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Case study 1:

Alcoholdehydrogenase (Adh) enzyme in fruit flies

Empirical Research on Allele Frequency Change by Selection

Douglas Cavener and Michael Clegg (1981) documented a cumulative change in allele frequencies over many generations in a laboratory-based natural selection experiment on the fruit fly (*Drosophila melanogaster*). Fruit flies, like most other animals, make an enzyme that breaks down ethanol, the poisonous active ingredient in beer, wine, and rotting fruit. This enzyme is called alcohol dehydrogenase, or ADH. Cavener and Clegg worked with populations of flies that had two alleles at the ADH locus: Adh^F and Adh^S. (The *F* and *S* refer to whether the protein encoded by the allele moves quickly or slowly through an electrophoresis gel.)

The scientists kept two experimental populations on food spiked with ethanol and two control populations of flies on normal, nonspiked food. The researchers picked the breeders for each generation at random. This is why we are calling the project a natural selection experiment: Cavener and Clegg set up different environments for their different populations, but the researchers did not themselves directly manipulate the survival or reproductive success of individual flies.

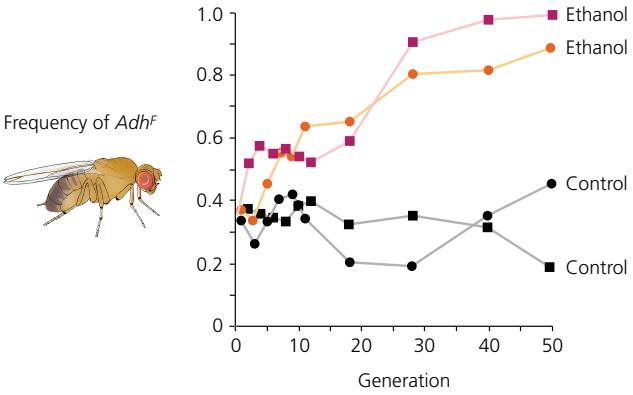

Figure 6.14 Frequencies of the allele in four populations of fruit flies over 50 generations The black squares and circles represent control populations living on normal food; the magenta squares and orange circles represent experimental populations living on food spiked with ethanol. From Cavener and Clegg (1981).

Every several generations, Cavener and Clegg took a random sample of flies from each population, determined their ADH genotypes, and calculated the allele frequencies. The results appear in Figure 6.14. The control populations showed no large or consistent long-term change in the frequency of the Adh^F allele. The experimental populations, in contrast, showed a rapid and largely consistent increase in the frequency of Adh^F (and, of course, a corresponding decrease in the frequency of Adh^S). Hardy–Weinberg conclusion 1 appears to hold true in the control populations, but is clearly not valid in the experimental populations.

Empirical research on fruit flies is consistent with our conclusion that natural selection can cause allele frequencies to change.

Can we identify for certain which of the assumptions of the Hardy–Weinberg analysis is being violated? The only difference between the two kinds of populations is that the experimentals have ethanol in their food. This suggests that it is the no-selection assumption that is being violated in the experimental populations. Flies carrying the Adh^F allele appear to have higher lifetime reproductive success (higher fitness) than flies carrying the Adh^S allele when ethanol is present in the food. Cavener and Clegg note that this outcome is consistent with the fact that alcohol dehydrogenase extracted from Adh^F homozygotes breaks down ethanol at twice the rate of alcohol dehydrogenase extracted from Adh^S homozygotes. Whether flies with the Adh^F allele have higher fitness because they have higher rates of survival or because they produce more offspring is unclear.

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Case study 2:

Fetal survival in malaria-infected mothers

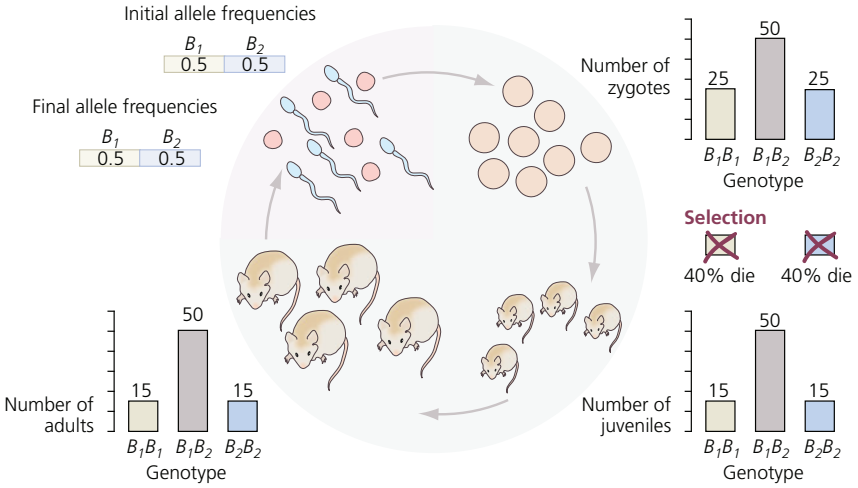

Figure 6.15 Selection can change genotype frequencies so that they cannot be calculated by multiplying the allele frequencies When 40% of the homozygotes in this population die, the allele frequencies do not change. But among the survivors, there are more heterozygotes than predicted under Hardy–Weinberg equilibrium.

We used strong selection in our numerical example to make a point. In fact, selection is rarely strong enough to produce, in a single generation, such a large violation of Hardy–Weinberg conclusion 2. Even if it does, a single bout of random mating will immediately put the genotypes back into Hardy–Weinberg equilibrium. Nonetheless, researchers sometimes find violations of Hardy–Weinberg conclusion 2 that seem to be the result of selection.

Empirical Research on Selection and Genotype Frequencies

Our example comes from research by Atis Muehlenbachs and colleagues (2008), working in the laboratory of Patrick Duffy, on genetic variation for the outcome of falciparum malaria during pregnancy. Falciparum malaria is caused by infection with the single-celled parasite *Plasmodium falciparum*. When a pregnant woman contracts the disease, the parasites invade the placenta via the mother's circulatory system (Karumanchi and Haig 2008). This triggers placental inflammation and may also interfere with placental development (Umbers et al. 2011). The potential complications include spontaneous abortion, premature delivery, low birth weight, and higher risk of infant death.

Pregnancy itself brings an increased risk of malaria infection, particularly a woman's first pregnancy (Karumanchi and Haig 2008). During a first bout of placental malaria, women develop antibodies that confer partial resistance during later pregnancies. Some 125 million women who live in areas affected by malaria become pregnant each year, and malaria infection during pregnancy is estimated to be responsible for an annual toll of 100,000 infant deaths (Umbers et al. 2011).

Muehlenbachs and colleagues (2008) suspected that the outcome of placental malaria hinges on the fetus's genotype at the locus encoding vascular endothelial growth factor receptor 1 (VEGFR1), also known as *fms*-like tyrosine kinase 1 (Flt1). Fetal cells in the placenta release a soluble form of this protein, sVEGFR1, into the mother's circulation. By interacting with vascular endothelial growth factor, VEGFR1 influences both placental development and inflammation.

Copies of the gene for VEGFR1 vary in the length of a two-nucleotide repeat in a region that is transcribed to mRNA but not translated. Alleles cluster into a short group (*S* alleles) and a long group (*L* alleles). Cultured cord blood cells with genotypes *SS* and *SL* produce more VEGFR1 than do *LL* cells.

COMPUTING CONSEQUENCES 6.4

Statistical analysis of allele and genotype frequencies using the χ^2 (chi-square) test

Here we use data from Muehlenbachs and colleagues (2008) to illustrate a method for determining whether genotype frequencies deviate from Hardy–Weinberg equilibrium. The researchers surveyed Tanzanian infants born to first-time mothers during malaria season. The genotype counts (provided by Atis Muehlenbachs and Patrick Duffy, personal communication) were

SS	*SL*	*LL*
16	50	10

The analysis proceeds in five steps:

1. Calculate the allele frequencies. The sample of 76 infants is also a sample of 152 gene copies. All 32 copies carried by the *SS* infants are *S*, as are 50 of the copies carried by the *SL* infants. Thus, the frequency of *S* is

$$\frac{32 + 50}{152} = 0.54$$

The frequency of *L* is

$$\frac{50 + 20}{152} = 0.46$$

2. Calculate the genotype frequencies expected under Hardy–Weinberg equilibrium. If the frequencies of two alleles are p and q, then the expected frequencies of the genotypes are p^2, $2pq$, and q^2. The expected frequencies among the infants are thus

SS	*SL*	*LL*
$0.54^2 = 0.29$	$2 \cdot 0.54 \cdot 0.46 = 0.5$	$0.46^2 = 0.21$

3. Calculate the expected number of infants of each genotype under Hardy–Weinberg equilibrium. This is simply the expected frequency of each genotype multiplied by the total number of infants, 76:

SS	*SL*	*LL*
$0.29 \cdot 76 = 22$	$0.5 \cdot 76 = 38$	$0.21 \cdot 76 = 16$

These expectations are different from the numbers observed (16, 50, and 10). The actual sample contains more heterozygotes and fewer homozygotes. Is it plausible that a difference this large between expectation and reality could arise by chance? Or is the difference statistically significant? Our null hypothesis is that the difference is simply due to chance.

Working with newborn babies of first-time mothers in Muheza, Tanzania, where malaria is a perennial scourge, Muehlenbachs and colleagues (2008) tested their hypothesis in part by using the Hardy–Weinberg equilibrium principle.

The researchers first determined the allele frequencies among 163 infants born from October through April, when the rate of placental malaria was at its annual low. The frequencies were

S	*L*
0.555	0.445

If the population of infants was in Hardy–Weinberg equilibrium, then multiplying these allele frequencies will allow us to predict the genotype frequencies:

SS	*SL*	*LL*
$0.555^2 = 0.308$	$2 \cdot 0.555 \cdot 0.445 = 0.494$	$0.445^2 = 0.198$

These predicted frequencies are, in fact, close to the actual genotype frequencies among the off-season infants:

SS	*SL*	*LL*
$\frac{49}{163} = 0.301$	$\frac{83}{163} = 0.509$	$\frac{31}{163} = 0.190$

The true frequency of heterozygotes is slightly higher than predicted, and the frequencies of homozygotes are slightly lower, but the discrepancies are modest. The infants thus conform to conclusion 2 of the Hardy–Weinberg analysis.

4. Calculate a test statistic. We will use one devised in 1900 by Karl Pearson. It is called chi-square (χ^2).

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

where the symbol $\sum$ indicates a sum taken across all the classes considered. In our data there are three classes: the three genotypes. For our data set

$$\chi^2 = \frac{(16 - 22)^2}{22} + \frac{(50 - 38)^2}{38} + \frac{(10 - 16)^2}{16} = 7.68$$

5. Determine whether the test statistic is significant. χ^2 is defined such that it gets larger as the difference between the observed and expected values gets larger. How likely is it that we could get a χ^2 as large as 7.68 by chance? Most statistical textbooks have a table giving the answer. In Zar's (1996) book. it is called "Critical values of the chi-square distribution."

To use this table, we need to calculate a number called the degrees of freedom for the test statistic. This value for χ^2 is the number of classes minus the number of independent values calculated from the data for use in determining the expected values. For our χ^2 there are three classes: the genotypes. We calculated two values from the data for use in determining the expected values: the total number of individuals, and the frequency of allele *S*. (We also calculated the frequency of *L*, but it is not independent of the frequency of *S*, because the two must sum to 1.) Thus the number of degrees of freedom is 1. (Another formula for calculating the degrees of freedom in χ^2 tests for Hardy–Weinberg equilibrium is

$$df = k - 1 - m$$

where k is the number of classes and m is the number of independent allele frequencies estimated.)

According to the table, the critical value of χ^2 for one degree of freedom and $P = 0.05$ is 3.841. This means there is a 5% chance under the null hypothesis of getting $\chi^2 \geq 3.841$. The probability under the null hypothesis of getting $\chi^2 \geq 7.68$ is therefore (considerably) less than 5%. We reject the null hypothesis and assert that our χ^2 is statistically significant at $P < 0.05$. (In fact, $P < 0.006$.)

The χ^2 test tells us that among infants born during malaria season, the alleles of the gene for VEGFR1 are not in Hardy–Weinberg equilibrium. This indicates that one or more assumptions of the Hardy–Weinberg analysis has been violated. By itself, however, it does not tell us which are being violated, or how.

Muehlenbachs and colleagues then determined the allele frequencies among 76 infants born from May through September, when the rate of placental malaria was at its annual high. The frequencies were nearly the same as among the off-season newborns:

S	*L*
0.539	0.461

If this segment of the population was, like their off-season counterparts, in Hardy–Weinberg equilibrium, then multiplying the allele frequencies will again allow us to predict the genotype frequencies:

SS	*SL*	*LL*
$0.539^2 = 0.291$	$2 \cdot 0.539 \cdot 0.461 = 0.497$	$0.461^2 = 0.213$

This time the predicted values are a poor fit to the actual frequencies:

SS	*SL*	*LL*
$\frac{16}{76} = 0.211$	$\frac{50}{76} = 0.658$	$\frac{10}{76} = 0.132$

There are substantially more heterozygotes than expected, and substantially fewer homozygotes. This discrepancy between prediction and data is statistically significant (see **Computing Consequences 6.4**). The genotypes of the infants born during peak malaria season are in violation of Hardy–Weinberg conclusion 2.

The discovery that genotype frequencies in a population are not in Hardy–Weinberg equilibrium may be a clue that natural selection is at work.

On this and other evidence, Muehlenbachs and colleagues (2008) believe the best explanation for the missing homozygotes is that they did not survive fetal development. A fetus's chance of surviving depends on both its own genotype and whether its mother contracts malaria **(Figure 6.16)**. If the mother does not contract malaria, *SS* infants do somewhat better than others. If, however, the mother does contract malaria, *SL* infants do substantially better than others. Overall, when malaria is common, heterozygotes survive at the highest rate. Consistent with this explanation, where malaria is absent, *S* alleles occur at high frequency.

Figure 6.16 Probability of fetal survival as a function of genotype and placental malaria Inferred from the patterns in maternal and newborn genotype frequencies in Muehlenbachs et al. (2008).

Changes in the Frequency of the *CCR5-Δ32* Allele Revisited

We are now in a position to give a more satisfying answer to the question we raised at the beginning of Section 6.1: Will the AIDS epidemic cause the frequency of the *CCR5-Δ32* allele to increase in human populations? The AIDS epidemic could, in principle, cause the frequency of the allele to increase rapidly, but at present it appears that it will probably not do so in any real population. This conclusion is based on the three model populations depicted in **Figure 6.17** (see **Computing Consequences 6.5** for the algebra). Each model is based on different assumptions about the initial frequency of the *CCR5-Δ32* allele and the prevalence of HIV infection. Each graph shows the predicted change in the frequency of the *Δ32* allele over 40 generations, or approximately 1,000 years.

The model population depicted in Figure 6.17a offers a scenario in which the frequency of the *Δ32* allele could increase rapidly. In this scenario, the initial frequency of the *CCR5-Δ32* allele is 20%. One-quarter of the individuals with genotype +/+ or +/*Δ32* contract AIDS and die without reproducing, whereas all of the *Δ32*/*Δ32* individuals survive. The 20% initial frequency of *Δ32* is approximately equal to the highest frequency reported for any population, a sample of Ashkenazi Jews studied by Martinson et al. (1997). The mortality rates approximate the situation in Botswana, Namibia, Swaziland, and Zimbabwe, where up to 25% of individuals between the ages of 15 and 49 are infected with HIV (UNAIDS 1998). In this model population, the frequency of the *Δ32* allele increases by as much as a few percentage points each generation. By the end of 40 generations, the allele is at a frequency of virtually 100%. Thus, in a human population that combined the highest reported frequency of the *Δ32* allele with the highest reported rates of infection, the AIDS epidemic could cause the frequency of the allele to increase rapidly.

At present, however, no known population combines a high frequency of the *Δ32* allele with a high rate of HIV infection. In northern Europe, many populations have *Δ32* frequencies between 0.1 and 0.2 (Martinson et al. 1997; Stephens et al. 1998), but HIV infection rates are under 1% (UNAIDS 1998). A model population reflecting these conditions is depicted in Figure 6.17b. The initial frequency of the *Δ32* allele is 0.2, and 0.5% of the +/+ and +/*Δ32* individuals contract AIDS and die without reproducing. The frequency of the *Δ32* allele hardly changes at all. Selection is too weak to cause appreciable evolution in such a short time.

In parts of sub-Saharan Africa, as many as a quarter of all individuals of reproductive age are infected with HIV. However, the *Δ32* allele is virtually absent (Martinson et al. 1997). A model population reflecting this situation is depicted in Figure 6.17c. The initial frequency of the *Δ32* allele is 0.01, and 25% of the +/+ and +/*Δ32* individuals contract AIDS and die without reproducing. Again, the frequency of the *Δ32* allele hardly changes at all. When the *Δ32* allele is at low

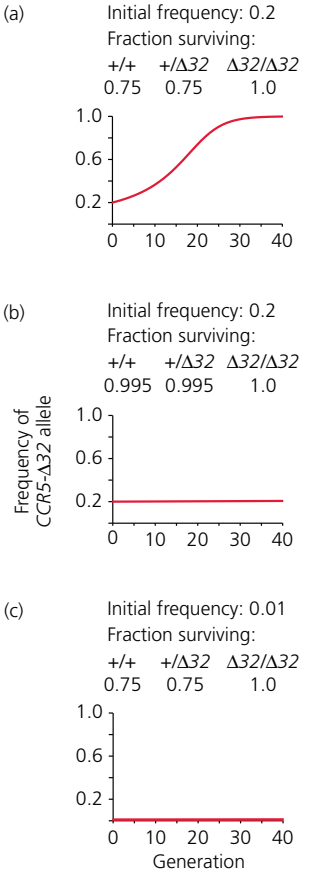

Figure 6.17 Predicted change in allele frequencies at the CCR5 locus under different scenarios (a) If the initial frequency of *CCR5-Δ32* is high and many people become infected with HIV, allele frequencies can change rapidly. (b) In Europe allele frequencies are high, but infection rates are low. (c) In parts of Africa the infection rates are high, but allele frequencies are low.

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Case study 3:

The case of flour beetles

hereditary diseases was weak. But what about the evolutionary logic behind compulsory sterilization? If the genetic assumptions had been correct, would sterilization have been an effective means of reducing the incidence of undesirable traits?

Before we try to answer this question, it will be helpful to address a more general one. How well does the theory of population genetics actually work? We developed this theory in Sections 6.1 and 6.2. The final product is a model of how allele frequencies change in response to natural selection (Figures 6.12 and 6.13, Computing Consequences 6.3 and 6.5). If our model is a good one, it should accurately predict the direction and rate of allele frequency change under a variety of selection schemes. It should work, for example, whether the allele favored by selection is dominant or recessive, common or rare. It should work whether selection favors heterozygotes or homozygotes. It should even predict what will happen when a particular allele is favored by selection under some circumstances and disfavored in others.

In this section, we will find out how well our model works. Using the theory we have developed to predict the course of evolution under different patterns of selection, we compare our predictions to empirical data from experimental populations. We then return to our question about the effectiveness of eugenic sterilization in changing the composition of populations.

Selection on Recessive and Dominant Alleles

For our first test, we focus on whether our theory accurately predicts changes in the frequencies of recessive and dominant alleles. Our example comes from the work of Peter Dawson (1970). Dawson had been studying a laboratory colony of flour beetles **(Figure 6.18)** and had identified a gene we will call the l locus. This locus has two alleles: + and *l*. Individuals with genotype +/+ or +/*l* are phenotypically normal, whereas individuals with genotype *l*/*l* do not survive. In other words, *l* is a recessive lethal allele.

Figure 6.18 Flour beetles, *Tribolium castaneum* Courtesy of Susan J. Brown, Professor/Kansas State University, Kansas.

Dawson collected heterozygotes from his beetle colony and used them to establish two new experimental populations. Because all the founders were heterozygotes, the initial frequencies of the two alleles were 0.5 in both populations. Because *l*/*l* individuals have zero fitness, Dawson expected his populations to evolve toward ever lower frequencies of the *l* allele and ever higher frequencies of the + allele. He let his two populations evolve for a dozen generations, each generation measuring the frequencies of the two alleles.

Dawson used the equations derived in Computing Consequences 6.3 and the method described in Computing Consequences 6.5 to make a quantitative prediction of the course of evolution in his populations. We can reproduce this prediction with a straightforward numerical calculation like the ones we performed in Figures 6.12 and 6.13. Imagine a gene pool in which alleles + and *l* are both at a frequency of 0.5. If we combine gametes at random to make 100 zygotes, we get the three genotypes in the following numbers:

+/+	+/*l*	*l*/*l*
25	50	25

Now we imagine that all the *l*/*l* individuals die and that everyone else survives to breed. Finally, imagine that each of the survivors donates 10 gametes to the new gene pool:

The 25 +/+ survivors together make 250 gametes: 250 carry +; none carry *l*.

The 50 +/*l* survivors together make 500 gametes: 250 carry +; 250 carry *l*.

This gives us 500 copies of the + allele and 250 copies of the *l* allele for a total of 750. In this new gene pool, the frequency of the + allele is 0.67, and the frequency of the *l* allele is 0.33. We have gone from the gene pool in generation zero to the gene pool in generation one. The frequency of the + allele has risen, and the frequency of the *l* allele has fallen.

To get from generation one's gene pool to generation two's gene pool, we just repeat the exercise. We combine the gametes in generation one's gene pool at random to make 100 zygotes—45 +/+, 44 +/*l*, and 11 *l*/*l*—and so on. The only problem with using pencil-and-paper numerical calculations to predict evolution is that chasing the alleles around and around the life cycle all the way to generation 12 is a tedious job.

With a computer, however, predicting how Dawson's population will evolve is quick and easy. We can use a spreadsheet application to set up the required calculations ourselves (see Computing Consequences 6.3 and 6.5), or we can use any of a variety of population genetics programs that are already set up to do the calculations for us. Such programs take starting allele frequencies and genotype fitnesses as input and use the model we have developed in this chapter to produce predicted allele frequencies in future generations as output. We encourage the reader to get one of these programs and experiment with it.

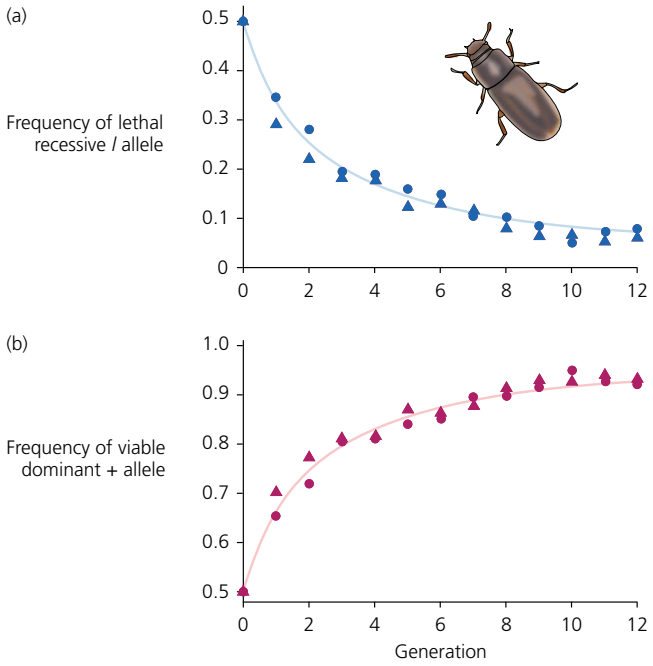

Figure 6.19 Evolution in laboratory populations of flour beetles (a) The decline in frequency of a lethal recessive allele (blue symbols) matches the theoretical prediction (blue curve) almost exactly. As the allele becomes rare, the rate of evolution slows dramatically. (b) This graph plots the increase in frequency of the corresponding dominant allele. Redrawn from Dawson (1970).

The prediction for Dawson's experiment appears as a curve in each of the graphs in Figure 6.19. The curve in the top graph predicts the falling frequency of the *l* allele; equivalently, the curve in the bottom graph predicts the rising frequency of the + allele. Our theory predicts that evolution will be rapid at first but will slow as the experiment proceeds.

Dawson's data appear in the graphs as colored circles and triangles. They match our theoretical predictions closely. This tight fit between prediction and data may seem unsurprising, even mundane. It should not. It should be astonishing. We

Empirical research on flour beetles shows that predictions made with population genetics models are accurate, at least under laboratory conditions.

COMPUTING CONSEQUENCES 6.6

An algebraic treatment of selection on recessive and dominant alleles

Here we develop equations that illuminate the differences between selection on recessive versus dominant alleles. Imagine a single locus with two alleles. Let p be the frequency of the dominant allele A, and let q be the frequency of the recessive allele a.

Selection on the recessive allele

Let the fitnesses of the genotypes be given by

w_{AA}	w_{Aa}	w_{aa}
1	1	$1 - s$

where s, called the **selection coefficient**, represents the strength of selection against homozygous recessives relative to the other genotypes. (Selection in favor of homozygous recessives can be accommodated by choosing a negative value for s.)

Based on Computing Consequences 6.3, the following equation gives the frequency of allele a in the next generation, q', given the frequency of a in this generation and the fitnesses of the three genotypes:

$$q' = \frac{pqw_{Aa} + q^2w_{aa}}{\overline{w}} = \frac{pqw_{Aa} + q^2w_{aa}}{p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa}}$$

Substituting the fitness values from the table above, and $(1 - q)$ for p, then simplifying, gives

$$q' = \frac{q(1 - sq)}{1 - sq^2}$$

If a is a lethal recessive, then s is equal to 1. Substituting this value into the preceding equation gives

$$q' = \frac{q(1 - q)}{1 - q^2} = \frac{q(1 - q)}{(1 - q)(1 + q)} = \frac{q}{(1 + q)}$$

A little experimentation shows that once a recessive lethal allele becomes rare, further declines in frequency are slow. For example, if the frequency of allele a in this generation is 0.01, then in the next generation its frequency will be approximately 0.0099.

Selection on the dominant allele

Let the fitnesses of the genotypes be given by

w_{AA}	w_{Aa}	w_{aa}
$1 - s$	$1 - s$	1

where s, the selection coefficient, represents the strength of selection against genotypes containing the dominant allele relative to homozygous recessives. (Selection in favor of genotypes containing the dominant allele can be accommodated by choosing a negative value of s.)

Based on Computing Consequences 6.3, we can write an equation that predicts the frequency of allele A in the next generation, p', given the frequency of A in this generation and the fitness of the three genotypes:

$$p' = \frac{p^2w_{AA} + pqw_{Aa}}{\overline{w}} = \frac{p^2w_{AA} + pqw_{Aa}}{p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa}}$$

Substituting the fitnesses from the table, and $(1 - p)$ for q, then simplifying, gives

$$p' = \frac{p(1 - s)}{1 - 2sp + sp^2}$$

If A is a lethal dominant, s is equal to 1. Substituting this value into the foregoing equation shows that a lethal dominant is eliminated from a population in a single generation.

Selection on recessive alleles versus selection on dominant alleles

Selection on recessive alleles and selection on dominant alleles are opposite sides of the same coin. Selection against a recessive allele is selection in favor of the dominant allele, and vice versa.

used a simple model of the mechanism of evolution combining the fundamental insights of Gregor Mendel with those of Charles Darwin to predict how a population would change over 12 generations. If the creatures in question had been humans instead of flour beetles, it would have meant forecasting events that will happen in 300 years. And Dawson's data show that our prediction was not just reasonably accurate, but spot on. If we had a theory that worked like that for picking stocks or racehorses—well, we could have retired years ago. Our model has passed its first test.

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Case study 4:

Overdominance in fruit flies

First imagine a recessive allele that is common: Its frequency is, say, 0.95. The dominant allele thus has a frequency of 0.05. By multiplying the allele frequencies, we can calculate the genotype frequencies:

AA	*Aa*	*aa*
$0.05^2 = 0.0025$	$2 \cdot 0.05 \cdot 0.95 = 0.095$	$0.95^2 = 0.9025$

Roughly 10% of the individuals in the population have the dominant phenotype, while 90% have the recessive phenotype. Both phenotypes are reasonably well represented, and if they differ in fitness, then the allele frequencies in the next generation may be substantially different.

Natural selection is most potent as a mechanism of evolution when it is acting on common recessive alleles (and rare dominant alleles). When a recessive allele is rare, most copies are hidden in heterozygotes and protected from selection.

Now imagine a recessive allele that is rare: Its frequency is 0.05. The dominant allele thus has a frequency of 0.95. The genotype frequencies are

AA	*Aa*	*aa*
$0.95^2 = 0.9025$	$2 \cdot 0.95 \cdot 0.05 = 0.095$	$0.05^2 = 0.0025$

Approximately 100% of the population has the dominant phenotype, while approximately 0% has the recessive phenotype. Even if the phenotypes differ greatly in fitness, there are so few of the minority phenotype that there will be little change in allele frequencies in the next generation. In a random mating population, most copies of a rare recessive allele are phenotypically hidden inside heterozygous individuals and thereby immune from selection.

As a final consideration in our discussion of dominant and recessive alleles, note that selection may favor or disfavor both kinds of variants. We emphasize this point because many people new to population genetics expect that dominant alleles are necessarily beneficial and thus tend to rise in frequency. While it is certainly true that some dominant alleles are beneficial, many others are deleterious. For example, Eileen Shore and colleagues (2006) identified a dominant mutation, located in a gene encoding a receptor for bone morphogenic protein, as the cause of fibrodysplasia ossificans progressiva, a rare and severely disabling condition in which skeletal muscle and connective tissue transform inexorably into bone. In all, some 30% of the alleles known to cause human diseases are autosomal dominants (López-Bigas et al. 2006). The terms *dominant* and *recessive* describe the relationship between genotype and phenotype, not the relationship between genotype and fitness.

Selection on Heterozygotes and Homozygotes

In our next two tests, we focus on whether our model can accurately predict what happens when selection favors heterozygotes or homozygotes. Both tests will use data on laboratory populations of fruit flies (*Drosophila melanogaster*).

Selection Favoring Heterozygotes

Our first example comes from research by Terumi Mukai and Allan Burdick (1959). Like Dawson, Mukai and Burdick studied evolution at a single locus with two alleles. We will call the alleles *V*, for viable, and *L* for lethal. This is because flies with genotype *VV* or *VL* are alive, whereas flies with genotype *LL* are dead. The researchers used heterozygotes as founders to establish two experimental populations with initial allele frequencies of 0.5. They let the populations evolve for 15 generations, each generation measuring the frequency of allele *V*.

So far, Mukai and Burdick's experiment sounds just like Dawson's. If it is, then our theory predicts that *V* will rise in frequency—rapidly at first, then more

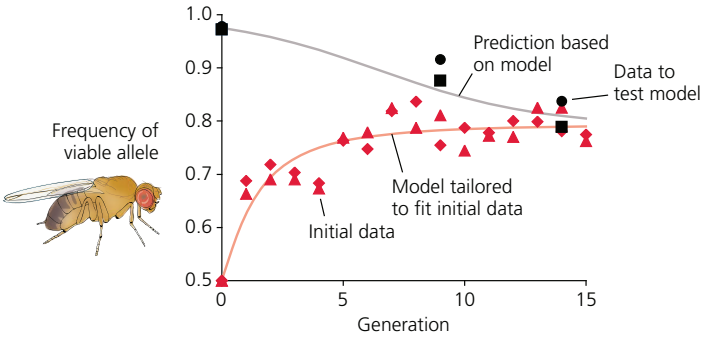

Figure 6.21 Evolution in four laboratory populations of fruit flies When homozygous, one allele is viable and the other lethal. Nonetheless, populations with a frequency of 0.5 for both alleles (red) evolved toward an intermediate equilibrium. The black populations represent a test of the hypothesis that heterozygotes enjoy the highest fitness. From data in Mukai and Burdick (1959).

slowly. By generation 15 it should reach a frequency of over 94%. But that is not what happened.

Mukai and Burdick's data appear in Figure 6.21, represented by the red symbols. As expected, the frequency of *V* increased rapidly over the first few generations. However, in both populations the rate of evolution slowed long before the viable allele approached a frequency of 0.94. Instead, *V* seemed to reach an equilibrium, or unchanging state, at a frequency of about 0.79.

How could this happen? An equilibrium frequency of 0.79 for the viable allele means that the lethal allele has an equilibrium frequency of 0.21. How could natural selection maintain a lethal allele at such a high frequency in this population? Mukai and Burdick argue that the most plausible explanation is **heterozygote superiority**, also known as **overdominance**. Under this hypothesis, heterozygotes have higher fitness than either homozygote. At equilibrium, the selective advantage enjoyed by the lethal allele when it is in heterozygotes exactly balances the obvious disadvantage it suffers when it is in homozygotes.

A little experimentation with a computer should allow the reader to confirm that Mukai and Burdick's hypothesis explains their data nicely. The red curve in Figure 6.21 represents evolution in a model population in which the fitnesses of the three genotypes are as follows:

VV	*VL*	*LL*
0.735	1.0	0

This theoretical curve matches the data closely.

Note that in this case the fit between theory and data does not represent a rigorous test of our model. That is because we examined the data first, then tweaked the fitnesses in the model to make its prediction fit. That is a bit like shooting at a barn and then painting a target around the bullet hole. Mukai and Burdick's flies did, however, provide an opportunity for a test of our model that is rigorous. And Mukai and Burdick performed it.

The researchers established two more experimental populations, this time with the initial frequency of the viable allele at 0.975. If the genotype fitnesses are, indeed, those required to make our model fit the red data points in Figure 6.21, then this time our model predicts that the frequency of the *V* allele should fall. As before, it should ultimately reach an equilibrium near 0.79. The predicted fall toward equilibrium is shown by the blue curve in Figure 6.21. Mukai and Burdick's data appear in the figure as blue symbols. The data match the prediction closely. Our model has passed its second test.

Research on fruit flies shows that natural selection can act to maintain two alleles at a stable equilibrium. One way this can happen is when heterozygotes have superior fitness.

Mukai and Burdick's flies have shown us something new. In all our previous examples, selection has favored one allele or the other. Under such circumstances

COMPUTING CONSEQUENCES 6.7

Stable equilibria with heterozygote superiority and unstable equilibria with heterozygote inferiority

Here we develop algebraic and graphical methods for analyzing evolution at loci with overdominance and underdominance. Imagine a population in which allele A_1 is at frequency p and allele A_2 is at frequency q. In Computing Consequences 6.3, we developed an equation describing the change in p from one generation to the next under selection:

$$\Delta p = \frac{p}{\bar{w}}(pw_{11} + qw_{12} - \bar{w})$$

$$= \frac{p}{\bar{w}}(pw_{11} + qw_{12} - p^2w_{11} - 2pqw_{12} - q^2w_{22})$$

Substituting $(1 - q)$ for p in the first and third terms in the expression in parentheses gives

$$\Delta p = \frac{p}{\bar{w}}[(1 - q)w_{11} + qw_{12} - (1 - q)^2w_{11} - 2pqw_{12} - q^2w_{22}]$$

which, after simplifying and factoring out q, becomes

$$\Delta p = \frac{pq}{\bar{w}}(w_{12} + w_{11} - qw_{11} - 2pw_{12} - qw_{22})$$

Now, by definition, the frequency of allele A_1 is at equilibrium when $\Delta p = 0$. The equation above shows that $\Delta p = 0$ when $p = 0$ or $q = 0$. These two equilibria are unsurprising. They occur when one allele or the other is absent from the population. The equation also gives a third condition for equilibrium, which is

$$w_{12} + w_{11} - qw_{11} - 2pw_{12} - qw_{22} = 0$$

Substituting $(1 - p)$ for q and solving for p gives

$$\hat{p} = \frac{w_{22} - w_{12}}{w_{11} - 2w_{12} + w_{22}}$$

where $\hat{p}$ is the frequency of allele A_1 at equilibrium. Finally, let the genotype fitnesses be as follows:

A_1A_1	A_1A_2	A_2A_2
$1 - s$	1	$1 - t$

Positive values of the selection coefficients s and t represent overdominance; negative values represent underdominance. Substituting the fitnesses into the previous equation and simplifying gives

$$\hat{p} = \frac{t}{s + t}$$

For example, when $s = 0.4$ and $t = 0.6$, heterozygotes have superior fitness, and the equilibrium frequency for allele A_1 is 0.6. When $s = -0.4$ and $t = -0.6$, heterozygotes have inferior fitness, and the equilibrium frequency for allele A_1 is also 0.6.

Another useful method for analyzing equilibria is to plot Δp as a function of p. Figure 6.20a shows such a plot for the two numerical examples we just calculated. Both curves show that $\Delta p = 0$ when $p = 0$, $p = 1$, or $p = 0.6$.

The curves in Figure 6.22a also allow us to determine whether an equilibrium is stable or unstable. Look at the red curve; it describes a locus with heterozygote superiority. Notice that when p is greater than 0.6, Δp is negative. This means that when the frequency of allele A_1 exceeds its equilibrium value, the population will move back toward equilibrium in the next generation. Likewise, when p is less than 0.6, Δp is positive. When

our model predicts that sooner or later the favored allele will reach a frequency of 100%, and the disfavored allele will disappear. By keeping a population at an equilibrium in which both alleles are present, however, heterozygote superiority can maintain genetic diversity indefinitely. For an algebraic treatment of heterozygote superiority, see **Computing Consequences 6.7**.

Selection Favoring Homozygotes

Our second example comes from work by G. G. Foster and colleagues (1972). These researchers set up experiments to demonstrate how populations evolve when heterozygotes have lower fitness than either homozygote. Foster and colleagues used fruit flies with compound chromosomes.

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Case study 5:

Frequency-dependent selection in Elderflower orchid

As in the first set of populations, the genotypes exhibit strong underdominance. This time, however, one kind of homozygote has much higher fitness than the other.

The algebraic analysis described in Computing Consequences 6.7 predicts an unstable equilibrium when the frequency of *C(2)* is exactly 0.8. If the frequency of *C(2)* ever gets above 0.8, then it should quickly rise to 1.0. Likewise, if the frequency of *C(2)* ever dips below 0.8, it should quickly fall to zero. Experimentation with a computer should allow the reader to reproduce this prediction.

The intuitive explanation is as follows. Heterozygotes are inviable, so the adults in the population are all homozygotes. Imagine first that *C(2)C(2)* individuals are common and *N(2)N(2)* individuals are rare. If the flies mate at random, then almost all matings will involve *C(2)C(2)* flies mating with each other, or *C(2)C(2)* flies mating with *N(2)N(2)* flies. Only very rarely will *N(2)N(2)* flies mate with their own kind. Consequently, most *N(2)N(2)* flies will have zero reproductive success, and the frequency of *C(2)* will climb to 1.0. Now imagine that there are enough *N(2)N(2)* flies present that appreciable numbers of them do mate with each other. These matings will produce four times as many offspring as matings between *C(2)C(2)* flies. Consequently, the frequency of *N(2)* will climb to 1.0 and the frequency of *C(2)* will fall to zero.

Foster and colleagues set up 13 mixed populations, with *C(2)* frequencies ranging from 0.71 to 0.96, then monitored their evolution for up to four generations. Predictions for the evolution of populations with initial *C(2)* frequencies of 0.75 and 0.85 appear as gray lines in the graph in Figure 6.23g. The data appear as purple lines. Qualitatively, the outcome matches the theoretical prediction nicely. In populations with higher initial *C(2)* frequencies, *C(2)* quickly rose to fixation, while in populations with lower initial *C(2)* frequencies, *C(2)* was quickly lost. The exact location of the unstable equilibrium turned out to be approximately 0.9 instead of 0.8. Foster and colleagues note that their *C(2)C(2)* flies carried recessive genetic markers, bred into them to allow for easy identification. They suggest that these markers reduced the relative fitness of the *C(2)C(2)* flies below the value of 0.25 inferred solely on the basis of their compound chromosomes.

Our model's predictions were not as accurate for Foster et al.'s experiments as they were for Dawson's and Mukai and Burdick's. Nonetheless, the model performed well. It predicted something we have not seen before: an unstable equilibrium above which the frequency of an allele would rise and below which it would fall. It predicted that the unstable equilibrium would be higher in Foster et al.'s second set of populations than in their first. And its predictions about the rate of evolution were roughly correct. Our model has passed its third test.

When heterozygotes have inferior fitness, one allele tends to go to fixation while the other allele is lost. However, different populations may lose different alleles.

Foster et al.'s experiments demonstrate that heterozygote inferiority leads to a loss of genetic diversity within populations. By driving different alleles to fixation in different populations, however, heterozygote inferiority may help maintain genetic diversity among populations.

Frequency-Dependent Selection

For our fourth and final test of population genetics theory, we will see whether our model can predict the evolutionary outcome when the fitness of individuals with a particular phenotype depends on their frequency in the population. Our example, from the work of Luc Gigord, Mark Macnair, and Ann Smithson (2001), concerns a puzzling color polymorphism in the Elderflower orchid (*Dactylorhiza sambucina*).

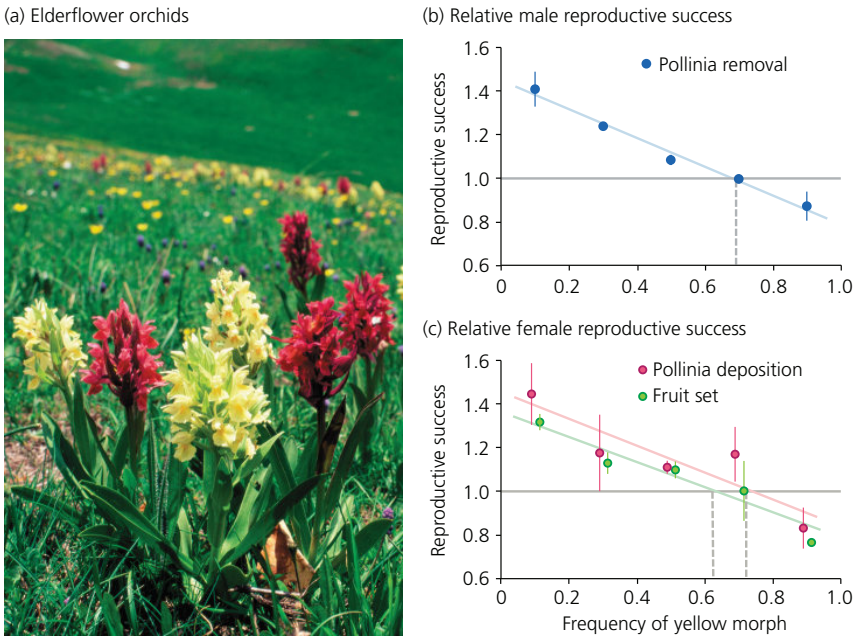

Figure 6.24 Frequency-dependent selection in Elderflower orchids (a) A mixed population. Some plants have yellow flowers, others have purple flowers. (b) Through male function, yellow flowers have higher fitness than purple flowers when yellow is rare, but lower fitness than purple flowers when yellow is common. (c) Through female function, yellow flowers have higher fitness than purple flowers when yellow is rare, but lower fitness than purple flowers when yellow is common. The dashed vertical lines show the predicted frequency of yellow flowers, which matches the frequency in natural populations. From Gigord et al. (2001).

Elderflower orchids come in yellow and purple (Figure 6.24a). Populations typically include both colors, though yellow is usually more common. The flowers attract bumblebees, which are the orchid's main pollinators. But the bees that visit Elderflower orchids are always disappointed. To the bees the orchid's colorful flowers appear to advertise a reward, but in fact they offer nothing. The puzzle Gigord and colleagues wanted to solve was this: How can two distinct deceptive advertisements persist together in Elderflower orchid populations?

The researchers' hypothesis grew from earlier observations by Smithson and Macnair (1997). When naive bumblebees visit a stand of orchids to sample the flowers, they tend to alternate between colors. If a bee visits a purple flower first and finds no reward, it looks next in a yellow flower. Finding nothing there either, it tries another purple one. Disappointment sends it back to a yellow, and so on, until the bee gives up and leaves. Because bumblebees tend to visit equal numbers of yellow and purple flowers, orchids with the less common of the two colors receive more visits per plant. If more pollinator visits translate into higher reproductive success, then the rare-color advantage could explain why both colors persist. Selection by bumblebees favors yellow until it becomes too common, then it favors purple. This is an example of **frequency-dependent selection**.

To test their hypothesis, Gigord and colleagues collected and potted wild orchids, then placed them in the orchids' natural habitat in 10 experimental arrays of 50 plants each. The frequency of yellow flowers varied among arrays, with two arrays at each of five frequencies: 0.1, 0.3, 0.5, 0.7, and 0.9. The researchers monitored the orchids for removal of their own pollinia (pollen-bearing structures), for deposition of pollinia from other individuals, and for fruit set. From their data, Gigord and colleagues estimated the reproductive advantage of yellow flowers, relative to purple, via both male and female function.

The resulting estimates of relative reproductive success, plotted as a function of the frequency of yellow flowers, appear in Figure 6.24b and c. Consistent with the researchers' hypothesis, yellow-flowered orchids enjoyed higher reproductive

Selection can also maintain two alleles in a population if each allele is advantageous when it is rare.

success than purple-flowered plants when yellow was rare and suffered lower reproductive success when yellow was common.

Gigord and colleagues calculated the relative reproductive success of yellow orchids as

$$RRS_y = \frac{2(RS_y)}{RS_y + RS_p}$$

where RS_y and RS_p are the absolute reproductive success of yellow and purple orchids. The relationship between relative reproductive success via male function and the frequency of yellow flowers is given by the best-fit line in Figure 6.21b. It is

$$RRS_y = -0.66F_y + 1.452$$

where F_y is the frequency of yellow flowers.

We can incorporate this relationship into a population genetics model. We might imagine, for example, that flower color is determined by two alleles at a single locus and that yellow is recessive to purple. We set the starting frequency of the yellow allele to an arbitrary value. We assign fitnesses to the three genotypes as we have before, except that the fitnesses change each generation with the frequency of yellow flowers. When we use a computer to track the evolution of our model population, we discover that the frequency of the yellow allele moves rapidly to equilibrium at an intermediate value. This value is precisely the allele frequency at which yellow flowers have a relative fitness of 1. We get the same result if we imagine that yellow flowers are dominant. Again the equilibrium value for the yellow allele is the frequency at which yellow and purple flowers have equal fitness.

The dashed vertical lines in Figure 6.24b and c indicate the predicted equilibrium frequencies Gigord and colleagues calculated for each of their fitness measures. The predictions are 61%, 69%, and 72% yellow flowers. The researchers surveyed 20 natural populations in the region where they had placed their experimental arrays. The actual frequency of yellow flowers, $69 \pm 3\%$, is in good agreement with the predicted frequency. Our model has passed its fourth test.

Gigord et al.'s study of Elderflower orchids demonstrates that frequency-dependent selection can have an effect similar to heterozygote superiority. Both patterns of selection can maintain genetic diversity in populations.

Compulsory Sterilization

We can use population genetics models to evaluate whether eugenic sterilization could have accomplished the aims of its proponents, had their assumptions about the heritability of traits been correct. The answer depends on the frequency of the alleles in question, and on the criteria for success.

The theory of population genetics, despite its simplifying assumptions, allows us to predict the course of evolution. Our four tests show that the model we have developed works remarkably well. So long as we know the starting allele frequencies and genotype fitnesses, the model can predict how allele frequencies will change, under a variety of selection schemes, many generations into the future. The requisite knowledge is easiest to get, of course, for experimental populations living under controlled conditions in the lab. But Gigord et al.'s study of Elderflower orchids shows that the model can even make fairly accurate predictions in natural populations. Given its success in the four tests, it is reasonable to use our model to consider the evolutionary consequences of a eugenic sterilization program. The proponents of eugenic sterilization sought to reduce the fitness of particular genotypes to zero and thereby to reduce the frequency of alleles responsible for undesirable phenotypes. Would their plan have worked?