fit of the reduced model (that is, the model without markers) under different compression levels followed the same trend as the statistical power of the full model for testing markers. Because the reduced model did not include marker effects, the computing time required to find the compression level with the best-fitting model was independent of the number of markers. For these reasons, the P3D model used an efficient strategy that determined the optimal clustering algorithm and compression level only once.

Similar to the residual approach, P3D eliminates the need to estimate population parameters separately for every marker. The advantage of P3D is that it does not lower statistical power regardless of the genetic architecture of the phenotypes. The P3D method works well for different numbers of QTNs and with various levels of heritability, dominance or epistatic effects.

Compressed MLM and P3D can be applied either separately or jointly and can also be used in combination with other approaches, such as the EMMA algorithm, to speed up the iteration process in the first step of P3D. The compressed MLM improves both computing speed and statistical power, whereas P3D improves computing speed without sacrificing statistical power. In addition, compressed MLMs can be applied at various compression levels. For an analysis in which statistical power is the top priority, the compression level with the best model-fit should be chosen; otherwise, a higher compression level may be chosen to reduce computing time. It should be noted that no trend has been identified to determine the compression level with the best model-fit across different datasets. The compression level that generated the best model-fit varied among phenotypes in the same population when the same kinship was used (**Supplementary Fig. 2**). Thus, for each new study, the compression level needed to be optimized using the reduced MLM.

The theoretical computing time reduction is faster by a factor of pc^3 for the joint use of compressed MLM and P3D, where p is the number of iterations and c is the compression level. When using proc mixed and proc cluster in SAS[26] on the three datasets, we showed that the computing time for the human dataset (largest sample size) decreased 19-fold with compressed MLM alone and 877-fold with compression with P3D at the compression level with the greatest statistical power (**Fig. 3**, bottom). Choosing a compression level that had power equivalent to that of the standard MLM reduced the computing time even more: computing time was 103-fold faster with compression alone and 7,582-fold faster with compression with P3D, respectively. For the human dataset with 1,315 individuals, the standard MLM (no compression, no P3D) took 821 s to screen one marker. (**Fig. 3**) At this speed, it would take 9,502 d (26 years) to analyze a GWAS with 1 million markers. The current methods (compression with P3D) took 0.34 s to screen a marker at the compression level of 3.8, which showed the highest statistical power, and at this speed, it would take only 2.7 d to screen one million markers. The increased speed is even more important for larger datasets (for example, one containing 5,000 individuals). This suggests that current GWAS datasets on several thousand of individuals at 500,000–1,000,000 markers could be analyzed by our methods within several days. We have made these methods available within an implementation of the software program TASSEL[27].

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

Z.Z. conceptualized the study, performed the data analyses and wrote the manuscript. E.E., M.A.G. and J.Y. participated in the data analyses and wrote the manuscript. P.J.B. implemented the two new methods in the TASSEL software package. C.L., H.K.T., D.K.A. and J.M.O. provided the human data and supervised its analyses. R.J.T. provided the dog data and supervised its analyses. E.S.B designed and supervised the project. All authors edited the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Standard MLM. A standard MLM for GWAS can be written by extending the notation of Henderson[15] as follows:

$$\mathbf{y} = W\mathbf{v} + X\boldsymbol{\beta} + Z\mathbf{u} + \mathbf{e} \quad (1)$$

where **y** is a vector of a phenotype; $\boldsymbol{\nu}$ and $\boldsymbol{\beta}$ are unknown fixed effects representing marker effects and non-marker effects, respectively; and **u** is a vector of size n (number of individuals) for unknown random polygenic effects having a distribution with mean of zero and covariance matrix of $G = 2K\sigma_a^2$, where K is the kinship (co-ancestry) matrix with element k_{ij} ($i,j = 1,2,\ldots,n$) calculated from either a set of genetic markers or pedigrees and σ_a^2 is an unknown genetic variance. W, X and Z are the incidence matrices for ν, β and **u**, respectively, and **e** is a vector of random residual effects that are normally distributed with zero mean and covariance $\mathbf{R} = I\,\sigma_e^2$, where I is the identity matrix and σ_e^2 is the unknown residual variance. The null hypothesis for the association test that is $\nu = 0$ and the alternative hypothesis is that $\nu \neq 0$. The test of the null hypothesis can be performed by either an F test or χ^2 test after the maximization of the following likelihood:

$$L(\mathbf{y}|\mathbf{v},\beta,\mathbf{u},\sigma_a^2,\sigma_e^2) \quad (2)$$

Compression. The form of the compressed MLM is the same as equation (1). The difference in content is that individuals in u are replaced by their corresponding groups, and kinship among individuals (K) is replaced by the kinship among groups ($\bar{k}$), which is defined as $\bar{k} = \{\bar{k}_{ij}\}$, where $i,j = 1$ to s, and where

$$\bar{k}_{ij} = \underset{h\in i,\, t\in j}{\text{average}}(k_{ht}) \quad (3)$$

Under the compressed MLM, the likelihood (L) is as follows:

$$L(y|\mathbf{v},\beta,\mathbf{u},\sigma_a^2,\sigma_e^2,C) \quad (4)$$

where **C** is the clustering results after using a clustering algorithm with s groups (where $s = 1,2,\ldots,n$).

P3D. The first step of P3D is to determine population parameters, including genetic variance (σ_a^2), residual variance (σ_e^2) and clustering result (C), by maximizing the following likelihood:

$$L(y|\beta,\mathbf{u},\sigma_a^2,\sigma_e^2,C) \quad (5)$$

Then, with the population parameters fixed as empirical Bayesian priors[23], the non-population parameters (v, β and u) are optimized for each marker by maximizing the following likelihood:

$$L(y|\mathbf{v}\,\beta,\mathbf{u},\hat{\sigma}_a^2,\hat{\sigma}_e^2,\hat{C}) \quad (6)$$

Equation (6) is maximized by solving equation (1) only once (no iteration) while holding those population parameters constant.

Observed data. We examined three genetic association datasets from human, dog and maize. Each dataset contained phenotype data and a set of genetic markers.

The human dataset was collected from 1,315 adult individuals (specifically, European Americans over 17 years old) who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study[28]. There were 637 genetic markers (388 microsatellite, or simple sequence repeat, markers and 259 SNP markers) scored on these individuals. All multiallelic simple sequence repeat markers were converted into biallelic markers by collapsing alleles into two states: the major allele and all other alleles. Measured phenotypes included height, physical activity, and serum triglyceride and cholesterol levels. Age and sex were also recorded for each individual. A prior study[28] found no significant population structure in this population and no statistically significant association between height and the genetic markers.

The dog dataset was based on 292 dogs from two breeds (Labrador retriever and greyhound) and their crosses (F_1, F_2 and two backcrosses). Hip dysplasia was indicated by Norberg angle measured on both the left and right sides. The lowest hip score (the minimum between the left and right measurements) was used in the analysis[29]. All dogs were genotyped with 23,500 SNPs at genome-wide coverage, of which 1,000 SNPs were randomly sampled for this study.

The maize dataset was composed of phenotype (flowering time scored as days to pollination), genotype (553 SNPs) and population structure (Q matrix) in 277 inbred lines[5]. No statistically significant association was found between the genetic markers and flowering time. This dataset is downloadable as a tutorial dataset of the TASSEL software package[27].

Simulation schemes. Two schemes were employed to simulate phenotypes each for the examination of compressed MLM and P3D. In both schemes, we used SNP marker data from the human, dog and maize datasets. Also, in each scheme, the population structure effect and impact of kinship were retained.

Scheme 1 was to add additional QTN effects to an observed phenotype[5]. This scheme was used to evaluate the compressed MLM approach on phenotypes with the original genetic architecture being retained. The added QTN effect contributed to only a small proportion of variation in that phenotype (0.03%–6.00%).

The QTN effect was represented in the unit of phenotypic standard deviation (k). The percentage of the total variation explained by the QTN (π) is a function of k and sample frequency (f) of the polymorphism at the QTN, defined as $1/(1+1/f\,(1-f)k^2)$[30]. Larger effects (a maximum of $k = 0.5$) were added for the dog and maize datasets, in which the sample sizes were smaller. Smaller effects (a maximum of $k = 0.2$) were added to the human dataset, which had a larger sample size sufficient to allow a small QTN effect to be detected. For a QTN with the largest effect ($k = 0.5$), the percentage of the total variation explained reaches a maximum value of 5.88% when $f = 0.5$. To facilitate comparison between datasets, we listed π at the $f = 0.3$. The genetic effect was assigned to all SNPs, one at a time, to produce replicates across all SNPs.

Scheme 2 was to simulate a phenotype with every known element, including the contribution of population structure, genetic effects (additive, dominance and epistatic) and residual effect. We used this scheme to examine whether P3D could work across traits with different genetic architecture. The general equation to simulate a phenotype (y) is as follows:

$$y = \text{population structure} + \text{additive} + \text{dominance} + \text{epistatic} + \text{residual} \quad (7)$$

where 'population structure' was based on the first five principal components, which were derived from all the genetic markers. The population structure explained 1% of the total phenotypic variation for humans, 25% for dogs and 25% for maize. 'Additive' is the sum of all additive effects for a known number of causal QTNs (5 or 20). The distribution of these QTN effects followed a geometric series[31]. The effect of the ith QTN was set as a^i, where $a = 0.92$. The proportion of the additive effect was defined by the narrow-sense heritability (h^2), which is the proportion of additive variance over the total variance (sum of additive and non-additive variances). Non-additive variance (dominance, epistatic and residual) was set to $V_a(1-h^2)/h^2$, where V_a is the additive genetic variance calculated among the total additive genetic effects across QTNs for each individual. Two levels of heritability were examined ($h^2 = 0.25$ or 0.5). 'Dominance' is the sum of dominance effects from all QTNs with a dominance effect of da^i for heterozygotes at the ith QTN, where d is the degree of dominance ($d = 0$, 0.25, 0.5 or 1). 'Epistatic' is the sum of pairwise interaction effects among all QTNs. The magnitude of the epistatic effect is indicated by the proportion of total variance explained by the epistatic effect (proportion of variance of 0, 0.05, 0.1 and 0.2). The 'residual' effect follows a normal distribution and has a variance to satisfy the contributions from additive, dominance and epistatic effects at the designated level. Simulations of the phenotypes were repeated 1,000 times. The non-causal SNPs were randomly sampled q times for each replicate, where q was set to the same number of QTN in each scenario ($q = 5$ or 20).

Statistical analysis. Proc mixed in SAS[26] was used to solve the MLM with variance components estimated by the restricted maximum likelihood algorithm. Model fit was examined with three criteria: negative log likelihood, adjusted Akaike information criterion and Bayesian information content.

For the analysis of the human dataset, the fixed effects were sex, age and the quadratic term of age in the evaluation of the observed phenotypes

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and phenotypes simulated under scheme 1. Similarly, breed (or fraction of Labrador retriever, for the crosses with greyhound) was the fixed effect in the analysis of the dog dataset, and population structure was the fixed effect in the analysis of the maize dataset. The first five principal components[6] derived from all genetic markers were fit as fixed effects for the phenotypes simulated under scheme 2.

Individuals or their corresponding groups were fit as a random effect. The kinship among individuals was estimated from the genetic markers by the approach of Loiselle *et al.*[32]. The individuals in each dataset were grouped based on their kinship by using proc cluster in SAS[26]. The genotypic effect of each genetic marker was fit as a fixed effect, one marker at a time. The association tests on the markers' genotypes were performed by conducting F tests.

URLs. Compression and P3D were implemented in SAS (**Supplementary Note**) and TASSEL[27] software package. The SAS code, standalone TASSEL program and demonstration date are available at http://www.maizegenetics.net/.

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