

10 Walking sticks: natural selection for cryptic coloration on different host plants

While she was a graduate student at the University of California, Christina Sandoval discovered a new species of insect. *Timema cristinae* is an inconspicuous stick insect that lives in the chaparral of Southern California. It is only about 2 cm long and it feeds mostly at night. During the day it remains still and hides by mimicking the branches and leaves of its host plant. Because they are such good mimics of the host plants they feed on they are called “stick insects” or “walking sticks”. Eggs hatch on ground and young climb into a nearby host plant. Sometimes they never leave that single plant.

Despite their inactivity, Sandoval noticed some very interesting differences between the insects. There were two color types. Some of the walking sticks were plain green while the others had a long white stripe on their back. Moreover, those two color morphs were associated with two different species of host plant, with one type found on one host plant and the other on the second host.

One of the first possibilities she considered was that the two forms were different species. Sandoval brought them back to the lab and found that the two types could interbreed freely, which showed that they were simply color variants of a single species of walking stick. **Why, then, were there two colors types? Why were they segregated on different host plant species?** She suspected that this was an example of natural selection at work. The striped form was favored on *Adenostoma* because it more closely mimicked the leaves of that host, whereas the unstriped form was more camouflaged on *Ceanothus*.

Adenostoma fasciculatum, commonly called “Chamise”, is a small shrub in the rose family (Rosaceae). It has narrow grey-green leaves. The other host is *Ceanothus spinosus*, “mountain lilac”, which is in the buckthorn family (Rhamnaceae). It is taller and has broader, brighter-green leaves. Both plant species can occur on the same hillside, but they tend to form patches where one or the other species is dominant. The species *Timema cristinae* can be found

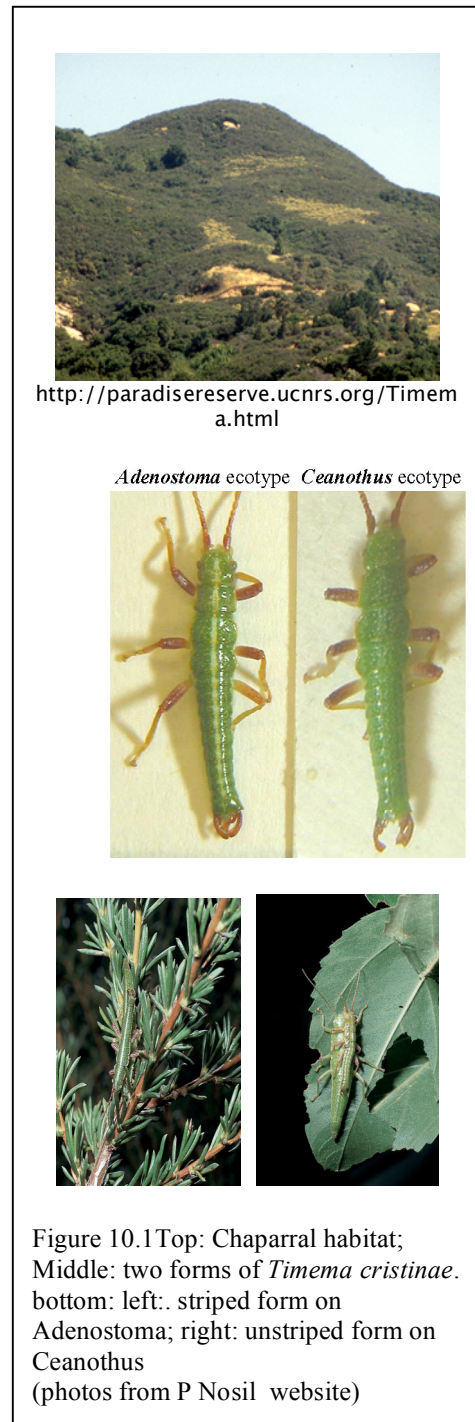


Figure 10.1 Top: Chaparral habitat; Middle: two forms of *Timema cristinae*. bottom: left: striped form on *Adenostoma*; right: unstriped form on *Ceanothus* (photos from P Nosil website)

on both host species, but Sandoval noticed that in populations where *Adenostoma* was abundant, the insects commonly had a long bright stripe on their back. In populations where *Ceanothus* was the major host plant, the insects commonly lacked that dorsal stripe.

Sandoval reared walking sticks of both color types in the laboratory to study the color polymorphism. When she made controlled crosses between the two types, she saw that the presence or absence of the stripe segregated as a single Mendelian locus with two alleles. Stripe x stripe matings always produced striped offspring, Unstriped x unstriped crosses occasionally produced a mixture of unstriped and striped offspring. She interpreted this to mean that the unstriped allele (U) was dominant to the striped allele (S).

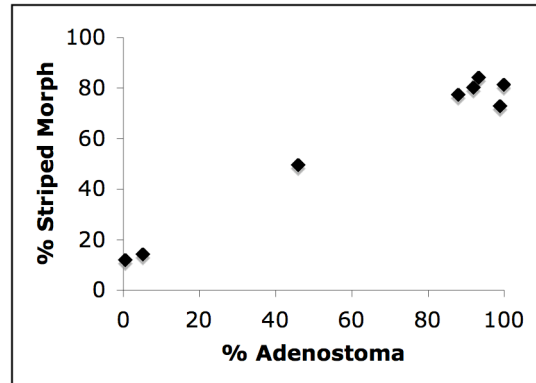


Figure 10.2 Sandoval (1994) found that the striped morph of *Timema* was most common in areas that had a high proportion of *Adenostoma* shrubs.

10.1 Evolutionary inferences

As is common in ecology and evolutionary biology, this is an example where we see an interesting pattern and we want to make inferences about the biological processes that have created that pattern. How do we start? There is a long list of possible explanations for the pattern, but we will concentrate on three:

- Natural selection favors the striped morph on *Adenostoma* because it is more cryptic.
- Natural selection favors the striped morph on *Adenostoma* for some other reason.
- The pattern is simply the result of random chance.

What kinds of evidence can we gather to see whether it supports the adaptive scenario?

Is the pattern repeatable? If the distribution of color morphs on the two host plants is caused by natural selection, then we ought to see the same pattern in numerous sites. While it might be possible that the distribution of insects at one location is simply the result of chance, it is unlikely that we would see the same pattern in many independent locations.

Sandoval and Patrick Nosil sampled insects on the two host plants in 15 different populations in the Santa Inez Mountains of California. The proportions of the striped and unstriped morphs differed slightly from site to site. But in 12 of those 15 samples the striped morph was more common on *Adenostoma* bushes and the unstriped morph was more common on *Ceanothus*, just as predicted.

Does the pattern make biological sense? This is the optimization argument for natural selection. Often, we start by creating a plausible scenario to see if our observations are consistent with other things we know about the natural history, such that we could plausibly

suppose that the striped form would have higher fitness on *Adenostoma*. We may note the differences in the leaf shape and color on the two plants and suppose that the striped form would be more cryptic on *Adenostoma*. We may compare this to other species that have been studied, and note the similarity between the color morphs of *Timema* and the color morphs of other insects. For example, if we knew that the main predator of these insects used its sense of smell to forage, that might cause us to reconsider our idea that the cryptic coloration was important for survival. However, for *Timema*, birds are the main predators and we know that birds use visual search to find prey. Thus we might expect from our knowledge of natural history that cryptic coloration would increase survival.

Sometimes this is called “adaptive storytelling” or “just-so stories”. It can be useful in helping to come up with hypotheses about possible adaptations and helping us to refine our ideas into a plausible scenario. But it can sometimes be overly seductive. If we are not careful, we can sometimes create elaborate stories that can reflect what we *want* to see, but may not reflect the real biological processes that are at work.

Is the pattern specific? Natural selection acts on particular phenotypes. From what we know about Mendelian inheritance, other phenotypic traits that are genetically independent of the stripe allele should segregate equally in the two morphs. If our hypothesis is that the stripe phenotype increases survival on *Adenostoma*, then we would expect to see a higher proportion of striped individuals on those host plants, but no necessary difference in other traits (size, body color, etc.). In fact, the insects collected from *Adenostoma* are smaller, and duller green in addition to having the stripe. So, selection for stripes may be only part of the story (or maybe the stripes are simply a byproduct of selection on an entirely different trait).

Can we see direct evidence of natural selection? Experimental manipulations generally provide the strongest evidence for natural selection, although they may be difficult or impossible to carry out for many species. Certainly we can never do experimental manipulations to identify potential adaptations in fossil species. But when they can be done, they provide uniquely powerful tests. There are several possible approaches, limited only by the imagination of the researcher.

One approach is to directly manipulate the phenotype. In a famous example, Malte Andersson artificially lengthened and shortened the tails of male widowbirds by clipping the tails of some birds and gluing the ends of the tails onto others. From those experiments he was able to show that males with long tails were more attractive to females and had higher mating success than males with shorter tails.

Another approach is to manipulate numbers, by moving individuals among populations or habitats. Sandoval chose that approach. She placed 100 marked walking sticks of the two color morphs on the two species of host plant. After 30 days she returned and found that frequency of the striped form had increased on *Adenostoma* whereas the frequency of unstriped morph had increased on *Ceanothus*. Her experimental manipulation showed that there was a causal connection between host plant and the persistence of the two morphs, although the precise mechanism was still a mystery.

10.2 Inferring the mechanism of selection

The circumstantial evidence for natural selection on the color of *Timema* walking sticks is strong. It is clear that the distribution of color morphs is not random across host plants. In all populations there is a higher frequency of striped morphs on *Adenostoma* than on *Ceanothus*. Moreover, that pattern matches our biological understanding because birds (the main predator) forage visually and the striped form appears to be more cryptic on *Adenostoma* than on *Ceanothus*. When Sandoval placed known numbers of striped and unstriped *Timema* on the two host plants, she found that the frequency of striped insects increased on *Adenostoma* while the frequency of unstriped morphs increased on *Ceanothus*.

While those data provide good circumstantial evidence for natural selection, they do not prove that the color variations are the result of natural selection through differential predation by birds. To say something about the mechanism of selection it is important to manipulate the birds as well.

Selection by birds. Patrick Nosil, a graduate student at Simon Fraser University, decided to test the hypothesis that selection by birds caused the differences in frequency of the two morphs on the two host plants.

His approach was to exclude predators from some bushes using chicken wire cages. He constructed the cages around bushes of *Ceanothus* and *Adenostoma*, and selected other nearby bushes of the two host plant species to serve as controls. He then placed 24 individuals on each bush, making sure that the frequency of the two color morphs was equal and that there were equal numbers of males and females. Each of those insects was marked on the underside of their abdomen with a felt-tip marker. That allowed him to exclude any recent immigrants from his estimates of survival, but the mark was hidden from predators. The holes in the wire cages were large enough that the walking sticks could move in and out of the enclosures, but birds were excluded.

After 24 days he returned to the bushes to see how many of the original marked individuals of each morph are still present. His results are present below:

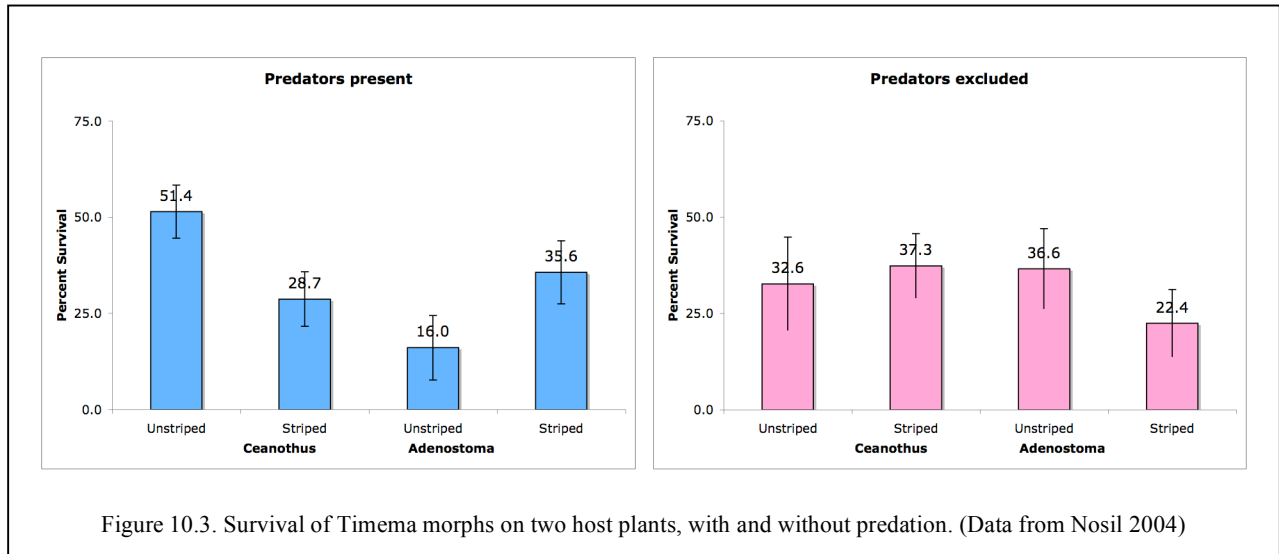
On the control bushes (left graph; predators present),

Which type had higher survival on *Ceanothus*? _____

Which type had higher survival on *Adenostoma*? _____

Do those results match our expectation? _____

What happened when birds were excluded?

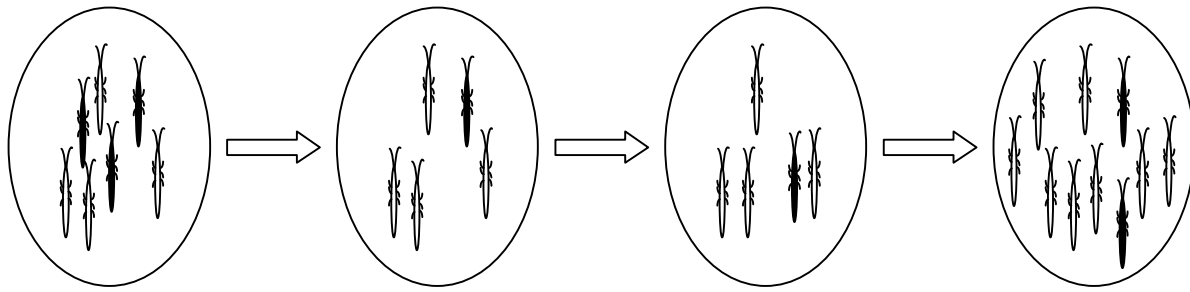


Nosil's mark-recapture experiment showed that there were differences in survival of the two types on the different host plants. The unstriped form had higher survival on *Ceanothus* whereas the striped form had higher survival on *Adenostoma*, just as Sandoval had guessed. However, the crucial bit of evidence was that when predators were excluded, those differences in survival disappeared. Without birds, all types had approximately equal survival. That experiment provides very powerful evidence that exposure to predators, not some other cause, is responsible for the differences in frequency of the two types on the different host plants.

So, we now know something about the forces of natural selection that act on the color morphs of *Timema*. But it is possible to go even further. How fast will the frequency of the two color morphs change as a result of differences in predation? What will be the long-term outcome of selection? Can the two color types coexist? For that we need a quantitative model of natural selection on this trait.

10.3 Modeling selection to predict changes in allele frequency

We will start with a verbal model of our understanding of natural selection on the walking sticks. We assume that at the start of our observations (generation 0) the unstriped allele (U) is present at some frequency p and the striped allele is present at frequency $q=1-p$. Those walking sticks are exposed to a period of predation by birds as they grow. Because the striped and unstriped walking sticks have different probabilities of survival, the frequency of the unstriped allele among the survivors will not be the same as at the start of the generation (we will call the new allele frequency p^*). Once the insects are mature, we assume the surviving adults mate at random to produce the offspring and start the next generation (generation 1). Because random mating does not change allele frequencies, the new allele frequency remains $p'=p^*$. Random mating will produce offspring genotypes in HW proportions, based on the new allele frequencies (p'^2 , $2p'q'$, and q'^2).



Generation 0

Initial allele frequency: p

Genotypes in HWE

Allele frequency after selection: p'

Selection removes some phenotypes, so no longer in HWE

Random mating does not change allele frequency

Generation 1

Allele frequency of the offspring is still p' .

Offspring genotypes are again in HWE

Calculate initial allele frequencies and genotype frequencies. As stated above, the presence of the stripe is controlled by a single locus with the unstriped allele (U) dominant to the striped allele (S). Because of the dominance of the unstriped allele, it is impossible to calculate the allele frequencies directly: unstriped walking sticks will be an unknown mixture of UU and US genotypes. However, if we assume that walking sticks mate at random with respect to the stripe locus then the genotypes should be in Hardy-Weinberg proportions. The unstriped walking sticks will be a mixture of homozygotes and heterozygotes, but we expect the relative proportions of the two types to be p^2 UU homozygotes and $2pq$ US heterozygotes. The recessive striped morphs will all have genotype SS and will have a frequency q^2 .

Table 10.1

Phenotype	Genotype	Expected Frequency
Unstriped	UU	p^2
	US	$2pq$
Striped	SS	q^2

So, how do we estimate the allele frequencies p and q ? The trick is to note that we are certain of the genotype of the striped morph and we expect its frequency to be q^2 . If the proportion of striped morphs in a population is Q , then the allele frequency of the striped allele is $q = \sqrt{Q}$. Then, once we know q , we can calculate the frequency of the unstriped allele as $p = 1 - q$.

Selection worksheet (long form)

Frequency of striped (SS) walking sticks is 0.09

Initial allele frequencies are $q = \sqrt{0.09} = 0.30$ and $p = 0.70$

Genotype	UU	US	SS
Phenotype	unstriped	unstriped	striped
Genotype frequencies (HWE)	p^2	$2pq$	q^2
Genotype frequencies ($p=0.7$)	0.49	0.42	0.09

As an example, let's assume we start with 1000 insects:

Number of newborns in the first generation (N=1000)	490	420	90
Survival probability (absolute fitness)	0.514	0.514	0.287
Relative fitness (w)	1	1	0.558

$$\bar{w} = p^2 w_{UU} + 2pq w_{US} + q^2 w_{SS}$$

Population mean relative fitness = 0.960

Expected number of individuals that survive to reproduce	251.86	215.88	25.83
Proportion	0.510	0.437	0.052

Selection has changed the relative abundance of color morphs. We can calculate the new allele frequency among the survivors:

$$p = \frac{N_{UU} + \frac{1}{2}N_{US}}{2N} = \frac{25.186 + 10.794}{49.357} = 0.729$$

The allele frequency among surviving adults is now $p'=0.729$. Notice also that the genotypes are no longer in HW proportions

Those surviving adults then mate at random among themselves. Random mating always produces Hardy-Weinberg proportions among the offspring genotypes, based on that new allele frequency.

Offspring genotype frequencies after random mating	$p'^2 = 0.531$	$2p'q' = 0.395$	$q'^2 = 0.073$
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The allele frequency of the newborns is still 0.729. Random mating does not change the allele frequency.

At the start of this second generation, the frequency of the U allele has increased from 0.7 to 0.729, due to the higher survival of unstriped walking sticks.

It is very important to notice that the selection coefficients are based on the phenotypes of the

walking sticks, not their genotypes. On *Ceanothus*, unstriped walking sticks are more camouflaged than striped walking sticks, so they suffer less predation by birds. Because U is dominant to S, *both* UU and US genotypes will be unstriped and both will have the *same fitness*. There is no direct selection favoring the U allele; there is only selection favoring the unstriped phenotype.

After a period of predation, the frequencies of the two morphs among the survivors (and therefore the frequencies of the U and S alleles) will have changed. The survival of the striped (SS) individuals is only 55.8% as high as the unstriped form, which decreases the frequency of the S allele in the population. For this example, the new allele frequencies are $p=0.729$ and $q=0.271$ (see worksheet, below).

Inheritance. Those surviving adults then mate to produce the offspring of the next generation. If we assume that mating is random with respect to their genotype at this locus, then the offspring will again be produced in Hardy Weinberg proportions *based on the new allele frequency in the parents that survive to reproduction*. So, we expect the new frequency of striped (SS) walking sticks to be $q^2 = 0.271^2 = 0.073$.

10.4 The general selection equation:

I have tried to spell out the process of natural selection in some detail, so the underlying logic will be clear. Notice that all that is happening is that differential survival changes the frequencies of striped and unstriped alleles among adults, compared to their initial frequency and those new allele frequencies determine the genotype proportions in the offspring.

In fact, it is possible to collapse all of this into one simple equation that will allow us to easily calculate the effect of selection on allele frequency. Assuming the walking sticks mate randomly with respect to the color morph, the genotypes will be in Hardy-Weinberg proportions: p^2 , $2pq$ and q^2 for UU, US and SS genotypes. The new allele frequency after selection (p') is simply:

$$p' = \frac{p^2 w_{UU} + pq w_{US}}{\bar{w}} \quad \text{eq. 10.1}$$

where

$$\bar{w} = p^2 w_{UU} + 2pq w_{US} + q^2 w_{SS} \quad \text{eq. 10.2}$$

As before, p is the frequency of the U allele and w_{UU} , w_{US} and w_{SS} are the relative fitnesses of the three genotypes.¹

¹ One way to think about equation 10.1 is that it is the average fitness of the U allele relative to the average fitness of the entire population. Remember, we can always calculate an average of any group by summing the probability of observing each type multiplied by its value: $\bar{x} = \sum \Pr(y = x) \cdot x$. Thus the population mean fitness (denominator) is the sum of the probabilities of observing each of the three genotypes (p^2 , $2pq$ and q^2) times their relative fitnesses (w_{UU} , w_{US} and w_{SS}). The mean fitness of the U allele (numerator) is the sum of

Using the data from Box ?? (selection worksheet) and equation 10.1, we get

$$p' = \frac{0.49 \cdot 1.0 + 0.21 \cdot 1.0}{0.96} = 0.729, \text{ exactly the same answer as above.}$$

10.5 Selection always increases the population mean fitness.

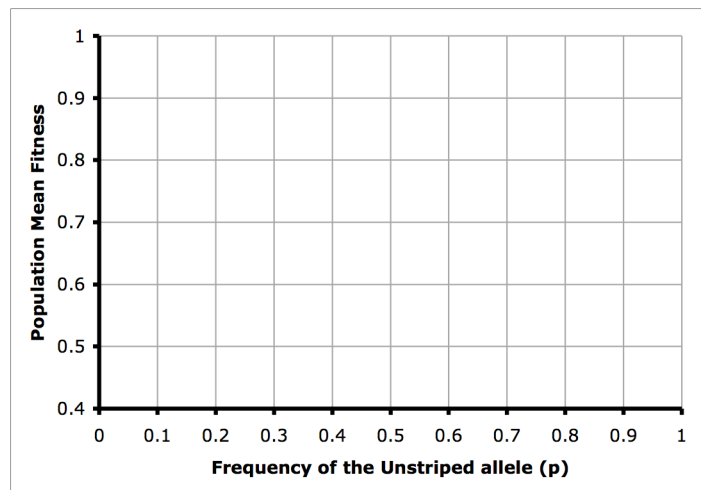
In the previous example, the population mean fitness at the beginning of generation 1 was $\bar{w} = 0.96$. Selection changed the allele frequency of the unstriped allele from $p=0.7$ to $p'=0.729$ at the start of generation 2.

Using the new allele frequency, what is the mean fitness of walking sticks at the start of the second generation?

$$\bar{w} = \underline{\hspace{2cm}}$$

This illustrates a general principle of natural selection: that selection always acts to increase the population mean fitness. R. A. Fisher called that the "fundamental theorem of natural selection". Unless some other evolutionary force is also acting on the population, natural selection will lead to a greater and greater degree of adaptation (higher mean fitness) to a particular environment.

Sketch a graph of the population mean fitness for populations with different frequencies of the unstriped allele. To make things easier, we already know the mean fitness when $p=0.7$ and 0.73 . What would be the mean fitness if all of the walking sticks were unstriped ($p=1.0, q=0$)? What would be the mean fitness in the population if all of the walking sticks were striped ($p=0$)? That should be enough to sketch an approximate graph.



This picture of the population mean fitness vs allele frequency in the population is often called the "adaptive landscape" because it forms hills and valleys of various shapes.

Hilltops are regions with high mean fitness. Natural selection always pushes the allele frequency in the population "uphill" towards a fitness peak.

In this example, what will be the eventual allele frequency in the population if it is always moving uphill on this adaptive landscape?

probabilities of observing a U allele (all of the alleles in the UU homozygotes and half of the alleles in the heterozygotes: p^2 and $1/2 \cdot 2pq$) times the fitnesses (w_{UU}, w_{US}).

Eventual allele frequency = _____

10.6 What are the allele frequency dynamics of this system?

What will be the equilibrium allele frequency in this system? By definition, the system will be at equilibrium when the allele frequency does not change. To find that equilibrium we need an equation for the change in allele frequency (Δp) and then we can solve for $\Delta p=0$.

The change in allele frequency (Δp) is

$$\Delta p = p' - p$$

$$\Delta p = \frac{p^2 w_{UU} + pq w_{US}}{\bar{w}} - p$$

Multiply the last term (p) by $\frac{\bar{w}}{\bar{w}}$ (to get a common denominator) and rearrange to get:

$$\Delta p = \frac{p((p w_{UU} + q w_{US}) - \bar{w})}{\bar{w}}$$

or, after some algebra,

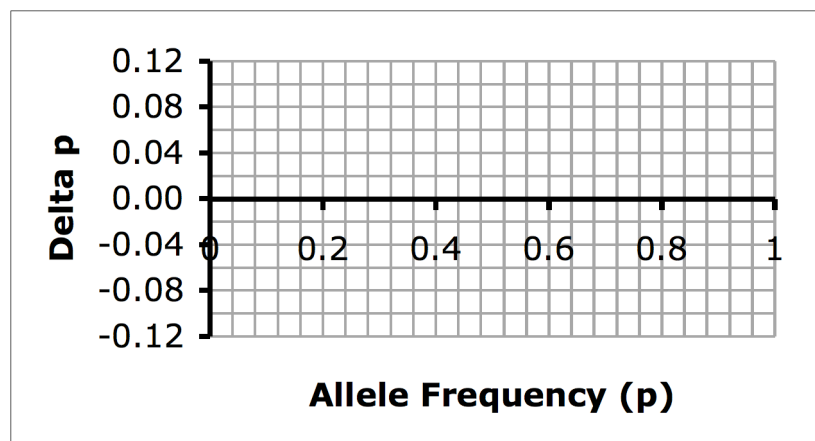
$$\Delta p = \frac{pq(p(w_{UU} - w_{US}) + q(w_{US} - w_{SS}))}{\bar{w}} \quad \text{eq. 10.3}$$

To understand this equation, notice that \bar{w} will always be a positive number. Therefore the direction of change in allele frequency depends only on the numerator of eq. 10.3. We want to find an equilibrium, which will occur whenever $\Delta p = 0$. That will happen when either $p=0$ or $q=0$ or the quantity in parentheses equals zero.

One of the easiest ways to see what happens is to graph Δp vs. p . Using the example data for selection on *Ceanothus* (where the relative fitnesses were $\{1, 1, 0.558\}$), here are some values for Δp , calculated using equation 10.3.

Sketch the graph of Δp vs. p .

p	Δp
0	0.000
0.1	0.056
0.2	0.079
0.3	0.083
0.4	0.076
0.5	0.062
0.6	0.046
0.7	0.029
0.8	0.014
0.9	0.004
1	0.000



By definition, there will be an equilibrium when $\Delta p=0$. This graph shows that there are two equilibria: $p=0$ and $p=1$.

If the starting frequency is $p=0.4$, will the U allele increase or decrease? _____

Note that Δp is positive for all values of p between 0 and 1, which means that the frequency of the U allele will always increase. No matter what the starting allele frequency is (assuming $p>0$), the frequency of the U allele will increase until it becomes fixed in the population.

You can see this by checking the stability of the two equilibria. First imagine that the population starts out fixed for the striped allele ($p=0$). It is an equilibrium because as long as there are no U alleles present, the frequency p will remain zero. We can check the stability by imagining there is some perturbation, i.e. some immigrants arrive that carry the U allele. The allele frequency p is now slightly greater than zero. What will happen? From the graph, Δp is positive, so the allele will increase in frequency, away from the equilibrium. The equilibrium at $p=0$ is an unstable equilibrium.

Now imagine a population that starts out fixed for the unstriped allele ($p=1.0$). Again there is a slight perturbation (immigrants arrive that carry the S allele) that makes p slightly less than 1. What will happen? From the graph, Δp is positive, so the allele will increase in frequency back towards that equilibrium, showing that the equilibrium at $p=1$ is a stable equilibrium.

What this all means is that eventually, no matter what the starting allele frequency is, we expect the population to eventually become fixed for the U allele and stay that way.

10.7 Assumptions of the selection model:

- The population is large (so the observed allele and genotype frequencies are exactly equal to the expected allele frequencies).
- There is no migration into or out of the population.
- There is no mutation.
- Mating is random (so offspring genotypes will be produced in HW proportions).

The assumptions of this selection model are very similar to our general assumptions used to derive the Hardy Weinberg Equilibrium. The ONLY HW assumption that we have altered is that the genotypes have different probabilities of survival.

10.8 Your turn:

Now calculate the changes in allele frequency in populations on the other host plant.

- In a population where the only host plant was *Adenostoma*, Patrick Nosil found 70 unstriped walking sticks and 322 striped walking sticks. What are the allele frequencies of the U and S alleles in this population? Using the survival data from figure 10.3a, calculate the expected changes in allele frequency for a population that is

feeding only on *Adenostoma*. What will be the frequency of striped walking sticks after 1 generation of selection on *Adenostoma*? What will be the frequency after 2 generations of selection? Make a graph of Δp vs. p . What is the stable equilibrium value of p ?

10.9 Further reading:

Sandoval, C.P. 1994. The effects of the relative scales of gene flow and selection on morph frequencies in the walking-stick *Timema cristinae*. *Evolution* 48:1866-1879.

Nosil, P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. *Proc. R. Soc. Lond. B.* 271: 1521-1528.

10.10 Questions and Problems:²

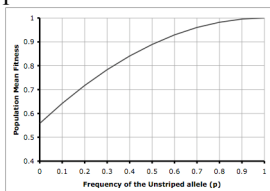
(will add a short set of exercises here)

Answers:

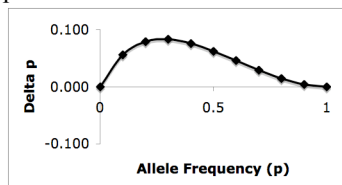
p 4: The unstriped form had higher survival on *Ceanothus* while the striped form had higher survival on *Adenostoma*, just as expected.

p 7. $W_{SS}=0.287$ W_{US} , $W_{UU} = 0.514$

p 10 mean fitness = 0.967



p 11



For the *Adenostoma* population, the frequency of the striped allele is $q = \sqrt{322/392} = 0.906$. $p = 1 - q = 0.094$. The relative fitnesses are .449 for the unstriped morph and 1.0 for the striped walking sticks. The new allele frequency will be $p' = 0.047$. The frequency of the striped *phenotype* (q^2) will be 0.909 after 1 generation and 0.956 after 2 generations of selection. Because Δp is always negative, the stable equilibrium will be $p = 0$.