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### Genetics and Hatcheries

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The term aquaculture includes a variety of different methods to propagate fish and shellfish as well as some plant species. Hatcheries, where fish and shellfish are artificially spawned and where embryos and early life stages are kept, are a prominent feature of aquaculture. Both freshwater and marine species are included, and usually some aspects of the process involve human intervention and artificial environments. The primary reasons for aquaculture throughout the world are economics and the demand for high-quality protein and fat, but hatcheries are also used to address conservation problems. Because humans are involved and artificial habitats are often used, there will be pressures on the cultured populations that potentially alter the gene pool. Cultured populations can reduce resources that are available to the wild populations because the cultured populations consume them. If cultured populations and wild populations interbreed, wild gene pools can be altered. These interactions can be important concerns. In this chapter, we examine some of the intersections of genetics and hatcheries.

Two of the questions that began this set of articles had to do with the genetic effects of aquaculture on fish (see Chapter 1. Even Fish Obey Mendel's Laws). Most aquaculture is accomplished by using hatcheries at some stage of production. The first question was, "Do hatcheries change the fish?" and its follow-up was "If they do, is that always bad?" The second question was, "Do hatchery fish harm wild stocks?" For much of the discussion in this chapter, we focus on salmon, but the general principles can be broadly applied to many other species.

### Purpose of hatcheries

We are now ready to address those questions, but first we need to ask, "What is the purpose of a hatchery?" The shortest answer is, "To extend habitat beyond what is available naturally." Think about it. Many hatcheries are used to increase the numbers of fish above their natural numbers. In those instances, it is obvious that either spawning or rearing habitat (or both) is insufficient to produce the desired number of fish. The increased numbers of fish can be used for put-and-take sport fishing, commercial harvests, conservation of depleted stocks, aquaculture, or other purposes. Whatever the reason, hatcheries supplement habitat. In hatcheries, the gametes are usually removed from the fish and combined to produce fertilized eggs, thereby removing normal mating choices that the fish themselves might make. Because the embryos are usually incubated under controlled (artificial) conditions, survivals are often much higher than would occur in nature where predation, ice scouring, floods, and other events reduce numbers. Some of those events may involve natural selection for processes that are important in natural production such as redd selection (redds are the "nests" in the gravel that fish—usually the female—excavate for their eggs) or tolerance of some environmental rigor. For species like coho, steelhead, sockeye, and chinook salmon that are usually fed and grown for substantial periods before they are released, the survival of juveniles is also generally higher in hatcheries than in nature.

### Intensive and extensive culture

There are two categories of culture, **intensive** and **extensive**. Intensive culture raises organisms in captivity and (in theory) they are never released into nature, unless they escape. Pen-reared Atlantic salmon are an example of intensive culture. Most intensive culture relies on captive domesticated brood stock. In some instances, the hatchery maintains the breeders, and in others growth to market size is done at the hatchery from an early life stage (seed) provided by suppliers, who maintain the brood stock. In contrast, extensive culture takes advantage of the natural environment, such as the ocean or a lake, for at least a part of the cultured organism's life cycle. Salmon ranching is an example of extensive culture; young salmon are released to sea where they grow and at maturity they return to their natal streams to perpetuate the cycle and to be harvested. Extensive culture can follow several different strategies or combinations of strategies: (1) hatcheries can be in remote locations, or they can release their young at sites far from wild populations of the same species and use returns or seed for brood stock; (2) hatcheries can be located near, but not on the same river system as, wild populations and take their brood stock from its returns; or (3) hatcheries can be located in the same system as a wild population and take brood stock from a mixture of hatchery and wild returns that escape harvest efforts. Fish that successfully make it past harvest activities are referred to as the "escapement" by fisheries scientists.

### Effects of intensive hatcheries

Now let's address one of the questions that brought us here, "Do hatcheries change the fish?" and "If they do, is that always bad?" Intensive hatcheries present several biological concerns. Some of the same concerns may also be issues with extensive hatcheries. The primary genetic concern is loss of genetic variation. Because maintaining brood stock can be expensive, brood-stock numbers maintained in hatcheries are often small, which means that only a small portion of the gene pool (a small sample size) may be used to perpetuate the stock. Random drift, which occurs as a result of small populations

(see Chapter 2. How Genes Vary in Populations), results in random fluctuations of allele frequencies from generation to generation. As a result of random drift, rarer alleles tend to be lost; and in persistently small populations, variation at many loci will eventually be lost. Also, small population sizes are often accompanied by an increase in **inbreeding**. Inbreeding can increase the incidence of the number of loci that are homozygous (have two copies of the same allele), including by random chance loci that are homozygous for unfavorable alleles (see sidebar 1).

### ***Alter gene pools***

One of the ways in which the gene pool can be diminished is by the spawning strategy used to produce hatchery fish. We cannot hope to replicate the mate selection that goes on in nature, but we can ensure that we maximize the numbers of parents (and their genes). One important practice is to use many males. Yes, it is possible for sperm taken from a single male to fertilize the eggs of 10 or more females. Why would one do that? It means that fewer fish would have to be handled. This approach might appear to increase efficiency, but the eventual cost in loss of genetic variation means that it is a false economy. The problem is that a single male would contribute half of the genes for all of the offspring and drastically reduce the genetic variation. A good rule is to use a one-to-one ratio of males to females. Another problem occurs when the sperm of multiple males is mixed together to fertilize eggs from multiple females. Several studies have shown that in such mixtures of sperm, the sperm of one male fertilizes a large proportion of the eggs, and sperm from other males may fertilize few or no eggs. In order to make sure that many males contribute, it is necessary to conduct single-pair (one male and one female) matings, or at least to use very small batches of fish.

Inadvertent selection can also alter gene pools of cultured species. In salmon culture there is a natural tendency to select large “pretty” fish for brood stock. However, being large or “pretty” does not necessarily translate to fitness in either the culture or natural environment. In addition, selection for similar phenotypes may restrict the gene pool. Another problem is that the numbers of returning fish cannot be predicted. As a result, there is a

tendency to select early returns as breeders in order to assure that there will be full incubators. The result of selecting the early fish can result in advancing the return time. We saw previously that return timing is a trait that is important to local adaptation. One consequence of altering return timing is that other timing-related traits, such as emigration timing, may also be changed (see Chapter 5. Fish Population du Jour). One example of inadvertent selection is the chinook salmon stock in Minter Creek in Washington. Prior to the 1970s, the middle of the return time was in mid October. In a little more than three decades, the return timing has been advanced to late August, nearly two months earlier (Figure 2). Think of the seasonal environmental changes that take place between August and October in western Washington! We can all think of strategies to reduce this effect. The simplest would be to take breeders throughout the return period. The first problem is hatchery capacity and conservation limitations on the numbers of fish a hatchery is allowed to release. So, why not take the eggs throughout the return period, and decide after the run is over which ones to keep? Well, it is illegal in Washington to discard eggs (and there would probably be a public outcry, anyway) and conservation concerns prevent people from moving eggs to another hatchery. Here is an instance that laws designed to conserve a stock are actually the cause of the problem!

### ***Domestication selection***

Another genetic process that can alter hatchery stocks is **domestication selection**. Domestication selection is especially likely to occur in closed hatchery stocks—ones that fail to incorporate new genetic material on a regular basis. To understand how phenotypic and genetic changes can occur, we need only to recall our previous discussions of local adaptation. It should be obvious that the habitats provided by hatcheries often bear little resemblance to those encountered by the wild populations. The manner in which particular fish are chosen as breeders and the incubation environment experienced by the embryos match virtually none of the conditions of the wild environment. If the juveniles are fed hatchery food and reared in freshwater hatchery ponds before release, the differences are even greater. Because of

## Sidebar 1

## INBREEDING

Most people have heard of inbreeding and think of it in terms of offspring that result from matings between relatives. The closer the relationship between the mates, the more inbred the offspring. Many human cultures and religions have taboos about inbreeding and some cultures even have specific rules for who can and can't marry.

The concern has a genetic basis. Most species have numerous deleterious alleles on their chromosomes, that are rare in the population as a whole. In humans, many different genetic diseases result from mutated alleles, which produce defective enzymes. Examples of these diseases are Tay-Sachs (1 of 3,500 in the Ashkenazi Jewish population—about 1 in 29 individuals in that population carry the allele); phenylketonuria (1 of 10,000); and galactosemia (1 of 60,000 births among Caucasians). Tay-Sachs is untreatable; phenylketonuria can be treated by removing the amino acid phenylalanine from the diet; and galactosemia can be treated by removing galactose or sugars that include a galactose molecule, like lactose, from the diet (galactosemia is different from lactose intolerance,

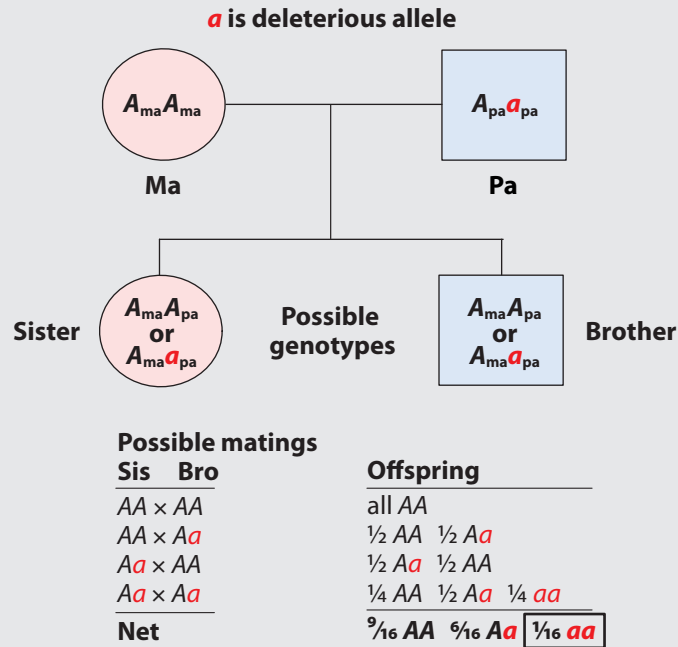


Figure 1. Tracking a recessive allele in a mating between close relatives, in this instance, a brother and sister.

which is also the result of an inherited defective enzyme). Most humans carry several recessive (see Chapter 1. Even Fish Obey Mendel's Laws) alleles that provide recipes for defective instructions for the protein or enzyme that they specify. Because each person carries two alleles that specify each enzyme (remember that we are diploid)

and only one is defective, they are not affected by the disease. Also, because most defective alleles are rare, it is unlikely that two unrelated individuals will both carry alleles for the same defective enzymes. Regardless of how rare a deleterious allele is, however, when related individuals mate, the chance that an offspring will inherit two copies

of a deleterious allele increases substantially. For instance in the mating of a brother and sister, the chances are 1 in 16 that a recessive allele will be homozygous in an offspring (Figure 1).

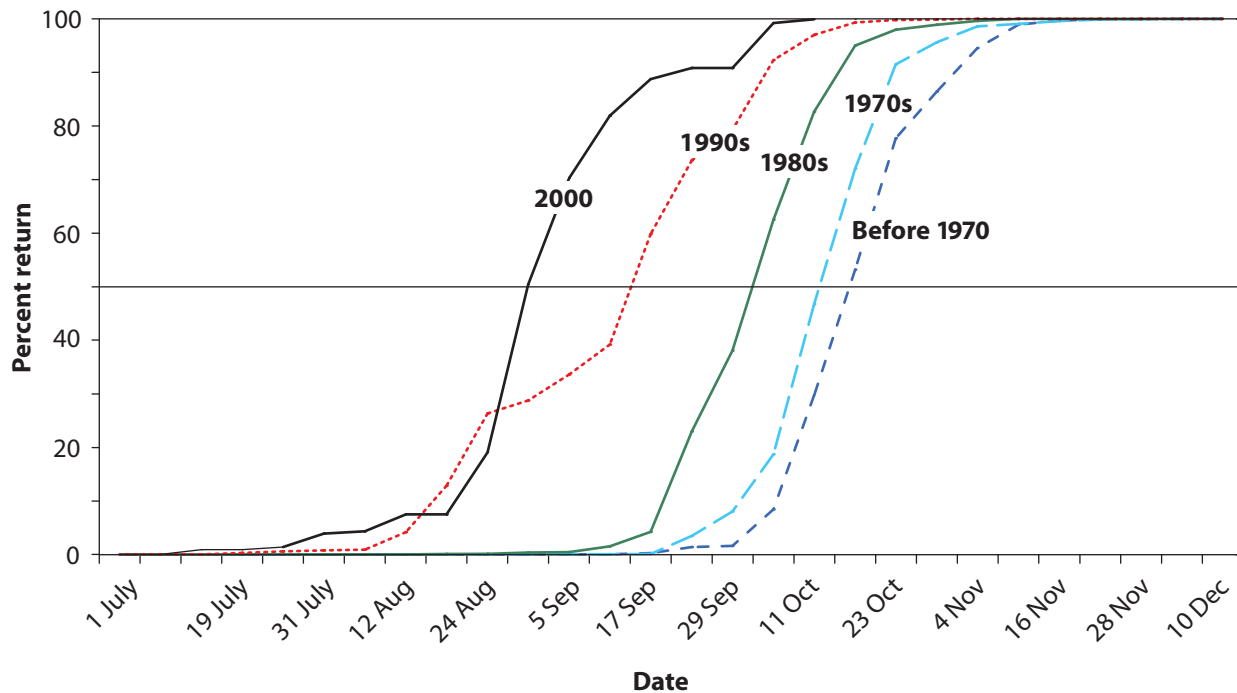
Let's follow the deleterious gene that **pa** carries in the figure. First we track alleles from parents to their children. Because both children receive an allele at a locus from each parent, they both have a chance to receive the same deleterious allele ( $a_{pa}$  from **pa** in the example). The chances that both receive the deleterious allele are 1 in 4 because the chances are  $\frac{1}{2}$  that the sister will inherit it and the chances are also  $\frac{1}{2}$  that the brother will inherit it. The chance that both brother and sister inherit the deleterious allele is  $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ . Now we look at the chances that both will pass the allele to a child. If they both carried the allele (which happens  $\frac{1}{4}$  of the time), the chance that the sister would pass the allele to the child is  $\frac{1}{2}$  (the other half of the time she would pass the normal allele). The brother would also pass the allele  $\frac{1}{2}$  of the time. So, the combined chance that both pass the allele is  $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ . But

remember that only  $\frac{1}{4}$  of the time did both brother and sister receive the deleterious allele, so the overall chance that a child from a brother-sister cross receives two copies of a deleterious allele at a locus is 1 in 16. However, both partners in any mating probably carry several loci that are heterozygous (see Chapter 1) for deleterious recessive alleles. Unfortunate results will occur in a child if even one of its loci is homozygous for a deleterious allele.

Matings between more distantly related relations have lower chances for producing offspring that are homozygous for deleterious alleles at a locus, but inbreeding can build up in a population and the buildup is faster in a small population than in a large population. A number of experiments have been conducted to determine the effect of inbreeding. Harold Kinkaid looked at growth and survival in inbred cultured rainbow trout. Relative to controls (fish from the population that were not purposely inbred), fish derived from brother-sister matings declined 17.4% in numbers, 22.3% in weight as young fish at

the size they were "planted" in a stream, and 36.6% in weight at that size, and 22.3% in weight at the time at which they would be caught by recreational fishermen. Two generations of brother-sister matings, which increased the extent of inbreeding another 50%, decreased the number of fish by 47.9%, weight at "planting" by 54.9%, and weight of catchable plants by 64.5%. In addition, the number of crippled fry increased in the inbred fish and their food conversion efficiency declined.

Similar experiments have been conducted in many species. There have been few exceptions to this pattern, and the exceptions are usually for large species that have been through severe population-size bottlenecks (see Chapter 2: How Genes Vary in Fish Populations). Survival through such bottlenecks presumably resulted in part from purging most of the deleterious recessives from the populations. Large populations do not have that ability, and it is likely that more populations perish than successfully pass through the bottleneck.



**Figure 2.** The change in timing of chinook salmon returns to Minter Creek in Washington over the last several decades. The graphs show the percentage of the total run of salmon entering Minter Creek that arrived from the ocean by each date. The date by which half of the salmon had returned changed from mid October in years before 1970, to late September in the 1980s, to late August in 2000. This apparently occurred because each year in the hatchery only early-returning fish contributed eggs and sperm to the next generation.

the differences, there is an enormous likelihood that a hatchery population will adapt to the hatchery conditions and that the genes that produce more successful hatchery phenotypes will increase in abundance. This process is called domestication selection.

Is domestication selection bad? As we said in Chapter 1, it depends. If the goal is to have a population that thrives under culture conditions, particularly in an intensive aquacultural setting, the answer is no. In fact, fish that are more suited to culture conditions can be produced more economically, and domesticating the stock may be one of the goals of intensive culture. In other culture strategies in which the cultured product has little or no opportunity to interbreed with wild populations, the conclusion about the influence of domestication will depend on its effect on the suitability of the cultured product for both intensive and extensive brood stocks and market. In some instances, fish fed

in culture can become very aggressive in their feeding habits. A voracious fish might be advantageous in a put-and-take sport fishery. In addition, some cultured fish that are released from salmon ranches return at different times or at different locations from wild populations and have little opportunity to interbreed or be harvested in mixtures with wild fish. This strategy can reduce both ecological and genetic interactions between hatchery and wild fish.

### **Disease**

Now it is time to include the second question, “Do hatchery fish harm wild stocks?” Unfortunately, the history of intensively cultured organisms shows that all too frequently they escape. The first concern about escapees and intensively cultured fish is not a genetic issue—it is a disease issue. Under crowded culture conditions, diseases can break out. Contact with diseased cultured fish can infect wild populations. Disease issues are usually foremost in fish culturists’

minds because diseases are ordinarily quite obvious. In contrast, most genetics issues are not immediately apparent, both because they are not readily visible and because they may generally reduce survivals without symptoms that diseases usually show. In addition, the severity of the genetic problems may take two or more generations to develop, and an effect such as inbreeding may grow incrementally worse over time.

### ***Interbreeding***

The extent of our concerns for the genetic effects of escapees from intensively cultured organisms on wild populations depends on the relative numbers and frequency of escape incidents. If they are rare and the numbers of escaped fish are small relative to the number of wild fish, the effect of gene flow may be small and maladaptive genes would probably be purged rapidly by natural selection. Unfortunately, there are many incidences of farmed Atlantic salmon escaping in Europe, and the numbers of escapees are large. There is substantial evidence that those cultured fish have contributed large numbers of offspring to nearby wild stocks. Furthermore, the original brood stock for those cultured Atlantic salmon was not exclusively from local populations, so the hatchery gene pool was not a result of local adaptation (except to culture conditions). In Ireland, some wild Atlantic salmon populations have been entirely displaced by farmed escapees.

### ***Outbreeding depression***

Another possible genetic impact is hybridization between wild and hatchery fish. Hybrids between fish of different populations can cause **outbreeding depression**, which is a reduction in the survival and overall productivity of the wild population. That is, outbreeding depression results in a loss of fitness. We have documented outbreeding depression in hybrids between pink salmon (see sidebar 2), and it has been documented in many other species, including largemouth bass.

### ***Competition with native species***

The final concern for escapees from intensive culture is not a genetic issue, but an ecological issue. Many cultured species are farmed outside their normal ranges. Two examples are Asian oysters (see Chapter

7. The Lowdown on Frankenfish) and Atlantic salmon. The native range of Atlantic salmon is Europe and northeastern North America, but they are being farmed intensively in western North America and in South America. So many Atlantic salmon have escaped their pens in British Columbia that they are caught offshore occasionally by anglers and commercial fishermen. They and their young have been observed in streams of British Columbia. So far there is no evidence that farmed Atlantic salmon can complete their life cycle in that the young have returned to spawn, and most attempts over the past century to introduce Atlantic salmon outside of their natural range have failed. But it is not unlikely that as a result of persistent escapes, Atlantic salmon will eventually colonize native habitat of Pacific salmon. At that time, we will have to deal with an exotic species that competes with native Pacific salmon species. Because of this concern, the state of Washington recommended that only monosex fish (unable to reproduce) be farmed in Washington (<http://www.wdfw.wa.gov/fish/atlantic/manage.htm>).

### **Effects of extensive culture**

Now let's consider the potential impacts of extensive culture, such as salmon ranching, on wild populations. For species ranched outside of their native ranges, we have to consider the strong possibility that they will colonize nearby habitat, as the pink salmon and other Pacific salmon did in the Great Lakes (see Chapter 3. History of a Salmon Population). Farming diploid (fertile) Asian oysters along the eastern seaboard of North America could also create this problem in the future as it already had done on the west coast (see Chapter 7). Most salmon ranching, however, occurs within the normal range of the species under culture. Some of the genetic concerns about the hatchery brood stock itself are similar to those we discussed for intensive culture. In particular, many hatcheries were originally stocked with available brood stock from other hatcheries rather than local fish, so there is a risk of inbreeding and the translocated brood stock may not perform as well in a new environment.

What about the effect on wild populations? If the culture facility and release sites are geographically remote, and therefore genetically isolated from wild

## Sidebar 2

**OUTBREEDING DEPRESSION**

In an agricultural context, we often hear about **hybrid vigor**. Hybrid vigor is the improvement in phenotypes that may occur in hybrids between genetically distinct strains, with improvement over the parental strains. This kind of effect is often observed, especially when inbred strains that have low amounts of variability are interbred. The enhanced characteristics result from the increased amount of genetic variability in the hybrid. The production of hybrid corn and tomatoes by interbreeding different inbred strains is a familiar example from modern agriculture. Hybrid vigor can result from interbreeding fish populations too, but not always.

What often gets left out of the descriptions of the wonders of hybrid vigor is that if the hybrids ( $F_1$ —see Chapter 1. Even Fish Obey Mendel's Laws) produce a second ( $F_2$ ) generation, the phenotypes of the second generation are often inferior to those of the parental strains as well as to the  $F_1$  hybrids. Indeed, hybrid tomatoes and corn are often not fertile; that's how seed companies make money selling you their seeds. Decreased fitness (ability to contribute genes successfully to the next generation) that results from hybridization is called **outbreeding depression**.

Outbreeding depression between two genetically divergent populations can occur in two ways—**ecological** and **genetic outbreeding depression**. First, if they inhabit different environments they may have become locally adapted to those environments. Hybrids between the two populations will have a mixture (50:50) of the genes of the two populations. The genes that the

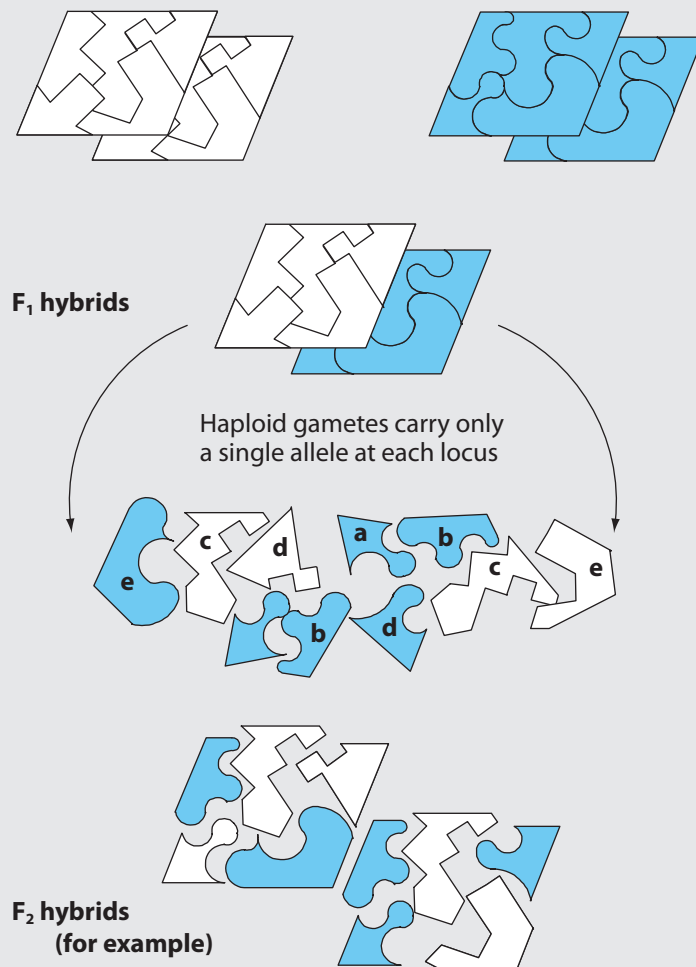
hybrid carries may be inappropriate in both parental habitats. The hybrids might be most successful in an intermediate environment (although I think it is unlikely that we humans could construct an intermediate environment that takes into account all of the features that are important to a fish). This decline is ecological outbreeding depression; the genetic mechanism results largely from locus-by-locus or piece-by-piece contributions to the phenotype.

The second mechanism that generates outbreeding depression results from the simultaneous accumulation of alleles at many loci that have coordinated functions. The assembly of alleles combines their effects and, most important, they interact with each other to generate a favorable genotype. In this instance, the whole is greater than the sum of the parts because the interactions between alleles at different loci (geneticists refer to it as **epistasis**) are an essential component of the phenotype. The assembly of alleles is referred to as a **coadapted genome**. In the first generation of hybridization, each offspring receives one intact coadapted genome from each parent. However, in the second generation as a result of Mendel's second law of segregation (see Chapter 1), the coadapted sets of alleles will be disrupted because the alleles segregate at random during gamete formation, and fitness may decline (Figure 3).

Because both first- and second-generation hybrids and controls must be examined to evaluate outbreeding depression in hybrids between populations, the experiments that are required to

measure outbreeding depression can take a long time. We have conducted a series of experiments that involve pink salmon, which has for salmon a very short 2-year generation time. A single study that was replicated in both even- and odd-year broodlines takes seven years, six to conduct the experiment and obtain data and another year to analyze and report the data. We are in the midst of our third experiment. Why conduct the experiments? We want to examine the possible effects of translocated stocks on wild populations and the interactions between hatchery and wild populations. Why so many experiments? One reason is that the results are unpredictable. Another reason relates to the nature of the scientific method. What do we mean? Keep reading!

The scientific method uses observations to formulate a "falsifiable" hypothesis. That means that the hypothesis must be constructed so it can be disproved. For example, if we are comparing the allele frequency distributions of two populations, even if we think the populations differ, our null hypothesis is that the samples from the populations that we are comparing are actually subsamples from one large population that has a particular allele frequency distribution. If through our analysis we determine that it is improbable that the two samples came from the same underlying distribution, we conclude that they differ. If we do not see differences, we do not conclude that they are the same; we conclude only that we could not detect a difference. Possibly with more powerful tests, we might see differences. It is critical to realize that

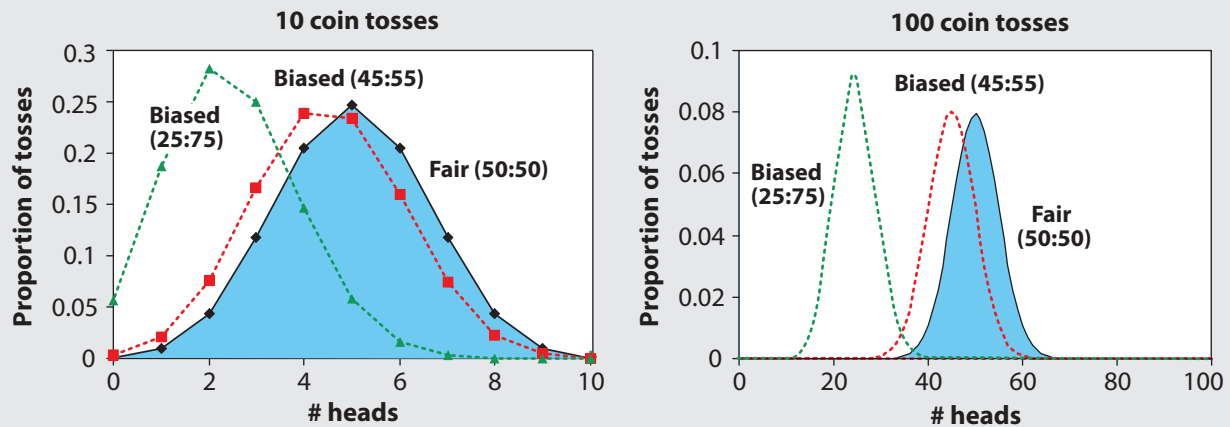


**Figure 3.** An analogy for genetic outbreeding depression. Genetic outbreeding depression results from disruption of coadapted alleles at multiple loci. Here two populations are represented by puzzles, the pieces of which represent alleles at different loci. The favorable interactions between alleles within a population are shown by the good fit of the pieces of the puzzle. Each individual in the population has two complete sets of the alleles because they are diploid. First generation (F<sub>1</sub>) hybrids receive one complete set of alleles from each puzzle and may not exhibit effects of outbreeding. In the second (F<sub>2</sub>) generation, however, each offspring receives two of each allele, regardless of the original parental source. The results may be a set of alleles that do not work well together.

just because we cannot delineate two populations by comparing them in some way, we are not justified in concluding that they are the same.

After we have constructed an appropriate hypothesis, we test it with appropriate experiments (or analyses). The challenge to scientists is that it is virtually impossible to “prove” a hypothesis. For example, consider a coin tossing experiment in which we want to determine if a coin is fair; that is, has equal chances of landing heads or tails. How do we go about testing it? First we have to formulate a hypothesis to test. Ordinarily we will hypothesize that it is fair (even if we have reason to think that it might not be) because that is a falsifiable hypothesis. Then we will test the hypothesis by tossing the coin some number of times and evaluating the results. Before we examine the results, let’s compare the possible results of a fair coin with the possible results of two different unfair (biased) coins—one that on average lands heads 25% of the time and tails 75% of the time, and another that lands heads 45% of the time and tails 55% of the time. For the fair coin, it should be obvious that most experiments will result in heads between 3 and 7 times. Go ahead and try it yourself! Sometimes, you may see 8 or 2 heads and even 9 or 1 head. All 10 heads or all 10 tails will be rare, but will occur every once in a while (one in 1,024 for each result).

We can plot the chances of seeing each result (Figure 4). If we compare the possible results of the fair coin (called a distribution) to those of the strongly biased coin, we will see that their distributions overlap between about 3 and 5 heads. This means that we could not distinguish between the coins (with a reasonable chance of being



**Figure 4.** The figures show the expected frequency that heads would result if coins were tossed 10 times or 100 times for a fair coin (50:50 chance of heads—black) and two biased coins: one on average lands heads 25% of the time (green) and the other on average lands heads 45% of the time (red).

correct) if those were our results for 10 tosses. If we compare the fair coin with the slightly biased (45:55) coin, we see that their outcomes overlap over most of the range. So what do we do? We increase the number of tosses! This experiment may take a bit more time if you do it yourself. The figure at the right (Figure 4) shows the sets of results (distributions) that we would expect if the coins were tossed 100 times. Notice that the results for the fair coin and strongly biased (25:75) coin differ substantially and overlap only at 36 to 39 heads, results that are unlikely for both coins. If we compare the fair coin with the slightly biased coin (45:55), however, we still see substantial overlaps. Only if we had less than 40 or more than 60 heads, would we be able to choose between the coins with reasonable confidence. However, that 5% edge can make a lot of money for Las Vegas. If we wanted to increase the power of our experiment to distinguish between a fair coin and the less biased coin, we might increase the sample size (number of tosses) to 1,000. For 1,000 tosses, there will still be reasonable doubt as to which coin

you have if you toss 473, 474, or 475 heads; however, all three results are unlikely for both coins. But what about a fair coin with a coin biased to have a 49:51 ratio of heads to tails? And we can keep on narrowing the difference! We hope you can see that when we say fair coin, we have to specify just how fair to be able to test it.

Now back to using the scientific method and testing hypotheses. We saw that it can be difficult to demonstrate that a coin is fair (i.e., prove a hypothesis). However, we also saw that some outcomes of coin tossing experiments could indicate that a coin is unlikely to be fair. For example, a single result of 0 or 1 or of 9 or 10 heads in an experiment that makes ten tosses is an unlikely result for a fair coin, but for outcomes of between 3 and 7 heads we cannot tell. By increasing the number of tosses to 100, we saw that we could detect smaller levels of bias, but between about 41 and 48 heads we could not differentiate reliably between 45:55 (biased coin) and 50:50 (fair coin). For both experiments there were clearly results that would reliably allow us to rule out the hypothesis that the

coin is fair, i.e., reject the hypothesis. But we can nearly always construct a hypothesis that is only slightly different from the null hypothesis. It may be difficult to resolve the two hypotheses, even with large sample sizes. The result is that it is very difficult to prove hypotheses, so most clever hypotheses that scientists test are ones that if disproved, leave only the alternative hypothesis; they are falsifiable. In the above example, we originally had reason to doubt if the coin was fair. The hypothesis that we constructed, however, was that it was fair. After the experiment, we could either reject the hypothesis and conclude that the coin is unfair or fail to reject the hypothesis. Beware! Failure to reject does not mean that the coin is fair, just that we did not see evidence of it. This is much like the criminal system in which a crook gets away with the crime because there is insufficient evidence that they committed the crime.

Gee, what a long-winded way to explain why we conducted three different outbreeding depression experiments with pink salmon. The bottom line is that the more genetically divergent two

populations are, the more likely it is that we can detect differences. We had been working with Auke Creek pink salmon, in part because pink salmon have a short two-year life cycle. Also, there are two genetically isolated populations in the same stream, but they return in different years, which makes experiments possible that involve genetically very different pink salmon. Because we did not know at the outset what kind of results we might observe, we wanted to look first for outbreeding depression in the most divergent populations that we could produce. To make such hybrids, we learned how to cryopreserve semen (freeze sperm). We hybridized even- and odd-year broodline pink salmon by freezing sperm from one broodline, holding it for a year on liquid nitrogen, and using it to fertilize eggs from the other broodline. For this first experiment, we assumed that after we released the fry we would have very few returning adult hybrids because a similar experiment had been previously conducted in Canada by Withler and Morley<sup>1</sup>; they recovered few hybrids. Surprisingly, nearly equal numbers of hybrids and controls returned to spawn. This was so unexpected that we had not planned for a second generation and were unable to follow through properly with those fish.

The successful return of hybrid fish prompted a pair of follow-up experiments (even-year males by odd-year females and odd-year females by even-year males), which we followed for two generations. In both experiments, the  $F_1$  hybrid and control fish had nearly identical survivals, which we measured as

returns of adults to the Auke Creek weir. In contrast, the survival of the  $F_2$  hybrids was only about 70% of the  $F_2$  control returns. Our null (falsifiable) hypothesis was that there would be no difference in survivals of hybrid and control fish. We were unable to reject that hypothesis for the  $F_1$  generation, but we rejected that hypothesis resoundingly for both  $F_2$  returns and concluded that the  $F_2$  hybrids between broodlines of pink salmon had lower survivals than the controls. The probable reason for the difference is outbreeding depression. Also, because the difference occurred in the second generation, but was not apparent in the first generation, it is likely that the genetic outbreeding depression rather than ecological outbreeding depression is the cause. That is, crossing the two lineages disrupted their coadapted genomes.

Our first definitive experiment demonstrated that outbreeding depression can occur in salmon. However, natural hybridization between broodlines is very unlikely to occur in the modern marine environment (but see chapters 3. History of a Salmon Population, and 5. Fish Population du Jour, especially the description of transplanting pink salmon to the Great Lakes). As a result, we conducted a set of experiments that were a bit more realistic, especially for concerns about stock translocations, which have been commonplace throughout the world for most of the last century. In these experiments, we used females from Auke Creek and produced hybrids with males from Pillar Creek on Kodiak Island and controls with males from Auke

Creek. We followed the hybrids and controls for two generations. In the first generation of the even-year broodline experiment (brood year 1996), the hybrid and control fish had similar survivals. In the second generation, the survival of hybrids was about 70% of the survival of controls. This pattern was similar to what we observed for crosses between broodlines, which was consistent with genetic outbreeding depression (disruption of coadapted genomes). Note that we are interpreting the results, but not stating that we “proved” them.

In the second (odd-broodline) experiment, we observed a reduction in survival of hybrids in both the first generation (hybrid survival was about 60% of the control survival) and the second generation (hybrid survival was about 75% of the control survival). The odd-broodline experiment suggests that both ecological and genetic outbreeding depression occurred.

So how critical to the “health” of the populations are the differences that we observed? If subsequent adult populations reflected the numbers of surviving progeny, the population would decline to 75% in one generation and to about 10% after eight generations if the effect persisted. There is no reason to think that recovery from genetic outbreeding depression would occur rapidly. It should be apparent that translocating stocks may be quite damaging to local populations of the same species, and the effect might persist for some time.

<sup>1</sup> Withler, F.C., and R.B. Morley. 1982. Use of milt from on-year males in transplants to establish off-year pink salmon (*Oncorhynchus gorbuscha*) runs. Can. Tech. Rep. Fish. Aquat. Sci. 1139. 49 pp.

populations, it is unlikely that the released fish would have a substantial genetic effect on wild populations. This sort of hatchery has been termed “segregated” because the artificially spawned hatchery population is genetically segregated from wild populations (<http://www.hatcheryreform.us> has technical discussion documents that describe segregated and integrated hatcheries in detail).

### ***Ecological problems***

There can, however, be conservation issues. The way in which fisheries are conducted can be hugely important because the survival of hatchery fish from fertilized egg to release into the wild usually is much higher than for wild populations. Although increased survival is not directly a genetic issue, it can cause an ecological problem. The harvests of well-managed commercial fish stocks are restricted to the ability of spawning adults to produce adult offspring. As a result, one of the goals is to ensure that a sufficient number of fish escape the commercial fishery to return to their natal streams to spawn. The problem is that the higher survival of embryonic fish produced in the hatchery, as compared to survival of embryos that are produced naturally, means that the hatchery fish can be exploited in the fishery at a higher rate than the wild fish and still produce an adequate number of returns to seed the next generation in the hatchery.

So what’s the problem? If both hatchery and wild fish are harvested together, harvesting at a rate that is most efficient for hatchery fish will overfish the wild populations. The wild populations will be unable to sustain their numbers and will decline. On the other hand, if the returning fish are harvested at a rate appropriate for the wild populations, there will be excess numbers of returning hatchery fish. Unfortunately, the solution to the problem is not as simple as waiting until the fish return to the hatchery to harvest them because for many salmon species the flesh quality deteriorates rapidly as the salmon complete maturation, and they become unmarketable. So why not just let them die? First, there will be public outrage at the “wasted fish” (even though the reason is a legitimate conservation issue). Second, disposing of the carcasses poses a large economic problem. And no, in the remote locations of many hatcheries, there is as yet no economically feasible alternative use for

the carcasses. Of course, one solution is to locate the hatchery where there are no wild stocks. Indeed, one of the strategies used in Southeast Alaska to increase chum salmon production has been to release young fish at remote sites where there are no (well, few) wild populations. The young fish can be imprinted to those sites by rearing them in seawater pens for a short period just prior to release. The fish return to those sites, where they are caught in fisheries designed to catch virtually all of them—so-called mop-up fisheries. A related strategy is to choose brood stock that return at a different time from the wild fish and to conduct separately managed fisheries. Timing differences would avoid the mixed stock fishery problem as well as potential genetic interactions, but the possibility of colonization would have to be monitored, and a brood stock that has appropriate timing might not be available. We will talk about yet another approach to the problem in a bit.

### ***Gene pool swamping***

Extensive culture that brings cultured and wild fish into contact can have problems other than passing disease from cultured to wild populations and possibly overfishing wild populations. If the culture facility is near wild populations, it is likely (inevitable) that some cultured fish will stray into the wild populations. If the cultured stock was developed from local populations, its gene pool may be preadapted for that area, and one would think that the effect of straying salmon interbreeding with wild salmon would be small. However, domestication selection may alter the hatchery gene pool by changing allele frequencies and those altered frequencies are what the wild population will receive. The impact of the strays depends on both their numbers and the extent of divergence between the cultured and wild populations. Regardless, if gene flow from the cultured population to the wild population is persistent and not countered by considerable gene flow in the other direction, the wild population will eventually be swamped by cultured genes. Even if the gene pool is not swamped, gene flow can move the genetic composition of the wild population away from the distribution of phenotypes that resulted from local adaptation. Some of the differences in phenotype can be subtle, but important, for example,

development rate (see Chapter 5. Fish Population du Jour). Coupled with different exploitation tolerances, this kind of hatchery system will often jeopardize wild populations.

### **Balance upset**

Another extensive strategy is a hatchery that is located in the same stream as a wild population. The brood stock is taken from a mixture of hatchery and wild returns and the wild population includes spawners that started life in the hatchery (an “integrated” hatchery program in the terminology of <http://www.hatcheryreform.us>). Of course, potential disease issues remain, but genetic issues diminish if the number of cultured salmon returning to spawn in the wild is restricted and a substantial proportion of wild-spawned salmon are incorporated into the hatchery brood stock each generation. If the numbers of returns are properly balanced, natural selection and local adaptation will continue to act on the total population and the extent of domestication selection will be limited. Unfortunately, success at low levels of culturing often leads decision makers to increase culturing levels, which could throw the wild-cultured fish ratio out of balance. The “balance” that allows natural selection to predominate over domestication requires that the hatchery release relatively small numbers of salmon; and in the short term, people are usually interested in harvesting large releases.

### **Habitat rehabilitation and artificial spawning channels**

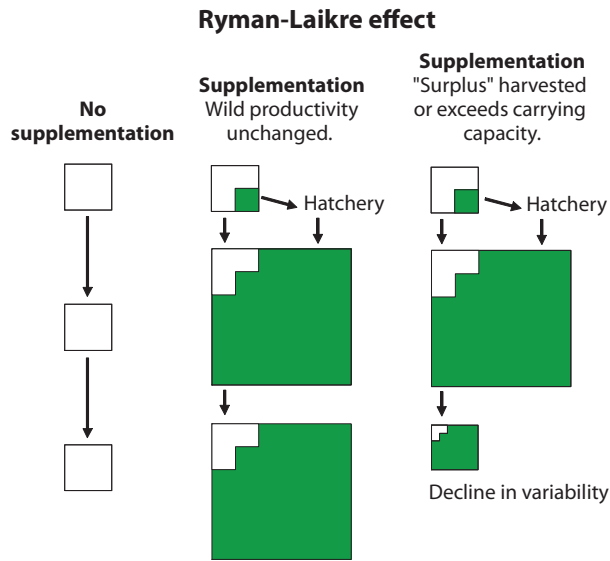
Two other strategies increase the amount of “natural” habitat. One is rehabilitation of previously used habitat that was degraded by natural or human processes. This approach would be ideal, if the habitat were restorable. Unfortunately, habitat rehabilitation usually means that land use must be taken from one use, especially agriculture, in order to pass back to the fish. The other strategy is development of artificial spawning channels, which provide additional spawning habitat where rearing habitat is not limiting. Notable successes have been observed for sockeye salmon spawning channels in the Babine Lake system in British Columbia. The same genetic concerns exist for these kinds of fish enhancement as

for other approaches if this “new” habitat is seeded by translocated stocks. However, if they are seeded naturally, the risks should be low. This approach also requires availability of appropriate land and water sources.

### **Pitfalls of culturing for conservation**

A final topic that belongs in this section concerns the possible snafus that can result from well-intentioned efforts to resuscitate a population that is in decline; that is, cultural activities conducted for conservation purposes. There are many reasons that wild populations may decline. Among the most common reasons are overfishing, habitat loss, and climate fluctuations. The salmon populations of the Columbia River in the Pacific Northwest are poster candidates for conservation issues. Chinook, chum, sockeye, and coho salmon, as well as steelhead, were all once very abundant in the Columbia River system. However, as a result of societal decisions in the last century, many dams were constructed to generate energy and provide water for agriculture. Not only do the dams interfere with the passage of returning fish, but many of the spawning grounds were converted from a free-flowing river to a series of water impoundments, which are no longer suitable for spawning or rearing. In addition, much of the water was dedicated to agriculture, which further reduced the habitat. But wait, there’s more! Many Columbia River salmon spend their marine lives along the Pacific Ocean coast to the north well into Alaska waters. Those fish are subjected to a gauntlet of fisheries that begin in Alaska, continue into British Columbia and along the Washington coast, and into the Columbia River. Independent of the problems that dams created and overfishing, the overall salmon production in the Pacific Northwest has declined since the 1970s as a result of (apparently) cyclical climate changes. Recently, a transitory increase in production occurred, but production decreased again. For all of those reasons, many Columbia River salmon populations have been listed as threatened or endangered under federal law.

Now we can get to the issues that involve genetics and conservation efforts. One idea to address the conservation problems of depleted populations is to



**Figure 5.** Ryman Laikre effect. Genetic variability can be reduced if supplementation does not successfully increase the numbers of individuals. The scenario at the right not only failed to increase numbers but also substantially reduced the available genetic variability. In the figure, the white area represents the proportion of "wild" genes or fish, and the filled area is the proportion of genes or fish that result from culture.

use hatcheries to supplement natural production. The rationale is that it might be possible to resuscitate a population by culturing a portion of the population in a hatchery until it is released as a smolt (the life stage at which salmon leave fresh water to enter the ocean and change physiologically in preparation for a saltwater existence prior to emigrating). In concept, the idea is sound because hatcheries extend the habitat capable of producing smolts. However, the assumption that is made (often implicitly) is that the overall survival and return of adults will be increased by the supplementation and, after a few cycles, the system will be self-sustaining without the hatchery.

The left column in Figure 2 demonstrates that the population will remain at a small size if no steps are taken to enhance it. In that instance, its gene pool will remain intact, except for random drift (see Chapter 2. How Genes Vary in Fish Populations). Clearly, the natural habitat can only carry the depleted number. Supplementation efforts culture a portion of the wild

returns. In a successful effort, the population size would increase and stay at the larger numbers, as is shown in the middle column of Figure 5.

There are two ways in which the assumption can go awry. The first is that if habitat is limiting, the population size will revert to the original low levels when hatchery production is stopped. The second problem is a harvest management problem. The public often has expectations that if there are increases in survival, much of that increase should be available to harvest. Both of these problems have genetic implications. The fraction of the endangered population brought into the hatchery can represent a large portion of the gene pool. Because the cultured portion has a much higher survival than the rest of the population, alleles from the cultured fish will increase in abundance relative to the genes in the rest of the population. If supplementation does not result in increased population numbers in the long term because natural habitat was not restored or because harvest keeps the population small, the rate of loss of genetic variation (a bottleneck effect) and rate of inbreeding will increase rapidly (see Chapter 2). This phenomenon is called the **Ryman-Laikre effect** (Figure 5). The consequence of heroic efforts to revive a population may be disastrous.

## Summary

Hatcheries are usually deployed to address particular problems—the reasons can be conservation, economics, or both. The success of a hatchery at solving the problem must be continually evaluated, and when the problem has been reversed or it is clear that the hatchery is not successfully addressing the problem, there should be a means to terminate its operation.

Hatchery practices, both purposeful and inadvertent, can alter the genetic composition of a cultured stock. Some changes are beneficial, but others may not be. If the stock is cultured intensively and there are few escapees, it is unlikely that they will harm wild populations. Hatchery practices can also change the gene pool of an extensively cultured stock. Some of those changes, in particular inadvertent selection, may not be as benign, but may not be obvious for several generations.

Intensive hatcheries culture organisms for their entire life cycle and either hold their brood stock or purchase seed organisms. As long as they do not escape, they are benign. However, escapees can spread diseases and alter the gene pools of local populations of the same species. Intensive culture practiced outside the native range of the cultured species can result in exotic introductions.

Extensively cultured organisms can affect wild populations negatively. If large numbers of cultured organisms persistently stray into wild systems, the wild gene pool will soon be swamped. Wild populations are often strongly adapted to local conditions. Even within a small stream, there may be multiple spawning aggregations that have adapted

to different environmental conditions. Finally, interaction between translocated fish (whether stocked or from an extensive culture facility) can be devastating to the wild population if outbreeding depression takes place.

Potential problems from wild-hatchery fish interactions can be reduced by using local populations as the source of brood stock and by releasing the fish at remote sites far from wild populations. Efforts should be made to monitor the influence of the hatchery releases on local wild populations. Ordinarily, such measures would require genetic and abundance surveys of local populations before the hatchery is initiated, and during the entire time that the hatchery is being operated.

