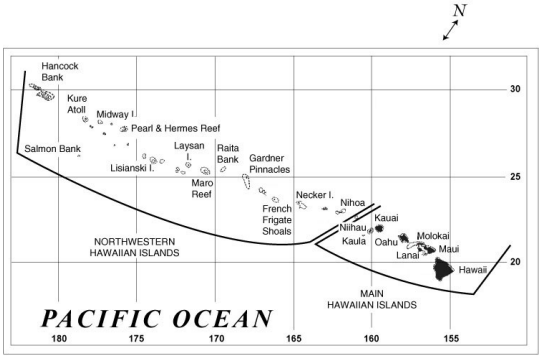
8 Genetics of Small Populations: the case of the Laysan Finch

In 1903, rabbits were introduced to a tiny island in the Hawaiian archipelago called Laysan Island. That island is only 187 ha in size, in the middle of the Pacific Ocean about 1000 km northeast of Hawaii. Despite the benign intentions for having rabbits on the island, the released rabbits quickly multiplied and devoured most of the vegetation.

Oceanic islands are often home to unique or endemic species that have evolved in isolation. Laysan was no exception. Well-known examples of island endemics include the finches on the Galapagos Islands and the dodo that was only known from the islands of Mauritius. On Laysan there were several species of birds and plants that were known only from that single island.

The introduced rabbits on the island destroyed the food supply for various species of land birds. Several endemic species of plants and animals were driven extinct, including the Laysan rail, the Laysan honeycreeper, the millerbird, and plants like the Laysan fan palm. Only 4 of 26 species of plants remained on the island in 1923, 20 years after the rabbits first arrived. Populations of other species declined to very small levels. One of the two surviving endemic land birds was a small finch called the Laysan finch, *Telespiza cantans*. After the rabbits were finally exterminated in 1923, the population of finches recovered on Laysan Island. Over the last four decades the average population size of on Laysan Island has been about 11,000 birds. Nevertheless, because of the continued risk of having only a single population on a single island, the US Fish and Wildlife Service transplanted some birds to another atoll in the Hawaiian archipelago called Pearl and Hermes reef. In 1967 approximately 100 birds were released on Southeast Island, of which 50 survived to found the new population. In 1968 two of those birds migrated to the nearby Grass Island, followed by 6 more in 1970. And in 1973 two birds migrated to another island in the atoll, North Island.

Figure 8.1. Laysan Island is about 800 miles NW of Hawaii.

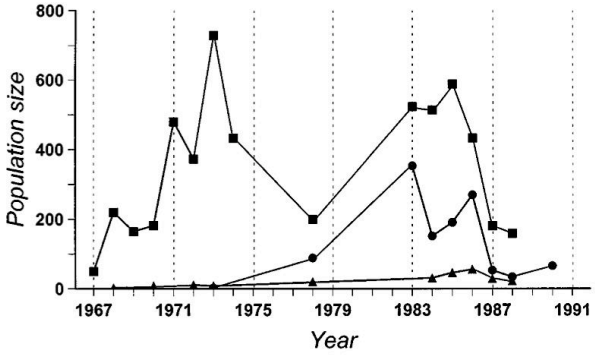


The Hawaiian Archipelago

Laysan Island

The islands of Pearl and Hermes are tiny coral atolls with a combined size of only 40 ha. The highest point of land is only 3 m above sea level and some parts are occasionally submerged. So the population sizes on those tiny islands have never gotten large in the 4 decades since they were founded. On Southeast Island the population grew rapidly and has since fluctuated around an average of about 350 birds, whereas the populations on North Island and Grass Island have hovered around 30-50. In contrast, the original population on Laysan averages about 11,000 birds.

Figure 2. Population size fluctuations on the smaller islands of Pearl and Hermes Reef.



Just as small populations of birds have a risk of extinction, there is always a chance of some alleles going extinct in any small population. Because the allelic diversity of a population is one important measure of the evolutionary potential of that population, conservation biologists have been very concerned about the rate of loss of genetic diversity in small populations. The population of Laysan Finches on Pearl and Hermes were all founded by small numbers of birds, and those populations have only increased to moderate size. What impact will small population size have on the genetic variation of those populations?

To answer that question we will first look at the sampling theory for alleles in finite populations and examine a classic laboratory experiment in some detail. After that, we'll return to the Laysan Finch and answer our question about the genetic consequences of small population size.

8.1 **Genetic Drift**

In deriving the Hardy Weinberg Equilibrium we assumed that the population size was large enough that we could ignore random sampling and look only at the expected frequency of alleles and genotypes. Under HWE we expect the allele frequency to remain constant. However, small samples of gametes from the gamete pool may deviate by chance from the sample as a whole. In finite populations there may be slight deviations in the number of A or a alleles that are actually passed on to the next generation, so allele frequencies may deviate slightly from the expected frequency, just

by chance. We call that process of random changes in allele frequency due to sampling in small populations **genetic drift**.

Genetic drift occurs whenever the population has finite size—in other words in all populations, all of the time. But drift is most important when population sizes are very small.

There are several ways that the sampling effects manifest themselves in small populations. The most important is that in any small number of trials the results may not exactly match the expectations. For example, imagine flipping a coin. Even though we expect to get "heads" half of the time, if we flip two coins there is a moderately high probability of getting either two heads or two tails. On the other hand if we flip 100 coins, the observed frequency of "heads" will be much closer to 0.5. In terms of population genetics, it is not unusual to find small deviations in allele frequency from one generation to the next in small populations, even though we expect allele frequency to stay constant. That sampling effect is genetic drift.

8.2 **Assumptions:**

We will keep all of the assumptions that were used in deriving the Hardy Weinberg equilibrium, EXCEPT now have finite population size. In particular, we will still assume that there are no fitness differences among alleles, that the population is closed, so there is no migration into or out of the population, and that there is no addition of genetic variation by mutation. And there is still random mating, so genotypes will be *approximately* in HW proportions at any given time. But in a finite population, the actual frequencies of genotypes and alleles will not always exactly match the HW expectations. The result is that, from the finite sampling process alone, allele frequencies will by pure chance deviate slightly from their expectations, which leads to a slow change in the allele frequencies over time.

Basic assumptions, modified for finite populations:

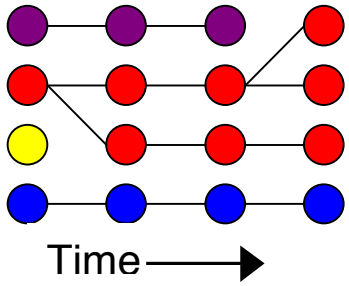
- Random mating. Individuals mate at random with respect to the locus in question.
- Closed population. There is no migration into or out of the population.
- No mutation.
- No selection. All individuals have the same expected survival and fecundity.
- Finite population size. This is the only assumption that has been modified.

8.3 **The sampling process**

To keep things simple, we will start by keeping track of gametes only (remember our conceptual model of a gamete pool?). We can do that because we assume there is random mating, which just means that gametes are chosen at random from the pool of possible gametes. If the population size stays constant, then each gamete will (on average) leave exactly 1 descendant. However, by chance some will be copied more than once and some will not leave any descendants at all.

Each time an allele fails to be copied, just by chance, that copy is lost from the population and leaves no further descendants. Eventually, if this process continues long enough, all alleles in a population can trace their ancestry to a single allele.

Figure 8.3.



Example: three simulations of drift in a tiny population with 2N=8 alleles. Assume that the population starts with two types of alleles (red and blue) and the initial frequency of the red allele is 3/8. To be explicit we'll give each founder allele a unique number. In the first generation all 8 of those alleles are present. We assume that these alleles are all selectively neutral, meaning that each allele has an equal probability of being copied each generation.

In each of those three replicates, all but one allele are eventually lost from the population. When only a single allele remains in the population we say that it has become "fixed" for that allele. Although the general pattern and time to fixation is similar in the three replicates, a different one of the eight original alleles was fixed in each simulation.

Each of the 8 initial alleles has an equal chance of being fixed in the population. Because the population starts with more blue alleles than red alleles, a blue allele is more likely to be the one that eventually goes to fixation.

Figure 8.4. Three simulations of drift in a small population with only 8 alleles.

Repicate 1	Replicate 2	Replicate 3
11222222555555555555	12222222244444444444	11111111111111111111
22222255555555555555	24244444444444444444	22311111111111111111
32222255555555555555	34444444444444444444	33331111111111111111
44445255555555555555	45554444444444444444	43333111111111111111
55555555555555555555	55554444444444444444	56333111111111111111
66555555555555555555	66555444444444444444	67363311111111111111
76555555555555555555	77665444444444444444	77663313111111111111
87655555555555555555	88666554444444444444	88776333333333111111
time ---->	time ---->	time ---->

If all of the initial alleles have an equal chance of being fixed and if we start with 3 red and 5 blue alleles, what is the probability that the population is eventually fixed for a blue allele?

_____

That thinking leads to a general result about genetic drift: the probability of fixation of an allele is equal to its frequency in the population.

$$\text{Prob(fixation)} = p \qquad \text{(eq 8.1)}$$

Alleles that start at high frequency have a higher probability of eventually going to fixation than alleles that start at low frequency.

Example: Every new mutation first occurs as just a single copy in just a single individual. When there are N individuals in the population there are 2N alleles, so the initial frequency of a new mutation is. $p = \frac{1}{2N}$.

In a population with 2N=100 alleles, what is the probability that the new mutation will eventually become fixed in the population? _____

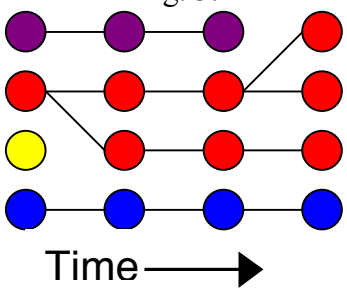
What is the probability that the new mutation will not become fixed (i.e. that it will go extinct)? _____

8.4 **Rate of decrease in heterozygosity**

Diversity is lost in all finite populations as one allele eventually goes to fixation and the others go extinct. One good measure of genetic variation is the heterozygosity (the proportion of heterozygotes in the population). How fast is that variation lost through drift?

Lets look again at the cartoon in Figure 8.3 of alleles being passed down through time in a tiny population. The two red alleles in generation 2 are said to be **identical by descent** because they are both copies of the same allele in the previous generation. The two red alleles in generation three are also identical by descent because both are copies of alleles that were already identical by descent in the generation 2.

Fig. 3.



The probability that two randomly chosen alleles are identical by descent (which we will abbreviate as *ibd*) is called the inbreeding coefficient or fixation index (F) of the population. Alleles can be ibd two ways: when they are new copies of the same allele in the previous generation, or when they come from different copies that were already ibd.

$$F_t = \begin{pmatrix}\text{ibd from the same allele}\\ \text{in the previous generation}\end{pmatrix} + \left[\begin{pmatrix}\text{copies of}\\ \text{different alleles}\end{pmatrix} * \begin{pmatrix}\text{probabilty the two different}\\ \text{alleles were already ibd}\end{pmatrix}\right]$$

If we choose an allele at random (let's say it is a copy of the red allele in the previous generation), what is the probability that a second randomly chosen allele will also be a copy of that *same* red allele? If there are N individuals there are 2N total alleles, so the probability that it comes from the same particular allele is 1 / 2N.

What if instead they are ibd from some previous event? The probability that two randomly chosen alleles come from different parent alleles is (1-1/2N) and the probability that those parents were already ibd is simply the inbreeding coefficient in the previous generation, F_{t-1}.

So our overall expression for the change in the inbreeding coefficient via drift is:

$$F_t = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_{t-1} \qquad \text{(eq 8.2)}$$

That is the probability that the two gametes are copies of the same allele in the previous generation (1/2N) or (+) they came from two different alleles (1-1/2N) and (*) those alleles were already *ibd* (F_{t-1}).

8.4.1 Heterozygosity

We started by looking at the increase in the fixation index because that is easy to visualize. But the math is actually a bit easier if we keep track of the heterozygosity instead. Since the expected heterozygosity (H) is the probability that two random alleles are not *ibd*,

$$H = 1\text{-}F \qquad \text{(eq 8.3)}$$

Recasting equation 8.2 in terms of H, you can show that

$$H_{t+1} = \left(1 - \frac{1}{2N}\right) H_t \qquad \text{(eq. 8.4)}$$

and the change in heterozygosity is

$$\Delta H = -\frac{1}{2N} H_t \qquad \text{(eq. 8.5)}$$

Each generation the average heterozygosity should decrease by a constant percentage, $\frac{1}{2N}$.

That means that after t generations of genetic drift, the heterozygosity will be:

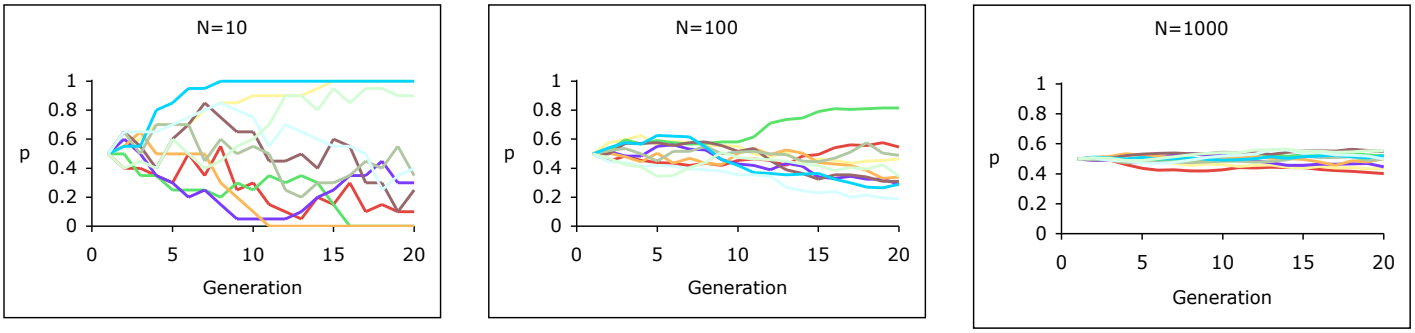
$$H_t = \left(1 - \frac{1}{2N}\right)^t H_0 \qquad \text{(eq. 8.6)}$$

8.5 **Divergence among populations**

The replicate populations in the simulations in figure 8.4 show another general feature of genetic drift. Even though all of the populations start with identical allele frequencies, they gradually diverge over time. If you start many replicate populations with two alleles A and a, and p is the frequency of allele A, then *p* of them will eventually become fixed for A and (1-p) will become fixed for allele a.

Replicate populations will tend to diverge in allele frequency as a particular allele gets fixed in some populations and goes extinct in others. As always, the effect of drift depend on population size. Large pops show 1) less variation in allele frequency over time 2) a longer time to fixation.

Figure 8.5. Change in allele frequency by drift among replicate populations of different sizes. In each case there are 10 replicate populations of a given population size, all starting with p=0.5. Even with a population size of 1000 the allele frequencies in the replicate populations do not remain exactly at p=0.5; however the changes in allele frequency are negligible compared to the changes in small populations.



Once an allele goes extinct, that allele is lost forever. So, if this process is allowed to continue long enough the allele frequency will eventually be either 0 or 1 in all populations.

8.6 **A laboratory study of genetic drift.**

One of the best tests of genetic drift comes from a laboratory experiment done by Peter Buri in the 1950s. He was a student of Sewall Wright at the University of Chicago. The design

of the experiment was elegantly simple: he set up over 100 replicate populations of 16 fruit flies and followed each population for many generations to examine the changes in allele frequency.

All of the populations started out with 8 males and 8 females that were all heterozygous for an eye color mutation. He could easily keep track of the different genotypes by their eye color. Homozygotes for the mutant *bw* allele had white eyes, homozygotes for the bw^{75} allele had bright red eyes and the heterozygotes had light orange eyes. Each generation he collected the first 8 males and the first 8 females in his sample, counted the numbers of each eye-color genotype, and used them as parents to start the next generation. In that way the population size was precisely maintained at 16 flies for the entire experiment.

Because he wanted to look at the neutral changes in allele frequency as a result of genetic drift, he was careful to show that the eye color locus had no measurable effect on the fitness of the flies. He did that by starting some large populations about 1000-2000 flies that had an equal frequency of bw and bw75 alleles and followed those populations for several weeks. There was no systematic change in allele frequency over that time period.

Figure 8.6. Allele frequency change in replicate populations of 16 fruit flies.

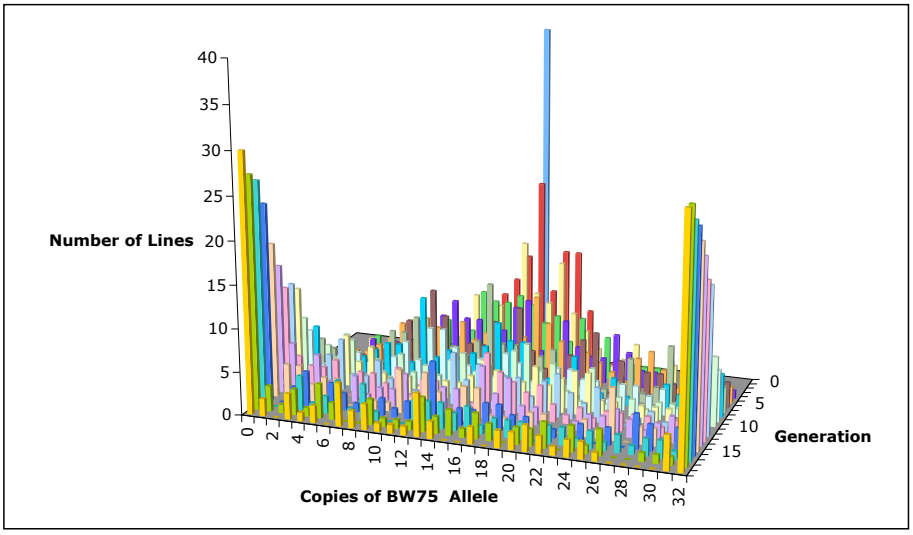


Figure 8.6 shows the results for one of his experiments. There were a total of 107 replicate populations. In each population of 16 flies there were a total of 32 alleles, so there could be anywhere between 0 to 32 copies of the bw75 allele in each population. At generation 0, all of the flies were heterozygous bw/bw75 so 107/107 populations had exactly 16 copies of the bw75 allele. In generation 1, most populations had intermediate numbers of the bw75 allele, but one population had only 7 copies and one population had as many as 22. By generation 10, some of the populations had lost the bw75 allele completely and others had lost the bw allele. By generation 19, many more of the populations had become fixed for one allele or the other.

- What do you predict the distribution to be if this experiment continued for many more generations?

- What is the allele freq at the beginning of the experiment? _____

- Approximately what is the average allele frequency (among all populations) at the end of the experiment? _____

Table 8.1. Changes in Heterozygosity over time during Buri's experiment.

	Average frequency of heterozygotes over all replicate lines	Expected Heterozygosity
Generation	**H**	**He**
1	0.514	0.500
2	0.464	
3	0.504	
4	0.456	
5	0.448	
6	0.428	
7	0.403	
8	0.402	
9	0.358	
10	0.348	
11	0.325	$H_{e,\ 11}$ =
12	0.305	
13	0.263	
14	0.255	
15	0.216	
16	0.202	
17	0.210	
18	0.197	
19	0.183	$H_{e,\ 19}$ =

The expected heterozygosity is 0.5 in generation 1 and there was a constant population of size N=16 flies. Using equation 8.6, what is the expected heterozygosity in generation 11, after 10 generations of drift?

He=_____

What is the expected heterozygosity in generation 19, after 18 generations of drift?

He=_____

How does that compare to the observed heterozygosity?

Buri's populations lost genetic variation much faster than would be predicted for a population of 16 flies. He repeated the whole experiment with a second series of populations and found essentially the same results.

8.7 **Effective population size**

Why don't the results of Buri's experiment match our expectations? When the results of an experiment don't match the expectations of the model, we need to go back and examine the model assumptions. In our idealized population, we modeled drift by assuming individuals were identical and mate completely at random (by that we meant that they produce an infinite number of gametes, from which we draw pairs of alleles at random). An implicit assumption of that model was that there were no sexes: we did not keep track of where the two random gametes came from. They could even have come from the same individual. In Buri's experiment (and most real populations we would be interested in) each individual must have one male and one female parent..

Another implicit assumption of that sampling process was that the number of offspring produced by each individual would come from a binomial distribution with mean=1. In many real populations, some individuals are more fecund than others. Large females may lay more eggs than small females, or dominant males may sire more offspring than subordinate males.

In either case, the **effective population size** (N_e) may be smaller than the actual number of individuals.

How can we calculate Ne? One way is to use equation 8.6, substituting and initial heterozygosity of 0.5 in generation 1 and the observed heterozygosity of 0.183 18 generations later and then solve for N.

$$0.183 = \left(1 - \frac{1}{2N_e}\right)^{18} 0.5$$

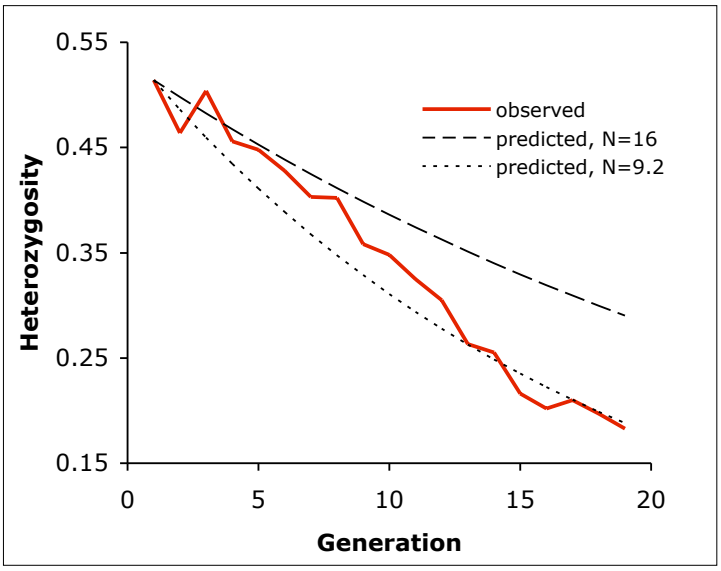
Take logs of both sides to get rid of the exponent:

$$\ln(0.183) = 18\ln\left(1 - \frac{1}{2N_e}\right) + \ln(0.5)$$

Solving for N yields N_e =9.2

The subscript e shows that the estimate of N is the *effective* population size. Buri's experimental populations all had 16 flies but they lost genetic variance *as if they had* only 9.2 flies in each. Thus we can say that the *effective population size* in this experiment was 9.2.

Figure 8.7 Observed and predicted decline in heterozygosity in Buri's experiment.



What are some of the biological reasons why Ne differs from N? We don't know the precise answer for Buri's flies, but theory has shown that several factors can influence the effective size of a population. Here are a few theoretical results:

8.7.1 Unequal sex ratio

When the number of males (N_m) and females (N_f) in a population are not equal, then the effective population size can be shown to be approximately

$$N_e \approx \frac{4N_m N_f}{N_m + N_f} \qquad \text{(eq. 8.7)}$$

- Assume there 1000 females (N_f=1000) but only a single male is responsible for all of the matings (N_m=1). What is Ne for that population?
- *Ne*=_____

8.7.2 Variation in family size

In many populations, some individuals will be highly fecund and others may not reproduce at all. That is especially true for animals with strong dominance hierarchies (such as wolves) where some individuals sire most of the offspring, and species with indeterminate growth (such as plants), where a few large individuals may be responsible for most of the seed production in the population. In that case there will be a much larger variance in family size that we assumed in our ideal population.

When there is variation in family size, Ne is approximately

$$N_e \approx \frac{4N-2}{v+2} \qquad \text{(eq. 8.8)}$$

where v is the variance in the number of offspring produced by different individuals.

- One estimate of variance in egg number for salmon was 69. In a population of 1000 spawners, how much smaller will the effective population size be than the apparent number of spawners?
  - N_e= _____

- What if you could ensure that all individuals have exactly the same number of offspring? (perhaps by managing a captive population in a zoo). In that case the variance in family size will be zero. What will be the effective population size?
  - N_e= _____

8.7.3 Variation in population size over time

When population size varies over time, the average effective size of the population is affected more by the periods when populations are small than when they are large. The average effective size is approximately the *harmonic mean* of population size:

$$\frac{1}{N_e} = Average\left(\frac{1}{N}\right)$$

so after t generations

$$\frac{1}{N_e} = \frac{1}{t}\left(\frac{1}{N_1} + \frac{1}{N_2} + \ldots + \frac{1}{N_t}\right)$$

The inverse of that equation will therefore estimate Ne:

$$N_e = \frac{t}{\frac{1}{N_1} + \frac{1}{N_2} + \ldots + \frac{1}{N_t}} \quad \text{(eq. 8.9)}$$

What if the population size is constant: i.e. if over 5 yrs N={100,100,100,100,100}?

Ne=_____

What if the population starts with a single individual but then immediately stabilizes at a larger size? i.e. over 5 yrs N={1,100,100,100,100}

Ne=_____

8.8 **How does this relate to the Laysan Finch?**

Biologists continued to study the Laysan Finches after they were introduced to Pearl and Hermes reef. In addition to censusing the population size on those islands, they occasionally collected blood samples from some of the birds to monitor any changes in genetic diversity in those populations.

Again the heterozygosity of a population is a good measure of genetic diversity. The observed heterozygosity is just the proportion of heterozygotes: the number of heterozygous individuals divided by the total number of individuals. When there are two alleles at a locus we have already seen that the expected proportion of heterozygotes under random mating is simply 2pq. But how do you calculate the expected heterozygosity when there are more than two alleles? With three alleles (p, q, r) there are three classes of heterozygotes, so you could just sum the frequencies: H=2pq + 2pr + 2qr. But with more alleles the number of different heterozygous combinations increases rapidly. For 5 alleles there are 10 possible heterozygotes to consider. When there are many alleles it is easiest to calculate the expected heterozygosity as the proportion of individuals that are *not homozygous*.

Each homozygous genotype has a frequency p_i^2, where p_i is the frequency of the i^{th} allele. All other genotypes are heterozygous. Therefore, the expected frequency of heterozygotes (using the not rule to find the frequency of genotypes that are *not* homozygous) is:

$$H_{\exp} = 1 - \sum p_i^2 \quad \text{(eq. 8.10)}$$

Table 8.2. Allele frequencies and heterozygosity for one microsatellite locus, for which 5 different alleles were observed.

		Alleles								
Locus Tc12B5E	**N**	**125**	**127**	**135**	**137**	**139**	**Number of heterozygotes**	H_{obs}	H_{exp}	N alleles in population
Laysan	44	0.295	0.034	0.545	0.114	0.011	25	0.568	0.602	5
Southeast	43	0.116	0.116	0.756	0.012	0	19	0.442	0.401	4
North	43	0.128	0	0.791	0.081	0	16			
Grass	36	0	0	1.000	0	0	0			

What are the observed and expected heterozygosities for North Island and Grass Island?

They looked at a total of 9 loci. Averaged across all of those loci, here is what they found.

Table 8.3

Island	**Average H**	**Ne**
Laysan	0.535	
Southeast	0.491	58.5
North	0.341	11.3
Grass	0.401	

Using the heterozygosity of the Laysan island population as the initial heterozygosity (H_0), and assuming that there have been approximately t=10 generations since the founding of the other three populations, what is the effective population size of each of the small islands? The arithmetic is a little tedious, but it is possible to calculate the effective population size on each island from equation 8.6, as in section 8.7, above. For example, on Southeast Island,

$$H_t = \left(1 - \frac{1}{2N}\right)^t H_0$$

$$Ln\left(\frac{H_t}{H_0}\right) = t \cdot Ln\left(1 - \frac{1}{2N}\right)$$

$$Ln\left(\frac{0.491}{0.535}\right) = 10 \cdot Ln\left(1 - \frac{1}{2N_e}\right)$$

$$\frac{-0.0858}{10} = Ln\left(1 - \frac{1}{2N_e}\right)$$

$$e^{\frac{-0.0858}{10}} = 0.9829 = 1 - \frac{1}{2N_e}$$

$$0.9914 = 1 - \frac{1}{2N_e}$$

$$N_e = \frac{1}{2(1 - 0.9914)} = 58.5$$

Using similar logic, what is the effective population size on Grass Island?

Remember that fluctuating population size is one of the factors that may affect Ne. Unfortunately we don't have yearly censuses of the population size on those islands. Here are the data that are available (from Fig 8.2).

Table 8.4	**Population size (N)**		
Year	**Southeast Island**	**North Island**	**Grass Island**
1967	50		
1968	223		2
1969	166		
1970	183		8
1971	480		
1972	375		8
1973	730	2	8
1974	436		
1975			
1976			
1977			
1978	202	92	16
1979			
1980			
1981			
1982			
1983	524	354	
1984	515	153	30

1985	591	194	46
1986	434	272	52
1987	181	56	26
1988	156	36	17
1989			
1990		67	
1991			
1992			
1993			
1994			
1995			
1996			
1997			
1998	350		30
Average of (1/N)	0.0045	0.065	
Ne	222	15.4	

Those data are pretty sparse. Still, we can calculate the harmonic mean of the counts that we do have and use that for a second (very rough) estimate of N_e.

What is the effective population size on Grass Island by this method?

_____

How do those estimates compare to the estimates of effective size based on changes in heterozygosity? _____

Finally, compare those estimates of N_e with the simulations of drift in Figure 8.5 to get a rough idea of how long those small populations are likely to maintain their genetic diversity if the populations continue as they are now.

Answers:

p 4. Prob(blue)=5/8
p 5. Prob(fixed)=1/2N; Prob(extinct)=1-1/2N
p 8. Eventually all lines will become fixed for one allele or the other. Starting allele frequency is 0.5; The *Average* allele frequency at the end of the experiment is still about 0.5
p 9. H(11) = 0.36 H(19) =0.28, both higher than the observed H.
p 11 Ne = 3.99
p 12 Ne (salmon) = 56.3; Ne (equal) = 2N-1; Ne (constant size) =100; Ne(bottleneck) =4.8
p 13 North Island: Ho=0.372 He =0.351; Grass Island: Ho=0 He=0
p 14 Grass Island Ne=17.6
p 16 Ne=9.6 -- not really close to the other estimate, but a similar order of magnitude.
For Ne of 10 they will lose most of their genetic variation in around 20 generations or so.