

ASSOCIATION MAPPING: A (VERY) BRIEF OVERVIEW

Introduction

One approach to understanding more about the genetics of quantitative traits takes advantage of the increasing number of genetic markers available as a result of recent advances in molecular genetics. Suppose you have two inbred lines that differ in a trait that interests you, say body weight or leaf width. Call one of them the “high” line and the other the “low” line.¹ Further suppose that you have a whole bunch of molecular markers that differ between the two lines, and designate the genotype in the “high” line A_1A_1 and the genotype in the low line A_2A_2 .² One last supposition: Suppose that at loci influencing the phenotype you’re studying the genotype in the “high” line is Q_1Q_1 and the genotype in the “low” line is Q_2Q_2 . Each of these loci is what we call a *quantitative trait locus* or QTL. Now do the following experiment:

- Cross the “high” line and the “low” line to construct an F_1 .
- Intercross individuals in the F_1 generation to form an F_2 .³
- “Walk” through the genome⁴ calculating a likelihood score for a QTL at a particular map position, using what we know about the mathematics of recombination rates and Mendelian genetics. In calculating the likelihood score we maximize the likelihood of the data assuming that there is a QTL at this position and estimating the corresponding additive and dominance effects of the allele. We then identify QTLs as those loci where there are “significant” peaks in the map of likelihood scores.

¹Corresponding to whether the body weight or leaf width is large or small.

²Since these are inbred lines, I can assume that they are homozygous at the marker loci I’ve chosen.

³Note: You could also backcross to either or both of the parental inbred lines. Producing an F_2 , however, allows you to estimate both the additive and dominance effects associated with each QTL.

⁴I forgot to mention another supposition. I am supposing that you either have already constructed a genetic map using your markers, or that you will construct a genetic map using segregation in the F_2 before you start looking for QTL loci.

The result is a genetic map showing where QTLs are in the genome and indicating the magnitude of their additive and dominance effects.

QTL mapping is wonderful—provided that you’re working with an organism where it’s possible to design a breeding program and where the information derived from that breeding program is relevant to variation in natural populations. Think about it. If we do a QTL analysis based on segregation in an F_2 population derived from two inbred lines, all we really know is which loci are associated with phenotypic differences *between those two lines*. Typically what we really want to know, if we’re evolutionary biologists, is which loci are associated with phenotypic differences *between individuals in the population we’re studying*. That’s where association mapping comes in. We look for statistical associations between phenotypes and genotypes across a whole population. We expect there to be such associations, if we have a dense enough map, because some of our marker loci will be closely linked to loci responsible for phenotypic variation.

Association mapping

So how does association mapping work? There are two broad approaches, one that is used in genome-wide association studies (GWAS) that is analogous to QTL mapping and one that looks for differences between “cases,” those that exhibit a particular phenotype of interest (e.g., a disease state in humans), and “controls,” those that don’t exhibit the phenotype of interest. Let’s talk about GWAS first.

Genome-wide association study

Principles

Imagine that we have a well-mixed population segregating both for a lot of molecular markers spread throughout the genome and for loci influencing a trait we’re interested in, like body weight or leaf width. Let’s call our measurement of that trait z_i in the i th individual. Let x_{ij} be the genotype of individual i at the j th locus.⁵ Then to do association mapping, we simply fit the following regression model:

$$y_i = x_{ij}\beta_j + \epsilon_{ij} \quad ,$$

where ϵ_{ij} is the residual error in our regression estimate and β_j is our estimate of the effect of substituting one allele for another at locus j , i.e., the additive effect of an allele at locus

⁵To keep things simple I’m assuming that we’re dealing with biallelic loci, e.g., SNPs, and we can then order the genotypes as 0, 1, 2 depending on how many copies of the most frequent allele they carry. So x_{ij} is the number of copies of A_1 individual i carries at locus j .

j .⁶ If β_j is significantly different from 0, we have evidence that there is a locus linked to this marker that influences the phenotype we're interested in, and we have an estimate of the additive effect of the alleles at that locus.

Notice that I claimed we have evidence that the locus is linked. That's a bit of sleight of hand. I've glossed over something very important. What we have direct evidence for is only that the locus is *associated* with the phenotype differences. As we'll see in just a bit, the observed association *might* reflect physical linkage between the marker locus and a locus influencing the phenotype or it could reflect a statistical association that arises for other reasons, including population structure. So in practice the regression model we fit is a more complicated than the one I just described. The simplest case is when individuals fall into obvious groups, e.g., samples from different populations. Then $y_i^{(k)}$ is the trait value for individual i . The superscript (k) indicates that this individual belongs to group k .

$$y_i^{(k)} = x_{ij}\beta_j + \phi^{(k)} + \epsilon_{ij} \quad .$$

The difference between this model and the one above is that we include a random effect of group, $\phi^{(k)}$, to account for the fact that individuals may have similar phenotypes not because of similarity in genotypes at loci close to those we've scored but because of their similarity at other loci that differ among groups. More generally, the model looks like

$$y_i = x_{ij}\beta_j + \phi_i + \epsilon_{ij} \quad .$$

where ϕ_i is an individual random effect where the correlation between ϕ_i and ϕ_j for individuals i and j , i.e., ρ_{ij} , is determined by how closely related they are. The degree of relationship might be inferred from a pedigree, if one is known, or from coefficients of kinship estimated from a large suite of genetic markers.

An example: warfarin maintenance dose

Shortly after World War II, warfarin was introduced for use as a rat poison. By the mid-1950s it was approved for medical use in the United States as a treatment for diseases in which blood clotting caused a significant threat of stroke. It is still in common use as a treatment for atrial fibrillation.⁷ Currently, determining the appropriate dose is done by closely monitoring the degree of anticoagulation, an INR of 2.5 ± 0.5 (<https://www.drugs.com/dosage/warfarin.html>). In an effort to identify genetic markers that could be used to choose an appropriate dosage, investigators at the University of Washington

⁶We can generalize the regression to allow us to estimate dominance effects too, but doing so only complicates the algebra without providing any additional insight.

⁷As it happens, my father took warfarin for more than 20 years before his death.

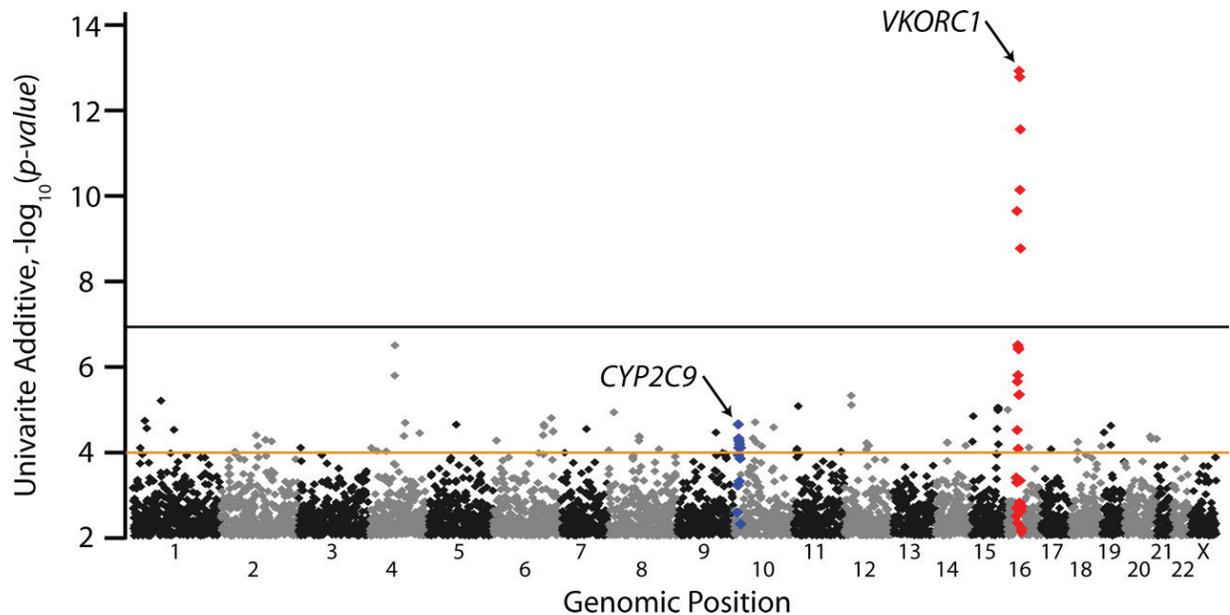


Figure 1: P -values from a genome-wide analysis of the association between SNP genotype and warfarin dose. The black line is the genome-wide level for statistical significance, 10^{-7} , and the brown line is the level, 10^{-4} at which SNPs identified in the index population were investigated in replicate populations (from [1]).

studied the relationship between the dose of warfarin patients were receiving and their genotype at 550,000 SNP loci [1].⁸ They identified two loci, *VKORC1* and *CYP2C9*, that were consistently associated with warfarin dose. *VKORC1* encodes the vitamin K epoxide reductase complex 1 enzyme, and *CYP2C9* encodes a cytochrome P450 (Figure 1). Differences at *VKORC1* account for approximately 25% of the variance in stabilized dose.⁹

⁸They log transformed warfarin dose (measured in milligrams per day) before the analysis.

⁹If you remember a little human physiology, vitamin K may ring a bell. “The name vitamin K comes from the German word ‘Koagulationsvitamin.’” (<https://www.webmd.com/vitamins/ai/ingredientmono-983/vitamin-k>, accessed 19 January 2019). Vitamin K plays an important role in blood clotting, so it makes sense that a locus encoding an enzyme related to vitamin K metabolism would have a strong association with the dose of warfarin needed to safely reduce blood clotting.

Case-control study

The GWAS approach I just described works well if the trait we're studying is continuous,¹⁰ but what do we do if the trait we're interested occurs in only two states, e.g., diseased vs. healthy? Let's suppose we have a set of "candidate" loci, i.e., loci that we have some reason to suspect might be related to expression of the trait. Now let's suppose we divide our population sample into two different sets: the "cases," i.e., those that have the disease,¹¹ and the "controls," i.e., those that don't have the disease. Let's further assume that each of our candidate loci has only two alleles.¹² Then for each of our candidate loci we can estimate the allele frequency for the population of cases, p_{case} , and for the population of controls, $p_{control}$. Then we simply ask, do we have evidence that p_{case} is different from $p_{control}$. If so, we have evidence that allelic differences at this locus are associated with different probabilities of falling into the case category, i.e., allelic differences at this locus are associated with a gene that influences development of the phenotype. As with our GWAS analysis, we have to be careful in interpreting this association. It *might* reflect physical linkage between the candidate locus and the gene influencing phenotypic development or it might reflect nothing more than a statistical association.

A digression into two-locus population genetics¹³

It's pretty obvious that if two loci are on the same chromosome and tightly linked, alleles at those loci are likely to be statistically associated with one another, but let's take a closer look at what being statistically associated means. We'll see that while tight physical linkage generally implies statistical association, the reverse isn't true. A statistical association can arise even if the loci are unlinked and independently inherited.

One of the most important properties of a two-locus system is that it is no longer sufficient to talk about allele frequencies alone, even in a population that satisfies all of the assumptions necessary for genotypes to be in Hardy-Weinberg proportions at each locus. To see why consider this. With two loci and two alleles there are four possible gametes:¹⁴

¹⁰With the caveats about interpreting the association that I mentioned earlier.

¹¹Please note that I'm using the phrase "have the disease" merely because it's convenient. Most of the applications of this approach have involved investigations of human disease, but the approach can be used for *any* binary phenotype, in which case the phrase "have the disease" can be replaced with the phrase "have the phenotype of interest."

¹²Just as with GWAS, this is a reasonable assumption, since we are probably dealing with SNP markers.

¹³**Note:** We'll go over only a small part of this section in lecture. I'm providing all the details here so you can find them in the future if you ever need them.

¹⁴Think of drawing the Punnett square for a dihybrid cross, if you want.

Gamete	A_1B_1	A_1B_2	A_2B_1	A_2B_2
Frequency	x_{11}	x_{12}	x_{21}	x_{22}

If alleles are arranged randomly into gametes then,

$$\begin{aligned} x_{11} &= p_1p_2 \\ x_{12} &= p_1q_2 \\ x_{21} &= q_1p_2 \\ x_{22} &= q_1q_2 \quad , \end{aligned}$$

where $p_1 = \text{freq}(A_1)$ and $p_2 = \text{freq}(A_2)$. But alleles need not be arranged randomly into gametes. They may covary so that when a gamete contains A_1 it is more likely to contain B_1 than a randomly chosen gamete, or they may covary so that a gamete containing A_1 is less likely to contain B_1 than a randomly chosen gamete. This covariance could be the result of the two loci being in close physical association, but as we'll see in a little bit, it doesn't have to be. Whenever the alleles covary within gametes

$$\begin{aligned} x_{11} &= p_1p_2 + D \\ x_{12} &= p_1q_2 - D \\ x_{21} &= q_1p_2 - D \\ x_{22} &= q_1q_2 + D \quad , \end{aligned}$$

where $D = x_{11}x_{22} - x_{12}x_{21}$ is known as the *gametic disequilibrium*.¹⁵ When $D \neq 0$ the alleles within gametes covary, and D measures *statistical* association between them. It does not (directly) measure the *physical* association. Similarly, $D = 0$ does not imply that the loci are unlinked, only that the alleles at the two loci are arranged into gametes independently of one another.

A little diversion

It probably isn't obvious why we can get away with only one D for all of the gamete frequencies. The short answer is:

There are four gametes. That means we need three parameters to describe the four frequencies. p_1 and p_2 are two. D is the third.

¹⁵You will usually see D referred to as the linkage disequilibrium. I think that's misleading. Alleles at different loci may be non-randomly associated even when they are not physically linked.

Another way is to do a little algebra to verify that the definition is self-consistent.

$$\begin{aligned}
D &= x_{11}x_{22} - x_{12}x_{21} \\
&= (p_1p_2 + D)(q_1q_2 + D) - (p_1q_2 - D)(q_1p_2 - D) \\
&= \left(p_1q_1p_2q_2 + D(p_1p_2 + q_1q_2) + D^2 \right) \\
&\quad - \left(p_1q_1p_2q_2 - D(p_1q_2 + q_1p_2) + D^2 \right) \\
&= D(p_1p_2 + q_1q_2 + p_1q_2 + q_1p_2) \\
&= D(p_1(p_2 + q_2) + q_1(q_2 + p_2)) \\
&= D(p_1 + q_1) \\
&= D \quad .
\end{aligned}$$

***D* in a finite population**

In the absence of mutation, D will eventually decay to 0, although the course of that decay isn't as regular as what is shown in the Appendix [2]. If we allow recurrent mutation at both loci, however, where

$$A_1 \xrightleftharpoons[\nu_1]{\mu_1} A_2 \quad B_1 \xrightleftharpoons[\nu_2]{\mu_2} B_2 \quad ,$$

then it can be shown [3] that the expected value of $D^2/p_1(1-p_1)p_2(1-p_2)$ is

$$\begin{aligned}
\frac{E(D^2)}{E(p_1(1-p_1)p_2(1-p_2))} &= \frac{1}{3 + 4N_e(r + \mu_1 + \nu_1 + \mu_2 + \nu_2) - \frac{2}{(2.5 + N_e(r + \mu_1 + \nu_1 + \mu_2 + \nu_2) + N_e(\mu_1 + \nu_1 + \mu_2 + \nu_2))}} \\
&\approx \frac{1}{3 + 4N_e r} \quad .
\end{aligned}$$

Bottom line: In a finite population, we don't expect D to go to 0, and the magnitude of D^2 is inversely related to amount of recombination between the two loci. The less recombination there is between two loci, i.e., the smaller r is, the larger the value of D^2 we expect.

This has all been a long way¹⁶ of showing that our initial intuition is correct. If we can detect a statistical association between a marker locus and a phenotypic trait, it suggests that the marker locus and a locus influence expression of the trait are physically linked. *But* we have to account for the effect of population structure, *and* we have to account for the effect of *past* population structure.¹⁷ Notice also that if the effective population size is large, D^2 may be very small unless r is very small, meaning that you may need to have a

¹⁶OK. You can say it. A *very* long way.

¹⁷I haen't told you why we need to account for population structure, but you'll see in just a moment.

Population	Gamete frequencies				Allele frequencies		D
	A_1B_1	A_1B_2	A_2B_1	A_2B_2	p_{i1}	p_{i2}	
1	0.24	0.36	0.16	0.24	0.60	0.40	0.00
2	0.14	0.56	0.06	0.24	0.70	0.20	0.00
Combined	0.19	0.46	0.11	0.24	0.65	0.30	-0.005

Table 1: Gametic disequilibrium in a combined population sample.

very dense genetic map to detect any association between any of your marker loci and loci that influence the trait you’re studying. As shown in the Appendix, it takes a while for the statistical association between loci to decay after two distinct populations mix. So if we are dealing with populations having a history of hybridization, teasing apart physical linkage and statistical association can become very challenging.¹⁸

Population structure with two loci

You can probably guess where this is going. With one locus I showed you that there’s a deficiency of heterozygotes in a combined sample even if there’s random mating within all populations of which the sample is composed. The two-locus analog is that you can have gametic disequilibrium in your combined sample even if the gametic disequilibrium is zero in all of your constituent populations. Table 1 provides a simple numerical example involving just two populations in which the combined sample has equal proportions from each population.

The gory details

You knew that I wouldn’t be satisfied with a numerical example, didn’t you? You knew there had to be some algebra coming, right? Well, here it is. Let

$$\begin{aligned} D_i &= x_{11,i} - p_{1i}p_{2i} \\ D_t &= \bar{x}_{11} - \bar{p}_1\bar{p}_2 \quad , \end{aligned}$$

where $\bar{x}_{11} = \frac{1}{K} \sum_{k=1}^K x_{11,k}$, $\bar{p}_1 = \frac{1}{K} \sum_{k=1}^K p_{1k}$, and $\bar{p}_2 = \frac{1}{K} \sum_{k=1}^K p_{2k}$. Given these definitions, we can now calculate D_t .

$$D_t = \bar{x}_{11} - \bar{p}_1\bar{p}_2$$

¹⁸Think about what this means for GWAS or case-control studies in human populations.

$$\begin{aligned}
&= \frac{1}{K} \sum_{k=1}^K x_{11,k} - \bar{p}_1 \bar{p}_2 \\
&= \frac{1}{K} \sum_{k=1}^K (p_{1k} p_{2k} + D_k) - \bar{p}_1 \bar{p}_2 \\
&= \frac{1}{K} \sum_{k=1}^K (p_{1k} p_{2k} - \bar{p}_1 \bar{p}_2) + \bar{D} \\
&= \text{Cov}(p_1, p_2) + \bar{D} \quad ,
\end{aligned}$$

where $\text{Cov}(p_1, p_2)$ is the covariance in allele frequencies across populations and \bar{D} is the mean within-population gametic disequilibrium. Suppose $D_i = 0$ for all subpopulations. Then $\bar{D} = 0$, too (obviously). But that means that

$$D_t = \text{Cov}(p_1, p_2) \quad .$$

So if allele frequencies covary across populations, i.e., $\text{Cov}(p_1, p_2) \neq 0$, then there will be non-random association of alleles into gametes in the sample, i.e., $D_t \neq 0$, even if there is random association alleles into gametes within each population.¹⁹

Returning to the example in Table 1

$$\begin{aligned}
\text{Cov}(p_1, p_2) &= 0.5(0.6 - 0.65)(0.4 - 0.3) + 0.5(0.7 - 0.65)(0.2 - 0.3) \\
&= -0.005 \\
\bar{x}_{11} &= (0.65)(0.30) - 0.005 \\
&= 0.19 \\
\bar{x}_{12} &= (0.65)(0.7) + 0.005 \\
&= 0.46 \\
\bar{x}_{21} &= (0.35)(0.30) + 0.005 \\
&= 0.11 \\
\bar{x}_{22} &= (0.35)(0.70) - 0.005 \\
&= 0.24 \quad .
\end{aligned}$$

Implications for GWAS

For GWAS this means that we have to be very careful to account for *any* population structure within our data if we want to interpret a *statistical* association between a locus and a trait

¹⁹Well, duh! Covariation of allele frequencies across populations means that alleles are non-randomly associated across populations. What other result could you possibly expect?

as indicating that the locus is *physically* associated with a locus influencing expression of the trait. In the example you'll work through in lab we do this by including an estimate of the genetic relatedness of individuals in our sample in the regression model. Specifically, in this regression equation

$$y_i = x_{ij}\beta_j + \phi_i + \epsilon_{ij}$$

ϵ is pure random error with a mean of 0 and a variance equal to the environmental variance. ϕ_i is a correlated error in which the residual for individual i is correlated with the residual for individual j and the degree of correlation is related to the degree of relationship between them.

References

- [1] Gregory M. Cooper, Julie A. Johnson, Taimour Y. Langae, Hua Feng, Ian B. Stanaway, Ute I. Schwarz, Marylyn D. Ritchie, C. Michael Stein, Dan M. Roden, Joshua D. Smith, David L. Veenstra, Allan E. Rettie, and Mark J. Rieder. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood*, 112(4):1022–1027, 2008.
- [2] W G Hill and A Robertson. Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics*, 38:226–231, 1968.
- [3] Tomoko Ohta and Motoo Kimura. Linkage disequilibrium at steady state determined by random genetic drift and recurrent mutation. *Genetics*, 63:229–238, 1969.

Appendix: Transmission genetics with two loci

I'm going to construct a reduced version of a mating table to see how gamete frequencies change from one generation to the next. There are ten different two-locus genotypes (if we distinguish coupling, A_1B_1/A_2B_2 , from repulsion, A_1B_2/A_2B_1 , heterozygotes as we must for these purposes). So a full mating table would have 100 rows. If we assume all the conditions necessary for genotypes to be in Hardy-Weinberg proportions apply, however, we can get away with just calculating the frequency with which any one genotype will produce a particular gamete.²⁰

²⁰We're assuming random union of *gametes* rather than random mating of *genotypes*.

Genotype	Frequency	Gametes			
		A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1/A_1B_1	x_{11}^2	1	0	0	0
A_1B_1/A_1B_2	$2x_{11}x_{12}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0
A_1B_1/A_2B_1	$2x_{11}x_{21}$	$\frac{1}{2}$	0	$\frac{1}{2}$	0
A_1B_1/A_2B_2	$2x_{11}x_{22}$	$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
A_1B_2/A_1B_2	x_{12}^2	0	1	0	0
A_1B_2/A_2B_1	$2x_{12}x_{21}$	$\frac{r}{2}$	$\frac{1-r}{2}$	$\frac{1-r}{2}$	$\frac{r}{2}$
A_1B_2/A_2B_2	$2x_{12}x_{22}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$
A_2B_1/A_2B_1	x_{21}^2	0	0	1	0
A_2B_1/A_2B_2	$2x_{21}x_{22}$	0	0	$\frac{1}{2}$	$\frac{1}{2}$
A_2B_2/A_2B_2	x_{22}^2	0	0	0	1

Where do $\frac{1-r}{2}$ and $\frac{r}{2}$ come from?

Consider the coupling double heterozygote, A_1B_1/A_2B_2 . When recombination doesn't happen, A_1B_1 and A_2B_2 occur in equal frequency ($1/2$), and A_1B_2 and A_2B_1 don't occur at all. When recombination happens, the four possible gametes occur in equal frequency ($1/4$). So the recombination frequency,²¹ r , is half the crossover frequency,²² c , i.e., $r = c/2$. Now the results of crossing over can be expressed in this table:

Frequency	A_1B_1	A_1B_2	A_2B_1	A_2B_2
$1 - c$	$\frac{1}{2}$	0	0	$\frac{1}{2}$
c	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total	$\frac{2-c}{4}$	$\frac{c}{4}$	$\frac{c}{4}$	$\frac{2-c}{4}$
	$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$

Changes in gamete frequency

We can use the mating table table as we did earlier to calculate the frequency of each gamete in the next generation. Specifically,

$$\begin{aligned}
 x'_{11} &= x_{11}^2 + x_{11}x_{12} + x_{11}x_{21} + (1-r)x_{11}x_{22} + rx_{12}x_{21} \\
 &= x_{11}(x_{11} + x_{12} + x_{21} + x_{22}) - r(x_{11}x_{22} - x_{12}x_{21}) \\
 &= x_{11} - rD
 \end{aligned}$$

²¹The frequency of recombinant gametes in double heterozygotes.

²²The frequency of cytological crossover during meiosis.

$$\begin{aligned}
x'_{12} &= x_{12} + rD \\
x'_{21} &= x_{21} + rD \\
x'_{22} &= x_{22} - rD \quad .
\end{aligned}$$

No changes in allele frequency

We can also calculate the frequencies of A_1 and B_1 after this whole process:

$$\begin{aligned}
p'_1 &= x'_{11} + x'_{12} \\
&= x_{11} - rD + x_{12} + rD \\
&= x_{11} + x_{12} \\
&= p_1 \\
p'_2 &= p_2 \quad .
\end{aligned}$$

Since each locus is subject to all of the conditions necessary for Hardy-Weinberg to apply at a single locus, allele frequencies don't change at either locus. Furthermore, genotype frequencies at each locus will be in Hardy-Weinberg proportions. But the two-locus gamete frequencies change from one generation to the next.

Changes in D

You can probably figure out that D will eventually become zero, and you can probably even guess that how quickly it becomes zero depends on how frequent recombination is. But I'd be astonished if you could guess exactly how rapidly D decays as a function of r . It takes a little more algebra, but we can say precisely how rapid the decay will be.

$$\begin{aligned}
D' &= x'_{11}x'_{22} - x'_{12}x'_{21} \\
&= (x_{11} - rD)(x_{22} - rD) - (x_{12} + rD)(x_{21} + rD) \\
&= x_{11}x_{22} - rD(x_{11} + x_{12}) + r^2D^2 - (x_{12}x_{21} + rD(x_{12} + x_{21}) + r^2D^2) \\
&= x_{11}x_{22} - x_{12}x_{21} - rD(x_{11} + x_{12} + x_{21} + x_{22}) \\
&= D - rD \\
&= D(1 - r)
\end{aligned}$$

Notice that even if loci are unlinked, meaning that $r = 1/2$, D does not reach 0 immediately. That state is reached only asymptotically. The two-locus analogue of Hardy-Weinberg is that gamete frequencies will *eventually* be equal to the product of their constituent allele frequencies.

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