Chapter 5: Advanced topics in quantitative genetics

In the preceding chapter we showed how the means and variances of quantitative traits were affected by allele frequencies and gene action, and described how heritability and additive genetic variance is measured using offspring-parent regression and sibling analysis. That discussion was based on single environments and single traits. In this chapter we expand on the basic knowledge in the last chapter to consider the effects of multiple environments and multiple traits. We will discuss phenotypic plasticity, in which traits are expressed differently in different environments. If different genotypes differ in the level or direction of plasticity, then genotype-environment interaction exists. We will then explain how genetic and phenotypic relationships among multiple traits can arise, and how these relationships can be studied using correlations. Artificial selection, selection of certain traits by humans, has produced all our domesticated plants and animals. We will discuss how artificial selection can be used as an alternative to offspring-parent regression and sibling analysis to study genetic variances and correlations. Finally, we will turn to QTL mapping, in which molecular markers are used to find the locations of genes contributing to genetic variation in quantitative traits.

5.1 Phenotypic plasticity and Genotype-environment interaction

Phenotypic plasticity can be defined as the same genotype producing different phenotypes in different environments. Figure 5.1 shows different reaction norms, which are extremely useful in understanding plasticity and genotype-environment interaction. Reaction norms depict the phenotypes produced by different genotypes within a population in two (or more) different environments. The environments are on the X-axis, and the Y-axis is the phenotypic values for each genotype. Each line connects the genotypic or breeding value (mean phenotype) in one environment to the genotypic or breeding value in the other environment. Exactly the same as estimating additive variance and heritability, these points represent genotypic values for clonal species, selfed or full-sib family means for highly selfing species, and breeding values or half-sib family means for sexual species. In the discussion that follows, we will focus on half-sib means (sire effects), but in each case clones or full-sib families could be substituted where appropriate based on the reproductive system of the organism. The spread of points along each vertical axis represents the corresponding variance, e.g. additive variance if breeding values are used. For the remainder of this section we will assume that the points represent breeding values from a half-sibling families. The normal curves along each vertical axis represent phenotypic mean and variance, that is, the distribution of individual measurements.

The two environments on the X-axis are different ‘macroenvironments’, for example, an open field versus forest, or a pond with fish versus a fishless one. Therefore, this is different from $V_E$, which is phenotypic variation within one genotype caused by ‘microenvironmental’ variation, that is, small random differences in the environment.
within one site (Figure 4.4). $V_E$ is not shown in Figure 5.1, because family means or breeding values are used; the environmental variance is part of the variance within these families (Chapter 4). The distinction between micro- and macroenvironments is sometimes tricky because they are often on a spatial continuum. For example, heading up a slope from a wetland to a ridge, the soil become progressively drier; the wetland and the top of the ridge are clearly different macroenvironments, but there may not be a clear dividing line between them.

**Figure 5.1.** Reaction norms for four different hypothetical situations. At the top of each panel is a summary of whether the reaction norms depict genetic variance, plasticity, or G X E for the trait. To the right of each panel is a summary of what a two-way ANOVA on the data would show; the asterisks denote the $P$-value for each effect, with more asterisks representing a smaller $P$-value, that is, a stronger effect. Factors without a significant difference between levels are denoted with n.s. for not significant. The sire effect would be replaced by a clones or full-sib families in clonal or selfing species respectively.
The steepness of the slope of the line for each family indicates the level of plasticity of that family. Figure 5.1A shows a population with no plasticity; the horizontal reaction norms indicate that each family produces the same phenotype in each environment. Note that the families differ phenotypically, indicating additive genetic variance that is equal within each environment. The steep slopes in Figure 5.1B indicate a high level of plasticity, that is, the phenotypes are very different between environments. Note that all the families respond to the two environments in exactly the same way -- they all decrease by the same amount -- which indicates that there is no genetic variation in plasticity. There is still equal genetic variation within each environment, as in Fig. 5.1A, but no differences in plasticity among families.

In Fig. 5.1C there is still roughly equal additive variance within each environment, but now there are large differences among families in plasticity, shown by the different slopes of the reaction norms. Therefore, there is additive genetic variation for plasticity; note that the lines cross, so that the phenotypic rank of the families is very different in the two environments. This is called a genotype-environment interaction (g-e), or sometimes genotype by environment interaction (G X E). This type of g-e is sometimes referred to as ‘crossing’ g-e, because the reaction norms cross. Figure 5.1D shows a different kind of g-e, often called ‘variance’ g-e, because the amount of genetic variance differs between environments, but the reaction norms rarely cross. In this example, there is significant additive variance in environment 2 but not environment 1. Note that in Figure 5.1C and D many individual families are plastic, that is, their means differ across environments, as reflected in their sloped reaction norms. However, because roughly equal numbers of families increase and decrease their phenotype when raised in environment 2 as compared to environment 1, on average the population is not plastic -- the means across all families do not differ between the two environments.

Also in Figure 5.1 are brief summaries of likely results from the analysis of variance for each dataset depicted. Shown are simplified results from two-way ANOVA; it is called two way because there are two main factors, sire (or clone or full-sib family) and environment. These factors are crossed (as opposed to nested) which means that individuals at each level of each factor are also represented at each level of the other factor (see box). Thus, there are members of each half-sib family (i.e., offspring of each sire) raised in each of the two environments. This is different from the nested ANOVA, where the dams were not mated to every sire, but rather each dam was mated to only one sire, so the dams were nested within individual sires.

The factors in the two-way ANOVA are called main effects, to contrast them with the interaction between them. In the ANOVA, the F-test for the main effects tests whether the phenotypic values at each level of that factor differs significantly when they are averaged over the levels of the other factor(s). For example, a significant Sire effect indicates that the sire family means differ from one another when all the sire’s offspring are averaged regardless of which environment they were raised in. This average for two environments is the midpoint of the reaction norm. Comparing Fig. 5.1 A and C, we see differences among the midpoints of the reaction norms in A, and therefore a significant sire effect, but little differences in the midpoints and a non-significant sire effect in C. In Fig. 5.1D the sire effect is significant but weaker because there is significant additive
variance in environment 2 but not environment 1, causing the midpoints of the reaction norms to be moderately different.

Similarly, the environment main effects tests overall or average levels of plasticity, and is based on a comparison of the phenotypic values in the two environments averaged across all sires. In Figure 5.1 there is an average difference only in panel B; in the others the average in each environment is about the same. The interaction in a two-way ANOVA tests whether different levels of one factor affect the dependent variable (phenotypic trait here) differently depending on the level of the other factor. In the two-environment experiment we have been describing, an interaction occurs when different families respond differently to the two environments, that is, there is genetic variation in plasticity. In other words, the reaction norms are not parallel. In Figure 5.1A and B, the reaction norms are parallel, so there is not a significant interaction. In contrast, Figure 5.1C and D show lots of variation in reaction norm slope and thus a highly significant interaction. This statistical interaction is why it is called genotype-environment interaction.

**Box: Two-way ANOVA**

Table 5.1 shows hypothetical data for a plant experiment in which 4 offspring from each of four sires are each grown in two different environments, sun and shade. Note the difference from nested ANOVA – here the offspring from all sires are grown in both environments, therefore the two main effects of sire and environment are crossed with each other. Recall from the previous chapter that in nested ANOVA, each sire is mated to a different set of dams; therefore, the same dams are not represented across all sires, so the dams are said to be nested within the sires.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Sire 1</th>
<th>Sire 2</th>
<th>Sire 3</th>
<th>Sire 4</th>
<th>$\bar{Y}_{environment}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun</td>
<td>63</td>
<td>61</td>
<td>64</td>
<td>66</td>
<td>62.75</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>64</td>
<td>67</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>59</td>
<td>66</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>61</td>
<td>62</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>$\bar{Y}_{Sire}$</td>
<td>62.5</td>
<td>61.25</td>
<td>64.75</td>
<td>62.5</td>
<td>66.8</td>
</tr>
<tr>
<td>Shade</td>
<td>65</td>
<td>66</td>
<td>71</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>64</td>
<td>69</td>
<td>69</td>
<td>66.8</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>67</td>
<td>70</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>62</td>
<td>68</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>
These data show evidence for differences among sire overall averages (bottom row of table) and a difference of about 4 mm between the leaf lengths in sun and shade (right hand column), but no evidence for an interaction between the two main effects. The means for each sire differ by approximately 4 mm between the sun and shade environments, suggesting that the offspring of all sires responded similarly to the two environments. These conclusions are supported by the ANOVA (Table 5.2). A reaction norm plot for these data would look very similar to Fig 5.1 B (but with only four reaction norms, one for each sire) where shade is on left and sun on right. We conclude from these data that there is additive genetic variation and plasticity for leaf length (significant main effects of sire and environment respectively), but no genetic variance for plasticity, that is, no genotype (sire) by environment interaction. If, for example, Sires 2 and 4 showed the reverse pattern, that is, leaves 4 mm longer in sun than in shade, then the results would look more like Figure 5.1C, with no significant main effects of Sire (averaged across environments) or environment (averaged across Sire), but a strong sire by environment interaction.

Table 5.2. ANOVA table for the data in Table 5.1. Recall from previous chapters that d.f. is degrees of freedom, SS stands for Sums of Squares, which is the variance at each level, MS is Mean Squares or SS/d.f., the F-ratio is the ratio of each MS to the MS for error, and the P-value is the probability that each variance (the difference among means) is greater than zero by chance alone. Note that the degrees of freedom for an interaction is the product of the d.f. for the two main effects in the interaction.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>1</td>
<td>132.0</td>
<td>132.0</td>
<td>30.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sire</td>
<td>3</td>
<td>71.6</td>
<td>23.9</td>
<td>5.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Sire*Environment</td>
<td>3</td>
<td>2.6</td>
<td>0.87</td>
<td>0.2</td>
<td>0.89</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>103.2</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An actual experiment like this using sexual species would be more complex than in this simplified example, because it would include dams nested within sires as well as more sires. Therefore, it is really a combination of a two-way ANOVA with crossed factors and a nested ANOVA. Additional main effects, nested or not, and interactions can be added using the same basic principles outlined here, with one caveat – there are no interactions between a main effect and a factor nested within that main effect. The details of more complex ANOVA analyses can be found in a basic statistics book, but the basic interpretation remains the same as in our simplified examples in Table 5.2 and Figure 5.1 -- a significant sire main effect is evidence for overall additive genetic variance, a significant environment main effect is evidence for plasticity, and a significant sire by environment interaction is evidence for additive variance for plasticity.

End Box.
Multiple subpopulations – differentiation vs. adaptive plasticity

If different subpopulations inhabit different macroenvironments, then these large environmental differences are likely to lead to differences in selection between subpopulations. In chapter 3 we stated that this situation is likely to lead to local adaptation and genetic differentiation at loci that affect adaptation to these local conditions. However, we also said that gene flow can be a potent force reducing differentiation, so that the evolution of local adaptation is constrained by high gene flow. This is because alleles that confer high fitness in one environment are being moved into the other environment and vice versa. An alternative route to adaptation to variable environments in the face of high gene flow is the evolution of adaptive plasticity. For this plasticity to evolve, there needs to be genetic variation for plasticity, that is, genotype-environment interaction.

Therefore, if two subpopulations differ in some phenotypic trait, it is important to know whether that difference is due to genetic differentiation or due to direct effects of the environment on the trait, that is, phenotypic plasticity. The simplest method to determine this is a common garden experiment, where individuals from both (or several) subpopulations are raised together in the same environment. If the phenotypic differences persist in the common environment, then this is good evidence for genetic differentiation between the subpopulations at loci that affect the trait(s). It is best to intersperse the individuals from the different subpopulations in the garden rather than grow them in adjacent plots to eliminate the possibility of microenvironmental differences causing a phenotypic difference between the subpopulations. Another potential pitfall with a common garden is that differences could be due to non-genetic maternal effects. For example, if one subpopulation of plants was in a higher nitrogen environment and thus had larger individuals, this size difference could persist in the common garden because the larger individuals also produced larger seeds. For this reason researchers often raise the individuals for two or more generations in the common environment before measuring the traits. Although this is necessary, it is best to do only one or two generations because selection to adapt to the common environment can occur in these intervening generations and thus reduce the phenotypic differences.

Another drawback to the common garden experiment is that if there is g-e interaction for the traits of interest, then the results are only valid for the environment used for the common garden. Because common garden experiments are rarely done in the natural habitat, it is difficult to know if any genetic differences or plasticity found would be expressed in the wild. For these reasons a more difficult technique, called a reciprocal transplant experiment, is superior. In a reciprocal transplant individuals from each subpopulation are raised in the habitat of each subpopulation (Figure 5.2), creating a common garden at each home site. If there are phenotypic differences between subpopulations within one of the sites, then this is evidence for genetic differentiation. If there are differences between the members of the same subpopulation when planted in different sites, then this is evidence for plasticity. These two outcomes are not mutually exclusive; there could be both differentiation and plasticity simultaneously. Because the reciprocal transplant is really multiple common gardens, the potential problems of microenvironmental variation and maternal effects still apply. However, since the natural
habitats of each subpopulation are used, the results are highly applicable to the natural situation.

<table>
<thead>
<tr>
<th>Habitat 1</th>
<th>Habitat 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 1 2 1 2</td>
<td></td>
</tr>
<tr>
<td>2 1 2 1 2 1</td>
<td></td>
</tr>
<tr>
<td>1 2 1 2 1 2</td>
<td></td>
</tr>
<tr>
<td>2 1 2 1 2 1</td>
<td></td>
</tr>
<tr>
<td>1 2 1 2 1 2</td>
<td></td>
</tr>
<tr>
<td>2 1 2 1 2 1</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.2**. Diagram of a reciprocal transplant between two environments (habitats). Individuals from both habitats (represented by the numerals 1 and 2) are raised interspersed within each of the habitats, creating a common garden at each site. The arrows depict the movement of individuals by the experimenter (perhaps as seeds) between habitats (straight arrows) and also placed back into the same habitat (curved arrows).

Given the names of these techniques, common garden and reciprocal transplant, it is clear that they were developed and have been used most in studies of plants. It is easier to conduct these studies in plants than it is in animals, because seeds or plants can be moved around and then stay wherever the experimenter places them. However, these techniques can be used in animals that rarely move or can be caged without affecting the relevant environmental variables. This is true in herbivorous insects, for example.

### 5.2 Correlations among traits

To this point we have dealt almost exclusively with single phenotypic traits. But organisms are not collections of isolated traits, but rather individuals are made up of genetically, functionally, developmentally, and physiologically interconnected traits. In addition, selection and the ecological forces causing selection act on whole organisms, not single traits. Therefore, we need a method to understand the genetics and evolution of groups of traits simultaneously; for this biologists use phenotypic, genetic, and environmental correlations among traits. As for most concepts in ecological genetics, the correlation has no meaning in an individual, but rather is a property of the population.

The **phenotypic correlation** ($r_p$) measures the degree to which two traits covary among individuals in the population. If two traits covary, it means that variance in one is related to variance in the other. A correlation is just a covariance that is standardized to range from –1 to 1 (see Appendix 1). Figure 5.3 shows examples of phenotypic correlations of various magnitudes; each point in the figures represents the phenotypic values for two traits measured in one individual, and all the points together are a random sample of the population. Figure 5.3A shows a strong positive correlation ($r_p = 0.85$), so that beetles with long elytra also are heavier, whereas beetles with short elytra are lighter.
Although there is some scatter in this relationship, generally you can predict the weight of a beetle fairly well from its elytral length and vice versa. Therefore, most of the variation in these two traits is not independent. Figure 5.3B shows a weaker positive correlation ($r_p = 0.54$); while there is a positive relationship between petal width and corolla tube length, the greater scatter means that there is more independent variation in the two traits. Figure 5.3 C and D show hypothetical ‘perfect’ correlations of 1 and –1, in which none of the variation in the two traits is independent of variation in the other trait.

The phenotypic correlation is made up of two components: the genetic and environmental correlations. The **genetic correlation** estimates the degree to which two traits are affected by the same genes (pleiotropy) or pairs of genes (linkage disequilibrium; see below). The **environmental correlation** ($r_E$) estimates the degree to which two traits respond to variation in the same environmental factors. The additive genetic correlation ($r_A$) is the correlation of breeding values, and is most important evolutionarily for the same reason as the additive genetic variance: it is passed on to
offspring. As we will see in section 5.3 and the next chapter, selection on one trait will cause an evolutionary change in a second trait that is not selected if there is an additive genetic correlation between the two traits.

The relative importance of $r_A$ and $r_E$ in determining the phenotypic correlation between two traits $X$ and $Y$ depends on the heritability of the two traits:

$$r_p = h_X h_Y r_A + e_X e_Y r_E$$ \hspace{1cm} (5.1)

where $h$ is the square root of the heritability, and $e^2 = 1 - h^2$, that is, the complement of heritability, or the proportion of phenotypic variance that is environmental and non-additive genetic. Equation 5.1 shows that if heritability is high, then the phenotypic correlation is determined mainly by $r_A$, whereas the environmental correlation is more important when heritability is low.

Note that here $r_E$ also includes correlation due to non-additive genetic effects, and lump both true environmental correlation and non-additive genetic correlation into $r_E$. However, as we discussed in the last chapter, in studies of natural populations it is often difficult to estimate additive variance and covariance, so full-sibling families are sometimes used. This estimates $r_G$, the broad-sense genetic correlation, which includes dominance and the effects of common environment.

To estimate genetic correlations and covariances, one uses exactly the same mating designs as are used for estimating genetic variance and heritability, that is, offspring-parent and sibling analyses. The only differences are that more than one trait is measured and the analyses are somewhat different. For offspring-parent analysis, instead of regressing the offspring values on the parental values for the same trait, the covariance of trait $X$ in the offspring and trait $Y$ in the midparents is an estimate of 1/2 the additive genetic covariance between the two traits. The opposite covariance is also calculated, that is, the covariance of trait $Y$ in the offspring and trait $X$ in the parents. This provides two estimates of the genetic correlation, which can then be averaged. The additive genetic correlation is calculated by standardizing these additive genetic covariances by the square root of the product of the two offspring-parent covariances for each trait:

$$r_A(1) = \frac{\text{cov}(X,y)}{\sqrt{[\text{cov}(X,x)][\text{cov}(Y,y)]}}$$

$$r_A(2) = \frac{\text{cov}(x,Y)}{\sqrt{[\text{cov}(X,x)][\text{cov}(Y,y)]}}$$ \hspace{1cm} (5.2)

where upper case refers to the midparent values and lowercase to the average of the offspring.

When using full or half sibling data, ANOVA can estimate covariance components as well as variance components, and the correlations can be constructed from these using equation A.4. A simpler method is to calculate the correlation of breeding values, especially when these are estimated using BLUP. Both these methods have advantages and disadvantages, and neither is clearly superior to the other.
Sources of genetic covariance among traits

Mendel’s law of independent assortment states that different traits are inherited independently. One of Mendel’s examples was that whether a pea seed was round or wrinkled was inherited independently from the seed’s color, either yellow or green. A genetic correlation between two traits is a violation of the law of independent assortment.

The two causes of genetic covariance (and thus correlation) are pleiotropy and linkage disequilibrium. Pleiotropy is defined as one locus affecting more than one trait (Figure 5.4). For example, genes affecting digestive enzyme activity or photosynthetic rates are likely to affect the sizes of many morphological traits. This hypothetical example emphasizes that the degree of pleiotropy depends on the level of the trait in the phenotypic heirarchy (see Chapter 1); higher level traits will have more pleiotropy than lower level traits. At higher phenotypic levels, there may be multiple pleiotropic loci affecting the same pair of traits, and some of these may be positively pleiotropic (that is, causing a positive genetic correlation) and some may be negatively pleiotropic.

![Figure 5.4](image)

**Figure 5.4.** Simplified diagram of pleiotropic gene action. All three phenotypic traits are oligogenic (affected by two loci), and loci 2 and 4 are pleiotropic, affecting two loci each.

For example, the \( W \) locus in morning glories (*Ipomoea purpurea*) determines the amount of floral pigment, with \( WW \) plants having dark flowers, \( Ww \) light flowers, and \( ww \) white flowers. The floral pigments are related to compounds that affect levels of herbivory. Simms and Bucher (1996) found that larvae of the herbivorous beetle *Charidotella* had a 50% survival rate on \( WW \) and \( Ww \) morning glories, but only 39% survival on \( ww \) (\( P = 0.04 \)). Therefore, the \( W \) locus has pleiotropic effects on both floral color and herbivore survival.

In addition to pleiotropic loci, linkage disequilibrium, defined as a non-random relationship between the alleles present at two or more loci, can cause genetic correlations. The best way to examine this is in gametes because they are haploid. Two loci \( A \) and \( B \) are in linkage disequilibrium if, for example, \( AB \) and \( ab \) gametes are more common than the other two combinations, \( aB \) and \( Ab \). This is why linkage disequilibrium is sometimes called gametic phase disequilibrium. It is more often called linkage disequilibrium because genetic linkage among loci helps maintain the non-random association between alleles against the randomizing effects of recombination.
Figure 5.5.
Diagram of meiosis showing an organism with N=2 pairs of chromosomes (one long and one short). The chromosomes that came from this organism’s father are shown in black and the maternal chromosomes in gray. Note that there was recombination between most of the maternal and paternal loci on the two separate chromosomes, because they lined up in the opposite orientation during Metaphase I. Because of crossing over during the Diplotene, in some cases there was recombination between loci on the same chromosome (denoted by gray or black sections in the chromosomes of the opposite shade).

Recombination can be defined as the mixing of maternal and paternal origin chromosomes into new combinations in gametes during meiosis. The evolutionary significance of recombination is that it produces a set of genetically variable, unique progeny from a single pair of
parents. There are two sources of recombination (Figure 5.5.). The first is during the Diplotene phase of meiosis, when homologous chromosomes pair up. During this pairing crossing over can occur, in which sections of the sister chromosomes (that is, the homologous chromosomes from each parent) are exchanged. The second source of recombination is in Metaphase I, during which there is a random probability that the maternal or paternal origin chromosomes of each chromosome pair will line up on either side of the metaphase plate. Therefore, if two loci, A and B, are unlinked (on different chromosomes), what is probability of recombination between them, that is, that the maternal A allele will wind up in a gamete with the paternal origin B allele? It is 0.5, due to the 50% chance of them lining up on the same side of the metaphase plate during Metaphase I. If A and B are on the same chromosome, that is, they are linked, the probability of recombination is proportional to the physical distance between loci on chromosome. This is because there is a random chance of crossing over at any site on the chromosome, so there is an increased probability of crossing over with increased distance. Tight linkage is when the loci are close together on the chromosome.

The probability of recombination or the recombination rate ($r$, sometimes referred to as $c$) between two loci varies between 0, which means there is no crossing over because they are the same gene, and 0.5, which means the loci are on different chromosomes. The recombination rate determines how fast a population in linkage disequilibrium returns to equilibrium after the process that created the disequilibrium ceases. If $r = 0.5$, then disequilibrium is halved each generation (Figure 5.6). Therefore, even if the loci are unlinked, there is only an asymptotic approach to equilibrium. Why doesn’t the population return to linkage equilibrium for unlinked loci after a single generation, as is the case for HWE? It is because by chance, half of the chromosomes from each parent line up on the same side of the metaphase plate, so half of the allele combinations remain the same each generation. Figure 5.6 shows that linkage is not necessary for linkage disequilibrium, but it does help maintain disequilibrium in the face of constant recombination – tighter linkage (lower $r$) slows the return to linkage equilibrium because recombination is less likely. This is why a more accurate term is gametic phase disequilibrium, but this is used less often in the literature than the term linkage disequilibrium. Figure 5.6 also illustrates why it is called a disequilibrium; after the non-random association is created, recombination tends to return the population to linkage equilibrium, that is, the combination of alleles at the two loci that is expected based on their frequencies.
Figure 5.6. Theoretical plots of the return to linkage equilibrium ($D_t/D_0 = 0$) under different degrees of physical linkage. $D_t/D_0$ is the degree of disequilibrium at generation $t$ relative to the initial level at generation 0, and $r$ is the per-generation recombination frequency between the loci. The curve for $r = 0.5$ is that for unlinked loci, that is, loci on different chromosomes, whereas the other three curves represent pairs of loci that are located on the same chromosome. The progressively lower recombination frequencies, and thus lower rates of decline of linkage disequilibrium, are due to decreasing physical distance between the pairs of loci.

**What can cause disequilibrium?**

The most common causes of linkage disequilibrium are selection for particular combinations of alleles (sometimes called epistatic selection, because fitness depends on interactions of alleles at different loci), a mixture of two populations with different allele frequencies, and drift.

The classic example of selection creating linkage disequilibrium is heterostyly in the English primrose (*Primula vulgaris*). The ‘pin’ morph has a long style and the anthers are placed well below the stigma in the middle of the corolla tube, while the ‘thrum’ morph has a short style, placing the stigma at mid-tube, and anthers placed up high at the opening of tube (Figure 5.7). The two ‘homostyle’ morphs, with the stigma and anthers at the same height, are extremely rare. Therefore, the genes for style length and anther placement are in linkage disequilibrium, with the pin and thrum combinations common and the homostyle combinations rare. This disequilibrium is thought to be caused by selection for increased outcrossing, as the pin pollen is more likely to be deposited on the stigma of a thrum plant and vice versa.
The anthers are shown in black, while the stigmas are stippled. The $G$ locus determines style length (short style dominant) and the $A$ locus determines anther position (higher anthers dominant). In both cases the thrum phenotype is produced by the dominant allele. The dashes in the genotypes signify that either allele may be present with the dominant to produce the phenotype. Modified from Ford 1975.

**Figure 5.7.** Cross sections of three types of primrose flowers, with their names and genotypes shown.

**Final words on mechanisms of genetic correlations**

Note that none of the examples of traits affected by pleiotropy and linkage disequilibrium above are quantitative traits; rather, they are simple polymorphisms. This is because genetic correlations among quantitative traits, while very important to evolutionary change, are difficult to study at a mechanistic level in natural populations. For oligogenic or polygenic traits, pleiotropy and linkage disequilibrium are not mutually exclusive. A pair of correlated traits might have some shared pleiotropic loci and other independent loci that are in linkage disequilibrium. Theoretical work indicates that tight linkage, inbreeding, extremely strong epistatic selection, or some combination of the three are necessary to maintain linkage disequilibrium. How often these conditions hold in nature is unknown.

For these reasons, we know very little about the mechanisms underlying genetic correlations in nature. Some insight is now being produced by QTL mapping (see below). Conner (2002) used the decline in linkage disequilibrium through recombination (Figure 5.6) to distinguish this mechanism from pleiotropy. Two large ($N_c \approx 600$, to minimize drift) replicate populations of wild radish were randomly mated for nine generations in the greenhouse. Each plant contributed equal numbers of offspring to the next generation, minimizing selection. Under these conditions, linkage disequilibrium should be greatly reduced unless linkage is very tight. Phenotypic and genetic correlations among six floral traits were then estimated in both replicate populations and in an equal-sized sample of the original natural population grown from stored seeds. The results (Fig 5.8) show no significant change in the correlations, indicating that the correlations are caused by pleiotropy or linkage disequilibrium with extremely tight linkage, unlikely in this case.
Figure 5.8. Each pair of bars represents correlations between the two traits on the X-axis; the lighter bar is the correlation in the original population, and the darker is the mean of the two populations that were random-mated for nine generations. The error bars are statistical 95% confidence intervals; the fact that they broadly overlap means the correlations did not change significantly due to the random mating. PetLen and PetWid are petal length and width, TubeLen is the length of the corolla tube, ShrtFil and LongFil are the lengths of short and long filaments, and Pistil is pistil length. Reprinted, with permission, from Conner 2002.

5.3 Artificial selection

Artificial selection refers to the process of selective breeding of plants and animals by humans to produce populations with more desirable traits. All of our domesticated species, whether crops, farm animals, or pets, were modified from wild species by humans practicing artificial selection. For example, many of our crop plants, such as wheat and corn, have seed heads that do not shatter and scatter the seeds, unlike their wild neighbors. Most pets have been selected to be more docile and thus better companions, and farm animals have been selected to produce greater quantity and quality of meat and milk. We are interested in artificial selection in this book because it causes human-directed evolution, and it is a very useful tool for measuring heritabilities and genetic correlations. Artificial selection is analogous to natural selection, and it was for
this reason that Darwin devoted most of the first chapter of the Origin of Species to artificial selection.

To perform artificial selection, the single phenotypic trait to be selected is measured in a population, and members of the population at one or both phenotypic extremes are chosen for breeding (Fig 5.9). The rest of the individuals do not contribute to the next generation. This is called truncation selection, because there is a discrete phenotypic value (the truncation point) above which the organisms have high fitness and below which they have zero fitness. In nature selection usually does not work this way, because fitnesses are continuously distributed (see next chapter). The strength of this selection is measured by the selection differential, $S$, which is the difference in the mean of the selected group and the mean of the entire population before selection (Fig 5.9). Note that this is not the same as the selection coefficient (Chapter 3), which is denoted by a lowercase $s$ and measures selection on genotypes, not phenotypes as does the selection differential.

Sometimes the selection differential is referred to as the change in the mean within a generation, that is, before versus after the selective event, which is the choice of which organisms to breed in artificial selection. The breeder and the evolutionary biologist is usually more interested in the change in the mean across generations, in other words, between the parental and offspring generations. This is short-term phenotypic evolution, and is called the response to selection, $R$, and is calculated as the mean of all offspring minus the mean of all parents (Fig. 5.9).

The magnitude of $R$ for a given strength of selection $S$ depends on the heritability. Recall from the last chapter that heritability is the proportion of the phenotypic variance that is caused by additive genetic variance, and that the latter is what is transmitted directly from parents to offspring. Selection within a generation (measured as $S$) acts on the phenotypic variance, and the change across generations $R$ depends on the product of $S$ and the fraction of this variance that is additive genetic, i.e., the heritability:

$$R = h^2S \quad (5.3)$$

This is often called the ‘breeder’s equation, and is a fundamental equation for phenotypic evolution. It clearly illustrates the fundamental point that $R$ increases when either the strength of selection ($S$) or the heritability ($h$) increases.
Figure 5.9. Two rounds of artificial selection with associated responses. The histograms represent the distribution of the phenotypic trait in the population, with the black bars representing the individuals selected for breeding to form the next generation. Dashed lines are means of entire population each generation, dotted lines are the means of the group of selected individuals, and the selection differential ($S$) is the difference between the two in each of the two generations with selection. The responses to selection ($R$) are the differences across generations in the population means (the dashed lines). Note that each of the two responses to selection is only slightly smaller than the selection differential that gave rise to it (i.e., those with the same subscripts), meaning that this is a highly heritable trait. In addition to the change in mean across generations ($R$) caused by this selection, note that it also reduced the variance in the population.

Palmer and Dingle (1986) selected for both increased and decreased wing length over nine generations in a natural population on milkweed bugs (*Oncopeltus fasciatus*). Wing length is associated with differences in migratory behavior in this insect. Fig. 5.10 shows two common ways of presenting artificial selection results. In Fig. 5.10A, the change in mean wing length is plotted against generation number for two replicates each of lines selected for long wings, short wings, and random-mated controls. Note the
strong and consistent response to selection over time. Fig. 5.10B shows the response as a deviation from the control means, and plots this against the cumulative selection differential rather than time. After nine generations, the long lines gained about 2mm of wing length from the total selection differential of 4mm; from a rearrangement of equation 5.3 we can calculate a realized heritability of about 0.5. The realized heritability is usually estimated as the slope of a linear regression of cumulative response across the generations on cumulative selection differential, i.e., the slopes in Fig. 5.10B. The short lines only lost about 1.2 mm of wing length from the 4 mm total selection differential, giving a realized heritability of about 0.3. This is called an asymmetrical response, and is common in artificial selection studies. There are many possible reasons for asymmetry (Falconer and Mackay 1996 pp. 211-215) and it is often hard to determine the cause in any particular experiment.
Figure 5.10. Responses to artificial selection on wing length in milkweed bugs (*Oncopeltus fasciatus*). There were two replicates each of lines selected for longer wings, lines selected for shorter wings, and random mated controls. Because females are larger than males results for the two sexes are separated. A. Responses plotted as wing length versus generation number in males and females separately. B. The same responses plotted as a cumulative deviation from controls versus cumulative selection differential (the latter is a measure of total selection imposed to that point). Reprinted, with permission, from Palmer and Dingle 1986.

If there are genetic correlations between the selected trait and other traits, then **correlated responses to selection** can occur, that is, an evolutionary response in the other unselected traits. This is because selection on one trait changes allele frequencies at pleiotropic loci or loci in linkage disequilibrium, thus changing the phenotypes of the correlated traits. Just as heritability determines the magnitude of the direct response to a given strength of selection on the same trait, the genetic correlation determines the magnitude of correlated response to selection on a correlated trait.

Correlated responses are common, indicating that genetic correlations are common. Palmer and Dingle found significant differences from controls in body length, head and thorax width, development time and fecundity. In all cases these were positive correlated responses indicating positive genetic correlations, that is, the lines selected for long wings were bigger in many dimensions, had higher fecundity (often correlated with body size), and took longer to develop compared to controls, whereas lines selected for short wings were smaller, less fecund, but developed faster. Correlated responses to natural selection will be discussed in the next chapter.

In the vast majority of artificial selection experiments a response to selection occurs, which means that additive genetic variation exists for most quantitative traits in natural populations. In fact, in selection experiments that have continued for many generations, the population mean in the selected lines is often moved far beyond the range of phenotypes that were present in the original population. For example, from a single presumed wild ancestor, the breeds of domestic dogs now vary over two orders of magnitude in weight. An experiment started in 1896 has selected continuously on oil content in corn, moving the population mean far outside the original phenotypic range (Fig. 5.11).

Figure 5.11. Results from the world’s longest continuous artificial selection experiment, selecting for increased and decreased oil content in corn kernels at the University of Illinois. IHO and ILO are the original high and low oil selection lines respectively. RHO and RLO are reverse selection lines, originated from the high and low lines but then selected in the opposite direction (i.e. for low oil content in the RHO line). SHO is a secondary high oil selected line started from the RHO line. Reprinted, with permission, from Dudley 1977.
At first glance it is hard to understand how selection could move the mean beyond the existing range, because selection can only act on available variation in the population, and only on the additive genetic portion of this phenotypic variation. The first answer to this apparent paradox is new mutation, which provides additional variation to select on. This may have been very important in animals and plants that have been domesticated and selected upon over millenia. However, even long-term experiments such as the corn oil study above do not encompass enough generations for mutation to be the only explanation for the large response.

Probably the more important reason that long-term experiments can move the mean beyond the original generation is that without strong directional selection, no individual will be homozygous for alleles that increase or decrease the trait at all loci affecting that trait. After a few dozen generations of strong artificial selection, however, many or most of the individuals in the population may be fixed at most or all loci, moving the population outside the original range. When this happens, additive variance for the trait will be depleted, the population will stop responding to selection, and will reach a selection plateau or limit to selection.

Plateaus are common in selection experiments, but often they are not caused by a depletion of additive variance. One line of evidence for this is that often when selection is relaxed, that is, the artificial selection is stopped for several generations, the traits evolve back towards their original mean. The fact that the traits can still evolve clearly shows that additive variance has not been entirely depleted. Because the lines spontaneously evolve in the opposite direction to the direction of artificial selection, this suggests that some form of natural selection in the laboratory caused the selection plateau. In other words, the artificial selection was opposed by an equal amount of natural selection in the opposite direction, halting any further response. Selection is often reversed in long-term artificial selection lines, that is, selection is imposed in the opposite direction as the original artificial selection (Fig. 5.11); a response to reversed selection is also excellent evidence for remaining additive genetic variance. On the other hand, artificial selection lines also been observed to remain at the selected mean for several generations even after reverse selection is imposed, suggesting that additive variance has been depleted in these cases. Another cause of selection plateaus is called an intrinsic limit; a good example is the low oil lines of maize (Fig 5.11), in which response is slowing only because the mean phenotype is nearing the intrinsic limit of no oil.

**Advantages and disadvantages of artificial selection**

Artificial selection has several advantages for determining genetic variation and covariation over the alternative methods of offspring-parent regression and sibling analysis (hereafter, referred to as single-generation methods). First and foremost, artificial selection directly answers whether the trait can evolve in response to selection, whereas single-generation methods are indirect. Artificial selection is simpler conceptually and practically than sibling analysis, and has greater statistical power for the
same number of individuals measured. This is primarily because artificial selection tests differences between line means, whereas sib analysis relies on variance and covariance components (Falconer and Mackay 1996). This means that artificial selection is a good choice for traits that are difficult to measure (e.g. physiology, behavior), because generally fewer individuals need to be measured at one time compared to single-generation methods.

Artificial selection also has some disadvantages compared to single-generation methods. Each artificial selection experiment can measure additive variance only for the selected trait and can only estimate genetic covariances between this trait and other measured traits. Therefore, a single artificial selection experiment cannot be used to measure the entire matrix of genetic variances and covariances among a group of traits \((G)\); a separate artificial selection experiment for each trait is necessary. In other words, artificial selection provides no information on the genetic covariance or correlation between two unselected traits, and does not provide a quantitative estimate of genetic variance or heritability for these traits. This information is necessary for making quantitative predictions of the speed of evolutionary change for several traits (e.g. Campbell 1996).

Artificial selection experiments are not practical for many organisms, because they typically require maintaining the organisms in the laboratory or greenhouse, sometimes for long periods, and controlled matings need to be performed. In some organisms single-generation experiments can be done relatively easily in the field. In monogamous birds, for example, offspring-parent regression has often been carried out with natural matings in natural populations (e.g. Schluter and Smith 1986; reviewed in Boag and van Noordwijk 1987). Since the magnitude of genetic variance and covariance can be strongly affected by the environment (genotype by environment interaction), confining the experiments to unnatural environments can be a serious shortcoming if quantitative estimates of variance are of interest. A final practical problem is that artificial selection is most efficient if the individuals that are measured can then be mated, so that traits that cannot be measured on live individuals pose more difficulty. This difficulty can be overcome by measuring some individuals and using their full-siblings to mate, but this will slow progress because the measured and mated individuals only share half of their genes.

### 5.4 QTL mapping

The techniques of statistical quantitative genetics described above and in the previous chapter provide great insight into the evolution of quantitative traits, particularly when combined with measures of the strength of natural selection on these traits (see next chapter). However, these techniques use statistical abstractions such as variances and correlations to represent the genome. Since statistical quantitative genetics treats the genome as a black box, it cannot directly address a number of important questions concerning the genetic architecture of phenotypic traits, such as how many genes affect a given trait, where those genes are located in the genome, how much variation in the phenotype is determined by individual gene loci, what is the mode of action of those genes (additive or dominant) what other traits are affected by these genes (pleiotropy),
how those genes interact with each other to produce the phenotype (epistasis), and what are the genetic mechanisms underlying genotype-environment interaction. Recent advances in molecular genetics combined with theoretical and statistical innovations have spawned a new technique that is a first step in breaking open the black box of the genetics of quantitative traits. This technique, called **quantitative trait locus (QTL) mapping**, locates regions of the genome containing genes affecting quantitative traits (reviewed in Lynch and Walsh 1998; Tanksley 1993). Once these regions have been identified, further work can determine what the genes actually code for, but this is still a very difficult undertaking.

The first step in QTL mapping is to create a genetic map, preferably covering the entire genome. This is the step that has been greatly facilitated by the proliferation of DNA-based genetic markers (Chapter 2), because earlier genetic maps were limited to small numbers of visible polymorphisms and allozymes. The goal is to have markers evenly and relatively closely spaced throughout the genome. To make a map, a **mapping population** is often created by crossing genetically divergent populations, most often inbred lines or closely-related species. A single individual from one population is mated to a single individual from the second population to create the F1 generation. The F1 tends to be highly heterozygous because the divergent parental populations are fixed for different alleles at many loci. It also has very high linkage disequilibrium, because the chromosomes from the parental populations have not had the opportunity to recombine. An F1 individual is then either self-fertilized (if inbreeding depression is not a problem) or mated to another F1 individual to create an F2 mapping population, or alternatively an F1 can be crossed with an individual from one of the parental populations to create a **backcross** population. In this second generation of sexual reproduction, recombination produces unique combinations of the genomes from the two parental populations in each F2 individual.

It is the frequency of recombination between markers from the two parents that is used to construct the map (Figure 5.12), because the frequency of crossing over increases with increasing distance on the chromosome. The **map distance** between two markers is usually measured in centiMorgans (cM), named after the pioneering geneticist T.H. Morgan. One cM is roughly equivalent to a 1% recombination rate. In spite of the fact that recombination frequency increases with increasing physical distance on the chromosome, the map distance is not equivalent to the **physical distance**, that is, the number of bases in the DNA, which is measured measured in kilobases (kb). This is because the rate of recombination per kb differs widely among different regions of the chromosome, different chromosomes, between the sexes, and among species.
Figure 5.12 Hypothetical diagram showing recombination among three marker loci on the same chromosome in an $F_2$ mapping population. Note that the parents are fixed for opposite alleles at all three loci, the ideal situation for mapping often achieved by using inbred lines or different species for the parents. Because the markers are linked, there is no recombination in 70% of the $F_2$ — they represent either the parental or the $F_1$ genotypes (nos. 1-7). Two of the $F_2$ individuals show recombination between the $A$ and $B$ marker loci (nos. 8 and 9), whereas only individual 10 shows recombination between the $B$ and $C$ loci. If these numbers were representative of a large sample of $F_2$ individuals, then the recombination fraction $r$ would be 0.2 between loci $A$ and $B$ and 0.1 between loci $B$ and $C$. Loci $A$ and $B$ are therefore about 20 cM apart and $B$ and $C$ about 10 cM apart; this is why on the diagram the former pair of loci are shown twice as far apart on the chromosome as the latter.

The result of this analysis is a genetic map (Figure 5.13) showing the positions of markers in various linkage groups, often with the distance in cM between markers shown. The linkage groups are groups of markers that have recombination frequencies less than 0.5, that is, they are on the same chromosome. In species that are not genetic model organisms, often it is not known which chromosome each linkage group corresponds to, and depending on the density of the map (that is, how many markers and
how close together), it is possible that two linkage groups are in fact on the same chromosome, and that some chromosomes may not be represented at all.

**Figure 5.13** Linkage map for four representative linkage groups (chromosome portions) from a backcross mapping population resulting from a cross between two monkey flower species (*Mimulus guttatus* and *M. platycalyx*). The position of each RAPD marker locus is depicted as a dash to the left of the line that represents the chromosome portion. The numbers indicate the map distances between each pair of adjacent markers in cM. Modified from Lin and Ritland (1997).

The second step is to test for associations between variability in DNA markers and variation in the phenotypic trait (Fig. 5.14). This step has been facilitated by a variety of new statistical techniques. The most common statistical test for QTL starts with a log-odds ratio or LOD score:

\[
LOD = \log_{10} \left( \frac{L_1}{L_0} \right)
\]

where \( L_1 \) is the likelihood that there is a QTL linked to a particular marker and \( L_0 \) is the probability of no QTL near that marker for the phenotypic trait under analysis. These are commonly presented as LOD plots for a given linkage group (Fig. 5.15). The horizontal line represents the statistical threshold; LOD scores above this threshold are taken as evidence that there is a QTL at that location on the linkage group, whereas LOD scores below the threshold are judged to be due to chance alone. There are a number of methods for setting the threshold, and clearly a LOD score that falls just below the
threshold should not be ignored entirely, because the statistical support for this is only a little worse than the support for a LOD score just above the threshold (e.g., fig. 5.15).

**Fig. 5.14.** Hypothetical raw data for QTL mapping. In this simplified example, genotypes at a codominant molecular marker locus (the black ellipses represent bands on an electrophoretic gel) are correlated with the size of a quantitative trait (stamen length, for example). There are nine individuals (nine lanes on the gel). Individuals that are homozygous for the upper band always have long stamens, homozygotes for the lower band always have short stamens, and heterozygotes have intermediate stamen length. Significant relationships between a marker and the trait indicate that a locus affecting stamen length is tightly linked to the marker.

**Figure 5.15** LOD plot showing QTL for lifespan on *Drosophila* chromosome 3. The X-axis is the map position along the chromosome in cM, the likelihood ratio is on the Y-axis, and the horizontal dashed line is the significance threshold. Note that some peaks lie just below the threshold, e.g. at about 140 and 300 cM. Reprinted, with permission, from Leips and Mackay 2000.

When there is an association between phenotypic variance and a given marker, then that is evidence that a locus that affects this trait (i.e., a QTL) is linked to that marker. These QTL are then placed on the genetic map (Fig 5.16). On this map, the positions of the QTLs on the genetic map are indicated by the horizontal line, and the length of the bars represent a confidence interval that indicates the statistical uncertainty in this position. The maps in figures 5.13 and 5.16 are from a study that mapped floral differences in a cross between *Mimulus guttatus*, which has a high rate of outcrossing, and *M. platycalyx*, which has a high rate of selfing (Lin and Ritland 1997; these are the
same two species mentioned at the end of chapter 2). Lin and Ritland found three gene loci that affect the lengths of the stamens on three different linkage groups, two loci that affect stigma-anther separation (a trait that affects selfing rate), and one locus affecting pistil length. The loci affecting the long and short stamens on linkage groups 2 and 6 are so close together that this likely indicates a pleiotropic locus affecting both traits on each of these linkage groups. The QTL affecting stamen lengths and pistil length on linkage group 1 are farther apart, but still within each other’s confidence intervals, so that this could be one pleiotropic locus, but perhaps more likely two to several linked loci.

![Fig 5.16. QTL positions for several floral traits in monkeyflowers. These are the same linkage maps as in Fig. 5.13 with the QTL added. The lengths of the bars for each QTL represent confidence intervals, meaning that there is statistical support for the QTL occurring somewhere in that interval. Modified from Lin and Ritland (1997).]

The percentage of phenotypic variance explained by each of these QTL, along with their LOD scores, are shown in Table 5.3. Note that the effect of each locus on the trait variance is moderate to fairly strong, ranging from 7.6 to 28.6 percent variance explained. Also note that the statistical support for each QTL, that is, the LOD score, generally increases with increasing effect of the locus on the phenotype.
Table 5.3. The percentage of phenotypic variance explained and LOD scores for each of the QTL in Figure 5.16. Modified from Lin and Ritland (1997).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Linkage group</th>
<th>% phenotypic variance explained</th>
<th>LOD score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long stamen length</td>
<td>1</td>
<td>17.7</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.6</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>13.0</td>
<td>3.35</td>
</tr>
<tr>
<td>Short stamen length</td>
<td>1</td>
<td>12.9</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.3</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.4</td>
<td>2.97</td>
</tr>
<tr>
<td>Stigma-anther separation</td>
<td>1</td>
<td>10.7</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.5</td>
<td>2.47</td>
</tr>
<tr>
<td>Pistil length</td>
<td>1</td>
<td>28.6</td>
<td>4.68</td>
</tr>
</tbody>
</table>

Lin and Ritland had a fairly small mapping population, consisting of 96 individuals, whereas theory suggests mapping populations of about 500 are necessary to increase power to detect QTL and reduce bias in the estimates of their effects (Beavis 1998). A study of floral QTL in a cross between two different species of *Mimulus* with 465 individuals in the mapping population was undertaken by Bradshaw et al. (1998). *M. cardinalis*, a species with tubular red flowers typical of hummingbird pollinated plants, was crossed with *M. lewisii*, whose flowers are pale with flat petals, likely adaptations to its bumblebee pollinators. This larger study found twice as many QTL per trait, with an average of almost four per trait compared to less than two Lin and Ritland’s, and a wider range of percent variance explained, from 3.3 to 84.3%.

Bradshaw et al. (1998) also reported that over two-thirds of their QTL exhibited evidence for dominance, with the rest being additive. Leips and Mackay (2000) provide more detailed information on mode of action in their study of QTL affecting lifespan in *Drosophila melanogaster* at two larval densities (Table 5.4). Recall from the previous chapter that $a$ measures the magnitude of the additive effect of the locus on the trait; therefore, this provides another measure (in addition to % variance explained) of the magnitude of effect of a locus. Also recall that $d/a$ measures the degree of dominance. The additive effects vary fourfold, from 1.48 to 6.18, while the degree of dominance varies from partial dominance ($d/a<1$) to strong overdominance for QTL 1 in males at low density ($d/a=3.7$). More interesting is the fact that both measures of mode of action vary across sexes for the same QTL. This is seen most strongly for QTL 1, which has weak additive effects but is strongly overdominant in males at low density, but has almost three times the additive effects but shows only partial dominance in females at low density. At high density it is not just a difference in mode of action of the same QTL; rather, the QTL affecting lifespan are completely different between males and females (i.e., QTL 2 and 3 in males versus QTL 4 in females).
Table 5.4. Mode of action and candidate genes for several QTL for lifespan in *Drosophila*. The additive effect of each QTL is given by $a$ and the degree of dominance by $d/a$. From Leips and Mackay 2000.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Density</th>
<th>QTL</th>
<th>$a$</th>
<th>$d$</th>
<th>$d/a$</th>
<th>Candidate genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Low</td>
<td>1</td>
<td>1.5</td>
<td>5.5</td>
<td>3.7</td>
<td><em>Adh</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>2.9</td>
<td>3.4</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.8</td>
<td>3.3</td>
<td>0.7</td>
<td><em>Pgm, Cat</em></td>
</tr>
<tr>
<td>Male</td>
<td>High</td>
<td>2</td>
<td>6.2</td>
<td>4.6</td>
<td>0.7</td>
<td><em>EF1α</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.4</td>
<td>5.8</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Low</td>
<td>1</td>
<td>4.2</td>
<td>3.2</td>
<td>0.8</td>
<td><em>Adh</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3.9</td>
<td>3.0</td>
<td>0.8</td>
<td><em>Sod</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/6</td>
<td>3.76</td>
<td>4.2</td>
<td>1.1</td>
<td><em>Pgm, Cat, Ide, Hsp 70</em></td>
</tr>
<tr>
<td>Female</td>
<td>High</td>
<td>4</td>
<td>5.2</td>
<td>5.5</td>
<td>1.1</td>
<td><em>Sod</em></td>
</tr>
</tbody>
</table>

Leips and Mackay also present evidence for genotype-environment interaction at single QTL, which has similarities to the genotype-sex interaction. Different QTL affect lifespan in males across the two densities, that is, QTL 1, 5, and 6 at high density and QTL 2 and 3 at low density. Other loci affect lifespan at both densities, but the mode of action changes (e.g., Fig 5.17). Note that at low larval density, the heterozygote for the QTL near marker 76B has the highest lifespan, that is, there is heterozygote advantage at this QTL, and the homozygous genotype from the Oregon parental line has lowest lifespan. At high density, however, the Oregon homozygote has the longest lifespan, and this QTL shows only weak partial dominance for lifespan, because the heterozygote is nearly intermediate between the two homozygotes. Therefore, the mode of action of this QTL for lifespan differs between environments, providing the first step in understanding the mechanisms underlying genotype-environment interactions.
Figure 5.17. Genotype by environment interaction at a single QTL affecting lifespan in *Drosophila*. Note that the 2b homozygote has the shortest lifespan of the three genotypes at low density and the longest at high density. Reprinted, with permission, from Leips and Mackay 2000.

Epistasis can also be detected with QTL analyses, although this is often difficult statistically due to the large number of possible interactions between different QTL. We can again turn to Leips and Mackay for an example (Fig. 5.18). Note that when the QTL at marker 76B is homozygous for the B allele, the 2b homozygote at the 50D QTL is associated with an eight day increase in lifespan compared to the heterozygote. However, when 76B has the OB heterozygous genotype, the 2b homozygote decreased lifespan by about five days relative to the heterozygote.

Figure 5.18. Epistasis between two QTL. The relative lifespans of the two genotypes at the 76B QTL is reversed depending on the genotype at the 50D QTL. Reprinted, with permission, from Leips and Mackay 2000.

An important thing to remember about all current QTL studies, especially those on natural populations, is that the resolution is still quite low – most studies have markers approximately every 10 cM or more. There can be many loci in 10 cM. Thus, the term QTL is a bit of a misnomer, because loci are not being identified, but rather gene regions that affect the trait of interest. The ultimate goal is to identify and determine the function of genes affecting the phenotype. There are several techniques that researchers are taking to achieve this goal, but to date we know little about individual gene loci affecting
quantitative traits. One of these techniques is the candidate genes approach. This technique is used in genetic model organisms, in which the molecular functions of genes have been identified using mutants, knockouts, and other genetic techniques. If one of these known genes maps to the same location as a QTL for a given quantitative trait, then this is a candidate gene for the quantitative trait. Some possible candidate genes for lifespan in Drosophila identified in the Leips and Mackay study are listed in Table 5.4. These include genes involved in basic metabolism (Adh, Pgm), breakdown of insulin (Ide), removal of toxic metabolic byproducts (Sod,Cat), protein synthesis (EF1α), and response to stress (Hsp 70). While each of these functions could easily be related to lifespan, and it is not hard to envision how their effects on lifespan could vary across environments, much more work will be necessary to determine whether and how each actually contributes to this phenotypic trait.

For correct inferences to be drawn from any QTL study it is very important to keep in mind how the mapping population was created. For natural populations to date, the most common mapping population is an F2 or backcross from a cross between species (e.g. Bradshaw et al. 1998; Lin and Ritland 1997). The QTL identified in this kind of study are those that differ between the two species, which are very important if much of adaptive divergence is associated with the creation of new species. The Leips and Mackay study discussed above was based on a cross between two laboratory strains that originated from different natural populations. What we do not know yet is whether the QTL identified in these studies (or in most QTL studies to date) are the loci responsible for segregating variation in natural populations, that is, those that underlie the response to selection. It seems likely that often they will be, especially in the cases of traits that are clearly adaptations, but that there are other important loci contributing to natural genetic variation that remain to be identified. A few studies have mapped QTL from crosses between divergent artificial selection lines; these identify the loci that are responsible for the response to selection and correlated responses, and were therefore at least some of the loci with segregating variation in the original population.

Readings questions:

Via 1994:
1. Define phenotypic plasticity and reaction norm.

2. Distinguish between noisy plasticity and adaptive reaction norms. What environmental conditions give rise to each, and what is the phenotypic response to these environments in the two types of plasticity?

3. Explain the meanings of each of the terms (main effects, interaction, error term) in the two-way ANOVA analyzing plasticity.

4. Sketch reaction norms for two environments depicting the following conditions:
   a. no plasticity
   b. plasticity but no variance in plasticity (i.e. no GXE)
   c. variance in plasticity
Which of the first three terms in the ANOVA (the two main effects and the interaction) would be statistically significant in each of your sketches?

5. Which of the cases in #4 allow for the evolution of a new average reaction norm (new form of plasticity) in response to new environments?

**Schluter and Smith 1986:**
1. Are there any possible sources of bias in the measurements of heritability that Schluter and Smith did not consider? If so, what specific effects would these biases have on the heritability estimates?
2. What basic method did they use to estimate genetic correlations?
3. How similar to each other are the phenotypic and the genetic correlations for the same pairs of traits? For what pairs of traits are there major differences between the phenotypic and genetic correlations? Use the corrected values throughout.

**Gromko et al. 1991:**
1. What trait did they artificially select? In what traits did they predict a correlated response would occur? Did they predict that the unselected traits would respond in the same or opposite direction as the selected trait?
2. Focusing on generation 10 of the Chain Cross population, are the correlated responses in the predicted directions? What table or figure contains these data?

**Via 1984:**
1. Why is genotype-environment interaction (g-e, same as GXE) important in agriculture? Why is it important in evolution?
2. Describe the experimental design used by Via. Pay particular attention to where the flies were collected from, how they were mated, and what host plants the offspring from each female were reared on.
3. From what data was the additive genetic variation (VA) calculated?
4. For which traits was there significant additive genetic variance? Significant GXE?
5. Why could GXE across the two host plants lead to genetic differentiation among the populations on the two host species (“host races”)? What is the evidence that host races have not formed in *Liriomyza*? What is one possible reason why this has not happened?

**Bradshaw et al. 1995:**
1. What is a QTL? What is the general technique used by Bradshaw et al. to find QTLs?
2. What phenotypic traits did they measure, and how do each of these affect pollination by bees and hummingbirds? Which phenotype for each trait is favored by bees? Which by hummingbirds?
3. Do their results provide evidence for pleiotropy? If so, what is the evidence, and for which trait pairs are there likely to be pleiotropic loci?

4. What is their main conclusion regarding the genetic basis of floral differences between the two species? Does their evidence support this conclusion? How does this conclusion affect our understanding of speciation?

References and further reading


Chapter 5 comment sheet
Email, give to Jeff in class, or campus mail (anonymous) to:
Jeff Conner
Kellogg Biological Station
133 Service Road

Which sections/pages/figures/concepts were unclear to you?

Which sections/pages/figures were particularly helpful?

Any errors?

Thanks for your help!