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Review: Population Structure in Genetic Studies: Confounding Factors and Mixed Models

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Abstract

A genome-wide association study (GWAS) seeks to identify genetic variants that contribute to the development and progression of a specific disease. Over the past 10 years, new approaches using mixed models have emerged to mitigate the deleterious effects of population structure and relatedness in association studies. However, developing GWAS techniques to effectively test for association while correcting for population structure is a computational and statistical challenge. Our review motivates the problem of population structure in association studies using laboratory mouse strains and how it can cause false positives associations. We then motivate mixed models in the context of unmodeled factors.

Introduction

Genetics studies have identified thousands of variants implicated in dozens of common human diseases (Manolio et al. 2009; Purcell et al. 2009; Stram 2013; Yang et al. 2010). These variants are locations in the human genome where genetic content differ among individuals in a population. A genome-wide association study (GWAS) seeks to identify genetic variants that contribute to the development and progression of a specific disease.

Association studies discover these genetic factors by correlating an individual's genetic variation with a disease status or disease-related trait. At the genome-wide scale, association studies typically focus on statistical relationships between single-nucleotide polymorphisms (SNPs) and disease traits. SNPs are the most common genetic variants underlying susceptibility to disease, and associated SNPs are considered to mark the region of a human genome that influences disease risk. A GWAS identifies a SNP as a significant, and therefore *associated*, variant when the specific genome sequence at the SNP is correlated with a disease trait or disease status. For example, a GWAS study may find that individuals with a specific sequence (or allele) at a SNP have higher blood pressure on average than individuals with a different sequence at the SNP. If a SNP has a significant correlation with a trait or disease status, the association study suggests that presence of the particular variant may increase an individual's risk for disease.

Typical analytical strategies for performing association studies rely on standard regression techniques, which assume the data have an identically and independently distributed (*i.i.d.*)

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property. If data have *iid*, all variables are mutually independent since each random variable shares the same probability distribution with others. Association study methodology was originally designed for populations comprised of unrelated individuals, and standard approaches assume this property is true (Risch and Merikangas 1996). However, the big genomic datasets available today inevitably contain distantly related individuals. This genetic relatedness prevents standard association studies from correctly identifying the causal variants and induces identification of many false positive associations (or *spurious associations*).

Two types of relatedness may produce high rates of false positive associations: population structure and cryptic relatedness. “Population structure” refers to different ancestry among individuals in a study. “Cryptic relatedness” exists when some individuals are closely related, but this shared ancestry is unknown to the investigators. Large (n=>5000) population cohorts inevitably contain individuals who have common ancestry from different populations. In either case, individuals who share ancestry are more related than individuals from different ancestries. These ancestry differences induce a self-organizing population structure effect, which causes the statistical methodology to assign strong association signals to variants that are not actually causal for the trait or disease. In many cases, applying standard association study techniques to population cohorts with population structure produces a high rate of false positive associations. These associations may appear to be significant, but they are driven by the cohort’s relatedness rather than variants that truly affect trait or disease risk.

Developing GWAS techniques to effectively test for association while correcting for population structure is a computational and statistical challenge. This challenge is relevant to human association studies as well as genetic studies in any organism, including model organisms such as mice. Mouse studies are widely used to study human disease and, because of the particular history of the laboratory mouse strains, have complex patterns of genetic relatedness that can cause false positives in association studies.

Over the past 10 years, new approaches using mixed models have emerged to mitigate the deleterious effects of population structure and relatedness in association studies (Zhou and Stephens 2012; Kang et al. 2008, 2010; Listgarten et al. 2012). These approaches were originally developed in the context of mouse studies and later applied to human studies. In this review, we explicitly characterize population structure as a confounding factor in order to explore the root cause of false positives in association studies. We trace the development of these methods in mouse studies and describe how these methods were adapted to human studies, particularly where they are applied to correct for population structure in large-scale genomic datasets.

Standard Genome Wide Association Studies (GWAS)

Genetic association studies attempt to identify single-nucleotide polymorphisms (SNPs) that are responsible for differences in trait or phenotype values within an individual. A SNP is a single position in the human genome sequence where individuals in the population have different

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genetic content. These differing forms of the same gene are referred to as alleles, and SNPs are the most common form of genetic variation.

SNPs are ideal targets for association testing, because they are the most common form of genetic variants and are so prevalent that they are correlated with other forms of variation. To conduct a typical single-SNP test, we first collect genetic information at the SNP in a set of individuals (referred to as genotypes). Next, we measure the association (or correlation) with the trait values (or phenotypes) of the individuals (see Figure 1a). In this Figure, it is intuitively clear that the first SNP appears to be associated, but the second SNP does not appear to be associated.

In order to evaluate if the association between a SNP and a phenotype is statistically significant, we can test two hypotheses using the collected data. The null hypothesis assumes a model where the SNP does not affect the phenotype (see Figure 1b). In this hypothesis, the phenotypes (y) are only affected by the population mean (μ) and the environment (e). Unless data indicate otherwise, we assume that the null hypothesis is true and the SNP does not influence the phenotype (i.e., the individual's disease risk).

An alternative hypothesis provides a model of the SNP being significantly associated with the phenotype (see Figure 1c). In this case, the phenotypes (y) are affected not only by the population mean (μ) and environment (e), but they are also affected by the genotype (x). In other words, presence of the SNP suggests an individual is likely to have the trait or disease risk. Here, the quantitative measurement of strength that the genotype has on the phenotype is referred to as the effect size (β). If the effect size (β) is equal to 0, we consider the two models equivalent. The SNP is determined to be significantly associated with the phenotype when the data fits the alternative hypothesis beyond a specific threshold.

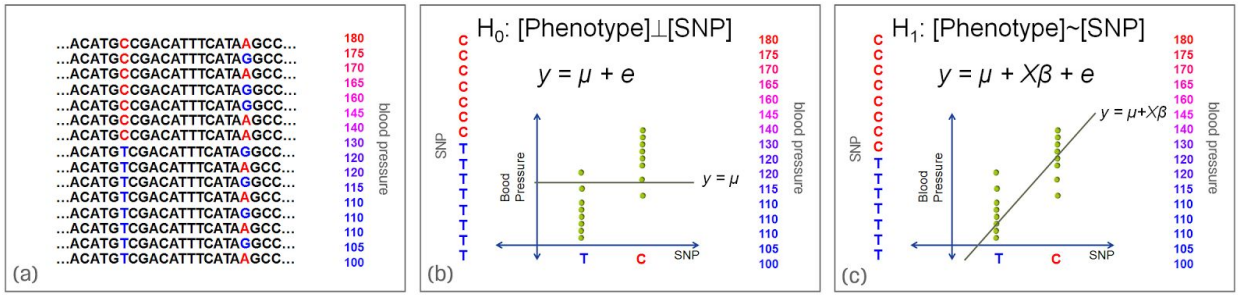

Figure 1. Standard genetic association study applied to blood pressure data. (a) The left SNP appears to be more strongly associated with blood pressure than the right SNP. (b) We test two hypotheses against each other to evaluate whether the association between a SNP and a phenotype is statistically significant. By default, a null hypothesis assumes that the SNP does not affect the phenotype. (c) If the data fits the alternative hypothesis beyond a certain threshold, the SNP is described as significantly associated with the phenotype.

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We mathematically express the null and the alternative hypotheses in order to perform a single-SNP test. We denote the kth genotype of the jth individual g_kj where the genotype is in the set {0,1,2}, which is the number of copies of the kth variant that the jth individual has on their two chromosomes. Here, a "0" denotes the genotype that does not contain the variant in either chromosome, while a "1" or "2" denotes the genotype presence in one or two of the chromosomes, respectively. In order to simplify the equations for association studies, we normalize the genotypes by subtracting the population mean and dividing by the variance. The frequency of a variant in the population is denoted as p_k, which is the average genotype frequency in the population. The normalized genotypes can be expressed as

$$X_{kj} \in \left\{ \frac{-p_k}{\sqrt{p_k(1-p_k)}}, \frac{1-p_k}{\sqrt{p_k(1-p_k)}}, \frac{2-p_k}{\sqrt{p_k(1-p_k)}} \right\}.$$

Once we have calculated the normalized genotypes, a typical single-SNP test can be used to identify variants associated with traits. A standard regression technique estimates the relationship among variables, including a dependent variable (y), any independent variables (x), and unknown variables (β). Using regression, these simple linear models can correlate the genetic variation with the trait, allowing us to assess whether the data best fits the null or alternative hypothesis.

The equation

$$y_j = \mu + \beta_k X_j k + e_j$$

models the phenotype, where j is an individual in the study. Here, the effect of each variant on the phenotype is β_k, the model mean is m, and the contribution of the environment on the phenotype is e_j. The environment's effect on a phenotype (e_j) is assumed to be normally distributed with variance σ_e^2, denoted $e_j \sim N(0, \sigma_e^2)$.

Converting known quantities into vectors allows us to scale these variants for genome-wide studies. In vector notation, we can model the phenotypes of all of the individuals

$$y = \mu 1 + \beta_k X_k + e \qquad (1)$$

with the phenotypes of all of the individuals in the dataset denoted as column vector y, a column containing the genotypes for the ith variant in the population denoted as x_i, and a vector containing the environments denoted as e. 1 is a column vector of 1s. We draw the random vector e from the distribution $e \sim N(0, \sigma_e^2 I)$. We note that each element of e is independent of the others, hence, the variance-covariance matrix is a diagonal matrix ($\sigma_e^2 I$).

Using the observed data, we can estimate the values of the population mean and the effect of the true variant by using the following equations:

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$$\hat{\mu} = \frac{1^T y}{N}$$
$$\hat{\beta} = (X_k^T X_k)^{-1} X_k^T y = \frac{X_k^T y}{N}$$
$$\hat{e} = y - \hat{\mu}1 - \hat{\beta} X_k$$
$$\hat{\sigma} = \sqrt{\frac{\hat{e}^T \hat{e}}{N-2}}$$

The reason the equations are so simple is because the genotypes are normalized. The resulting value is the association between an SNP and phenotype. We can then test the significance of this association by using the statistic

$$S_k = \frac{\hat{\beta}_k}{\hat{\sigma}} \sqrt{N} \sim N(\frac{\beta_k}{\sigma_e *} \sqrt{N}, 1) \tag{2}$$

This statistic is normally distributed with a mean that depends on the effect of the SNP on the trait, the environmental variance, and the number of individuals. The variance of the statistic is 1. If the SNP does not have an effect on the trait, the the statistic will follow the null distribution

$$S_k = \frac{\hat{\beta}_k}{\hat{\sigma}} \sqrt{N} \sim N(0, 1)$$

which is a standard normal distribution. We can then use this null distribution to determine whether the association is significant. This statistic is significant with a significance level of α_s if

$$\Phi(S_k) < \alpha_s/2 \text{ or } \Phi(S_k) > 1 - \alpha_s/2$$

, in which case the variant is considered to be associated (see Figure 2). We use the notation α_s to denote the significance level that we need to achieve at any SNP which in human studies is typically $5 * 10^{-8}$.

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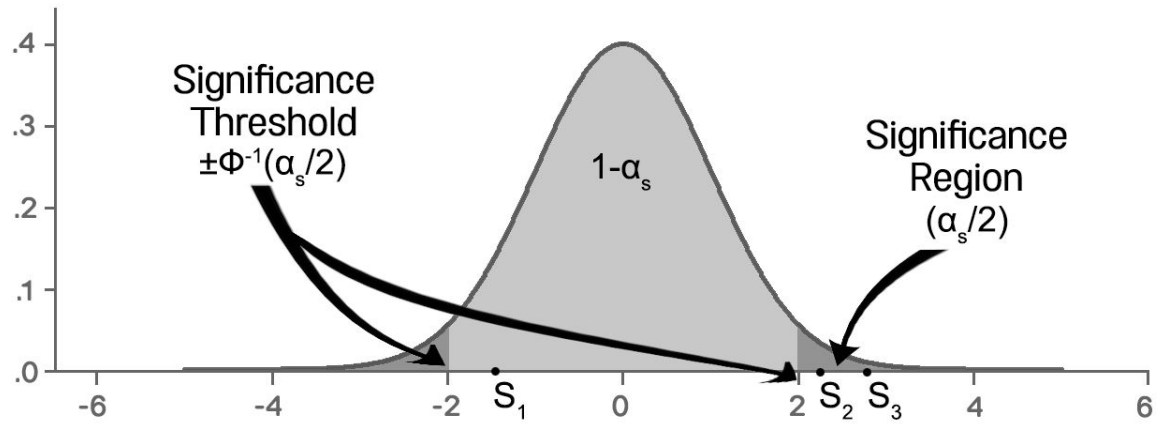

Figure 2. Significance testing in association studies. The null distribution is shown which is the standard normal distribution and the expected distribution of the association statistics under the assumption that the effect size is 0. For each variant, that association statistic in equation (3) is computed and its significance is evaluated using the null distribution. If the statistic falls in the significance region of the distribution, the variant is declared associated. In this example, S1 is not significant and S2 and S3 are significant. The exact location of the threshold is dened as the location on the x-axis where the tail probability area equals the significance threshold (s). This is denoted using the quantile of the standard normal $\phi^{-1}(x)$.

True Genetic Model

Theoretically, the single-SNP test will tell us if a SNP is responsible for the differences we observe in an individual's trait or phenotype expression values. However, this simple linear model is an unrealistic model for identifying variants associated with traits in today's large genomic datasets that contain a high degree of relatedness. In real populations, the true effect of a single SNP is influenced by multiple variants that are affecting the trait. A 'hypothetical' true genetic model takes into account the effect of all SNPs on the trait.

Here, the vector notation

$$y = \mu 1 + \sum_{i=1}^{M} \beta_i X_i + e$$

models the phenotypes of all the individuals in the dataset denoted as column vector y. Again, the effect of the ith variant on the phenotype is β_i, the mean is μ, and the contribution of the environment on the phenotype is denoted by e. Here, the number of variants is M.

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The true genetic model takes into account the true effect of all SNPs, including the effect of the SNP being tested for association with a trait. When testing SNP k, we are using equation (1) the actual data is generated from

$$y = \mu + X_k\beta_k + \sum_{i \neq k} \beta_i X_i + e \tag{2}$$

In applying the simple linear model to data, we observe a mismatch between the model used for testing and the assumed underlying generative model. Here, any term that is missing in the testing model when compared to the generative model is called an unmodeled factor. The unmodeled factor is exactly $\sum_{i \neq k} \beta_i X_i$.

In this case, the unmodeled factor is the effect of variants in a genome other than the variant being tested. This factor can significantly affect the results of an association study. If the individuals in the study are related to each other, the unmodeled factor may produce a high rate of false positive associations. In an association study, relatedness among individuals is referred to as population structure.

Over the past few years, there have been many methods which have been developed to mitigate the effect of population structure in association studies. One of the most commonly utilized approaches today, mixed models, was originally popularized in mouse studies and is now the standard approach for analyzing human GWAS studies. In this review, we motivate the problem of population structure in association studies utilizing laboratory mouse strains and how it can cause false positives associations. We then motivate mixed models in the context of unmodeled factors .

An example of Population Structure Confounding from Mouse Genetics

Genetic mapping using inbred strains of mice provides a good example of why it is necessary to control for population structure. Mice strains pose particular problems that mixed models are developed to solve, and the basic ideas behind mixed models can be clearly demonstrated with mice genetics. Today's classical inbred laboratory mouse strains descend from a relatively small number of genetic founders (mostly fancy mice originally kept as pets) and are characterized by several population bottlenecks (Frazer et al. 2007; Yang et al. 2007). A second group of laboratory strains are referred to as "wild-derived" strains. These strains are mouse strains captured from the wild and inbred mice. These strains were never kept as pets and do not share the population history of classical laboratory strains. A simple way to visualize the relationship between multiple ancestral groups and traits in the mouse genome is by using a phylogenetic tree that can be computed from the genetic information (Figure 3). This tree visualizes the genetic relationships between 32 classical inbred strains and 6 wild derived strains (we had genetic variant information at 140,000 SNPs for each strain).

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We observe that the two groups are close to each other in the phylogeny and there is a long branch length (denoted with a dotted line), which represents the many genetic differences between the groups. We also have the measurements for the body weight and liver weight for each of the strains. Not surprisingly, the body weights of the classical strains are much larger than the body weights of the wild derived strains (Figure 4). This is due to the different selective pressure on the two groups.

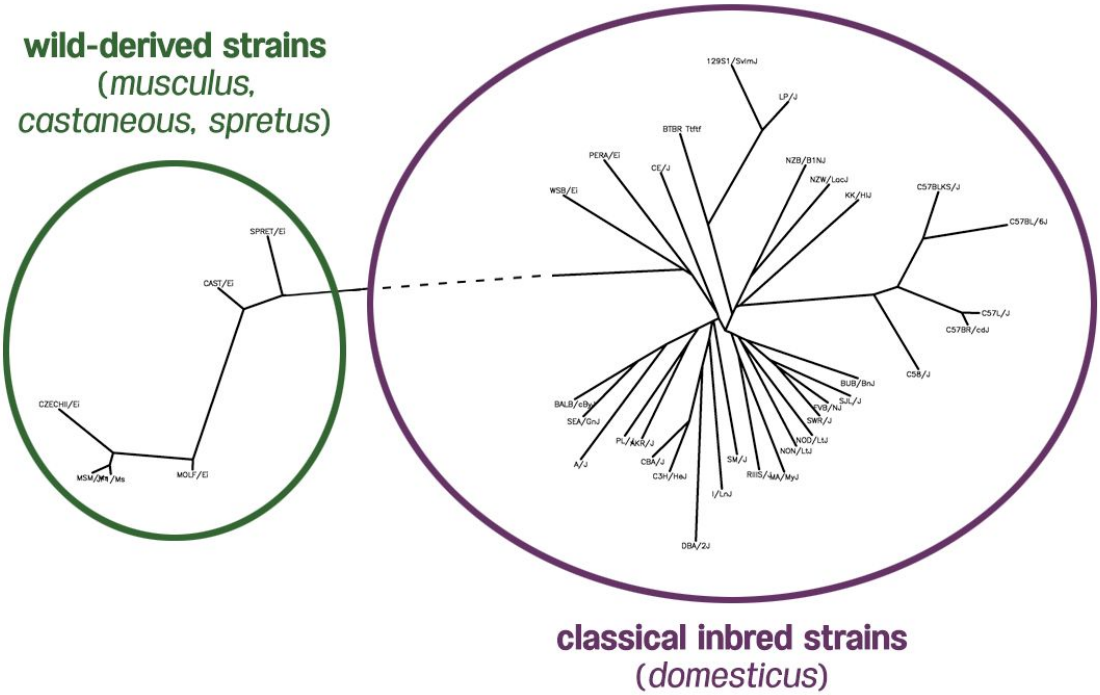

Figure 3. Phylogeny of 38 inbred mouse strains using 140,000 mouse HapMap SNPs. Green strains represent wild-derived non-domesticus mice, and purple strains represent classical inbred mice.

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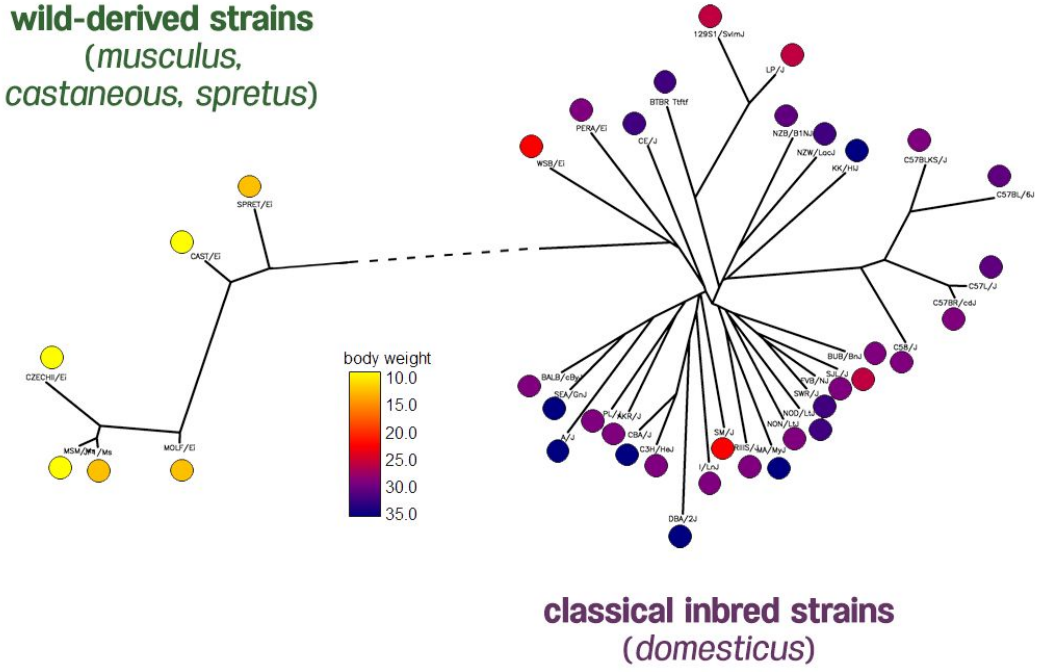

Figure 4. Body weight phenotypes of 38 inbred mouse strains from the Mouse Phenome Database generated by The Jackson Laboratory. The distribution of mice body weights shows two clades of mice have very different body weights.

We attempted to use this dataset to identify which genetic variants are associated with body weight by applying the linear model described above to the 140,000 SNPs. In general, we expect association study results to indicate very few significant associations between particular SNPs and a trait. One common way to visualize the results of an association study is through a Manhattan plot. In a Manhattan plot, the mouse genome is plotted against the x-axis, and the measure of significance of correlation between the genome and trait is plotted against the y-axis. Each red spike represents a SNP at a particular genomic position, and the height of the spike represents the strength of the association. The green horizontal line represents the significance threshold. Any SNP which crosses this line is considered a significant association. We expect to observe a Manhattan plot similar to the one in Figure 5, where there would be a number of SNPs affecting the phenotypes and thus at a few locations in the genome, would we observe signals that cross the threshold, but most of the SNPs will not be associated with the phenotype.

Another way to visualize the results of an association study is with a cumulative p-value distribution plot (b) and a quantile-quantile (Q-Q) plot (c), graphical techniques for determining if multiple datasets come from populations with common distribution. Here, the cumulative p-value

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distribution plot shows the quantiles of the p-values, which assess the probable significance of association between a genotype and trait; the Q-Q plot shows the distribution of the same data log-transformed. Since we expect most SNPs not be to associated, most p-values will be uniformly distributed and only a small fraction of the SNPs to have signals stronger than expected at the tail of the distribution. This will result in a cumulative p-value distribution close to the diagonal line (Figure 5b) and a Q-Q plot that follows the line for the beginning of the curve (as shown in Figure 5c).

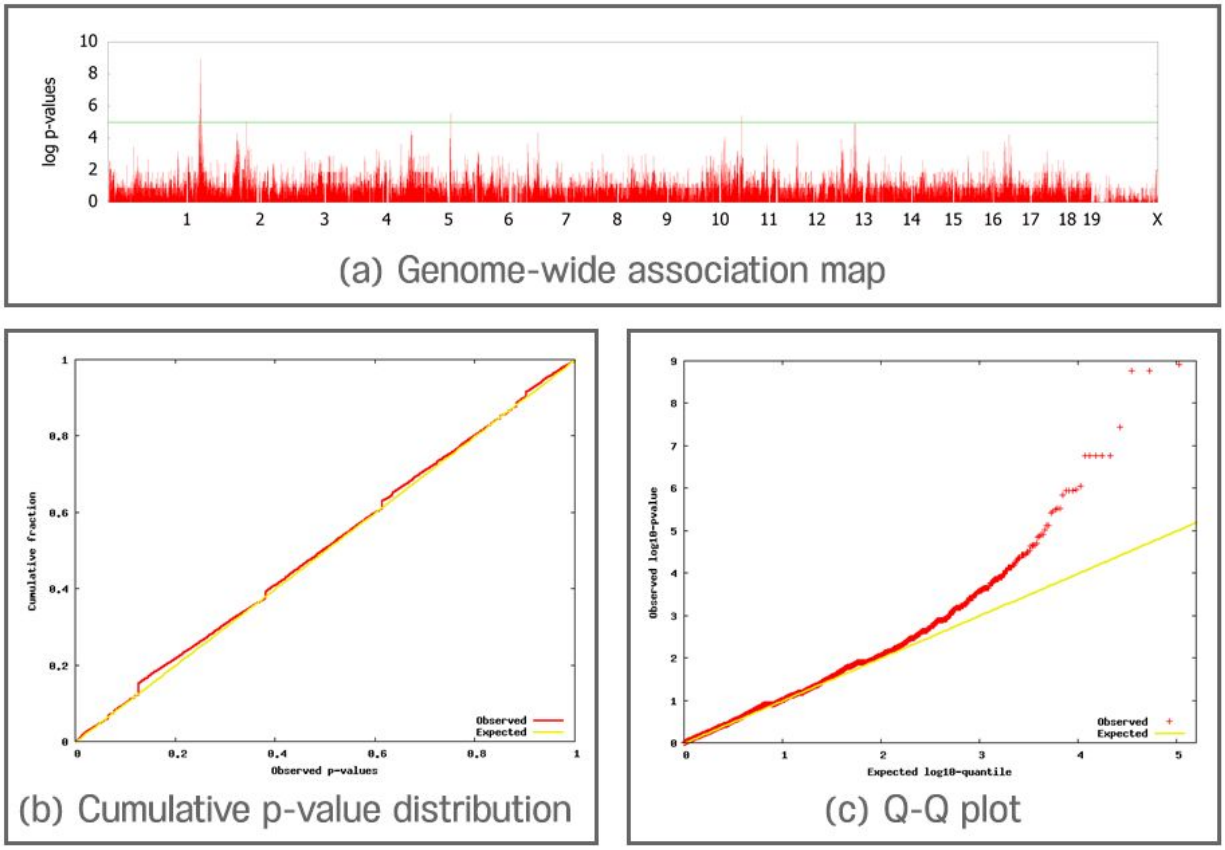

Figure 5. Expected distribution in a typical (a) Manhattan plot, (b) genome-wide association plot, and (c) Q-Q plot.

However, when we applied standard linear models to the inbred mouse dataset, we observed strong signals in many locations in the genome (Figure 6a). The cumulative p-value distribution and the Q-Q plots are shown in Figure 6b and 6c. In our results, we observe that nearly 50% of the SNPs are significantly associated with the phenotype. There are far more significant associations (red line) than expected associations (yellow line).

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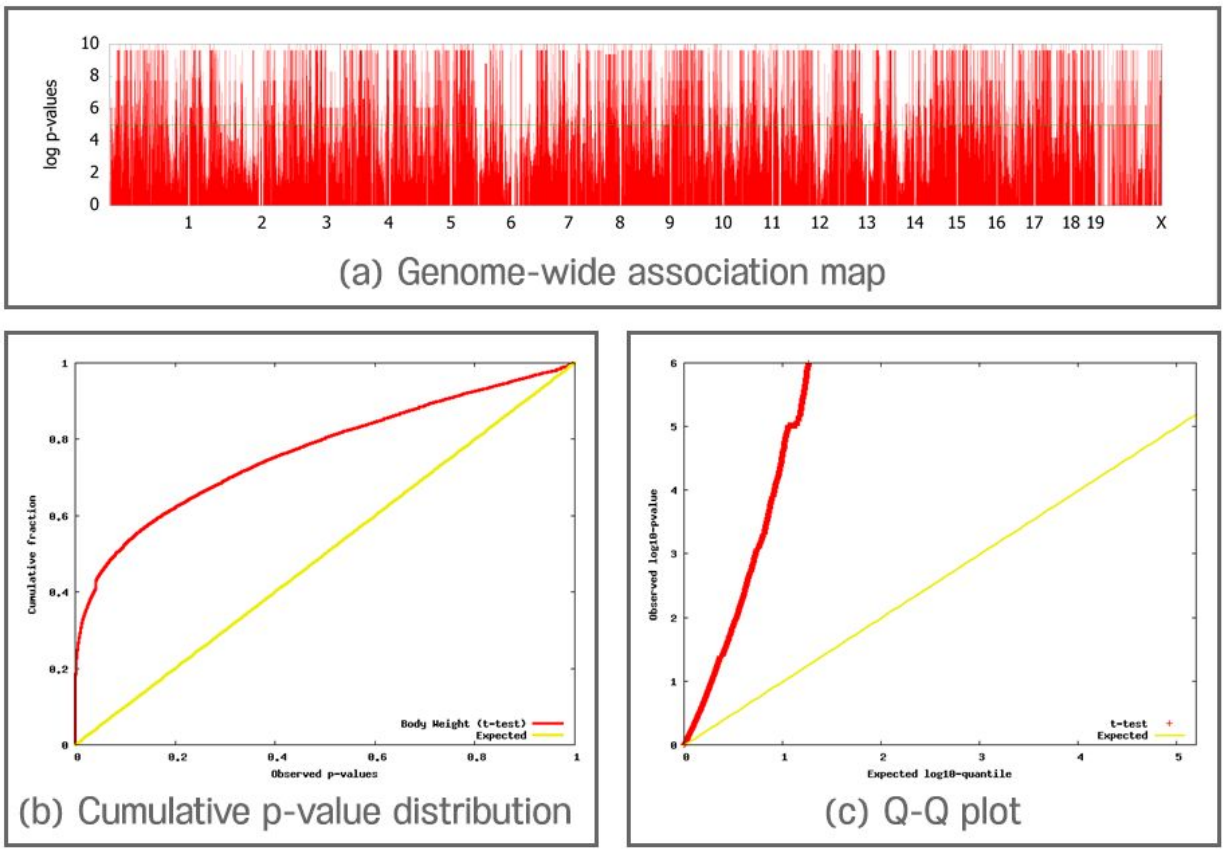

Figure 6. Observed distribution in a (a) Manhattan plot, (b) genome-wide association plot, and (c) Q-Q plot.

Why We Observe False Positives in Mouse Genetic Studies

We can explain why we observe the excess amount of strong association by examining the data for one of the red peaks from the Manhattan plot (Figure 6) in Figure 7a. Here, the big circles are body weight values, and the small circles are genome-wide SNPs. When we look at the distribution of body weight values and SNPs, it appears that green SNPs correspond to mice with small body weight and pink SNPs correspond to mice with heavy body weight. Clearly there is a very strong correlation between the SNP and the body weight and it is no surprise that we observe a very significant p-value.

However, if we lay the phylogenetic tree over the pattern of SNPs and body weight values, we see that the separation of the population into classical and wild derived strains is strongly correlated with the body weight and the SNP differentiates these two groups. The length of each branch in the tree corresponds to the amount of genetic differences between the two groups separated by the branch. The long branch length between the two classical and wild strains signifies that there are many SNPs that separate these two groups and each of them has a

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strong signal. This correlation between groups causes the large amount of observed associations.

Clearly there are genetic differences between these two groups that affect body weight, but not every genetic difference between the two groups affects body weight. However, the simple linear model will associate every SNP that separates these two groups with body weight. Thus, most of the associations that we observe are for SNPs that are not actually affecting body weight. These associations are referred to as spurious associations.

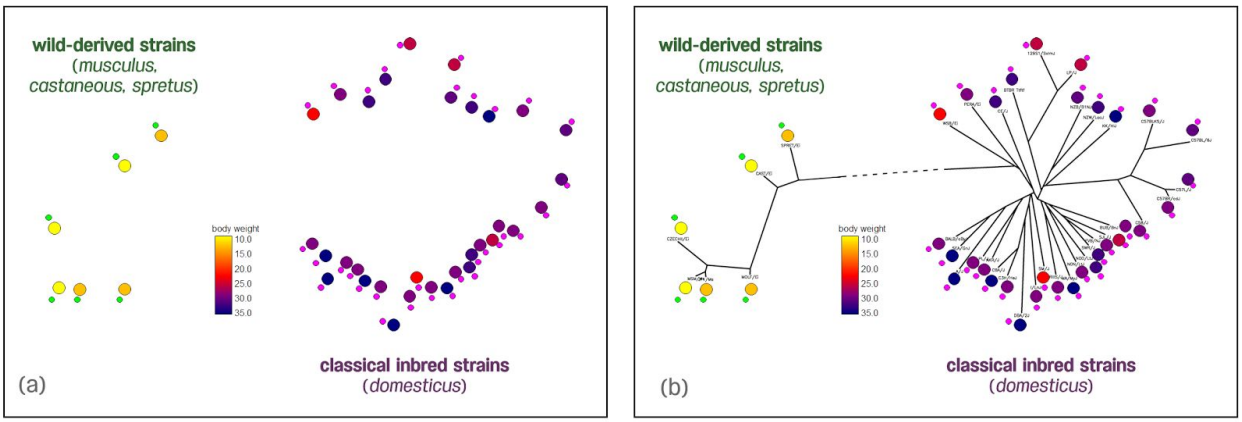

Figure 7. Body weight phenotypes of 38 inbred mouse strains from the Mouse Phenome Database. (a) When we look at only SNP distribution, it appears that green SNPs correspond to small mice and purple SNPs correspond to large mice. (b) When we look at SNP distribution and phylogeny together, we see that many SNPs segregate two clades due to a long, shared breeding history.

Another way to understand the effect of population structure on association is through graphical models. We consider SNPs and traits in Figure 8a. In general, we will perform an association test on a SNP. If we observe an association, this gives evidence that the SNP affects the trait. On the other hand, if we don't observe the association, this suggests that either the SNP does not affect the trait, or that the effect is too small for our study to detect. However, if there is population structure present (Figure 8b), there will be many SNPs directly correlated with population structure (straight dark line) due to shared histories. In addition, phenotypes, such as body weight, are also highly correlated with the population structure (straight dark line). This will induce correlation between many SNPs and the phenotype (dotted line) including but not limited to the SNPs that are actually responsible for variants.

This phenomenon of association due to relatedness is exactly related to Equation (3). Here, the genetic history shared between mouse strains is the unmodeled factor $\sum_{i \neq k} \beta_i x_i$. Since the

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shared genetic history is missing from the testing model, we consider population structure the unmodeled factor.

Using Mixed Model Methods to in Mouse Association Studies

We have shown that population structure can bias association study results. Our mouse examples show that we must correct for population structure in order to accurately identify specific genetic variants involved in disease risk. Several challenges presently limit usefulness of genome association studies for implicating genetic variants. First, unmodeled factors are not known and cannot be accounted for in computational methods that match traits with phenotypes. Second, we do not know the exact ways that unmodeled factors interact with population structure to bias output. Finally, many studies ignore dependency among these unmodeled factors.

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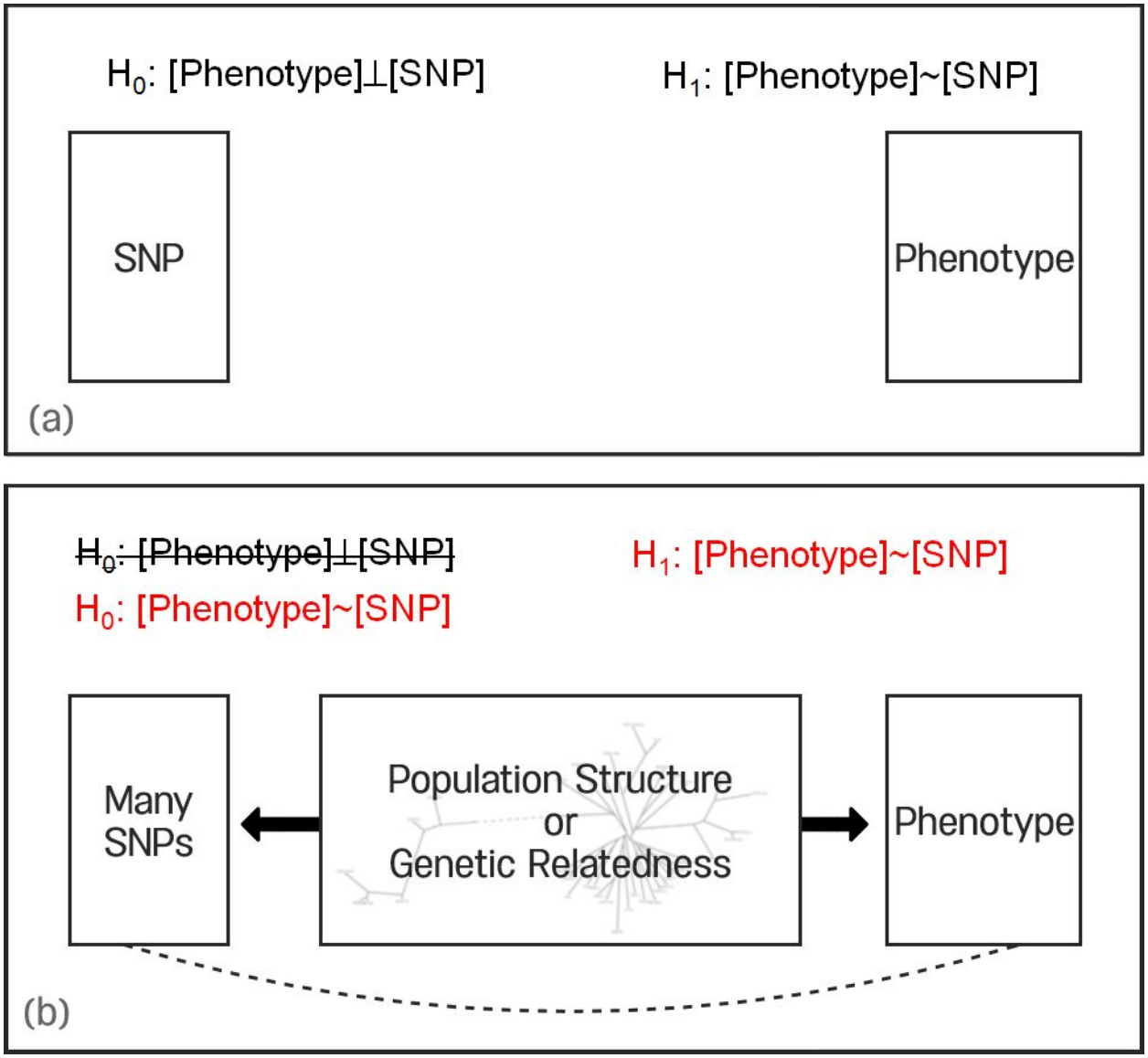

Figure 8. (a) The SNP and Phenotype are independent under the null hypothesis (H_0) and correlated under the alternative hypothesis (H_1). (b) In the case of population structure, the structure will influence many SNPs and the phenotype. In this case, correlation between SNPs and the phenotype will be induced in both the null and alternate hypothesis.

The effects of these SNPs are the unmodeled factor in the equation shown in equation (3) and they confound our ability to perform association studies. There are many SNPs that lie on the long branching line (Figure 7, dashed line) and affect the phenotype. While we cannot know which specific SNPs comprise the unmodeled factor, we can use available knowledge about similarities between the genomes of individuals in our studies to estimate the unmodeled factor.

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TRUE MODEL

$$\mathbf{y} = \mu + X_k\beta_k + \underbrace{\sum_{i \neq k} X_i\beta_i}_{\text{UNMODELED FACTORS}} + \mathbf{e}$$

Strain	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
B6	A	C	C	G	T	A	A	G	C	T
CAST	T	G	T	C	A	C	A	A	T	G
CAUSAL SNPS		↑		↑		↑		↑		↑

(a)

Strain	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
B6	A	C	C	G	T	A	A	G	C	T
C3H	A	C	C	G	A	A	A	G	C	T
DBA	A	C	C	G	A	A	T	G	T	T
129S1	A	G	C	G	T	C	T	G	C	T
CAST	T	G	T	C	A	C	A	A	T	G

(b)

Figure 9. In a true model of association, the unmodeled factors cannot be known. We can estimate the unmodeled factors when (a) genomes are similar or (b) genomes share few causal variants.

Using our mouse example, we consider two different strains, B6 and C3H. These two strains are both classical inbred mice derived from domesticated mice and have similar genomes. In Figure 9a, we show a toy example considering the genomes of the two strains. Here, the genomes are very similar; nine out of ten SNPs are shared between B6 and C3H. In our example, let us assume that the even numbered SNPs are causal variants that affect the phenotype. For those variants, their corresponding effect size (β_i) will be non-zero. We neither know the actual effect sizes nor the resulting value for the unmodeled factor. However, because they share the same allele as these SNPs, we do know that the two strains will have a similar value for the unmodeled factor.

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Next, we consider two very different strains pairwise (Figure 9b): the classic inbred mouse strain B6 and the wild mouse strain CAST. In this case, the strains have different alleles present at many SNPs. If any of these SNPs affect the trait, the value of the unmodeled factor will differ by the effect size. Thus, we expect the two strains to have different values for the unmodeled factor.

The amount of pairwise sharing of alleles between strains can be used to capture the similarity between the values of the unmodeled factor among strains. In order to do this, we make a matrix that contains all SNPs shared between the paired genomes (Figure 10). This matrix allows us to "model" the values of the unmodeled factors among the individuals in our study, and it shows us which pairs have similar sharing of alleles and which pairs have dissimilar values.

The principle underlying mixed models is that we incorporate this "model" of unmodeled factors into the association test. We incorporate the unknown factors into the model of association using what is called a "random effect" or a variance component. Our model is called a "mixed model," because it combines a random effect to model population structure with the effect sizes of the SNPs we are testing (referred to as "fixed effects").

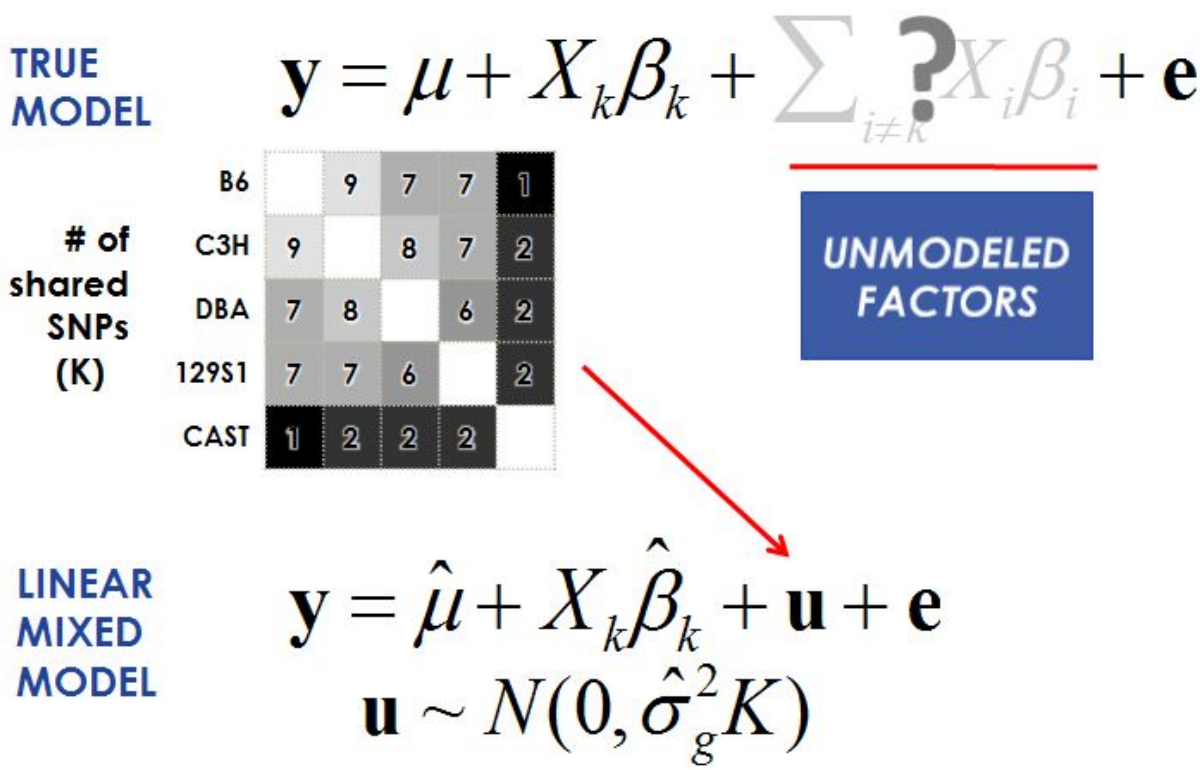

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Figure 10. The true model ignores dependency among unmodeled factors and produces false associations. The mixed model reduces false associations by accounting for the dependency among SNPs correlated with phenotypes due to population structure.

One key step in using a mixed model to identify causal variation is to establish these fixed parameters and random effect components. A linear mixed model (LMM) uses the information from the matrix to account for the unmodeled factor. We extend the simple, hypothetical true model

$$y = \mu 1 + \beta_k X_k + e$$

to include a term that captures the unmodeled factors. The term u in

$$y = \mu 1 + \beta_k X_k + u + e$$

is a random vector that depends on the amount of shared genome in terms of pairwise differences. In practice this can be computed from the genotypes using the equation $K = XX^T/N$ and we assume that $u \sim N(0, \sigma^2 K)$. Each entry of K estimates the pairwise similarity between the genomes of the individuals in the study which follows the intuition of Figures 9 and 10.

The standard estimation equations above cannot be used to estimate the values of the parameters because due to the random effect u, the phenotypes of the individuals are no longer independent of each other which is an assumption of the previous methods.

However, if we know the values of σ_g^2 and σ_e^2, we can then apply the following "mixed model trick". We note that the phenotypes will follow the distribution

$$y \sim N(\mu + \sum \beta_i X_i, V)$$

where $V = \sigma_g^2 K + \sigma_e^2 I$. If we transform the then multiply the phenotypes and genotypes by $V^{-\frac{1}{2}}$, we then get

$$V^{-\frac{1}{2}} y \sim N(V^{-\frac{1}{2}} 1\mu + \sum \beta V^{-\frac{1}{2}} X_i, I).$$

In the transformed data, the individuals are now independent of each other and we can apply the estimation equations presented above to estimate the values for β and the association statistics.

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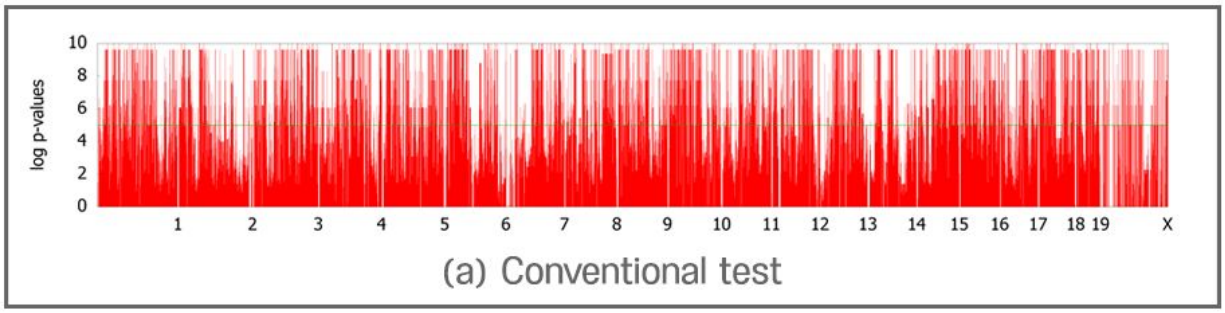
In this case, we assume that the β_i values are drawn from a normal distribution with a mean zero as effect size and σ_e^2 as the variance.

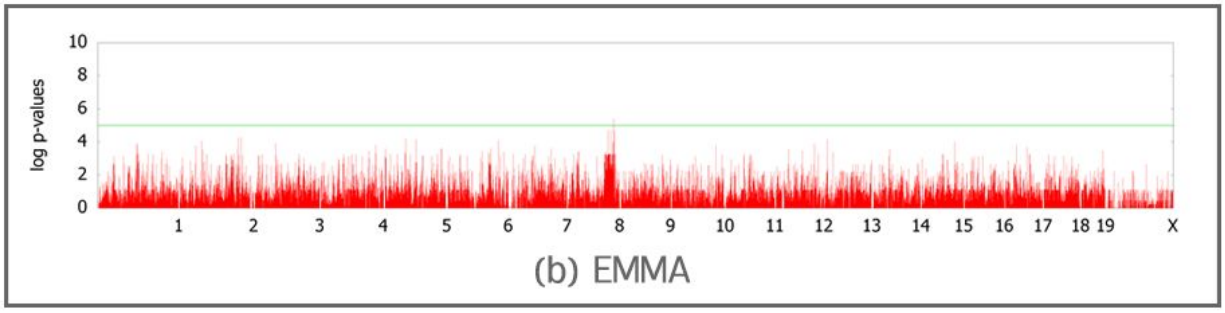
Estimating the values of σ_g^2 and σ_e^2 is a difficult computational problem referred to as estimating the variance components. We developed Efficient Mixed Model Association (Kang et al. 2008), an efficient algorithm for estimating these parameters. Since we first presented EMMA, many other groups have developed similar efficient algorithms (Kang et al. 2010; Lippert et al. 2011; Zhou and Stephens 2012).

We applied EMMA to the same mouse association data analyzed using a standard LMM approach (see Figure 6). With these computational improvements, we almost completely reduced the inflation of false positives and obtained nearly uniform p-value distribution for most SNPs (Figure 11). Here, the strongest peak, which is not significant, falls into a region of the genome on chromosome 8 which is known to be associated with body weight. These regions are referred to as Quantitative Trait Loci (QTL).

We also applied EMMA to other phenotypes from the same mouse strain datasets including a liver weight phenotype. Here, we see that the inflation of false positives is reduced and a strong signal at chr2 is more pronounced after the correction (Figure 12). Here, EMMA correctly identifies the QTLs for Lvrq1 (liver weight), Orgwq2 (organ weight), Splq1 (spleen weight), Hrtq1 (heart weight), Lbm1 (lean body mass). These SNPs are not correlated with population structure, and correcting for the background population structure helps the mixed model correctly prioritize signal strength. Studies have since revealed that the chr2 region falls into known QTLs for liver weight (Rocha et. al. 2004).



(a) Conventional test



(b) EMMA

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Figure 11. (a) The conventional GWAS test applied to mouse body weight phenotypes produces numerous false positions. (b) The mixed model approach using EMMA almost completely reduced the inflation of false positives and identifies a strong peak (chr8) that falls into a known body weight QTL.

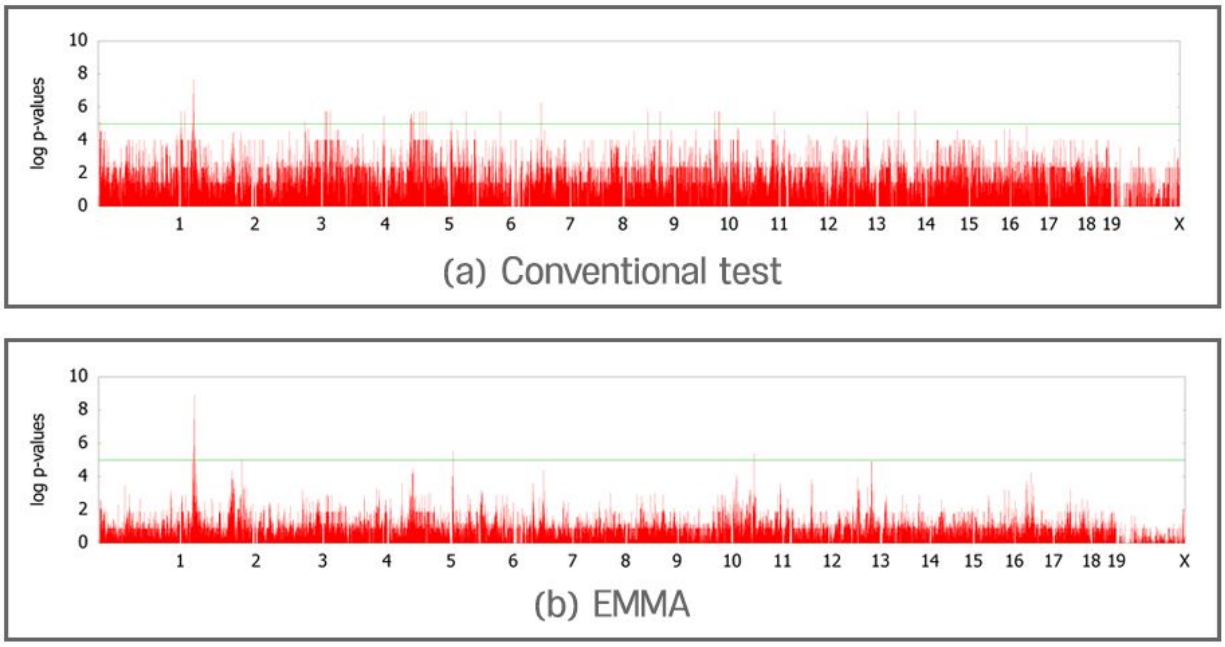

Figure 12. (a) The conventional GWAS test applied to mouse liver weight phenotypes produces numerous false positions. (b) The mixed model approach using EMMA reduces inflation of false positives and correctly produces a stronger signal at chr2, a region that is located in known QTLs for liver weight.

Population Structure and Mixed Models in Human Association Studies

At the time that mixed models were starting to be used in mouse studies, the problem of relatedness in human studies was becoming apparent and was causing difficulties in analyzing human GWAS studies. At that time, there was no single approach to handle relatedness and instead different types of relatedness were explicitly modeled and association study methods were adapted to those scenarios. There is an entire class of methods designed to handle relatedness when there are closely related individuals in the genetic study and the genetic relationships are known. These include methods for multigenerational families, twins, and siblings (Freimer and Sabatti 2004; Van Dongen et al. 2012).

A complication in human association studies is when the relationships are unknown. One of the most common types of relatedness among individuals in human studies is due to ancestry. Ancestry refers to the population that an individual descended from. Many individuals are admixed, which means they are descended from ancestors in different populations. If an

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association study contains individuals from different populations or differing degrees of admixture, the individual will have different degrees of relatedness among them. In other words, individuals with the same ancestry are slightly more related to each other than individuals with different ancestries. It is well documented that these ancestry differences can induce false positive associations (Helgason et al. 2005). Association studies that analyzed individuals with differences in ancestry typically utilized an approach to predict the ancestry for each individual and then incorporated this information as a covariate in the model (Pritchard et al. 2000). An alternate approach was to estimate principal components over the genotype data, which could be interpreted as a proxy for association studies and included in the model as covariates (Price et al. 2006). In the human genetics literature, ancestry differences are sometimes referred to as population structure. In this review, we use the term ancestry differences separately from the term population structure, which we use to describe the general phenomenon of relatedness in a sample.

A second type of relatedness is cryptic relatedness (Voight and Pritchard 2005). Since GWAS are applied to extremely large samples, there are often individuals included in the study who happen to be related--but this relatedness is unknown the both the individuals and the investigators. Cryptic relatedness is typically handled by screening the association study for related individuals and computing the genetic similarity between each pair of individuals.

A general purpose approach to correct for population structure or any type of confounding in association studies is genomic control (Devlin and Roeder 1999; Bacanu et al. 2002). The idea behind genomic control is that we can measure the extent that population structure (or other confounders) is affecting the association statistics by examining the cumulative p-value distribution plot . Specifically, we consider the deviation of the plot from what is expected at the median. Since we expect the vast majority of variants not to be associated with the trait, we expect the median observed p-value to be close to 0.5. Typically, due to population structure the observed median p-value will be more significant. Genomic control computes a correction factor referred to as λ, which is a scaling factor used to scale all of the observed p-values so that the corrected median p-value is then 0.5. The λ is on the χ^2 scale meaning that the median p-value is converted to a χ^2 value and the ratio is computed relative to the χ^2 value corresponding to a p-value of 0.5 which is 0.545. The observed association p-values are converted from p-values to χ^2 statistics, scaled by λ and then converted back to p-values. We can also use the value of the λ as a measure of the extent of the effect of confounding on the association statistics. Genomic control λ's are widely utilized to compare different correction approaches. A λ of 1.0 shows that there is no inflation. A value greater than 1.0 is evidence that there is inflation of the association statistics. Typically the 95% confidence interval of the λ in GWAS studies is 0.02. Thus, any lambda of 1.03 or higher suggests that there is some inflation. We note that more recent thinking about polygenicity or the amount of causal variants for a trait suggest that there are many more causal variants than originally expected and the λ values should actually be higher than 1.0 (Yang et al. 2011). We discuss this perspective in the Discussion.

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While in the literature, ancestry differences and cryptic relatedness are referred to as distinct phenomenon, in fact they can be thought of as different degrees of relatedness in the sample. Consider in Figure 13a which shows a potential pedigree that relates all of the individuals in an association study sample. Ancestry differences can be thought of relatedness near the top of the tree (Figure 13b) and cryptic relatedness can be thought of relatedness in a more recent portion of the tree (Figure 13c).

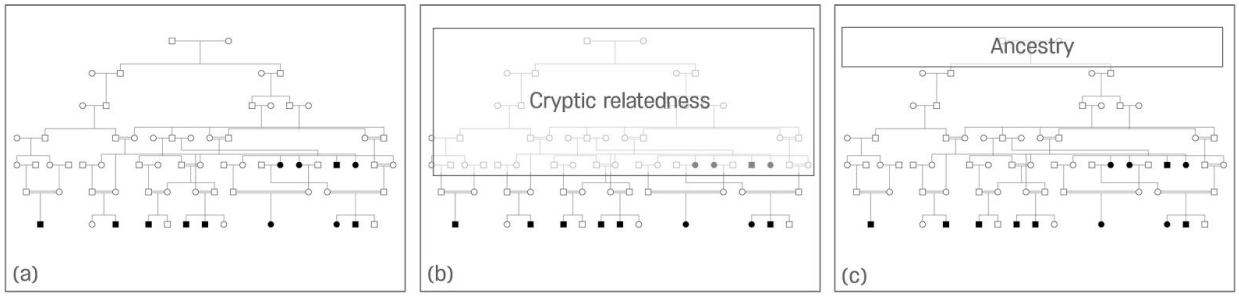

Figure 13. All of the individuals in a genetic study are somehow related through a large pedigree or family tree (a). This tree can produce two forms of hidden relatedness: cryptic relatedness (b) and ancestry (c), where the box represents the level of the pedigree that causes that type of relatedness..

Mixed models can handle nearly arbitrary genetic relationships between individuals and this made them a natural approach to apply to human studies. The advantage of mixed models is that they could be applied without needing to explicitly identify the ancestry and relatedness within the sample. They also enabled the analysis of datasets with particularly complex genetic relationships such as isolate populations where the population is descended from a small number of founder individuals (Kenny et al. 2010). For isolate populations, the previous methods were not able to fully account for the population structure.

In human studies, mixed models were first applied to the Northern Finnish Birth Cohort (Sabatti et al. 2009) where they were applied to 331,475 SNPs in 5,326 individuals who were phenotypes for 10 traits (Kang et al. 2010). These traits include C-reactive protein (CRP), triglyceride (TG), insulin plasma levels, (INS), diastolic blood pressure (DBP), body mass index (BMI), glucose (GLU), high-density lipoprotein (HDL), systolic blood pressure (SBP) and low density lipoprotein A (LDL). Individuals within this cohort both have some ancestry differences due to their origin from different parts of Finland as well has having genetic relationships between them.

Table 1 shows the results of mixed models on the traits. Each entry in the table shows the λ value for the analysis of that phenotype. The first column shows the results of the uncorrected analysis. We can see that there are very large λ factors, particularly for height. In fact, the associations with height were not reported in the original Sabatti et al. (2009) manuscript

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because of this reason. The second column shows the λ factors after eliminating cryptically related individuals. This was done by computing the pairwise relationships between individuals and filtering out one of any pair that was closely related. This filter resulted in filtering out 611 individuals. The third column shows the λ factors after using 100 principal components as covariates. Each component included decreases the λ and using 100 components is an absurdly large number of components, well beyond what is typically utilized in any type of association study. This was done to show the limit of the principal component approach in correcting for population structure. The last column shows the λ for mixed models. Each of these λ values are within the 95% confidence interval around 1.0 suggesting the mixed models can correct for all of the population structure including cryptic relatedness and ancestry differences in the sample. As shown in Table 1, only mixed models adequately correct for population structure in this sample.

Table 1. Results of analysis (λ values) on NFBC66 data.

Traits	Uncorrected	IBD<0.1	100PC	EMMAX
BMI	1.036	1.028	1.024	1.001
CRP	1.012	1.020	1.020	0.994
DBP	1.033	1.025	1.029	1.010
GLU	1.045	1.025	1.030	1.009
HDL	1.054	1.041	1.037	1.003
INS	1.026	1.026	1.015	1.005
LDL	1.093	1.089	1.040	1.002
SBP	1.063	1.054	1.021	1.004
TG	1.024	1.021	1.018	0.999
HEIGHT	1.193	1.152	1.080	1.002

Mixed models became important in human GWAS analysis because the estimates of σ_g^2 and σ_e^2 can be used to estimate the heritability of the trait which suggested that common variants explain a large proportion of the variance of complex traits than previously thought(Purcell et al. 2009; Yang et al. 2010; Eskin 2015).

Discussion

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Over the past decade, association studies have identified thousands of variants implicated in dozens of common human diseases. The traditional approach to association studies assumes that individuals are unrelated to each other. However, in practice, individuals in genetic studies are related to each other in complex ways. In this review, we demonstrate how these relationships cause false positives in the association studies and how mixed models can correct for these confounding genetic relationships.

This review covers only the basic principles of mixed models and population structure. Since the original EMMA paper in 2008, mixed models have become an active research area. Many groups have published papers exploring various aspects of mixed models and their application to complex genomic problems.

Many approaches have been developed to improve the efficiency of mixed models, including the methods Fast-LMM (Lippert et al. 2011) and GEMMA (Zhou and Stephens 2012). More recently, a method called BOLT-LMM (Loh et al. 2015) was developed for scaling analyses to handle cohorts in the hundreds of thousands of individuals.

Another direction of method development has been extending mixed models to handle case control studies. These approaches typically assume a liability threshold model where there is an underlying continuous phenotype; if the phenotype is above a threshold, the individual has a disease and if it is below, the individual does not have the disease (Zaitlen et al. 2012). These types of studies are also complicated by a phenomenon of selection bias, because the cases are oversampled from the population. At present, such mixed model extensions to case/control setting result in challenging computational problems (Hayeck et al. 2015; Weissbrod et al., 2015).

Some mixed models are developed based on observation of a particular bias inherent to standard approaches. Namely, that the SNP being tested was used in the computation of the kinship matrices (Listgarten et al. 2012). This bias motivated the idea that, when applying mixed models, the kinship matrix should not contain the SNP being tested. As a result, the Leave One Chromosome Out (LOCO) approach constructs a different kinship matrix for testing each chromosome, leaving out the SNPs on the chromosome being tested (Yang et al. 2014). This approach is also motivated by the observation that many complex traits are highly polygenic, suggesting that there are hundreds (if not thousands) of loci that influence some traits (Yang et al. 2011). Some traits, such as height, are known to be highly polygenic. In this case, it is not clear what the actual value of λ should be for a polygenic trait as it is expected to have a contribution from both confounding effects as well as polygenicity. More recently, a method called LD score regression has been developed that attempts to differentiate between these two components (Bulik-Sullivan et al. 2015).

From their origins in non-human organisms to powering large scale human genome wide association studies today, mixed models play an important role in the analysis of genetic data,

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particularly in correcting for population structure. Research in improving and extending mixed model approaches is now an active research area in the field.

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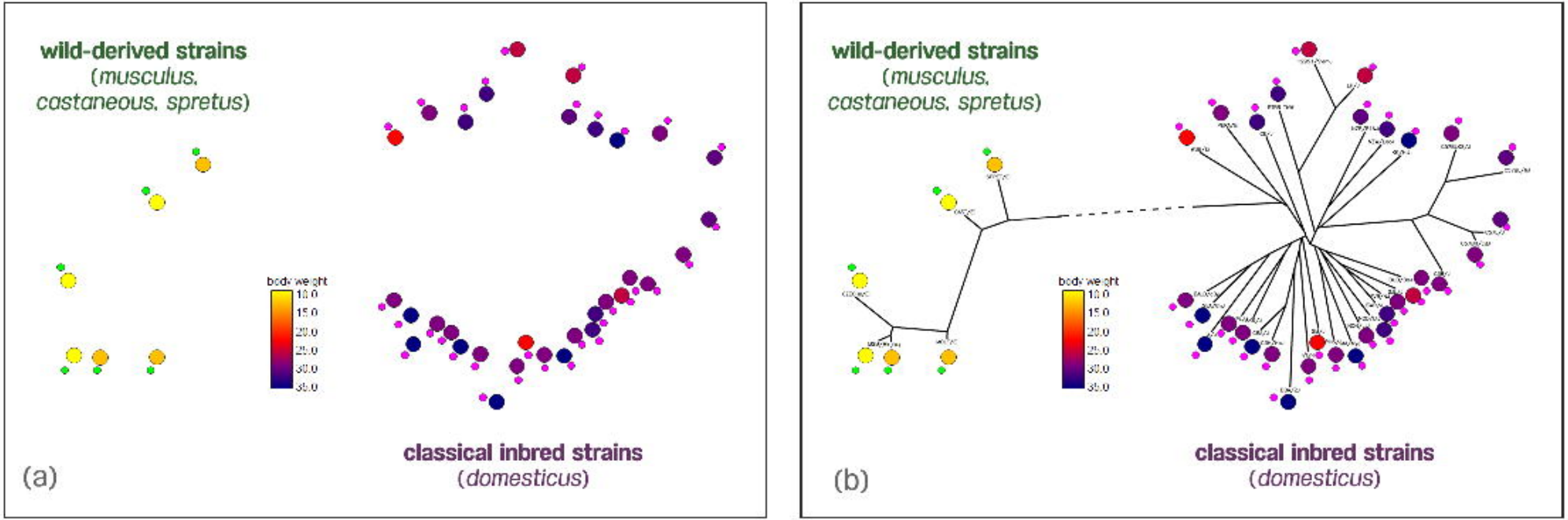
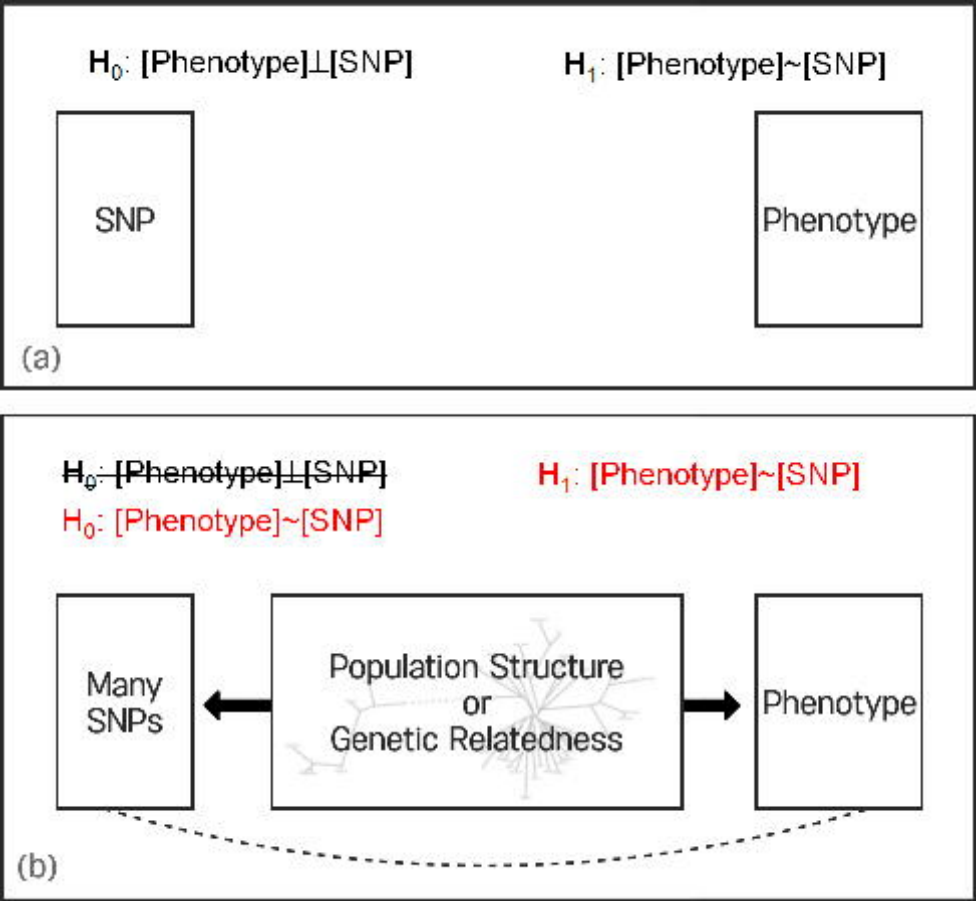

wild-derived strains
(musculus,
castaneous, spretus)
body weight
10.0
15.0
20.0
25.0
30.0
35.0
classical inbred strains
(domesticus)
(a)
wild-derived strains
(musculus,
castaneous, spretus)
body weight
10.0
15.0
20.0
25.0
30.0
35.0
classical inbred strains
(domesticus)
(b)


H0: [Phenotype]⊥[SNP]
H1: [Phenotype]~[SNP]
SNP
Phenotype
(a)
H0: [Phenotype]⊥[SNP]
H1: [Phenotype]~[SNP]
H0: [Phenotype]~[SNP]
Many SNPs
Population Structure or Genetic Relatedness
Phenotype
(b)

TRUE MODEL

$$\mathbf{y} = \mu + X_k\beta_k + \underbrace{\sum_{i/k} X_i\beta_i}_{\text{UNMODELED FACTORS}} + \mathbf{e}$$

Strain	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
B6	A	C	C	G	T	A	A	G	C	T
CAST	T	G	T	C	A	C	A	A	T	G
CAUSAL SNPS		↑		↑		↑		↑		↑

(a)

Strain	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
B6	A	C	C	G	T	A	A	G	C	T
C3H	A	C	C	G	A	A	A	G	C	T
DBA	A	C	C	G	A	A	T	G	T	T
129S1	A	G	C	G	T	C	T	G	C	T
CAST	T	G	T	C	A	C	A	A	T	G

(b)

TRUE MODEL

$$\mathbf{y} = \mu + X_k\beta_k + \sum_{i \neq k} ? X_i\beta_i + \mathbf{e}$$

UNMODELED FACTORS

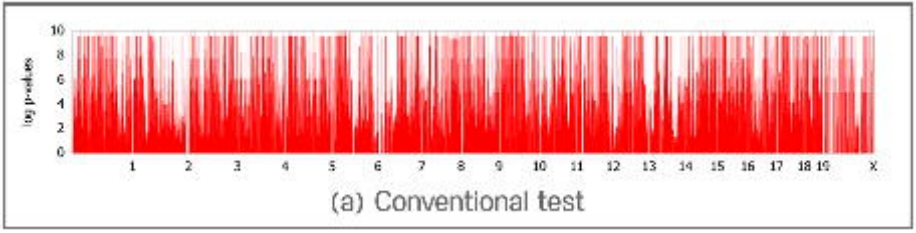
# of shared SNPs (K)

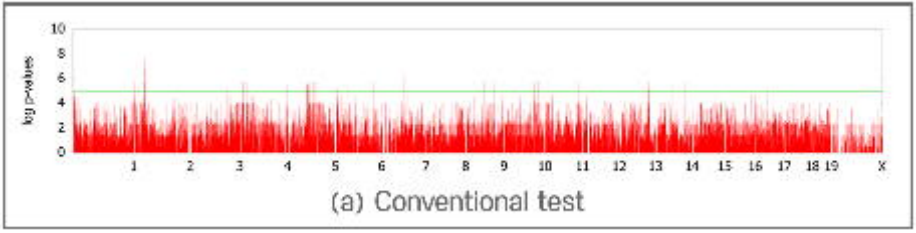
	B6	C3H	DBA	129S1	CAST
B6		9	7	7	1
C3H	9		8	7	2
DBA	7	8		6	2
129S1	7	7	6		2
CAST	1	2	2	2	

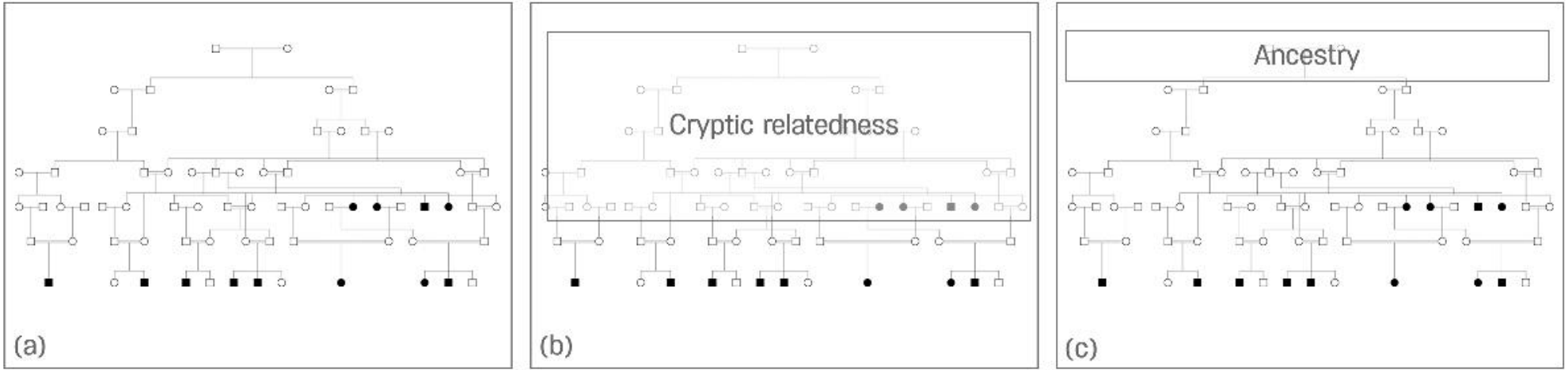
LINEAR MIXED MODEL

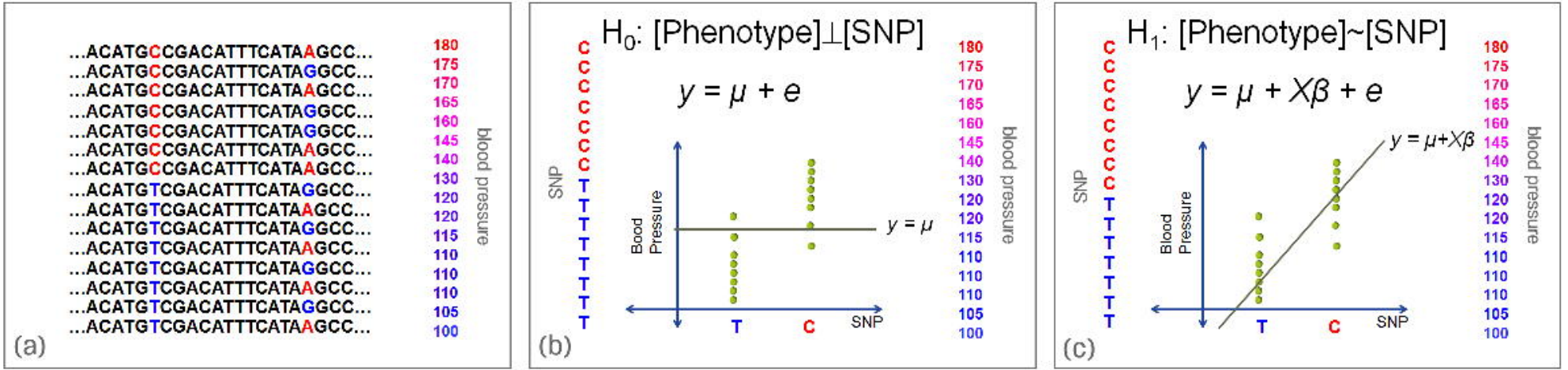
$$\mathbf{y} = \hat{\mu} + X_k\hat{\beta}_k + \mathbf{u} + \mathbf{e}$$

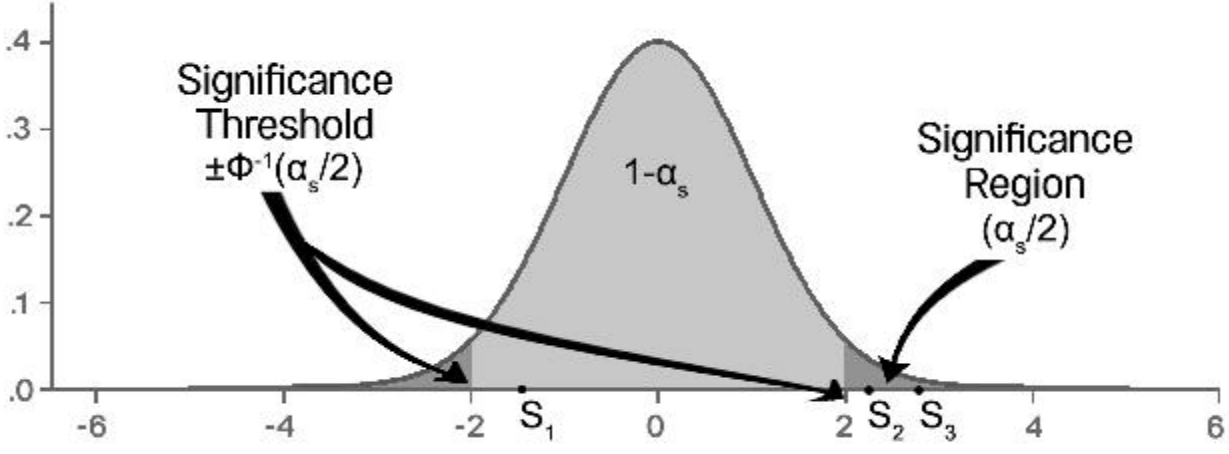
$$\mathbf{u} \sim N(0, \hat{\sigma}_g^2 K)$$

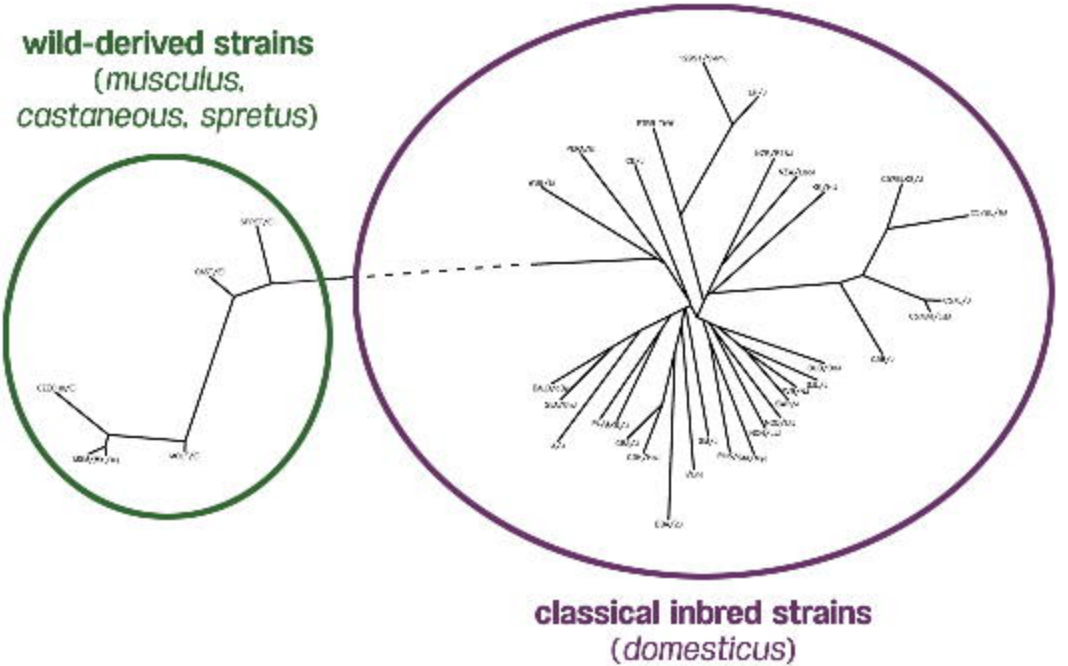

log p-values
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
(a) Conventional test
log p-values
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
(b) EMMA

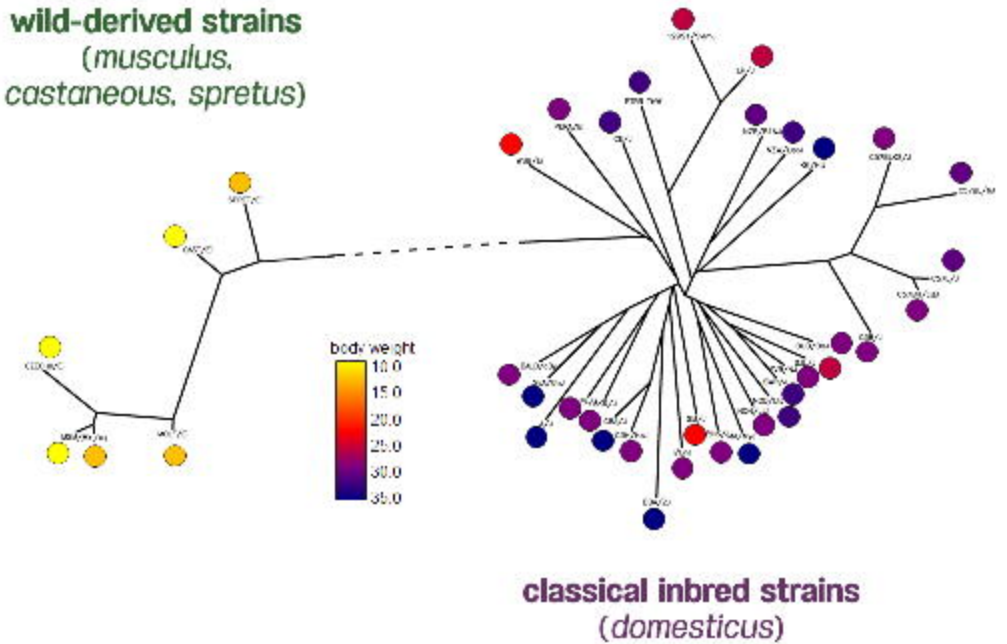

log p-values
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
(a) Conventional test
log p-values
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
(b) EMMA


Cryptic relatedness
Ancestry
(a)
(b)
(c)


...ACATGCCGACATTTCATAAGCC... 180
...ACATGCCGACATTTCATAGGCC... 175
...ACATGCCGACATTTCATAAGCC... 170
...ACATGCCGACATTTCATAGGCC... 165
...ACATGCCGACATTTCATAGGCC... 160
...ACATGCCGACATTTCATAAGCC... 145
...ACATGCCGACATTTCATAAGCC... 140
...ACATGTCGACATTTCATAGGCC... 130
...ACATGTCGACATTTCATAAGCC... 120
...ACATGTCGACATTTCATAGGCC... 120
...ACATGTCGACATTTCATAAGCC... 115
...ACATGTCGACATTTCATAGGCC... 110
...ACATGTCGACATTTCATAAGCC... 110
...ACATGTCGACATTTCATAGGCC... 110
...ACATGTCGACATTTCATAAGCC... 105
...ACATGTCGACATTTCATAAGCC... 100
blood pressure
(a)
H0: [Phenotype]⊥[SNP]
y = μ + e
SNP
Bood Pressure
y = μ
T
C
SNP
blood pressure
(b)
H1: [Phenotype]~[SNP]
y = μ + Xβ + e
SNP
Blood Pressure
y = μ+Xβ
T
C
SNP
blood pressure
(c)


Significance
Threshold
$\pm\Phi^{-1}(\alpha_s/2)$
$1-\alpha_s$
Significance
Region
$(\alpha_s/2)$
.4
.3
.2
.1
.0
-6
-4
-2
S_1
0
2
S_2
S_3
4
6


wild-derived strains
(musculus,
castaneous, spretus)
classical inbred strains
(domesticus)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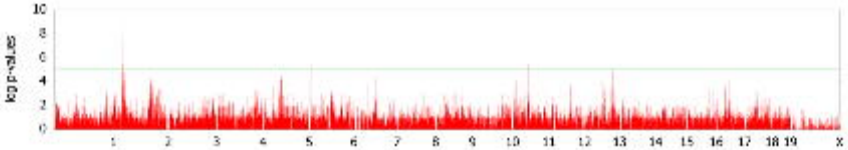
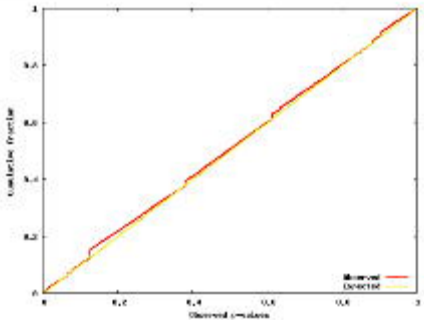
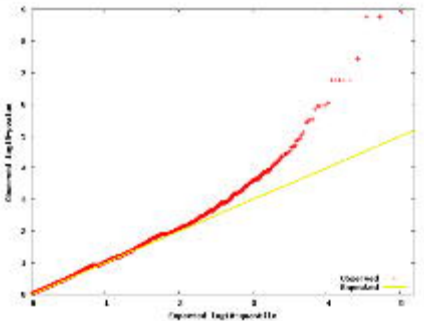


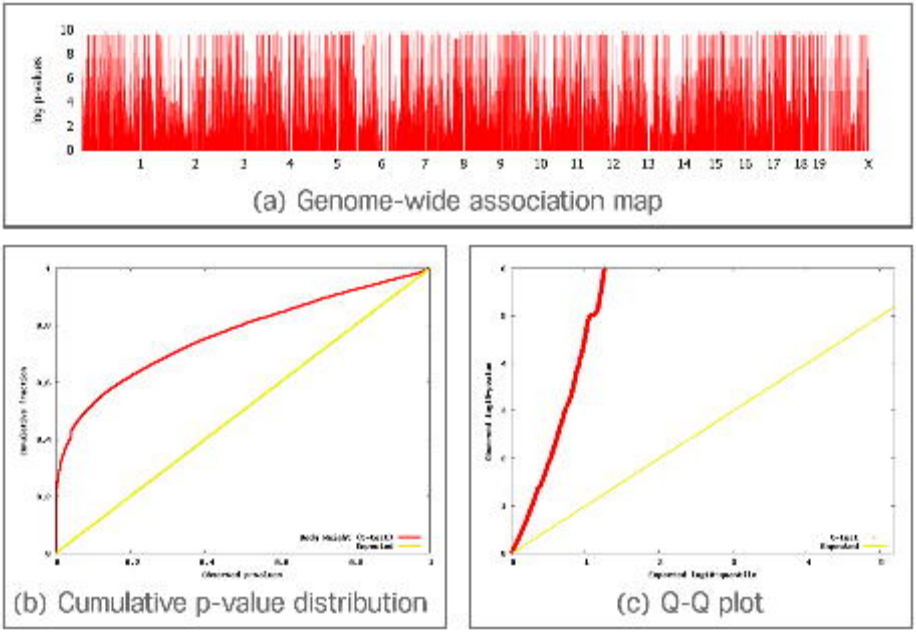
(a) Genome-wide association map



(b) Cumulative p-value distribution



(c) Q-Q plot



(a) Genome-wide association map

(b) Cumulative p-value distribution

(c) Q-Q plot