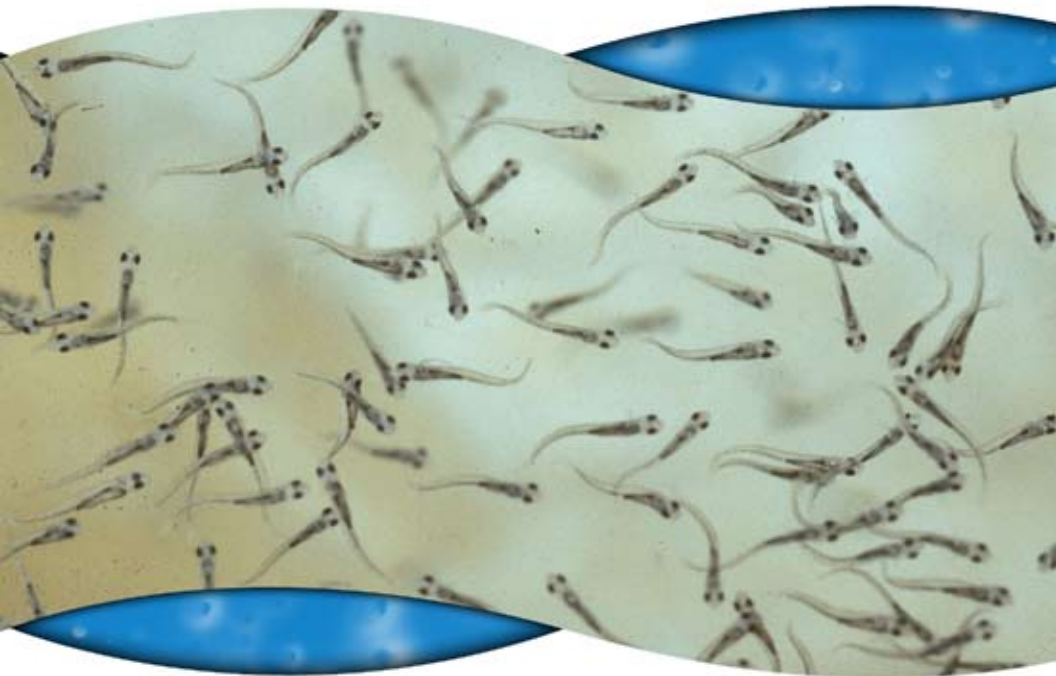


GENETIC GUIDELINES for FISHERIES MANAGEMENT

by Anne R. Kapuscinski and Loren M. Miller

Second Edition



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INTRODUCTION

* Note: Technical terms found in the glossary are highlighted in the text upon first usage.

This manual is a primer for fishery and hatchery managers. It is also oriented to fisheries decision-makers who would like to gain a basic understanding of the role of genetics in addressing problems and opportunities in their work but do not intend to become practicing fish geneticists. This manual is relevant for hatcheries used in fishery stocking programs and **captive aquaculture**, while focusing on the former application. The manual includes three major topics:

- 1) biological principles underlying the genetics of fish,
- 2) genetic tools and their application to fish populations, and
- 3) genetic issues in fisheries management.

A glossary of technical terms is provided. The most significant changes in this second edition of Genetic Guidelines are in Chapter Two: Genetic Tools for Fisheries Applications. Updated information reflects recent advances in genetics, especially in DNA analysis and genomics, which are increasing our understanding of genetic mechanisms that affect the biology fish in the wild and captivity.

Another important development since the first edition is publication of a population genetics textbook targeted at fisheries professionals, *Population Genetics: Principles and Applications for Fisheries Scientists* (Hallerman 2003). This manual provides a useful companion to the textbook by giving a briefer and broader overview of topics in population genetics, selective breeding, and modern biotechnology. The textbook provides greater depth in population genetics and provides numerous references to the primary literature.

Perpetuation of a resource is the common goal of all fisheries management programs. Genetic factors affect this goal because fish are the products of their genes, the environment, and interactions between the two. The genetics of fish, in connection with the environment, determine the quality and persistence of a fishery resource. Fisheries managers must realize that implementation of regulations, stocking strategies, and other management activities affect the genetic make-up of fish stocks. Management activities that impact the genetics of fish stocks include: 1) maintenance of a fishery with adequate natural

reproduction, 2) regulation of a fishery through fish size or gear restrictions, 3) enhancement of a fishery with marginal natural reproduction by stocking, 4) rehabilitation of a depleted fishery by stocking or control of harvest.

Often, managers have concentrated on manipulation of non-genetic, environmental aspects of fisheries (e.g., harvest control, stocking, and pollution abatement). There are at least two problems with this approach. First, a primary determinant of the quality of fishery resources (genetics) has been neglected entirely. Second, management activities inadvertently affect the genetic make-up of fish stocks. Management costs due to the effect of management activities on the genetics of fish stocks have not been considered. This last point is particularly unfortunate because relatively small and inexpensive changes in management practices may affect substantial improvements in the genetic integrity of a stock.

Genetic variation is an important aspect of the genetic makeup of managed fish stocks. The long-term utility of genetic variation is to give populations the ability to adapt to changing environments. The genetic diversity in a population is a finite resource that can be used up. Humans can intentionally or inadvertently "spend" the genetic diversity in populations through activities aimed at relatively short-term goals. The long-term effect on the perpetuation of free-roaming fish populations is often uncertain and could be detrimental in many cases. It is important, therefore, to rationally integrate genetic conservation into the management of fisheries resources. This manual presents the foundational information for such integration.

CHAPTER ONE: GENETIC PRINCIPLES

OVERVIEW

In this section we describe the biology underlying the genetics of fish. Individual topics are explained in sufficient detail to allow subsequent discussion of genetic issues in fisheries and hatchery management. Individuals interested in a more complete treatment of any particular issue should consult a recent text. Snustad and Simmons (2003) provide good overview of all areas of genetics. Hartl and Clark (1997) is useful for general population genetics while the textbook edited by Hallerman (2003) applies population genetic principles specifically to fisheries issues. Falconer and MacKay (1996) is a general reference for quantitative genetics and breeding and Tave (1993) and Lutz (2001) give a thorough treatment of genetics and breeding of fish. The volume edited by Turner (1984) is a good reference for topics related to evolutionary genetics of fish.

MOLECULAR GENETICS AND CYTOGENETICS

Molecular genetics and **cytogenetics** are foundations for the genetics of individuals and populations. Molecular genetics is the study of genetic processes at the molecular level. Cytogenetics is the study of genetics at the level of **chromosomes** in cells. The field of molecular genetics has grown tremendously in recent years and, as a result, many new techniques are available for study and manipulation of genes in fishes. Some familiarity with molecular genetics and cytogenetics is essential to understand genetic processes and to appreciate the values of new tools.

DNA

All of the genetic information in an individual fish is contained in molecules of **deoxyribonucleic acid (DNA)**. Molecules of DNA are composed of subunits called **nucleotides**. Each nucleotide contains a compound called



Figure 1. DNA. Nucleotides in one strand are paired with nucleotides in the opposite strand. Adenine (A) pairs only with thymine (T), and cytosine (C) pairs only with guanine (G). Together, the two strands form a double helix. During replication the strands unwind, and serve as templates for the synthesis of two new DNA molecules. (From Strickberger 1976)

a **base**. There are four kinds of nucleotides in DNA because there are four different bases (adenine, guanine, thymine and cytosine). DNA molecules, as shown in Figure 1, consist of a long ladder of paired nucleotides. A natural twist in the ladder gives the DNA molecule a **double helix** structure.

Nucleotides form **base pairs** in the double helix in a specific manner (Figure 1). Where thymine is found in one strand of the helix, only adenine will be found in the same position of the opposite strand. Similarly, where guanine is found in one strand, only cytosine will be found in the same position of the opposite strand. The two strands of the helix are said to be complementary because of the way nucleotides form base pairs.

Whenever a cell divides, the DNA must be replicated in order to provide each daughter cell with a complete set of genes. An advantage of complementary base pairing is evident during **replication** of the DNA molecule. During replication, the two strands of the DNA helix are separated by **enzymes** so that each strand is available to serve as a template for a new molecule (Figure 1). Individual nucleotides are affixed to each template. Two complete and identical DNA molecules result. The complementary pairing of bases ensures that the replication of DNA is essentially error free.

EXPRESSION OF GENES AT THE MOLECULAR LEVEL

A **gene** is a sequence of nucleotides occupying a specific position (**locus**) on a DNA molecule. The end product of a **structural gene** is a specific chain of **amino acids** called a polypeptide (a subunit of a **protein**). The **one gene - one protein concept** provides a useful definition for a structural gene: a structural gene is the DNA that codes for a single protein, or more accurately, a single polypeptide. A second class of genes codes for molecules (certain types of **RNA**) that are involved in protein synthesis but are not themselves translated into proteins. Each gene of both classes contains coding DNA sequences and regulatory sequences that do not code for specific molecules but regulate the functioning of the gene, such as **promoter** sequences. **Regulator genes**, a special type of structural gene, produce proteins that regulate the expression of another gene, thus allowing additional spatial or temporal control of gene expression.

The many genes found on each chromosome are interspersed among long regions of **non-coding DNA** that do not code for or regulate production of gene products. In humans, for example, one entire set of chromosomes is approximately three billion base-pairs of DNA, but only 1% of this sequence codes for proteins and only a few percent has direct coding or regulatory function. Possible functional roles for this DNA are undetermined, and it has even been termed “junk DNA” by some scientists. Non-coding DNA provides many useful genetic markers of inheritance that are used by fish geneticists (see Chapter Two: Genetic Tools for Fisheries Applications).

The Genetic Code

Sequences of nucleotides in structural genes are the templates for amino acids in proteins. The genetic code is a **triplet code** because nucleotide triplets code for individual amino acids. A triplet of nucleotides is called a **codon**. The same genetic code is shared by almost all organisms.

Figure 2 illustrates the correspondence between nucleotides, codons, and amino acids in proteins. Note that the order of amino acids in the protein is the same as the order of the respective codons in the DNA molecule.

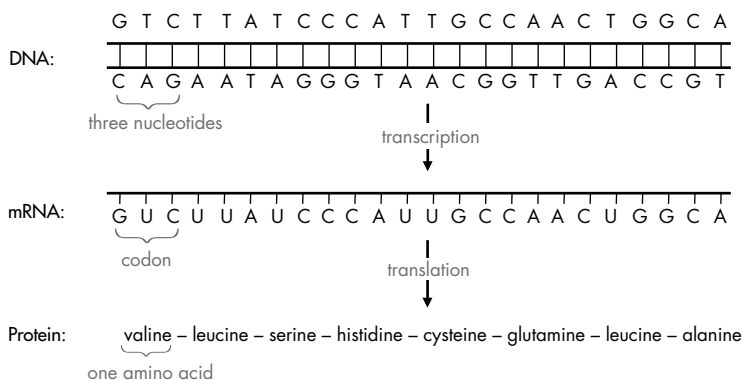


Figure 2. Transcription and translation, a diagrammatic illustration of how genetic information passes from DNA to mRNA to protein. DNA is used as a template (the lower DNA strand in this figure) for transcription of mRNA. The mRNA is used as a template for protein synthesis during translation. Three nucleotides in DNA specify one codon in mRNA and one amino-acid in the final protein. Note that thymine (T) in DNA is replaced by uracil (U) in mRNA. (After Strickberger 1976)

Protein synthesis

Genetic control of biological processes begins with the synthesis of proteins. An intermediate molecule (**messenger RNA**) and two processes (**transcription** and **translation**) are involved (Figure 2). Messenger RNA (**mRNA**) is a chain of nucleotides in a single strand, much like a single strand of the DNA helix. During transcription, a strand of RNA is produced that is complementary to one strand in the DNA helix; all of the sequence information in the DNA is transferred to RNA. This property makes RNA an appropriate template for protein synthesis. During translation, enzymes link individual amino acids together using the mRNA as a template. The resulting chain of amino acids is shaped by other enzymes to form the final protein product.

ORGANIZATION OF GENETIC INFORMATION IN CELLS

Two types of DNA are found in eukaryotic cells: **nuclear** and **cytoplasmic DNA**. Nuclear DNA is found in chromosomes located in the **nucleus** of a cell. Cytoplasmic DNA is found outside of the nucleus in various **organelles**

(e.g., **mitochondria**) within the **cytoplasm** of the cell. All of the cytoplasmic DNA in an individual fish is thought to be inherited from the mother through the cytoplasm of the egg. The sperm of the male is thought to contribute no cytoplasm to the fertilized egg. Many genetic tools and issues of interest to hatchery and fisheries managers involve nuclear DNA. Information about cytoplasmic DNA should not be neglected, however. **Mitochondrial DNA (mtDNA)** has been used extensively, particularly for stock identification and systematics. Furthermore, the suitability of an organism for a particular environment depends on both its nuclear and cytoplasmic DNA.

Chromosomes — structure

Most of the DNA in fish is packaged in chromosomes that reside in the cell nucleus. Each chromosome contains a single strand of DNA. Prior to and during cell division, the chromosomes are condensed. The DNA in **condensed chromosomes** is coiled so that the chromosomes assume a characteristic form and occupy a minimal amount of space. (Chromosomes condense just before cell division and are easy to see using a light microscope).

Heterochromatin are regions of a condensed chromosome that stain more darkly than other regions when chromosomes are prepared for microscopic examination. Heterochromatin gives stained chromosomes a banded appearance with alternating dark (heterochromatin) and light bands (Figure 3). Heterochromatin bands can be useful as markers for stock identification.

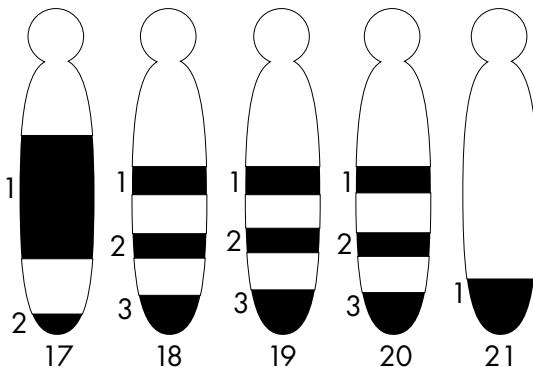


Figure 3. Diagram of chromosomes 17 - 21 of chinook salmon showing location and relative size of stained bands called Q bands because they are visualized using the chemical stain quinacrine. The banding patterns may vary between individuals and between populations. (From Phillips et al. 1985)

Chromosomes – number

The number of chromosomes in **somatic cells** (cells that are not eggs or sperm) differs widely among species but is relatively constant among individuals of the same species (Table 1). The number of chromosomes in a cell is normally an even number (denoted $2n$) because both parents contribute an equal number of chromosomes to their progeny. The number of chromosomes contributed by each parent through either the egg or sperm is called the **haploid** number and is denoted by n . Normal individuals of most species are said to be **diploid** because they have $2n$ chromosomes in each somatic cell. Polyploidy (duplication of entire chromosomes sets so that they are $4n$, $8n$, etc.), however, was important in the evolutionary history of some fish families (e.g., trouts [Salmonidae], and suckers [catostomidae]), and still exists in some groups (e.g., sturgeon and paddlefish [Acipenseriformes]).

Table 1. The diploid ($2n$) chromosome number of various fish species. Variation within species may be real or a byproduct of the difficulty counting small fish chromosomes. All data from FishBase (www.fishbase.org).

Species	Diploid ($2n$) chromosome number
Nile tilapia	40-44
Walleye	48
Northern pike	50
Fathead minnow	50
Pink salmon	52-54
White sucker	98-100
Atlantic sturgeon	99-112
Sea lamprey	168

The chromosomes in a haploid cell (egg or sperm) differ from one another in size, shape, banding patterns, and in the genes that they carry. In a cell of a diploid individual, pairs of **homologous chromosomes** are distinguishable. Homologous chromosomes originate from the **gametes** (eggs and sperm) of each parent but have the same general shape and carry the same genes.

Variation in the ploidy of individuals is common but is usually associated with reduced viability and fertility in species that are normally diploid. Individuals with a single haploid complement of chromosomes are haploid or **monoploid**; those with more than two haploid complements are **polyploid**. There are various types of polyploids. **Triploids**, for example, have $3n$ chromosomes and **tetraploids** have $4n$ chromosomes.

Triploid individuals are usually sterile because the triploid genome cannot be split into two parts with equal numbers of chromosomes; inviable gametes result. Tetraploid genomes can be split into two equal parts (each with $2n$ chromosomes) and viable tetraploid species of fish are common. Tetraploidy is thought to have played an important part in fish evolution (Turner 1984).

Aneuploidy is another kind of variation in chromosome number in which there are extra or too few copies of a single chromosome. Individuals with three copies of a particular chromosome are said to be **trisomic**; those with four copies are said to be **tetrasomic**. Aneuploidy in animals usually results in deformity, sterility, and loss of viability.

Sex chromosomes

Sex chromosomes are the major determinants of sex in fish. Chromosomes that are not principal determinants of sex are called **autosomes**. Many, but not all, fish have a pair of sex chromosomes.

Chromosome aberrations

Chromosome abnormalities occur when chromosomes break and reunite at the broken ends. Multiple breaks may occur on the same chromosome, resulting in deficiencies (loss of chromosome segments), inversions (inverted segments of chromosomes), and duplications (presence of multiple copies of a segment). Multiple breaks on different chromosomes may result in a translocation (a segment of a chromosome inserted into the body of another, nonhomologous chromosome). Duplications and deficiencies of chromosome segments are usually lethal. Translocations and inversions, for example those found in sockeye salmon (Thorgaard 1978), are not necessarily detrimental.

CHROMOSOMES DURING GROWTH, GAMETOGENESIS, AND FERTILIZATION

Chromosomes are the vehicles of genes. Consequently, the behavior of chromosomes during the life cycle of an organism is very important.

Growth

Cells must divide to replace senescent cells or increase in number. Cell division for growth involves duplication (rather than splitting) of whole chromosome sets because daughter cells are diploid and genetically identical to each other and their precursor.

Mitosis is the division of the cell nucleus and cytoplasm. **Chromosome duplication** is completed before mitosis begins. Figure 4 shows the sequential

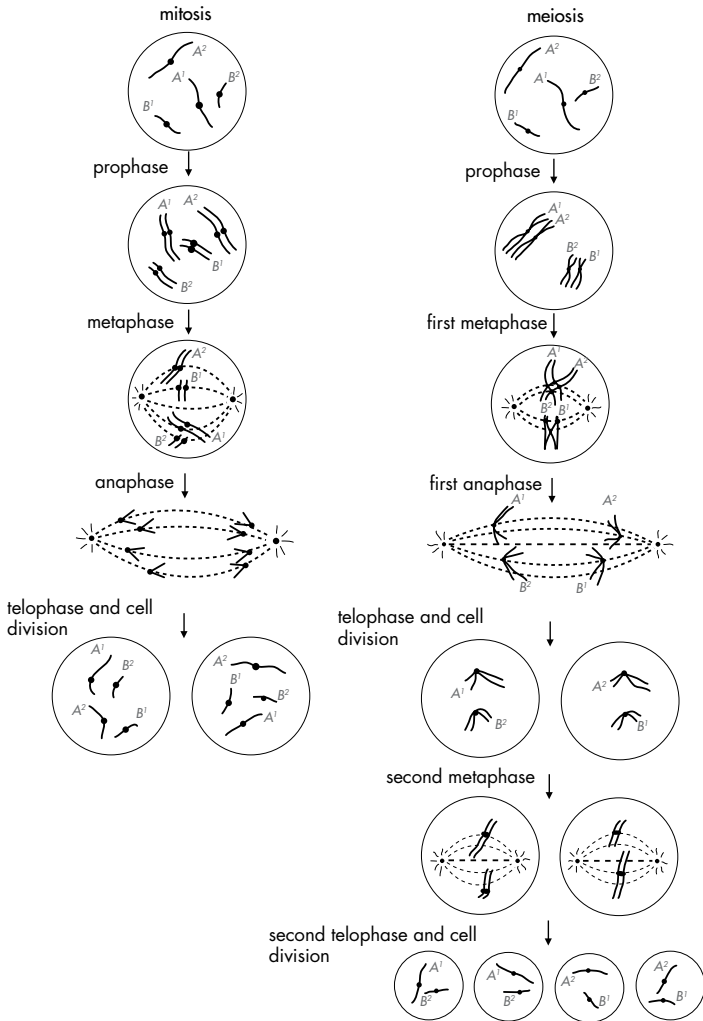


Figure 4. Mitosis and meiosis. Mitosis produces normal diploid (2n) somatic cells. Meiosis produces haploid (n) germ cells that mature into gametes (eggs and sperm). An important feature of meiosis is that homologous chromosomes form pairs that may cross over at the first metaphase. Chromosome number is reduced from 2n to n after the first meiotic metaphase. (From Strickberger 1976)

stages of mitosis: **prophase**, **metaphase**, **anaphase**, and **telophase**. **Cytokinesis**, the division of the cytoplasm to complete formation of two daughter cells, occurs during telophase.

Gametogenesis — segregation and independent assortment

Gametogenesis is the production of gametes. In gametogenesis, the diploid ($2n$) number of chromosomes in somatic cells is reduced to the haploid (n) number. Reduction in the number of chromosomes is accomplished through **meiosis**. Two meiotic cell divisions are required (Figure 4). The chromosome number is reduced during the first meiotic division. The second meiotic division leads to eggs or sperm.

Gametogenesis has two important consequences, **segregation** and **independent assortment**, that are known as **Mendel's principles**. The two products of the first meiotic division each contain a single chromosome from every homologous pair found in the parent cell. Homologous chromosomes and their respective genes are said to segregate during gametogenesis because pairs of homologous chromosomes separate into different daughter cells. Independent assortment means that non-homologous chromosomes of maternal and paternal origin segregate randomly so that each gamete receives a mixture of maternal and paternal chromosomes. Because of segregation and independent assortment, the chromosomes in a single germ cell are a complete haploid set and are random mixtures of maternal and paternal chromosomes.

Crossing over

During **crossing over**, pieces are exchanged between homologous chromosomes (Figure 5). Crossing over is an important source of genetic variation because genes of maternal and paternal origin come to reside on the same chromosome, a phenomenon known as **recombination**. Chromosomes in an individual's gametes may differ substantially from chromosomes in the individual's somatic cells because of recombination.

Fertilization

The genetic composition of an organism is determined at **fertilization** when the egg and sperm unite. At fertilization, the diploid ($2n$) condition is normally restored and the cytoplasmic DNA of the egg becomes the cytoplasmic DNA of the offspring. An abnormal chromosome number after fertilization may result from: a) union of an unreduced germ cell ($2n$) with a normal germ cell (n) resulting in a triploid ($3n$), or b) **hybridization** between two species, one with haploid number n and the other with haploid number n' , producing an offspring with $n+n'$ chromosomes.

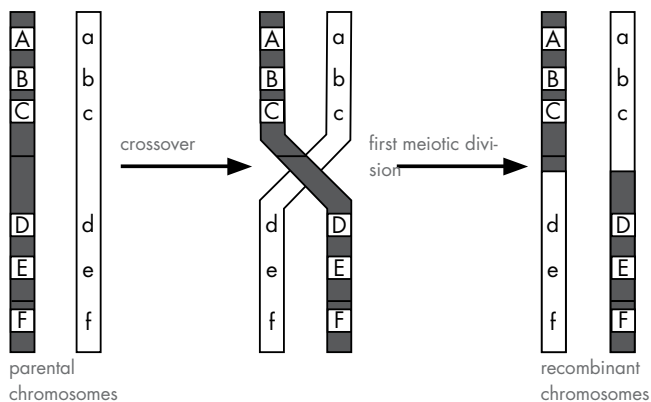


Figure 5. Crossing over between homologous chromosomes during meiosis results in new combinations of alleles on individual chromosomes. (After Strickberger 1976)

GENETICS OF INDIVIDUAL ORGANISMS

Genetics at the molecular and cellular level is the foundation of genetics in individual organisms. Similarly, the genetics of individual fish is the foundation for the genetics of broodstocks and populations.

PHENOTYPE AND GENOTYPE

Every individual has both a **phenotype** and a **genotype**. The genotype is the specific set of genes carried by the individual. The phenotype is the set of characteristics (e.g., morphological, physiological, behavioral) expressed by the individual. The phenotype is produced by the genotype in combination with the environment.

Description of the genotype

There are two representatives of every gene (called **alleles**) in a normal diploid individual because alleles of the same gene occupy the same place (locus) on both homologous chromosomes. The genotype is the set of alleles an organism carries at one or more loci in an organism.

Consider a gene with two alleles denoted A and a . Three genotypes are possible in a diploid individual: AA , Aa , and aa . Individuals with two copies of the same allele (i.e., those with AA or aa) are **homozygous** while those with different alleles (Aa) are **heterozygous**. In a triploid individual the number of

potential genotypes is larger than in a diploid because all possible combinations of the two alleles taken three at a time (e.g., *aaa*, *aaA*, *aaA*, etc.) must be counted.

There may be any number of different alleles for a single gene. The number of possible genotypes depends on the number of alleles. For example, with three alleles (*A*, *a*, *a'*) six genotypes are possible in a population of diploid individuals: *AA*, *Aa*, *Aa'*, *aa*, *a'a'*, and *aa'*.

We can describe the genotype of a single individual at several loci. Consider two loci, each with two alleles (*A* and *a* at one locus, *B* and *b* at the other locus). There are nine possible genotypes: *AABB*, *AABb*, *AAbb*, *AaBB*, *AaBb*, *Aabb*, *aaBB*, *aaBb*, and *aabb*. It is apparent that relatively few alleles at only a few loci can generate an enormous amount of genetic diversity. The potential for genetic diversity increases as either the number of heterozygous loci, number of alleles, or ploidy of an organism increases.

Description of the phenotype

The phenotype of an organism is some detectable attribute. The attribute or **trait** may be physical (e.g., size), physiological (e.g., ability to osmoregulate in salt water), or behavioral (e.g., water temperature preference). The attribute of interest may be a **qualitative trait** or a **quantitative trait**. A qualitative trait can be described without measurement (e.g., albinism, presence or absence of dorsal spines, and sex). Quantitative traits are described by a count or measurement (e.g., scales in the lateral line or total weight of a fish).

Phenotype, genotype, and the environment

Geneticists study phenotypic variation in order to make inferences about genotypic variation. However, it must be remembered that the phenotype is the product of the environment, the genotype, and the interaction between the two. A particular trait in an organism may or may not be determined primarily by the genotype. For example, a fish that is starved cannot express its genetically determined potential for growth.

QUALITATIVE TRAITS

Qualitative traits are often controlled by a relatively small number of loci and alleles. Furthermore, different genotypes may produce distinct phenotypes so that phenotypic variation among individuals can be attributed easily to variation in genotype. It is often possible to learn how a qualitative trait is inherited because the underlying genetics are relatively simple. Qualitative traits that have a simple genetic basis are called **Mendelian traits**.

Phenotypic expression of qualitative traits

Consider a fictitious locus that controls the presence or absence of dorsal fin rays (a qualitative trait) in a fish. There are two alleles: D , associated with normal development of dorsal fin rays and d , associated with absence of dorsal fin rays. The three possible genotypes are DD , Dd , and dd . The phenotypic expression of these genotypes depends on **dominance** (interaction between alleles at the same locus), **epistasis** (interactions between genes at different loci), **penetrance**, and **expressivity**.

Dominance relationships

A **dominant** allele is one that is always expressed phenotypically, regardless of the genotype. A **recessive** allele is one that is expressed only in individuals who are homozygotes for the recessive allele. A locus with two alleles and complete dominance produces only two phenotypes; a heterozygote is identical to a dominant homozygote. **Codominant** alleles are expressed equally in the heterozygote. With **semidominance**, or **incomplete dominance** both alleles are expressed in the heterozygote but not to the same extent. See Table 2 for example relationships between genotype and fin ray development under these different dominance relationships.

Penetrance and expressivity

Genotypes that are not always expressed phenotypically have incomplete or partial penetrance. Genotypes that are expressed to different degrees in different individuals have variable expressivity (Table 2). Partial penetrance and variable expressivity illustrate that the pathway from genotypes to phenotypes is often modulated by environmental conditions and genetic background.

Inheritance: independent assortment and segregation

The principles of independent assortment and segregation (Mendel's principles) govern the inheritance of all genes but these principles are particularly evident in the inheritance of qualitative traits. Consider two loci each with two alleles. The first locus controls development of dorsal fin rays as in the previous example. The second locus controls pigmentation with allele P associated with normal pigmentation and allele p with albinism.

Each haploid gamete has one complete set of chromosomes and therefore, one complete set of alleles because homologous chromosomes and alleles segregate during gametogenesis. The principle of segregation asserts that an individual with genotype Dd produces equal numbers of two types of gametes, one containing D and the other containing d (Table 3). Using a simple tool called a Punnett square, we can then predict the genotypes and phenotypes of offspring when gametes unite during fertilization (Figure 6).

Table 2. Relationships between genotypes and phenotypes with different dominance relationships, penetrance and expressivity for a fictitious locus that controls the presence or absence of dorsal fin rays in a fish.

Allelic relationship	Genotype	Phenotype
Dominance	DD	normal fin rays
	Dd	normal fin rays
	dd	fin rays absent
Codominance	DD	normal fin rays
	Dd	fewer normal fin rays
	dd	fin rays absent
Incomplete dominance	DD	normal fin rays
	Dd	abnormal fin rays
	dd	fin rays absent
Partial penetrance	DD	normal fin rays
	Dd	normal fin rays
	dd	some with normal fin rays, some absent
Variable expressivity	DD	normal fin rays
	Dd	normal fin rays
	dd	range from normal-abnor- mal-absent

Table 3. Expected gamete haplotype frequencies for an individual with genotypes *Dd* and *Pp* at two genes. The first two rows illustrate segregation at each gene separately. If the two genes assort independently, the gamete haplotype frequencies for both genes are products of the individual gene expectations, e.g., 50% *D* x 50% *P* = 25% *DP* (row 3). If alleles *D* and *P* are linked tightly on the same chromosome (depicted as *DP/dp* in row 4), they will pass together into the same gamete, as will alleles *d* and *p* on the other homologous chromosome.

Genotype of parent	Gamete haplotypes	Genetic principle
Gene 1: <i>Dd</i>	50% <i>D</i> ; 50% <i>d</i>	segregation
Gene 2: <i>Pp</i>	50% <i>P</i> ; 50% <i>p</i>	segregation
Both genes: <i>DdPp</i>	25% <i>DP</i> ; 25% <i>Dp</i> ; 25% <i>dP</i> ; 25% <i>dp</i>	independent assortment
Both genes: <i>DP/dp</i>	50% <i>DP</i> ; 50% <i>dp</i>	complete linkage

		Male gamete haplotype	
		D (50%)	d (50%)
Female gamete haplotype	D (50%)	DD (25%) normal fin rays	Dd (25%) normal fin rays
	d (50%)	Dd (25%) normal fin rays	dd (25%) undeveloped fin rays

Figure 6. Punnett square depicting the expected genotype and phenotype frequencies in offspring of two heterozygous (Dd) parents. The row and column headings depict the 50:50 probability of producing either D or d gamete haplotypes. The boxes depict the probability of each offspring genotype when the gametes unite randomly during fertilization. The expected genotypic ratios are 25% DD ; 50% Dd ; 25% dd . The expected phenotypic ratio is 75% normal fin rays; 25% undeveloped if D is a dominant allele, because both DD and Dd individuals would develop normal fin rays.

The principle of independent assortment asserts that alleles of different loci assort independently when gametes are produced. Consider an individual with genotype $DdPp$, where the first gene affects fin rays (as in Figure 6) and the second affects pigment. If alleles at the two loci assort independently, then equal number of gametes with **haplotypes** DP , Dp , dP , and dp will be produced (Table 3). If two individuals with genotype $DdPp$ mate, a 4 x 4 Punnett square can be used to predict the simultaneous inheritance of both traits in offspring (Figure 7). Independent assortment of genes on different chromosomes should come as no surprise. It is surprising, however, that the principle of independent assortment holds generally for genes far from each other on the same chromosome, because of crossing over during meiosis.

Genes on the same chromosome that do not assort in a completely independent manner are **linked** and the strength of the linkage is measured by the degree to which independent assortment is observed. Consider two linked genes carried by an individual with genotype $DdPp$ (DP on one homologous chromosome and dp on the other homologous chromosome). Independent assortment (no linkage) would result in gametes with equal numbers of each haplotype listed above. Gametes with only two haplotypes (DP and dp) would be produced in the complete absence of independent assortment (complete linkage) (Table 3). With incomplete linkage all four haplotypes would be produced but with an excess of DP and dp and a deficiency of dP and dP .

		Male gamete haplotype			
		DP	Dp	dP	dp
Female gamete haplotype	DP	DDPP normal fins pigment	DDPp normal fins pigment	DdPP normal fins pigment	DdPp normal fins pigment
	Dp	DDPp normal fins pigment	DDpp normal fins albino	DdPp normal fins pigment	Ddpp normal fins albino
	dP	DdPP normal fins pigment	DdPp normal fins pigment	ddPP no fin rays pigment	ddPp no fin rays pigment
	dp	DdPp normal fins pigment	Ddpp normal fins albino	ddPp no fin rays pigment	ddpp no fin rays albino

Figure 7. Punnett square depicting the expected genotype and phenotype frequencies in offspring of two heterozygous parents at two loci ($DdPp$). The expected phenotypic ratios are 9:3:3:1, that is, nine individuals with normal fin rays and pigment (dominant for both traits) for every three with normal fin rays and albinism or no fin rays and pigment (dominant for one trait, recessive for the other), for every one with no fin rays and albinism (recessive for both traits).

Independent assortment of genes on the same chromosome depends on the exchange of alleles between homologous chromosomes (recombination) during the first meiotic division of gametogenesis. The probability of crossover and the degree of linkage between two genes depend on the distance between them on the chromosome. With greater distance between genes, the probability of crossover is increased and the degree of linkage is decreased.

Sex linked genes

Sex linked genes are located on sex chromosomes. Sex linkage creates a unique pattern of inheritance. For example, in an XY-male system, genes on the Y-chromosome occur only in males while genes of the X-chromosomes occur in two copies in females but only once in males. A male always inherits his X-chromosome from his mother, while a female inherits one X-chromosome from each parent.

SEX DETERMINATION

Sex determination in fishes is **polygenic**, that is, controlled by more than one gene (Kallman 1984). Two types of genes are involved. The first type, called **superior sex genes**, are found on sex chromosomes. The second type, called

male factors and **female factors**, are found on either autosomes or sex chromosomes and are less important than superior sex genes in the determination of sex.

Superior sex genes are the principal determinants of gender. The sex of an individual can usually be predicted from the combination of sex chromosomes that it carries. Certain combinations of male or female factors, however, may overwhelm the influence of the superior sex genes, thus reversing the sex of an individual. Interactions among male factors, female factors, and superior sex genes result in the diverse array of sexual phenotypes exhibited by fish (e.g., **hermaphroditism** and its special case, **sex reversal**).

QUANTITATIVE TRAITS

Quantitative traits are described by a count or measurement. Traits that can be described by a simple count, such as the number of dorsal fin rays on a fish, are **meristic traits**. Variation among individuals in a meristic trait is discrete. A fish, for example, may have seven or eight dorsal fin rays but cannot have 7.5 dorsal fin rays. Quantitative traits described by a measurement, such as body weight, are continuously variable. For example, a fish may weigh 7, 8 or 7.5 grams. Many of the important traits in fish are continuously variable.

Genetic control of quantitative traits

Genetic control of quantitative traits differs from the genetic control of qualitative traits in at least four ways: 1) a large number of genes typically control a single quantitative trait, 2) environmental influences can have substantial effects on the phenotype so that differences in the environments of individuals with the same genotype may result in different phenotypes, 3) the effect of any single gene is usually small, and 4) single genes may affect more than one trait (**pleiotropy**). Because of the environmental influences and the large number of genes that affect a quantitative trait it is not feasible to consider genes individually.

Phenotypic and genotypic value

It is usually impossible to specify the genotype or genotypes that produce a particular quantitative trait. Genotypes and phenotypes for quantitative traits are linked so loosely that geneticists distinguish between the **genotypic value** and **phenotypic value** of an individual. The phenotypic value of an individual is determined by taking a measurement. For example, the phenotypic value for body weight of a 250 gram catfish is 250 grams. The genotypic value of an individual is the mean phenotypic value of individuals with the same genotype. By averaging over a large number of individuals with the same genotype,

extraneous sources of environmental and genetic variation are controlled. Genotypic value is an estimate of the phenotypic value conferred, on average, by a particular genotype.

Phenotypic expression of quantitative traits

The phenotypic value (P) of an individual for a quantitative trait is determined by genes, the environment, and the interaction of genes with the environment through the following formula:

$$P = G + E + G \times E$$

G = genotypic value

E = **environmental effect**

GxE = **genotype-environment interaction**

The relative importance of each of these factors varies from trait to trait. The genotype-environment interaction arises from the possibilities that the same environment will have different effects on different genotypes or that the same genotype may be expressed differently (have different phenotypes) in different environments.

The genotypic value (G) can be calculated by the summation of additive effects (A) and non-additive effects (D and I):

$$G = A + D + I$$

A = **additive** effects (the cumulative contribution of alleles at all the loci governing a quantitative trait)

D = dominance (resulting from interaction among alleles at the same locus)

I = epistasis (I) due to interactions among loci

Therefore:

$$P = A + D + I + E + G \times E$$

Additive effects

Additive effects of individual alleles are important because they contribute to the **breeding value** of individuals and are passed to progeny in a predictable manner. The breeding value of an individual is judged by the mean phenotypic value of its progeny (Falconer and Mackay 1996). It is impossible to determine the additive effect of an allele by examining a single individual because the effect is obscured by the other factors that contribute to the phenotype.

Non-additive genetic effects

Non-additive genetic effects include the dominance relationships described above for qualitative traits, **overdominance**, and epistasis. Dominance and epistatic effects are not passed from parents to progeny because the diploid genotypes of parents are dismantled when haploid gametes are produced. Non-additive effects in the progeny depend on only the diploid genotype and environment of the progeny; they cannot be predicted from non-additive effects observed in the parents.

Overdominance

Overdominance (or **heterozygote advantage**) occurs when the phenotypic value of heterozygotes is greater than the phenotypic value of homozygotes. Consider a locus with two alleles that controls growth rate in a fish. If the growth rate of one homozygote is 10 units while the growth rate of the other homozygote is 20 units, then the predicted growth rate of the heterozygote would be between 10 and 20 units. With overdominance, the actual growth rate of the heterozygote would be greater than 20 units.

MECHANISMS FOR INTRODUCTION OF GENETIC DIVERSITY

Genetic diversity can be induced by **mutation**, recombination, variation in chromosome number, migration and hybridization. Mutation is a change in DNA resulting from an error in replication during cell division. These errors may be accelerated by exposure to **mutagens** (e.g., radiation and chemicals). Recombination creates new combinations of alleles for genes on the same chromosome. Variation introduced by recombination may be important when genes are linked so that sets of alleles are inherited as a unit and the alleles interact strongly with one another. Variation in chromosome number was an important event in the evolution of the salmonids (Allendorf and Thorgaard 1984). Migration can introduce new or lost alleles into isolated populations. Hybridization between different species can result in a new organism. Hybridization has been used for management purposes (e.g., splake, a cross between lake trout and brook trout) but may also contribute to the endangerment of species (Allendorf et al. 2001).

GENETICS OF POPULATIONS AND STOCKS

The rational management of hatcheries or fisheries requires an understanding of the genetics of groups (**populations** and **stocks**) of fish. In the following section we discuss the genetics of qualitative and quantitative traits in populations. Most of the concepts are explained in the context of **idealized**

populations. Managers will note that real stocks of fish often differ substantially from these idealized populations. The principles developed for idealized populations, however, can be applied to real stocks. It is easier to discuss genetic issues that relate to real stocks after considering those same issues in the context of ideal populations. Let us consider an ideal population that has: 1) infinite size, 2) random mating, 3) non-overlapping generations (individuals born in one generation do not mate with individuals born in another generation), 4) equal numbers of males and females, 5) no migration into or out of the population, 6) no mutation, and 7) no **natural selection**.

GENOTYPIC FREQUENCIES

The genetic constitution of a population could be described if the genotype of every individual in the population were known. Consider an ideal population of 100 individuals and just one locus with two alleles (A and a). If 25 individuals were AA , 50 individuals were Aa , and 25 individuals were aa , then the **genotypic frequencies** of AA , Aa , and aa would be 0.25, 0.5, and 0.25, respectively.

The description is more complicated when more than one locus or more than two alleles are included. Consider an additional locus with just two alleles (B and b) and the same genotypic frequencies. The following example completely describes the genotypes at both loci in the population.

Note that the loci in this example exhibit independent assortment because the frequency of a combined genotype is the product of the frequencies of the genotypes at each locus. For example, the frequency of $AABB$ (0.0625) is the product of the frequency of AA (0.25) and the frequency of BB (0.25)

genotype	frequency
$AABB$	0.0625
$AABb$	0.125
$AAbb$	0.0625
$AaBB$	0.125
$AaBb$	0.25
$Aabb$	0.125
$aaBB$	0.0625
$aaBb$	0.125
$aabb$	0.0625
total =	1.000

The example included nine combined genotypes because there were two loci, each with three individual genotypes. If there were three alleles at each loci, then there would be six individual genotypes for each locus and 36 combined genotypes.

The number of possible combinations for a large number of alleles and loci is staggering. Epistatic interactions among the many combinations of alleles at many loci are one of the important sources of genetic variation in populations.

ALLELIC FREQUENCIES

Allelic frequencies for codominant genes are easily obtained from genotypic frequencies. Consider the genotypes AA , Aa , and aa in the last example. There are 25 individuals with AA (50 A alleles), 50 individuals with Aa (50 A alleles and 50 a alleles), and 25 individuals with aa (50 a alleles). There are 200 alleles in total, 100 of A and 100 of a . The allelic frequency of A is $100 / 200$ or 0.5 and the frequency of a is $100 / 200$ or 0.5.

HARDY-WEINBERG PRINCIPLE

Genotypic frequencies in real populations can be predicted from the allelic frequencies only under the conditions of **Hardy-Weinberg equilibrium**. Consider a large idealized population of diploid organisms and a single locus with two alleles, A and a , with frequencies of p and q , respectively.

According to the Hardy-Weinberg principle, the frequency of any genotype in the population after one generation of random mating is the product of the parental allelic frequencies. Genotypic frequencies after one generation of random mating are given by terms in the expansion of $(p + q)^2 = p^2 + 2pq + q^2$. For example, the frequency of the genotype AA is the probability that a sperm with allele A will find an egg with allele A . The probability that a particular sperm or egg carries allele A is p (the frequency of A) so that the probability of a union between two gametes with A is $p \times p = p^2$. The frequencies of the other genotypes are obtained in the same way. The genotype Aa appears in two ways, either a sperm with allele A unites with an egg with allele a (with probability $p \times q$) or a sperm with allele a unites with an egg with allele A (with probability $q \times p$). The genotypic frequency for heterozygotes is the sum of the two probabilities is $(p \times q) + (q \times p) = 2pq$.

Allelic frequencies in an idealized population do not change from one generation to the next. Genotypic frequencies also remain constant after the first generation of random mating and are said to be in Hardy-Weinberg equilibrium.

The Hardy-Weinberg principle holds for loci with more than two alleles. If alleles A , a , and a' have frequencies p , q , and r , respectively, then the frequencies of all possible genotypes are given by terms in the expansion of $(p + q + r)^2$.

CHANGES IN ALLELIC AND GENOTYPIC FREQUENCIES

In contrast to idealized populations at Hardy-Weinberg equilibrium, real stocks and populations of fish experience changes in allelic and genotypic frequencies.

Two types of processes cause these changes: **dispersive processes** (**inbreeding** and **genetic drift**) and **systematic processes** (mutation, migration and selection). Dispersive processes cause changes in allelic and genotypic frequencies that are random in amount and direction of change. Systematic processes cause changes that are consistent and predictable. The allelic and genotypic frequencies of any population are due to the combined effects of inbreeding, genetic drift, mutation, migration, and selection.

Dispersive and systematic processes have different effects on the genetics of populations. Dispersive processes cause loss of alleles, increased homozygosity, and loss of genetic diversity. Selection, a systematic process, often but not always, reduces diversity, whereas mutation and migration increase genetic diversity in populations and counteract the loss of genetic diversity by the other processes.

Dispersive processes

The allelic and genotypic frequencies of real populations, especially small populations, do not remain the same from one generation to the next, even in the absence of natural or artificial selection, because of genetic drift and inbreeding. The term inbreeding does not always imply intentional inbreeding by an animal breeder. It also can refer to matings between related individuals that occur by chance in randomly mating populations or, importantly, to the increased relatedness among all individuals in a closed population over time.

The consequences of genetic drift and inbreeding are always loss of alleles and genetic diversity and increased homozygosity. The average rate at which alleles and genetic diversity are lost depends on the size of the population. In large populations the effects of genetic drift and inbreeding may be counteracted by systematic processes that increase genetic diversity, i.e., mutation and immigration. In small populations the effects of genetic drift and inbreeding overwhelm systematic processes so that losses of diversity outpace any gains. Another important consequence of genetic drift is genetic differentiation among subpopulations derived from a single, larger population. The allelic frequencies for the subpopulations will diverge over time as genetic drift operates independently in each subpopulation (Figure 8).

Genetic drift

It is easy to illustrate genetic drift with an extremely small, hypothetical population. Consider a single locus with two alleles (A and a) and a population with four members, two females and two males. One male and one female are AA while the other male and female are aa . The initial frequency of allele A is $p = 0.5$ and the initial frequency of a is $q = 0.5$. If the male with genotype aa

died before mating and only the female with genotype AA managed to rear her young, all the progeny would arise from the mating between the AA male and the AA female. Allelic frequencies for the progeny would be $p = 1.0$ and $q = 0$; the progeny would be composed of homozygous individuals belonging to just one family. The loss of one male and one female is very significant in a population of only four individuals; the similar proportional loss in a population of several thousand individuals would generate a much smaller effect.

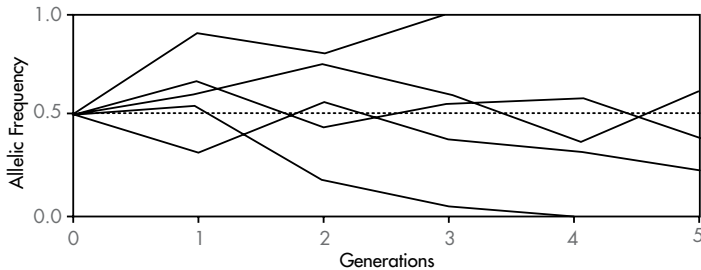


Figure 8. Genetic divergence in five fictitious lake trout populations over six generations. Five populations were derived from a single hatchery population by stocking over five separate reefs. The initial frequencies of both alleles at the locus were 0.5. The y-axis in the figure shows the frequency of one of the alleles. Allelic frequencies diverge over time as genetic drift operates independently in each population. Note that the allele shown becomes fixed (frequency = 1) in one population and is completely lost (frequency = 0) in another. (After Falconer and Mackay 1996)

Random events during gametogenesis and fertilization also contribute to genetic drift. Consider a male and a female, both heterozygotes (Aa). Inheritance principles (see Figure 6) allow us to predict a 0.25 probability of having an offspring with genotype AA . If this pair has two offspring (i.e., replace themselves), there is a $0.25 \times 0.25 = 0.0625$ probability that both would be AA , and allele a would be lost. The same probabilities apply to their having offspring with genotype aa and loss of allele A , for a combined probability of 0.125 that an allele would be lost by chance alone. These random events will have their greatest effect in small populations that have few families to balance occasional aberrations.

Inbreeding

Inbreeding is the mating between related individuals. The consequence of inbreeding is an increase in homozygous individuals because related indi-

viduals share alleles inherited from their common ancestors. It is useful to distinguish inbreeding within one breeding period and that which occurs over time in a population. Within one breeding period, inbreeding is an exception to random mating that increases the number of homozygous individuals above their expectation described by the Hardy-Weinberg principle. Over time in a real population, some families may fail to breed or fail to successfully rear their young so that entire families are eventually lost. Other families may find optimal spawning and rearing habitat and have high reproductive success. As some families are lost and others contribute disproportionately many offspring, the probability of a mating between related individuals increases and the population becomes increasingly inbred, *relative to the population at an earlier time*. This inbreeding occurs even if mating is random during each breeding period. Thus, all populations will lose alleles and genetic diversity if other processes do not compensate for these losses.

Systematic processes

Mutation, migration, and selection are systematic processes that change allelic frequencies. The rates and direction of allelic frequency changes can be predicted quantitatively if certain information (e.g., mutation and migration rates) is available. In the absence of such detailed information, the qualitative results of systematic processes can be predicted.

Mutation

Mutation is the source of new alleles. A new allele may be entirely novel or it may be the same as some other allele that previously existed or already exists in the population.

Migration

Movement of individuals between populations with different allelic frequencies can change the allelic frequencies of the populations involved. Immigration of reproductive adults into very small populations can counteract the loss of genetic diversity due to inbreeding and genetic drift by contributing new or rare alleles to the recipient population. A relatively low rate of immigration is sufficient to counteract the effects of genetic drift and inbreeding (Allendorf and Phelps 1981). The impact of immigration on genetic diversity is an important issue for fisheries management because stocking and transplantation of fish are artificial forms of immigration.

Selection

Selection occurs whenever reproductive success depends on genotype. Individuals with certain genotypes produce more progeny than do individuals with other genotypes so that the genes of the former group increase in fre-

quency with each generation. Natural selection is operating if the environmental factors affecting reproductive success (e.g., predation, disease, temperature) are not controlled by humans. Artificial selection occurs whenever humans select the individuals that will breed successfully. Selection, whether natural or artificial, can cause changes in allelic and genotypic frequencies.

EFFECTIVE POPULATION SIZE (N_e)

The **effective population size (N_e)** of a real population is the size of an ideal population that would experience genetic drift and inbreeding at the same rate as the real population. In an ideal population (i.e., with random mating, no migration, no selection, balanced sex ratio, and non-overlapping generations) the rates of inbreeding and genetic drift depend on the number of individuals in the population. In any real population, however, the reproductive success of individuals varies. For example, some individuals will be too young or old to breed, sex ratios may be unequal, some females may be more fecund than others, some individuals may not mate and some progeny will not be viable. These inequities in reproductive success make the effective population size smaller than the number of individuals in the real population.

INBREEDING COEFFICIENTS (F)

Inbreeding coefficients measure the extent and rate of inbreeding and genetic drift (divergence) in wild and hatchery populations of fish. Inbreeding coefficients (denoted as F) are related to the probability that uniting gametes contain identical alleles derived from a common ancestor. This probability increases (the value of F increases) as alleles are lost from populations by inbreeding or genetic drift. Inbreeding coefficients measure the current level of inbreeding and genetic drift relative to a base population, usually the condition of the population at some previous time. The inbreeding coefficient in the base population is assumed to be zero. Values of F can range from zero (no inbreeding or divergence from the base population conditions) to one (complete inbreeding or divergence).

A base population must be specified by the investigator and should reflect the history of the population and the management objective. If, for example, the objective is to monitor the loss of genetic diversity in a hatchery broodstock, then the condition of the broodstock at the time it was established in the hatchery is the appropriate base population. The inbreeding coefficient at the time the broodstock was established would be zero and inbreeding coefficients (measured from a pedigree, whereby relationships to a common ancestor can be determined, see Hallerman [2003]) would be calculated to determine how

much inbreeding had occurred since the broodstock was established.

Wright (1978) distinguished inbreeding within populations from divergence among populations using hierarchical F-statistics. The coefficient F_{IS} measures inbreeding within populations (IS = individual relative to subpopulation), as indicated by heterozygote deficiencies compared to Hardy-Weinberg expectations, and is closely related to the inbreeding coefficient measured by pedigree analysis. The coefficient F_{ST} (also called the **fixation index**) measures the divergence among isolated populations due to genetic drift (ST = subpopulation relative to total population). It is a type of inbreeding coefficient because fish within an isolated population are more closely related (inbred) relative to one another than to those from other isolated populations.

The fixation index F_{ST} would apply to determining how much genetic divergence had occurred between subpopulations of fish that were derived from the same broodstock but planted into different lakes at about the same time. In this case, the condition of the broodstock at the time the lakes were stocked is the appropriate baseline. The coefficient F_{ST} would measure how much divergence due to genetic drift had occurred since the subpopulations were established. The fixation index also is used to assess genetic differentiation among wild populations of fish (see Stock Identification, page 45).

The rate of inbreeding is the amount of new inbreeding that occurs in each generation. Not surprisingly, the mean rate of inbreeding (ΔF) depends on the effective population size:

$$\Delta F = 1 / (2N_e)$$

FITNESS

Fitness is a measure of the reproductive success of individuals or populations. Fitness is a quantitative trait that is the product of many genes and the environment interacting throughout the lifetime of an individual. **Fitness-related traits** have important effects on reproductive success (e.g., survival, growth rate, size at sexual maturity, and disease resistance). The environment can have tremendous influence over fitness. Examples of environmental factors that affect fitness of wild fish populations include availability of suitable forage, predator-prey interactions, fishing mortality rates, and availability of suitable spawning habitat. The fitness of a hatchery population is also affected by environmental factors (e.g., water quality, temperature regimes, loading densities, and feed quality)

Individual fitness

A practical measure of an individual's fitness is the number of progeny from that individual that survive to reproduce. Individuals in real populations have different levels of fitness due to differences in viability, fecundity, and other factors.

Population fitness

The fitness of a population is determined by the fitness of all members. Population fitness is generally used as a relative measure. The fitness distributions of two populations can be compared to determine their relative health or vigor. The distribution of individual fitness levels in a single population may change over time so that it may be useful to compare the fitness distributions of a single population at two points in time. The fitness of a population depends on the environment so that the fitness of a hatchery or wild stock may change when the fish are planted in new habitats or when major changes occur in the environment.

Inbreeding depression

Inbreeding depression is a loss of fitness associated with inbreeding. The loss of fitness may be due to decreased heterozygosity at gene loci showing overdominance, or superior fitness of heterozygous individuals. A second mechanism for loss of fitness may be **fixation** of **deleterious alleles** at loci with dominance for fitness traits. Deleterious alleles have a negative effect on fitness; an allele is fixed when every individual in the population is homozygous for that allele. Inbreeding depression is difficult to demonstrate in natural populations but is commonly observed in domestic animals and laboratory populations that are inbred.

Heterosis

The opposite of inbreeding depression is **heterosis**, or increased fitness in crosses between genetically different populations. Heterosis is attributed to increased heterozygosity at loci with overdominance and masking of deleterious recessive alleles at loci with dominance for fitness traits. Also called **hybrid vigor**, it is the increased value of a quantitative trait (e.g., growth rate) in hybrid progeny relative to the two parental lines.

QUANTITATIVE GENETICS OF POPULATIONS

The description of a quantitative trait for an entire population involves taking a measurement from every member of the population or from a representative sample. The distribution of phenotypes in the population can then be described by the mean and variance of the measurements. The mean and

variance of a quantitative trait in a population are fundamental quantities in quantitative genetics.

Components of variance

Analysis of phenotypic variance is essential to understanding and managing the genetics of quantitative traits in populations. The same factors that determine the phenotypes of individuals cause phenotypic variance in a population. Consequently, the total phenotypic variance in a population (V_p) can be partitioned into genetic and environmental components:

$$V_p = V_G + V_E + V_{G \times E}$$

V_G = variance due to purely genetic differences among individuals

V_E = variance due to environmental differences among individuals

$V_{G \times E}$ = variance due to the interaction between genetic and environmental differences

The ratio V_G / V_p indicates how much of the total phenotypic variation in a population is due to genotypic differences among individuals. The genetic component of phenotypic variance is composed of additive (V_A), dominance (V_D), and epistatic effects (V_I):

$$V_G = V_A + V_D + V_I$$

Additive genetic variance indicates how much of the total phenotypic variation is passed from one generation to the next in a predictable manner. It is the property most often exploited by plant and animal breeders in artificial selection programs. The ratio V_A / V_p is the **heritability (h^2)** of a phenotypic trait.

Heritability (h^2)

Heritability (V_A / V_p), denoted as h^2 , describes the contribution of additive genetic effects to the phenotypic variance of a trait in a population. Heritability values can be used to predict the phenotypic values of progeny from the phenotypic values of the parents because additive genetic effects are passed from one generation to the next. Values of h^2 may range from zero, when there is no additive variance, to one when the phenotypic variance is due entirely to additive genetic effects ($V_A = V_p$). The higher the heritability, the greater will be the resemblance among parents, progeny, and other related individuals.

Heritabilities measured in one environment may not apply in another. The heritability of a particular trait in a single population depends on the environ-

ment (because $h^2 = V_A / V_P$ and $V_P = V_G + V_E + V_{G \times E}$). For example, if an environmental change causes an increase in phenotypic variance, then the heritability will decrease because $h^2 = V_A / V_P$ will be smaller. The heritability of a trait in one environment may be different from the heritability for the same trait and the same population after an environmental change. It would be unwise, for example, to formulate a breeding program for largemouth bass in the northern U.S. state of Minnesota on the basis of heritabilities that were estimated in the southern state of Florida.

Estimation of variance components and heritability

The heritability and variance components for a trait can be estimated in a number of ways. Most methods involve comparison of related individuals (e.g., parent and offspring, mean of both parents and offspring, half-sibs or full-sibs) in an analysis of variance. Details vary among procedures but the ideas underlying the various methods are similar (Falconer and MacKay 1996).

Selective breeding

Selection, response to selection, and heritability are discussed most often in the context of intentional breeding (artificial selection) in captive aquaculture, such as propagating fish for food or hobby aquaria. It is important to remember that these concepts also underlie natural selection.

Individuals used for breeding in artificial selection programs are usually obtained by breeding all individuals with phenotypic values greater than or less than some cutoff value. The difference between the mean value of the individuals selected for breeding and the mean of the original population is the **selection differential (S)**. The expected **response to selection (R)** from one generation of artificial selection is easy to calculate once the heritability (h^2) and selection differential (S) are known:

$$R = h^2S$$

The actual response to selection will differ from the predicted value due to chance, errors in estimation of h^2 , changes in environmental conditions, and inbreeding.

CHAPTER TWO: GENETIC TOOLS FOR FISHERIES APPLICATIONS

OVERVIEW

Four categories of genetic tools and techniques that are useful in the management of fisheries and fish hatcheries are described. Tools in the first category apply genetic markers to the study of natural fish populations, including topics such as hybridization, stock identification, parentage, and forensics. Tools in the second category are used to understand the structure and function of entire genomes, and those in the third category are used for breeding and management of hatchery broodstocks in stocking programs or captive aquaculture operations. The fourth category is biotechnological tools for creating fish with novel genotypes.

TOOLS TO STUDY NATURAL POPULATIONS

Molecular genetic tools are useful in the management of natural populations. They are applicable to studies at the level of species, populations, and individuals. Since **genetic markers** are often used to resolve genetic variation, the main laboratory techniques for assaying genetic markers, and common applications of genetic markers in the study of natural fish populations are worth understanding.

GENETIC MARKERS

All existing genetic variation could be uncovered by complete DNA sequencing of all individuals in a sample, but this is infeasible. Instead, geneticists use markers that reveal underlying sequence differences. Genetic markers are phenotypic characteristics (e.g., proteins, chromosome bands, sequences of nucleotides) that can be used to infer the genotype of individuals. Genetic markers occur naturally and are passed from one generation to the next without human intervention so that it is not necessary to tag fish. They do not increase the

probability of capture by nets as physical tags might. A variety of biochemical and molecular genetic markers that have been applied to studies of fish are described below along with a discussion of the advantages and limitations of each (see also Liu and Cordes [2004], Hallerman [2003], and Carvalho and Pitcher [1994]).

Proteins (allozymes)

Genetic variation can be detected as **polymorphic** proteins. Differences in DNA sequences can lead to differences in the amino acid composition of proteins that alters their structure and charge. Using gel **electrophoresis** of homogenized tissue extracts, these slightly different forms of a protein can be separated by electrical current and visualized with appropriate histochemical stains (see Electrophoresis, page 39). The proteins used most often in electrophoretic studies are enzymes. Polymorphic enzymes – with the same general function but different chemical structures – that are encoded by one or more loci are called **isozymes**. Isozymes that are products of different alleles at the same locus are called **allozymes**.

Advantages

Until recently, geneticists used protein electrophoresis as their primary tool for assessing genetic variation in fish populations. Large amounts of allozyme data are available for numerous fish species, providing a reservoir of information to which new data can be compared. A large number of loci and fish can be examined in a relatively short time and at moderate expense. Protein electrophoresis has been proven useful for species and population discrimination (stock identification).

Limitations

The genetic variation detected by protein electrophoresis may be inadequate to detect differences among populations and individuals, a problem that limits allozyme studies of some species (e.g., northern pike, yellow perch). Protein electrophoresis can only detect genetic variation in structural genes expressing proteins detectable by histochemical stains. These genes are a small subset of all structural genes, and structural genes themselves make up a small portion of the DNA of higher organisms. Furthermore, because of the redundancy of the DNA code, not all changes in a gene lead to changes in the charge of a protein; thus some genetic variation goes undetected. Sampling requirements are also a drawback of protein studies. To prevent degradation of enzymes, tissue samples must be immediately and continuously frozen, which complicates collection and storage of samples. In addition, sampling is often lethal to the fish because muscle, eye and liver are common sources of tissue.

Mitochondrial DNA (mtDNA)

Because of its unique characteristics, and its frequent use by population geneticists, mitochondrial DNA (mtDNA) warrants a discussion separate from that for nuclear DNA. Mitochondrial DNA occurs as a circular loop of 16,000–20,000 base pairs (bp) in fish. It is considered haploid because each mitochondrion typically contains the same copy of mtDNA (as opposed to the homologous pairs of chromosomes in nuclear DNA). It is almost always inherited directly from mother to offspring without recombination so the mtDNA genome is essentially a clone of the mother's mtDNA. Variation in mtDNA sequence is often detected directly by sequencing or indirectly by cleaving mtDNA into fragments with enzymes that cut at specific sequence recognition sites (see Restriction Enzymes, page 42).

Advantages

Mitochondrial DNA variation is relatively well-characterized for many fish species. Because mtDNA is maternally inherited and does not undergo recombination, genetic drift more rapidly leads to population differences in mtDNA than in nuclear DNA. Its use in combination with nuclear DNA markers can tell us about sex-specific differences in behaviors such as migration. Each cell contains multiple copies of the mtDNA genome so that DNA amplification via the **polymerase chain reaction (PCR)** (see Polymerase Chain Reaction, page 42) of small and degraded samples might be more feasible with mtDNA primers than with some nuclear DNA primers. Universal primers for PCR developed for use among diverse taxa have effectively amplified fish mtDNA.

Limitations

Like other DNA techniques, mtDNA analysis is more expensive than allozymes and requires more specialized equipment. Its maternal inheritance may conceal genetic processes mediated by males. For example, gene flow may be induced by dispersal of males among populations with sedentary females. Because of the lack of recombination, the mtDNA genome is treated as only a single locus, which limits statistical power in some applications. Recently, the assumed near-neutrality of mtDNA variation has been questioned, which may affect the interpretation of mtDNA data in biodiversity and conservation studies (Bazin et al. 2006).

Nuclear DNA (nDNA)

The DNA of the cell nucleus makes up the vast majority of an organism's genome. Nuclear DNA is composed of 0.3–4.0 billion bp in bony fishes (Ohno 1974) dispersed over numerous chromosomes (e.g., 25 pairs in northern pike, 22 pairs in Nile tilapia, 42 pairs in brook trout). One way to characterize

nDNA markers is according to whether they detect genetic variation at single loci or simultaneously at multiple loci.

Single-locus nDNA markers

Data from **single-locus nDNA markers** are readily amenable to genetic analyses and modeling because inheritance of these markers is easily interpretable. A popular type of single-locus marker used in fisheries studies is **microsatellite DNA**. Microsatellites, also known as short tandem repeats (STRs), are segments of nuclear DNA composed of 1-6 base-pair repetitive sequences (e.g., ACACACAC...). The repetitive sequence is prone to replication error, which leads to high rates of mutation and thus high levels of genetic variation due to differing numbers of repeats. These various alleles are detected as size differences in PCR-amplified DNA as determined by gel electrophoresis (see Electrophoresis, page 39). **Single nucleotide polymorphisms (SNPs)** are markers based on single nucleotide changes in DNA sequence (e.g. AACTGC v. AAGTGC). Hundreds of SNPs can be assayed at one time using **DNA chip** technology (see DNA Chips, page 43). As sequence data accumulate for fish species, this new type of marker may come to the forefront for genetic studies.

Multi-locus nDNA markers

Multi-locus nDNA markers are efficient because they simultaneously screen multiple regions of DNA for genetic variation. These markers, however, are less powerful or unsuitable for some genetic analyses because it is not possible to discern all alleles and loci. Multi-locus techniques produce differently sized fragments of DNA that are separated by gel electrophoresis (see Electrophoresis, page 39). Gel 'bands' are scored based on whether a given sized fragment is present or absent when compared across individuals. The degree of band sharing indicates genetic similarity. Specific techniques include **multi-locus DNA fingerprinting, randomly amplified polymorphic DNA (RAPD)**, and **AFLP**. In contrast to single-locus techniques, no prior sequence information is needed to use multi-locus DNA markers.

Advantages

Nuclear DNA techniques can uncover great amounts of genetic variation because the nuclear genome is much larger than that of mtDNA, and as opposed to allozymes, nDNA techniques can assess variation in all genes and in the much more abundant non-coding DNA regions. Because nDNA is composed of independently assorting chromosomes, and because recombination further rearranges the genome, nDNA provides many independent loci for characterization of genetic variation. Multiple independent loci increase statistical power in genetic studies. Samples can be obtained non-lethally, and when using PCR, very small and even degraded tissues (fin clips, archived scales) are suitable for genetic analyses.

Limitations

Nuclear DNA techniques can be expensive and often require special equipment and expertise. Single-locus techniques often require species-specific DNA sequence information, which is lacking for many species. Population genetic databases are also lacking for many species, so each new marker type requires a survey of populations to understand the geographic distribution of genetic variation. Repeatability problems have been encountered while using RAPDs and they have generally fallen out of favor. The reliability of RAPDs must be clearly established on a case-by-case basis before they are used.

LABORATORY TECHNIQUES FOR ASSAYING GENETIC MARKERS

Electrophoresis

Electrophoresis has been the workhorse of molecular genetic techniques for many years. Originally it was used to assay genetic variation in polymorphic proteins (allozymes). Protein electrophoresis involves separation of the proteins from a homogenized tissue sample in a slab-like gel that is subjected to an electrical field. The proteins are separated on the basis of small differences in electrical charge and molecular size. After the proteins have been separated, they can be stained in such a way that allozymes are clearly visible on the gel as bands.

Genotypes of individual fish at protein loci can be easily determined by examining gel banding patterns. Consider a fictitious system of two allozymes, one monomorphic and the other polymorphic (Figure 9A). Four fish have been analyzed electrophoretically. There are no differences among individuals in the banding patterns on the gel for allozyme one because the allozyme is monomorphic. We can infer that all of the individuals in the sample are homozygous at the first locus.

The differences among individuals in the banding patterns for allozyme two are due to genetic differences among individuals. We note three distinct bands in the patterns for allozyme two and infer that there are three alleles (say a , a' , and a'') at the locus in question. Individuals with just one band must be homozygotes because both alleles produce the same band. Individuals with two bands must be heterozygotes because each allele produces a different band. We could expect to see the banding patterns characteristic of all possible genotypes (aa , $a'a'$, $a''a''$, aa' , aa'' , $a'a''$) in sufficiently large samples.

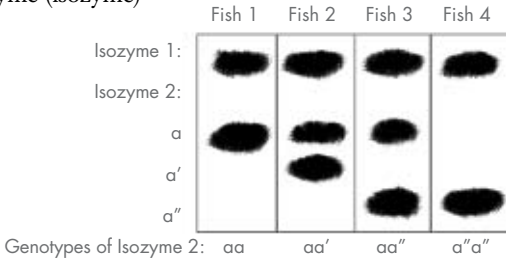
Electrophoresis is also applied to DNA analyses. DNA electrophoresis involves separation of differently sized DNA fragments in a gel matrix. The DNA fragments may be the result of PCR amplification, digestion of larger

fragments by restriction enzymes, or a combination of both. Negatively charged DNA is drawn through a gel made of agarose or acrylamide. The gel acts like a sieve so that smaller fragments pass quickly through the gel and are separated from slower moving larger fragments. The DNA in the gel is visualized by staining with ethidium bromide, which fluoresces under UV light, or by labeling with radionucleotide or fluorescent molecules.

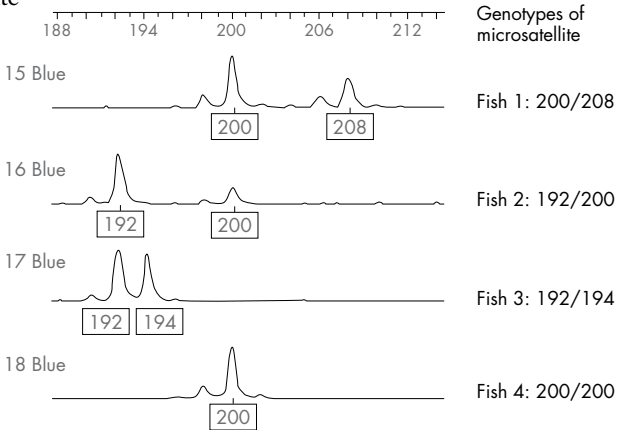
Genotypes of individual fish at single-locus DNA markers also can be easily determined by examining gel patterns. The size of each fragment is determined using a DNA size standard simultaneously run in the gel. Consider a microsatellite DNA marker in the same sample of four fish. To create the image in Figure 9B, PCR-amplified DNA was labeled with fluorescent tags before gel electrophoresis. Advanced DNA analysis equipment scanned the gel during electrophoresis and converted fluorescent signals to the graphical image seen in the figure. The tall peaks correspond to bands in the gel. In this case, each band represents the DNA from one of a pair of chromosomes. Individuals with one band are homozygotes, that is, the PCR fragments from both chromosomes are the same size. Heterozygous individuals have two bands because the DNA sequence on each chromosome has a different number of microsatellite repeat units, leading to differently sized PCR fragments.

Multi-locus DNA markers are interpreted differently. Now consider AFLP analysis of the same four fish (Figure 9C). Each individual will have a few to dozens of bands on the gel. Each band represents one region of DNA that was selectively cut with a restriction enzyme and amplified with a specific PCR primer. Sequence differences can affect whether or not the DNA is cut or amplified, resulting in different banding patterns. The size of each band is determined and individuals are scored for the presence or absence of bands. Copy number generally cannot be determined so homozygotes (+/+) and heterozygotes (+/-) are indistinguishable; they can only be distinguished from individuals without bands (-/-).

A. Allozyme (isozyme)



B. Microsatellite



C. AFLP

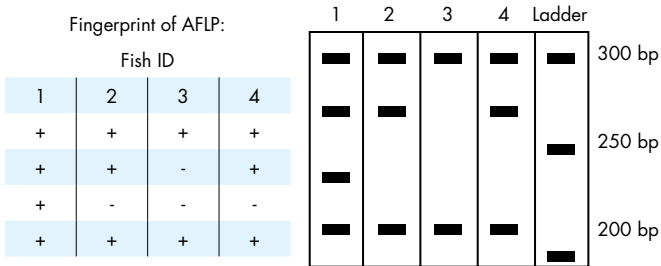


Figure 9. Illustrative examples of gel electrophoresis: (A) two isozyme loci. Isozyme 1 is monomorphic: all four fish are homozygous for one allele. Isozyme 2 is polymorphic; fish 1 and 4 are homozygous for different alleles and fish 2 and 3 are heterozygous. (B) a microsatellite DNA locus. Only fish 4 is homozygous. (C) an AFLP multi-locus DNA fingerprint. Most fish have different AFLP fingerprint patterns.

Restriction enzymes

Certain enzymes (**restriction endonucleases** or restriction enzymes) cleave DNA at single characteristic locations called **recognition sites**, which are enzyme-specific. Although restriction enzymes are commonly used for the cutting processes of recombinant DNA techniques, their recognition sites also can be used as genetic markers because there are differences in recognition sites among individuals and stocks (Awise and Saunders 1984). These differences are detected by two approaches. In one approach, the starting point is purified whole mtDNA or PCR-amplified fragments of mtDNA. One or more restriction enzymes are applied and the DNA is cleaved. The cleaved fragments are separated by gel electrophoresis to detect **restriction fragment length polymorphisms (RFLPs)**. Differences in fragmentation patterns reflect genetic differences among samples. For example, if whole mtDNA – a 16,000 bp circular molecule – is cut at three recognition sites, it might result in three fragments of 10,000, 4,000 and 2,000 bp. If a mutation at one site prevents recognition and cleavage, only two fragments of 10,000 and 6,000 bp might result. In the second approach to detecting RFLPs, the starting point is purified genomic DNA. The DNA is again cleaved with enzymes and separated by size using gel electrophoresis. If this DNA was visualized directly, there would be so many fragments of all sizes that they would appear as one continuous streak on the gel. Instead, a DNA probe is used to highlight a subset of fragments, which appear somewhat like the bar codes used on merchandise. Different banding patterns represent sequence differences at restriction sites. This was the original approach known as “DNA fingerprinting.”

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) has revolutionized molecular genetic studies. In PCR, a specific DNA segment (either mtDNA or nDNA) is amplified until millions of copies are obtained. This large amount of DNA can then be visualized or further manipulated for many purposes ranging from analyzing genetic variation to cloning. This amplification of DNA allows genetic analyses from non-lethally collected, very small and even degraded tissues (e.g., fin clips, archived scales, and archaeological collections). The PCR relies on short synthetic pieces of DNA, called primers, which bind specific sequences and provide a starting point or ‘prime’ of the amplification of a target fragment. The primers are added to a cocktail including the nucleotide components of DNA and the polymerase enzyme that drives DNA replication. Through successive cycles of heating and cooling, double-stranded DNA is separated and replicated, leading to an exponential increase in copy number (Figure 10). Primers may need to be developed for each species or closely related species because they are sequence-specific and the homologous sequence in the organism’s genome may differ across species.

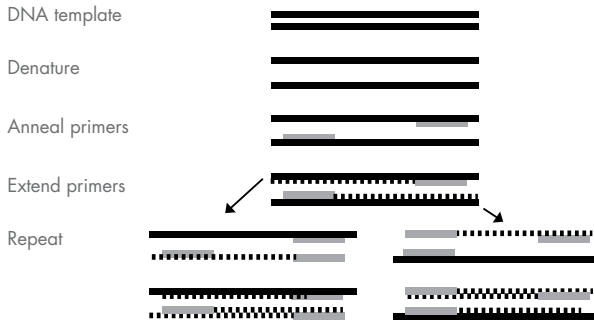


Figure 10. Diagrammatic representation of the polymerase chain reaction. (PCR) (modified from Park and Moran 1994). The DNA template is heat-denatured into its two strands followed by cooling, which allows a pair of synthetic primers to bind (anneal) to the complementary strands. The strands are extended by a DNA polymerase enzyme, filling in the complementary strands of the template. The copied DNA strands become templates for the next replication. The process is repeated 25-45 times resulting in exponential replication of the target sequence flanked by the primer pair.

DNA chips (microarrays)

One of the most exciting advances in molecular biology is DNA chip technology, or **microarrays**. Microarrays can be used to detect genetic variation in the genome and to detect gene expression. To detect variation, arrays of short DNA segments are spotted on the surface of a chip. These DNA segments have the alternative sequences complementary to single nucleotide polymorphisms (SNPs). Sample DNA is hybridized to the chip and will bind only to its exact complement, indicating its sequence. Microarray experiments (in humans) have contained thousands of SNPs, so the data generated from a single array can be extensive. Fish microarrays are not nearly as advanced but will become increasingly feasible as more fish genomes are sequenced.

APPLICATIONS OF GENETIC MARKERS

Genetic markers have been applied to natural populations to help us better understand genetic and evolutionary processes and to provide an alternative to physical tags as tracking tools. This section describes the common applications of genetic tools to the management of fish populations, but is not exhaustive.

Hybridization

Genetic markers can identify hybrids when physical characteristics are insufficient. Hybridization may affect the performance of fish (e.g., first generation black and white crappie hybrids grow faster than parental species, Buck and Hooe 1986) or may threaten the existence of species (e.g., cutthroat trout from the east slope of the Rocky Mountains are threatened by hybridization with introduced rainbow trout, Allendorf et al. 2001). Many genetic markers can help differentiate species. For example, researchers have identified 12 isozyme loci (Dunham et al. 1994) and mtDNA markers (Travnichek et al. 1997) that distinguish black and white crappies. We recently developed four microsatellite DNA markers for crappies using PCR allowing non-lethal and archived samples to be used for hybrid studies of these species (L. Miller, unpublished data).

Box 1. Interpreting hybrid data at the population level can be complicated. Consider two lakes with hybrids between species A and B. The frequency of alleles specific to species A at five markers averages 0.30 in both lakes. In Lake 1, one of every five individuals is homozygous for allele A at all five loci and one of every five is heterozygous at all loci. The remaining three of five individuals are homozygous for allele specific to species B. In Lake 2, individuals have 0.5 heterozygous genotypes at the five loci. The biological interpretation of the hybrid data differs for each lake. In Lake 1, two species exist and only F₁ hybrids are produced. They are probably sterile; thus no advanced-generation hybrids are detected. Lake 2 has a hybrid swarm in which many descendants of hybrids exist and distinct species have been lost.

Typical genotypes at five species-specific genetic markers

	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5
Lake 1: sterile F ₁ hybrids					
Fish 1	AA	AA	AA	AA	AA
Fish 2	AB	AB	AB	AB	AB
Fish 3	BB	BB	BB	BB	BB
Fish 4	BB	BB	BB	BB	BB
Fish 5	BB	BB	BB	BB	BB
Lake 2: hybrid swarm					
Fish 1	BB	BB	BB	BB	BB
Fish 2	AB	AB	AB	AB	AB
Fish 3	AA	AB	BB	BB	BB
Fish 4	AB	AB	BB	AA	BB
Fish 5	AB	BB	AB	AB	BB

Limitations

Using species-specific markers, it is easy to detect hybrids but more difficult to determine their ancestry. A first generation hybrid (F1) will be heterozygous at all loci because it receives one chromosome from each parental species. Advanced generation hybrids (F_x, crosses between hybrids or backcrosses with parental species) can be distinguished from F1s because, due to recombination, some loci will be heterozygous while others are homozygous. Many loci are needed to distinguish among various F_x generations and to avoid misclassifying F_x individuals as F1s or parental species (Epifanio and Philipp 1997). Furthermore, interpretation of hybrid data at the population level is not always straightforward (see Box 1).

Stock identification

One of the first and most important applications of genetic markers in fisheries was the identification of distinct populations, or stock identification. Genetic markers may allow managers to determine if samples of fish are from different populations and to determine the relative contribution of stocks to a mixed stock fishery. Allozymes and mtDNA have often proven sufficient to identify stock structure in many species over varying geographic scales. Nuclear DNA markers are often effective for species with low allozyme or mtDNA variation (e.g., northern pike, Senanan and Kapuscinski 2000) and for fine geographic scales (e.g., brook trout in Quebec, Angers et al. 1995; yellow perch in Lake Michigan, Miller 2003). Typically, samples of fish from two or more alleged stocks are obtained. The sample frequencies of alleles at several markers are determined and the frequencies for different samples are compared statistically, for example, by a chi-square test. If there are significant differences in marker frequencies between samples, then there is evidence that the samples were drawn from different populations.

Many researchers assess genetic population structure using Wright's F_{ST}, or the fixation index. The fixation index estimates the proportion of genetic variation attributable to differences among populations. Its range is 0-1.0, with higher values indicating greater genetic differences. A value significantly greater than zero indicates genetic structure, i.e., that one or more of the samples in the comparison were drawn from genetically differentiated populations.

Another common statistical approach is to compute the **genetic distance** between all pairs of samples (Wright 1978). A **cluster analysis** (Sneath and Sokal 1973) of the genetic distance measurements is then used to group the samples that are similar and to separate the samples that are dissimilar. Samples assigned to the same group may belong to the same or similar populations. The results of such an analysis can be summarized in a figure called a **dendrogram** (Figure 11).

If different populations contribute to a mixed-stock fishery, samples from the fishery can be partitioned according to their source by comparing sample genotypes to baseline genotypes of potentially contributing source populations (e.g., Shaklee et al. 1999). As variations on this theme, genetic markers can be used to identify distinct populations for collection of broodstock (e.g., Pacific salmon from different runs within a stream, Olsen et al. 2000) and for distinguishing stocked versus naturally produced fish.

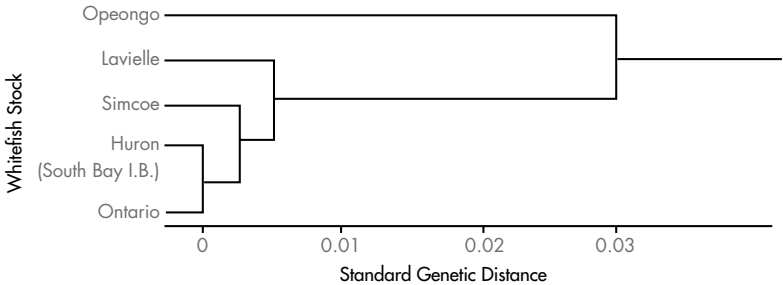


Figure 11. A dendrogram of the standard genetic distances for five southern Ontario lake whitefish stocks, produced by cluster analysis of electrophoretic data. The Huron and Ontario stocks are most closely related. The Opeongo stock is the most genetically divergent stock of the group. (From Ihssen et al. 1981)

Limitations

None of the genetic tools allow managers to determine unequivocally if samples of fish are from the same population. Genetic techniques for population identification rely on the existence of observable genetic differences between populations. In the absence of observable genetic differences these techniques provide no information about stock structure. There may be an absence of observable differences because only a few genetic markers were examined or the type of marker showed few polymorphisms.

In addition, lack of detectable genetic structure does not preclude biologically meaningful stock structure (Waples 1998). Only a few migrants per generation may be necessary to eliminate most evidence of genetic structure yet these few migrants may not, for example, be sufficient to quickly rebuild a depleted fishery in a region. Information obtained by other means, such as tagging experiments and analysis of growth and reproductive characteristics may suggest the presence of stock isolation at a level important to the fisheries manager.

Conversely, Allendorf and Phelps (1981) stress that statistically significant differences in the frequencies of genetic markers can exist among stocks even

when there is considerable migration between the stocks. Temporal replication of samples helps confirm the stability of observed population structure, which strengthens the case for limited migration between populations (Waples 1998).

Results of stock identification studies can be misleading if samples are not representative of the populations from which they were taken. Consider a sampling program that involved capture of fry emerging from spawning grounds. Very large samples might be obtained that consisted of the progeny of just one or a few matings. Statistically significant differences in marker frequencies among the samples would then be due to differences among a few families, not due to differences among populations (Allendorf and Phelps 1981).

Parentage

Recent development of many new, highly variable DNA markers has increased the use of genetic markers as tracking tools. An application in fisheries has been determining parentage for fish stocked into or produced in the wild. Parentage assignment can be used to track stocked fish when physical tagging is infeasible (e.g., fry stocking, Eldridge et al. 2002) and importantly, it allows for comparisons of individual reproductive success in the natural environment (e.g., Garant et al. 2001). Parentage analysis (and the related paternity or maternity analysis) can be straightforward. An offspring must get one of its alleles from its mother and one from its father. If the genotypes of all potential parents are known, an offspring's genotype is simply compared to parental genotypes to see if it is compatible. Of course an offspring may be compatible with many potential parents at any one locus. When several highly variable loci are used it is possible to uniquely assign individuals to their parent pair.

Limitations

The ability to uniquely assign parentage depends on characteristics of the genetic markers, the size of the population, and the sample of adults. Loci with large numbers of alleles and high heterozygosity are the most effective. The fewer the adults, the easier it is to assign offspring, and knowing the sex of adults or information about potential mates (e.g., known hatchery crosses) reduces the number of possible parent pairs. Finally, sampling a greater proportion of the adults provides greater assignment accuracy, but techniques exist to determine the likelihood that unsampled adults could have contributed offspring to a sample (Marshall et al. 1998).

Forensics

Genetic tools can assist in the identification of fish parts and products. Species-specific markers can be developed for the species of concern. The markers are applied to the tissue of interest and a match is determined. As examples,

allozymes were used to identify illegally possessed catfish, red drum and spotted seatrout fillets (Harvey 1990), and mtDNA markers have been developed to identify the species of commercial caviar (Birstein et al. 1999). The Barcode of Life project entails sequencing a common DNA region in all organisms to create a database of genetic information that will be a useful reference for species-level forensics. A subgroup is working on fish species (www.fishbol.org). Other forensic applications are possible. In many species there is enough variation among populations so that the geographic source of a sample could be determined. We are using a set of eight microsatellite DNA markers to verify the wild source population of walleye being reared in pond aquaculture (L. Miller, unpublished data). In another use, geneticists detected fraud during a fishing competition, again using microsatellite DNA data (Primmer et al. 2000). Using statistical approaches, they confidently excluded the possibility that a 5.5 kg Atlantic salmon originated from the contest location in Finland. The accused later confessed to purchasing the salmon at a fish shop.

GENETIC MARKERS, SELECTIVE NEUTRALITY, AND ADAPTIVE VARIATION

Genetic markers are not generally useful for determining if a particular population is well adapted to a particular environment. In fact, for many applications it is assumed that genetic markers are **selectively neutral**, that is, selection does not favor one individual over another with variant alleles at a marker. This allows geneticists to analyze data as a balance between mutation, drift, and migration without the complicating effects of selection. This is important for many applications like those described in the previous section. For example, consider a stock identification study of two populations in complete isolation. Drift might fix alternative alleles of a neutral locus in each population and we would identify the populations as distinct stocks. In contrast, selective forces may fix the same allele in each population if the allele conferred greater fitness in each environment. We would not detect stock isolation at such a locus.

Although selectively neutral genetic markers have many applications in fisheries management, effective fisheries conservation will require additional emphasis on the adaptive significance of genetic variation. Assessment of quantitative genetic variation in important life history traits will inform us about adaptive differences among and evolutionary potential within populations (Hard 1995, Reed and Frankham 2001). Recent advances in genetic technologies bring us closer to linking quantitative genetic variation with its underlying molecular causes.

TOOLS FOR GENOMICS

Genomics goes beyond the study of a few genes to the study of structure and function of entire genomes. Tools for genomics are at the cutting edge of genetic studies for all types of organisms and are just starting to find applications in fisheries research; limitations, advantages, and applications are not yet clear. Genomics tools will be used to improve aquaculture production and to understand adaptation and evolution in natural populations. The best information on this rapidly advancing area of genetics often can be found on the Web (e.g., www.ncbi.nlm.nih.gov; www.animalgenome.org/aquaculture). Reviews relevant to fisheries include Melamed et al. (2002), Cossins and Crawford (2005), and Wilson (2005).

GENETIC MAPPING

Genetic mapping is the ordering of genetic markers according to their positions on chromosomes. To create a genetic map (also known as a linkage map), pedigreed families are genotyped at numerous polymorphic markers. Marker alleles on different chromosomes will be inherited independently by the offspring. Marker alleles on the same chromosome will be inherited together unless a recombination event separates them. The closer two markers are on a chromosome, the more likely they will be inherited together. Markers inherited together are said to be linked. The order of markers along a chromosome is determined by the strength of the linkage among them. When enough markers are genotyped, a cluster of linked markers will result for each of the species' chromosomes. Genetic maps are the guides for hunting down genes. They are used to show how inheritance of chromosomal regions associates with inheritance of traits. Genetic maps are often combined with physical maps of chromosomes to more narrowly define gene positions. Genetic maps are being developed for a number of fish species including rainbow trout (Young et al. 1998), tilapia (Agresti et al. 2000), and catfish (Liu et al. 1999).

QUANTITATIVE TRAIT LOCI (QTLs)

Genetic maps provide the basis for identifying the multiple genes underlying quantitative traits; traits important for fish production and fitness (e.g., growth, cold tolerance, flesh quality). Researchers have identified QTLs for such traits as rapid embryonic development in rainbow trout (Sundin et al. 2005), temperature tolerance in carp (Sun and Liang 2004) and rainbow trout (Jackson et al. 1998), and disease resistance in Atlantic salmon (Moen et al. 2004). To detect **quantitative trait loci (QTLs)**, pedigreed families are genotyped at numerous markers spaced along all of their chromosomes. The

co-inheritance of markers and trait values is examined in offspring generations. The analysis determines how much of the variation in a trait can be explained by inheritance of alternative alleles at polymorphic markers. The markers themselves are usually not thought to be part of the underlying gene, but rather linked to a nearby polymorphic gene on the same chromosome. Once a chromosomal region has been located for a QTL, other techniques are necessary to refine its position and actually identify the gene responsible for the trait variation. Alternatively, breeders can select broodstock with QTL marker alleles for superior traits, knowing that the linked gene will usually be co-inherited with the marker (see [Marker-Assisted Selection](#), page 63).

GENOME SEQUENCING

The ultimate genetic map is the complete sequence of an organism's genome, giving the order of A's, G's, C's, and T's along each chromosome. Complete genome sequences have been obtained for humans and a number of other agricultural or model organisms. Work on fish genome sequencing is proceeding, primarily with model species used for comparative genomic and organismal development research (e.g., zebrafish, www.sanger.ac.uk/Projects/D_rerio). Complete genome sequences will assist in identifying all genes in an organism and in making the link between QTL identification and genetic mapping.

MICROARRAY EXPRESSION ANALYSIS

With increasing numbers of genes being discovered, the next step is to learn more about how they function in fish cells and when they are expressed. To detect gene expression, DNA segments from genes are spotted onto DNA chips. Sample mRNA from expressed genes is isolated from tissues. The mRNA (actually cDNA derived from it) is hybridized to the chip and will bind to its corresponding genes. The presence or absence of a signal from the gene spots will indicate whether the gene was being actively expressed in the tissue.

[Microarray expression analysis](#) is still in its infancy but many applications can be foreseen. For example, diseased and healthy fish can be compared to determine which genes are playing a role in the disease condition. Fish from different regions can be compared after exposure to a common environmental condition (e.g. temperature) to see which genes or environmental factors contribute to local adaptation.

Roberge et al. (2006) used microarrays to compare gene expression patterns of 3557 genes in farmed and wild Atlantic salmon. They examined farmed salmon from Norway and Canada. The farmed salmon were derived from the

wild stocks and selectively bred for characteristics like increased growth for 5-7 generations. Changes in gene expression reflected the effects of selective breeding and inadvertent selection in the aquaculture environment that contributed to domestication of the stocks. Similar changes in gene expression were found in both farmed stocks for several genes, providing insight into the molecular basis for parallel evolution (i.e., similar responses to selection) in the independent broodstock programs.

TOOLS FOR BREEDING IN CAPTIVE AQUACULTURE

Tools discussed in this section are the classic breeding techniques for improvement of quantitative traits in animals. These tools are used in intentional selective breeding in captive aquaculture. Many of the tools (e.g., **mass selection**, **family selection**, **multiple trait selection**) make use of additive genetic variation for improvement of fish strains. Others (e.g., hybridization) make use of nonadditive genetic variation. Several techniques (e.g., **rotational line crossing**, **outcrossing**) can be used to lessen inbreeding in broodstocks. Some techniques (e.g., **indirect selection**, **sib selection**, **progeny selection**) can be used when the phenotype is difficult to measure or, as in the case of dressed weight, cannot be measured without killing the fish. Finally, molecular genetic tools can enhance traditional breeding techniques in a process known as marker-assisted selection. The tools described in this section can be applied to the improvement of one phenotypic trait or to the simultaneous improvement of several traits (multiple trait selection). Generally, fisheries managers avoid intentional selective breeding in hatchery stocking programs due to the potential loss of fitness when fish are released into the wild (see Chapter 3).

ESTIMATION OF HERITABILITY (h^2)

Artificial selection programs that make use of additive genetic variance require heritability estimates for the traits involved. Heritability estimates allow breeders to estimate the expected response to a selection program as well as time and cost required to reach the desired goals.

One of the easiest methods for estimating the heritability of a trait is to compare the mean phenotypic value of **full-sibs** (individuals that have the same two parents) to the mean phenotypic value of their parents (**mid-parent mean**) in a regression analysis. In a randomly mating population, the slope of the line obtained from a regression of full-sib means on mid-parent means is exactly h^2 , the heritability (Figure 12).

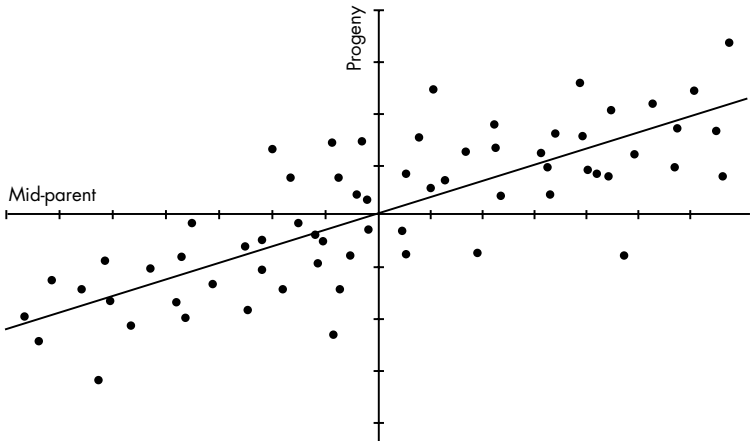


Figure 12. Estimation of heritability by regression of the phenotypic value of the progeny on the mean phenotypic value of the parents (mid-parent mean). In this fictitious example, the heritability (h^2) of six-month-weight in a hatchery population of lake trout is estimated by the slope (b) of the regression line; $b = h^2 = 0.33$. (After Falconer and MacKay 1996)

Heritabilities for many commercially-important and fitness-related traits in fish have been calculated and are reviewed by Gjedrem (1983) and Kinghorn (1983) (Table 4). Generally, heritabilities are lower in fish than in domestic animals and poultry.

Table 4. Average heritability estimates for five traits in Atlantic salmon from multiple studies reviewed in Gjedrem (1983).

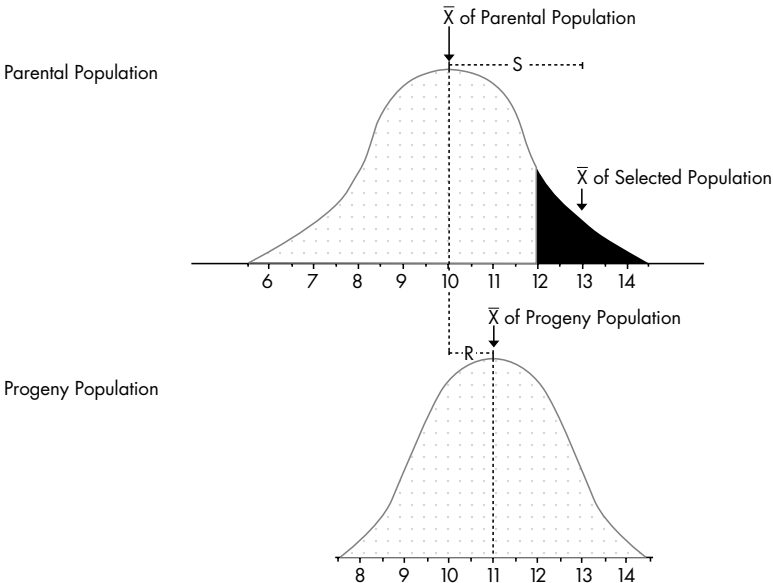
Trait	Heritability
Adult body weight	0.36
Adult body length	0.41
Mortality	0.11
Meatiness	0.16
Age at maturation	0.71

MASS SELECTION

Mass selection (also called **individual selection**) is the simplest form of artificial selection. The best individuals are selected from a population for breeding and the remaining individuals are discarded. The progeny of the selected individuals are usually better, on average, than the original population. Mass selection can be repeated in each new generation until the desired change in the mean phenotypic value of the population is obtained.

Predicting the response to mass selection

Improvement due to mass selection is predictable (Figure 13) using the familiar formula $R = h^2 S$ introduced in Chapter 1 (see Selective Breeding page 34). If one generation requires x years then the expected response to breeding is R / x units per year. Note that the sign and direction of R is the same as the sign and direction of S . Note also that the response to selection is less than the selection differential because h^2 is essentially always less than one. The **intensity of selection** is a standardized measure of the selection differential, computed by dividing the selection differential by the standard deviation from the original population. In general, the number of individuals that can be used for breeding decreases as the selection differential increases or the size of the population decreases.



Selection Differential = $S = 13 - 10 = 3$ grams

Heritability = $h^2 = 0.33$

Response = $S \times h^2 = 3 \times 0.33 = 1$ gram

Figure 13. An example of response to mass selection. A population of lake trout had a mean weight of 10 grams at six months of age. The adults selected for breeding (the dark section of the parental population) had a mean weight of 13 grams at six months of age. The selection differential $S = 13 - 10 = 3$ grams. The heritability for weight at six months of age is 0.33, so the predicted response to one generation of mass selection is $R = S h^2 = 3 \times 0.33 = 1$ gram.

Pitfalls in the prediction of response

It is important to remember that the expected response to a unit of selection differential (S) will change if the heritability changes. Heritability ($= V_A / V_P$) will change if the environment (which contributes to the total phenotypic variance V_P) or the amount of additive genetic variation (V_A) in the population changes. Estimates of the heritability for a trait are usually obtained under experimental conditions that are unlike the environmental conditions in a production-scale hatchery or rearing facility. Consequently, estimates of heritability obtained under experimentally controlled conditions may not apply in the environment of a hatchery or rearing facility. If the heritability estimate is inappropriate, then calculation of the expected response to selection will be misleading. Changes in the environment of the production facility (e.g., water temperature, feeding) will also affect heritability and the response to selection.

A final note of caution is that heritabilities are population specific. The amount of additive variance, phenotypic variance, and the heritability for a trait varies from population to population. The predicted response to selection for a population may be unreliable if the heritability estimate was obtained from a different population.

Advantages

Mass selection is simple and works well when the heritability is high (> 0.3) and the population is large so that large selection differentials can be employed.

Limitations

Mass selection is ineffective when the heritability is low (< 0.3) or the population size and selection differential are small. Large selection differentials cannot be used with small populations because few individuals would be used as parents and inbreeding would result. Under circumstances of low heritability and small populations, other breeding schemes (e.g., family selection) perform better.

FAMILY SELECTION

Family selection is used when the heritability for mass selection is low. Family selection involves choosing entire families, usually groups of full-sibs or **half-sibs** (half-sibs are individuals that share one parent), rather than individuals for breeding. Families are selected on the basis of their mean phenotypic value. Phenotypic values of individuals are ignored except in calculation of the means for families.

The heritability for family selection is often larger than the heritability for

mass selection because most of the variance among family means is genetic variance. Environmental variance among family means is reduced by raising the families in similar environments and by averaging over a large number of individuals in calculation of the family mean.

The predicted response to one generation of family selection is given by a familiar looking formula:

$$R = S' h^2'$$

S' = difference between the mean of the selected families and the mean of the population

h^2' = heritability for family selection

The number of families that a breeder can maintain is usually small so that the families used for breeding must be selected from a relatively small group. Consequently, selection differentials used in family selection programs are usually smaller than those used in mass selection programs. The increased heritability is usually sufficient to offset the reduction in selection differential.

Advantages

Family selection can produce an acceptable response to selection when the heritability for individual selection is low.

Limitations

Increased complexity and the resources required to rear a large number of families are the principal limitations of family selection. Detailed records must be kept and families of fish must be maintained separately. Rearing families separately could be circumvented by using genetic markers to identify families but the costs may be prohibitive. Inbreeding may be an obstacle if the effective size of individual families is small.

PROGENY AND SIB SELECTION

Progeny and sib selection are variations of family selection. Progeny selection involves selection of individuals for breeding on the basis of the mean phenotypic value of their progeny. The “families” in the case of progeny selection are groups of progeny (either full- or half-sibs). The principal advantage of progeny selection is that relatively small families can be used to determine which families should be used for broodstock. After the families are selected, the size of the best families can be increased by allowing the original parents to continue breeding. The principle disadvantage of progeny selection is lack

of speed. The response to selection per unit of time is low because of the time required to breed and evaluate families.

Sib selection is useful when the phenotypic value of an individual (e.g., carcass weight) cannot be determined without destroying the fish. Sib selection involves the selection of broodstock on the basis of the mean phenotypic value of full- or half-sibs. The difference between family selection and sib selection is that the phenotypic value of individuals selected as parents in sib selection are not included in calculations of the family mean.

WITHIN FAMILY SELECTION

Within family selection is useful when phenotypic differences among families are due primarily to environmental factors, rather than genetic differences among families. The mean phenotypic values of families are ignored for within family selection. Instead, the best individuals from each family are selected and used as broodstock.

Advantages

Within family selection allows a breeder to make use of information about families when the variation among families is due largely to environmental factors. For example, consider families of fish in a hatchery raised at different water temperatures. The average size of families in the coldest water would be smaller than the average size for those in warm water. Most of the variation in average size among families would be due to environmental differences (i.e., water temperature) rather than genetic differences. Another advantage is that inbreeding is minimized. Consider an example involving a number of families and within family selection. In every generation, two breeders are obtained from each family so that there is very little chance that any family will fail to contribute progeny to the next generation. The rate of inbreeding is decreased because the probability of losing any particular family is reduced. Within family selection effectively doubles the effective size of the population (Falconer and MacKay 1996). Consequently, only half as much space is required to maintain a population with a given effective population size under a program of within family selection.

Limitations

A disadvantage is that heritabilities for within family selection are usually lower than for mass selection. It is possible to combine family selection and within family selection in a program of **combined selection** in which only the best individuals from the best families are bred. The advantage of combined selection is increased response because the additive variance among individuals as well as within families is exploited.

SELECTION INVOLVING MULTIPLE TRAITS: INDEX SELECTION AND SELECTION FOR MERIT

The **breeding value** of an individual in an artificial selection program is determined from the mean phenotypic value of its progeny. The simplest estimate of breeding value is based on a single trait. Additional information about the breeding value of an individual can be obtained from traits that are **genetically correlated** with the trait under selection as well as from relatives. Two traits, say X and Y, are genetically correlated if the breeding value of an individual for trait X is correlated with the breeding value of the individual for trait Y. Genetic correlation results, in part, from pleiotropy (single genes that affect more than one trait). Information from genetically correlated traits as well as from relatives can be combined to form an index that is used to decide whether or not an individual should be kept for breeding.

Index selection

An index is the weighted sum of all the available information about breeding value:

$$I(i) = b_1 P_1(i) + b_2 P_2(i) + b_3 P_3(i) + \dots$$

$I(i)$ = index for the value of individual i

$P_j(i)$ = the phenotypic value for the j^{th} correlated character or relative

b_j = a weighting factor that reflects the importance of the j^{th} character in the index

The weighting factors are estimated in such a way that the correlation between the index for individuals and their breeding values is maximized (Falconer and MacKay 1996).

Selection for merit

Breeders often want to improve several traits simultaneously. The relative importance of each trait can be used to construct an index for merit that takes each of the important traits into account. The merit of an individual can be used to determine its value in a breeding program.

For example, if three traits affect the commercial value of a fish, then a reasonable expression for merit would be:

$$M(i) = a_1 C_1(i) + a_2 C_2(i) + a_3 C_3(i)$$

$C_j(i)$ = breeding value of individual i for the j^{th} trait

a_j = weights reflecting the relative economic gain expected from one unit of improvement in trait j

Index selection and selection for merit can be combined:

$$a_1 C_1(i) + a_2 C_2(i) + a_3 C_3(i) + \dots = b_1 P_1(i) + b_2 P_2(i) + b_3 P_3(i) + \dots$$

The weights b_j are chosen so that the correlation between the index on the right side of the equation and breeding value for merit on the left side of the equation is maximized.

Advantages

Index selection uses all available and relevant information. The standard deviation of index values and selection intensity can be used to predict the response to selection.

Selection for merit can be used to improve a number of characteristics simultaneously. The effort devoted to improving any single trait is proportional to its relative importance. Merit can be expressed in any units that are convenient and meaningful to the breeder. For example, merit can be expressed in dollars if all of the characteristics considered affect economic value.

Limitations

The determination of the traits to include in an index and the estimation of coefficients for the index are complex tasks, a large amount of information is required. The coefficients for an index can change as selection proceeds, gene frequencies are altered, and the genetic correlations between characters change. Coefficients for merit can change as the breeding goals or market conditions change.

Independent culling

Independent culling involves choosing individuals for breeding on the basis of a set of independent cutoff values; a different cutoff value is used for each trait under selection. For example, consider a breeding program designed to improve three phenotypic traits in a fish stock: age at maturity, body weight at maturity, and fat content of flesh. The breeder might decide to discard fish that mature at ages greater than one year, weigh less than 0.5 kg at maturity, and have less than 5% fat content. A fish that failed to meet any one of these criteria would be discarded, even though that fish might exceed the cutoff values for the other two traits. A disadvantage of independent culling is that all of the traits are assumed to have equal importance. Independent culling and **tandem selection** are simpler than index selection but not as efficient.

Tandem selection

Tandem selection involves selection for one trait in the first generation, a sec-

ond trait in the second generation, a third trait in the third generation and so on. A breeding program that requires five generations of selection for each of three traits would take at least $5 \times 3 = 15$ generations to complete. If the generation time is two years then a total of $5 \times 3 \times 2 = 30$ years will be required. The principal disadvantage of tandem selection is a slow rate of progress.

Indirect selection

Indirect selection is a simple form of index selection based on a pair of genetically correlated traits. Individuals are selected for breeding on the basis of their phenotypic value for one trait, called a **secondary character**, in order to affect improvement in another, genetically correlated trait. Indirect selection is efficient when the heritability of the secondary character and the genetic correlation between the secondary and primary characters are high.

Indirect selection is useful when the heritability of the primary character is low, the primary character is difficult or expensive to measure, or is expressed in only one sex. A breeder may, for example, wish to increase fecundity in a population of fish. Fecundity cannot be determined for males and can only be determined in mature, relatively old females. If the breeder knows that another character, say growth rate, is genetically correlated with fecundity, then males as well as females can be selected for breeding and the selection process can occur while the fish are relatively young.

INTENTIONAL INBREEDING

The purpose of inbreeding is to increase homozygosity and decrease heterozygosity. Individuals in completely inbred strains are homozygous and genetically identical. Inbred lines are genetically stable; allelic frequencies remain the same from one generation to the next even when effective population size is small because virtually all of the genetic variation has been eliminated. Breeders use inbreeding primarily to generate lines for use in the production of hybrids). Inbred lines (e.g., white mice) are used in scientific work when genetic uniformity is important.

Advantages

Intentional inbreeding is a traditional and well understood method for producing lines with reduced heterozygosity. Inbreeding combined with artificial selection can be used to minimize inbreeding depression.

Limitations

Inbreeding programs require careful design and control, and many years if the generation time is long. It may be difficult or impossible to completely avoid

inbreeding depression. Inbred lines are often more susceptible to environmental variation than are non-inbred lines.

HYBRIDIZATION

Hybrids are the progeny of parents from different lines, strains (intraspecific hybrids), or species (interspecific hybrids). The mean phenotypic value of hybrids is often greater than the value of either parental line. Breeders cross lines in order to produce superior hybrid individuals. Superiority of hybrids is called hybrid vigor or heterosis and is due to heterozygosity at many loci. Interspecific hybrids are often sterile and may not display hybrid vigor (Blanc and Chevassus 1979).

The primary genetic characteristics of hybrid lines are high levels of heterozygosity and genetic uniformity. When two completely inbred (homozygous) lines, different at every locus are crossed, the progeny are completely heterozygous and genetically identical. Phenotypic uniformity, due to underlying genetic uniformity, is an important characteristic of some hybrids.

The characteristics of hybrids depend on the parental lines. The best parental lines differ widely in genetic composition, are vigorous themselves, and yield superior hybrids when crossed. Great effort is often devoted to breeding parental lines used to produce hybrids.

Combining ability

The **combining ability** of a line is a measure of the quality of the hybrids that can be expected when the line is used in a cross. **General combining ability** is the mean value of the progeny obtained when the line is crossed to a large number of other lines. **Specific combining ability** is a measure of the value of the hybrids obtained when two specific lines are crossed. General combining ability is an estimate of the total genetic variation (VG) present in the line for the given trait; specific combining ability estimates the nonadditive component of the genetic variation (Falconer and MacKay 1996).

Reciprocal crosses

A **reciprocal cross** involves mating the females of line A with the males from line B and mating the males of line A with the females from line B (the former cross is the reciprocal of the latter). The phenotypic value of the hybrids will often depend on which reciprocal cross was employed. Differences between the hybrids obtained from reciprocal crosses depend on the parental line that was the source of the females because of maternal effects. **Maternal effects** on progeny depend on the mother, not on the genotype of the progeny.

Egg size and quality are examples of important maternal effects in many fish species. Maternal effects may be due to environmental causes (e.g., differences in nutrition) or to genetic causes (e.g., genes for large eggs).

The hybrids produced by females of one line might be better, on average, than the hybrids obtained from the reciprocal cross. Therefore, it is possible to consider the combining ability (general or specific) of males and females separately. If the combining abilities of males and females differ, a breeder may decide to cross females of one strain with males of another but not vice-versa.

Advantages

Advantages of hybridization include increased vigor and phenotypic uniformity in the crossbred progeny. It is not necessary to maintain hybrid strains because they can be produced at any time as long as the parent strains exist.

Limitations

Hybridization is an optimum breeding program only when hybrids show heterosis. Heterosis depends on nonadditive genetic variation. Consider a breeder who wants to improve the commercial value of a fish stock and can maintain several lines derived from the base population. The breeder could select for combining ability in the lines and market hybrids (a hybrid breeding program) or select for phenotypic value within lines and market surplus stock (a line breeding program). If nonadditive genetic variance in the original population was negligible, then the hybrid breeding program would give poorer results than the line breeding program. Conversely, if there was a great deal of nonadditive genetic variance and little additive genetic variance in the base population, then the hybrid breeding program would perform better than the line breeding program.

Hybrid lines cannot be maintained by allowing hybrids to reproduce; they must be produced from parental lines in every generation. Consider a population of identical hybrids with genotype Aa at one locus. If the hybrids were allowed to reproduce, then the progeny would have genotypes AA , Aa , and aa in the proportions 0.25, 0.5, and 0.25, respectively. The genetic uniformity and extreme heterozygosity of hybrids is lost after a single generation of random mating.

OUTCROSSING

Outcrossing involves breeding individuals from one strain to superior individuals from unrelated lines or strains to bring new genes into a selection program. Outcrossing differs from hybridization in that progeny of crosses are used in the ongoing selective breeding program.

Advantages

This technique utilizes the additive genetic variation in both strains and any heterosis resulting from their combination. Additionally, new genes from the unrelated lines become available to the selection program and inbreeding is reduced.

Limitations

Outbreeding programs are necessarily larger in scope than simpler selection programs. Individuals from unrelated but suitable strains may not be available. It may be difficult to evaluate the suitability of unrelated strains of fish.

ROTATIONAL LINE CROSSING

Rotational line crossing involves sequential crosses among three or more separate lines (Figure 14). In the first generation females from line A are mated to males from line C, females of line B are mated to males of line A, and females of line C are mated to males of line B. This mating scheme is repeated in each succeeding generation. Specific recommendations for the implementation of rotational lines crosses in hatcheries are given by Kincaid (1977) and Hynes et al. (1981).

Advantages

Rotational line crossing reduces the rate at which inbreeding accumulates within the strains. This advantage, however, depends on the presence of a high level of genetic diversity in the starting broodstock and on the maintenance of genetic diversity in subsequent generations. Kincaid (1977) suggests starting each line from different strains and using at least 50 fish of each sex to advance the generation for each line.

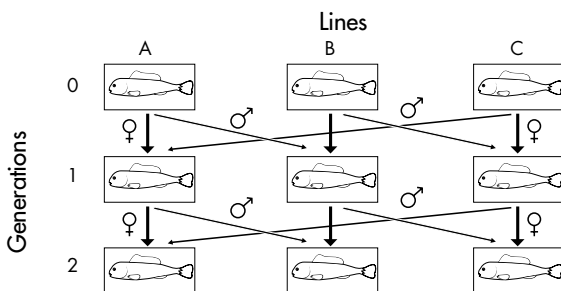


Figure 14. Rotational line crossing scheme using three separate lines. Each box represents a group of fish from a separate line. Bold, vertical lines show the source of females used for breeding. Thin, diagonal lines show the source of males mated with those females. This scheme minimizes further inbreeding and genetic drift within the lines. [After Kincaid 1977]

Limitations

Rotational line crossing makes the handling of hatchery stock more complex.

MARKER-ASSISTED SELECTION (MAS)

A new addition to the breeder's toolkit combines traditional artificial selection with genetic marker data. When a QTL for an important production trait has been identified, a closely associated genetic marker can be used to choose parents with alleles linked to superior performance for the trait, a process known as marker-assisted selection (MAS) (Poompuang and Hallerman 1997). The actual gene or how it functions does not have to be known so long as it is tightly linked to the marker so that recombination will not disassociate the marker and functional gene.

Advantages

MAS can increase the efficiency of traditional selection programs. Breeders can be assured that selected broodstock achieved their characteristics because of superior genes rather than environmental conditions.

Limitations

Genetic markers suitable for MAS typically are associated with single genes with major effects on traits of interest. MAS is not effective for imposing selection upon the many additional genes with small effects that may be dispersed throughout the genome. Cumulatively, these many genes of small effect may account for much of the genetic control over some traits.

MAS requires tight linkage between the marker allele and the actual gene contributing to the trait; recombination can link the superior MAS marker allele with an inferior allele at the functional gene.

GENETIC TOOLS TO PRODUCE NOVEL TRAITS

Fish geneticists have gone beyond classic breeding to develop other genetic methods to produce fish with novel traits (ABRAC 1995; NRC 2002; Scientists' Working Group on Biosafety 1998). These techniques involve:

- deliberate gene alterations such as changes in genes, transposable elements, non-coding DNA (including regulatory sequences), synthetic DNA sequences, and mitochondrial DNA
- deliberate manipulations of chromosome numbers and fragments
- deliberate hybridization between taxonomically distinct species

The term, modern biotechnology, is sometimes used to refer to this entire suite of tools. Modern biotechnology, however, can also refer only to gene transfer (deliberate gene changes) that yields transgenic organisms.

There are potential hazards in the use of any tool that creates “new” fish with traits never or rarely found in nature. An important hazard is the possible effect of these fish on wild fish populations and natural ecosystems (NRC 2002; Kapuscinski 2005; Kapuscinski et al. 2007). Novel fish produced by chromosomal manipulations or by inter-specific hybridization have been used in a limited number of food aquaculture systems and, more rarely, in fish stocking programs. The first applications for transgenic fish should be in well confined aquaculture operations to reduce escapes of these fish into natural waters (Kapuscinski 2005; Mair et al. 2007).

GENE TRANSFER

Gene transfer is the technique that is often thought of when one hears the term genetic engineering or genetic modification. Gene transfer involves insertion of recombinant DNA into the native DNA of a recipient organism to create a **transgenic organism**. The recombinant DNA consists of a new, man-made combination of genes derived from the same species, a different animal species, from a plant or bacterium, or even from human DNA. The key advantage of gene transfer is that the transgenes can confer novel traits not obtainable by artificial selection or hybridization. Research scientists and commercial companies have developed transgenic fish with a growing diversity of new traits (Kapuscinski 2005; Nam et al. 2007), Table 5 lists a few examples. Gene transfer is also being applied to modify traits of oysters and other mollusks, shrimp and other crustaceans, and algae and other aquatic plants (NRC 2004; Nam et al. 2007).

To create a transgenic organism, scientists first isolate the structural gene (or genes) and promoter sequences they hope will confer new “traits” to the fish. Next, they recombine the structural gene and promoter to produce a novel transgene and then produce multiple copies of the transgene, usually by introducing it into bacteria, which copy and reproduce the transgene (i.e., “clone” the transgene) as the bacteria reproduce. Next, scientists insert the transgene into the target animal’s native DNA, using one of several different techniques. In one method, genetic material is inserted directly into newly fertilized fish eggs, where it may become part of the fish’s own DNA. Another method involves application of a brief electrical pulse to newly fertilized fish eggs; the pulse induces formation of pores through which the DNA gains access to the eggs. Next, scientists check to ensure the desired transgene has been success-

fully integrated into the native DNA of the fish and determine if it functions as expected. Finally, the transgenic fish are crossed for several generations to ensure production of a stable transgenic line in which every individual carries a functioning transgene.

Table 5. Examples of transgenic fish with novel traits. In 2006, these transgenic lines were in commercial use (U), approaching commercial consideration (A), or in early research (R).

Species	Engineered Trait (Inserted Transgenes)	Proposed Application	Status
Zebrafish (<i>Danio rerio</i>)	Fluorescent red or green body color ¹ (fluorescent protein gene derived from jellyfish or sea anemone + zebrafish muscle-specific promoter)	Hobby aquarium market	U
Atlantic salmon (<i>Salmo salar</i>)	Increased growth rate and food conversion efficiency (FCE) ^{2,3} (Chinook salmon growth hormone gene + ocean pout antifreeze protein promoter)	Aquaculture (human food)	A
Hybrid tilapia (<i>Oreochromis hono- rum</i> x <i>O. aureus</i>)	Increased growth rate and FCE ⁴ (tilapia growth hormone gene + viral promoter)	Aquaculture (human food)	A
Common carp (<i>Cyprinus carpio</i>)	Increased growth rate and FCE ⁵ (grass carp growth hormone gene + carp beta-actin promoter)	Aquaculture (human food)	A
Mud loach (<i>Misgurnus mizolepis</i>)	Gigantism, increased growth rate and FCE ⁶ (mud loach growth hormone gene + mud loach beta-actin promoter)	Aquaculture (human food)	R
Channel catfish (<i>Ictalurus punctatus</i>)	Enhanced bacterial resistance ⁷ (moth cecropin B gene + viral promoter)	Aquaculture (human food)	R
Goldfish (<i>Carassius auratus</i>)	Increased cold tolerance ⁸ (ocean pout antifreeze protein gene + its promoter)	Aquaculture (ornamental fish, model for food fish)	R
Nile tilapia (<i>O. niloticus</i>)	Biofactory production of blood clotting factor ⁹ (human gene for clotting factor VII + tilapia vitellogenin promoter)	Human medicine	R
Medaka (<i>Oryzias latipes</i>) – model species	Produce male-only offspring ^{10,11} (‘interference RNA’ that binds to and blocks expression of native gene for aromatase, thus blocking development of female gonads)	Biological control of aquatic invasive species, such as common carp	R

- ¹ Gong, Z., H. Wan, T.L. Tay, H. Wang, M. Chen, and T. Yan. 2003. Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. *Biochemical and Biophysical Research Communications* 308(1): 58-63.
- ² Cook, J.T., M.A. McNiven, G.F. Richardson, and A.M. Sutterlin. 2000. Growth rate, body composition and feed digestibility/conversion of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* 188: 15-32.
- ³ Hew, C.L. and G.L. Fletcher. 1996. Transgenic salmonid fish expressing exogenous salmonid growth hormone. U.S. Patent & Trademark Office. Patent #5,545,808.
- ⁴ Martinez, R., J. Juncal, C. Zaldivar, A. Arenal, I. Guillen, V. Morera, O. Carrillo, M. Estrada, A. Morales, and M.P. Estrada. 2000. Growth efficiency in transgenic tilapia (*Oreochromis sp.*) carrying a single copy of an homologous cDNA growth hormone. *Biochemical and Biophysical Research Communications* 267: 466-472.
- ⁵ Fu, C., W. Hu, Y. Wang, and Z. Zhu. 2005. Developments in transgenic fish in the People's Republic of China. Scientific and Technical Review (Office International des Épizooties) 24: 299-307.
- ⁶ Nam, Y.K., H.J. Cho, Y.S. Cho, J.K. Noh, C.G. Kim, and D.S. Kim. 2001. Accelerated growth, gigantism, and likely sterility in autotransgenic triploid mud loach *Misgurnus mizolepis*. *Journal of the World Aquaculture Society* 32(4): 353-363.
- ⁷ Dunham, R.A., G.W. Warr, A. Nichols, P.L. Duncan, B. Argue, D. Middleton and H. Kucuktas. 2002. Enhanced bacterial disease resistance of transgenic channel catfish *Ictalurus punctatus* possessing cecropin genes. *Marine Biotechnology* 4(3): 338-344.
- ⁸ Wang, R., P. Zhang, Z. Gong, and C.L. Hew. 1995. Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. *Molecular Marine Biology and Biotechnology* 4(1): 20-26.
- ⁹ Hwang, G., F. Muller, M.A. Rahman, D.W. Williams, P.J. Murdock, K.J. Pasi, G. Goldspink, H. Farahmand, and N. Maclean. 2004. Fish as bioreactors: transgene expression of human coagulation factor VII in fish embryos. *Marine Biotechnology* 6: 485-492.
- ¹⁰ Thresher, R.E., L. Hinds, P. Grewe, and J. Patil. 2002. Genetic control of sex ratio in animal populations. International Publication Number WO 02/30183 A1. World International Property Organization.
- ¹¹ Kapuscinski, A.R. and T. J. Patronski. 2005. Genetic methods for biological control of non-native fish in the Gila River Basin. Contract report to the U.S. Fish and Wildlife Service. University of Minnesota, Institute for Social, Economic and Ecological Sustainability, St. Paul, Minnesota. Minnesota Sea Grant Publication F 20. Available at www.seagrant.umn.edu (accessed May 25, 2006).

INDUCED POLYPLIIDY

The production of triploid fish is of interest to fisheries managers because most triploids are sterile (Benfey 1999, Felip et al. 2001). Sterile fish do not divert energy to reproduction and thus grow to larger size. It may be possible to use sterile fish for management purposes (e.g., weed control, control of overabundant forage fish) with little risk to the natural system because reproduction will not occur. Some suggest that aquaculture of sterile triploid fish could provide 'biological confinement' reducing ecological risks posed by non-native species, selectively bred lines, or transgenic fish escaping from captivity (ABRAC 1995; NRC 2004). Interspecific triploid hybrids sometimes have higher survival rates than diploid interspecific hybrids (Chevassus et al. 1983; Scheerer and Thorgaard 1983).

Triploidy in fish is usually induced by treatment of fertilized eggs with temperature or pressure shocks just before the second meiotic division so that the maternal contribution to the embryo is 2n chromosomes. The haploid sperm combines with the unreduced diploid eggs to produce triploid progeny. Another method is to first induce tetraploidy and then mate tetraploid individuals (which are fertile) with diploid individuals to produce offspring that are all triploid.

These techniques have several limitations. Induction of triploidy is not 100% effective; fertile diploids are usually produced as well. Separation of the fertile individuals from the sterile individuals is feasible but requires specialized equipment and properly trained technicians. Methods for verifying ploidy levels in fish and shellfish were reviewed by the National Research Council (NRC 2004) and Mair et al. (2007). Mating tetraploid fish with diploid fish should yield 100% triploid individuals, but viable tetraploid lines have been produced in only a few species (reviewed by Mair et al. 2007). Triploid fish, especially males, might produce a limited number of potentially fertile gametes (Devlin and Donaldson 1992; Dunham 2004). These limitations are a significant disadvantage when it is important to be sure that all fish are sterile. Finally, the growth and health-related traits of triploids may be compromised compared to normal diploid fish (Benfey 1999; Jhingan et al. 2003).

GYNOGENESIS AND ANDROGENESIS

Gynogenesis is the production of viable progeny with all-maternal inheritance. Development of the embryo is initiated when irradiated sperm penetrate the egg. Radiation inactivates the DNA in the sperm so that the DNA in the progeny comes only from the mother (Thorgaard 1986). **Androgenesis** is

the production of viable progeny with all-paternal inheritance. In the case of androgenesis, the DNA of the egg is inactivated by radiation (Thorgaard 1986). These methods can be used to produce sterile triploids, as described in the previous section, or fertile diploids as described next. Dunham (2004) reviewed these methods and their applications, primarily in research.

Diploid gynogenesis

Diploid gynogenesis occurs in fish when sperm fertilize eggs but do not contribute DNA to the embryo. The progeny are diploid but all the genetic information in the developing embryo comes from the mother. Natural gynogenesis is rare. Induced gynogenesis is a genetic tool of potential use to fish breeders. Gynogenesis can be used to generate inbred lines of all female fish.

Diploid gynogenesis in fish can be induced by sperm in which the DNA has been deactivated by radiation. Development of a haploid embryo is initiated when a deactivated sperm penetrates an egg but does not fertilize it. A temperature or pressure shock is applied to the egg after development begins just prior to the second meiotic division. The shock prevents the second meiotic division from occurring so that the germ cell remains diploid. Another method of restoring the diploid state involves use of pressure shock to suppress the first mitotic division in haploid embryos. The remainder of development is normal if the treatments have been successful.

Diploid androgenesis

Diploid androgenesis occurs when the egg does not contribute DNA to the embryo; all of the genetic material in the embryo comes from the sperm. Development of a haploid embryo occurs when a nonirradiated sperm penetrates an irradiated egg. The diploid state is restored by using a pressure shock to block the first mitotic division in the haploid embryo. Androgenesis may have several advantages over gynogenesis for generating inbred lines of fish (Parsons and Thorgaard 1985). For example, less time may be required to produce inbred lines for species in which males mature earlier than females.

PRODUCTION OF MONOSEX POPULATIONS

Technologies that allow production of monosex populations are potentially useful because one sex may be more valuable than the other and because reproduction can be controlled in monosex populations. There are at least three ways to produce fish that are all one sex. The first method involves hybridization of two closely related species. The second method involves the use of **hormones** to artificially reverse sex (**sex-reversal**). The third method, induced gynogenesis (discussed above), is possible when the female is the **homogametic** sex.

There are a few examples of interspecific hybridization of tilapia to produce monosex populations (Hulata 1983; Mair et al. 1991). Crossing males from a male-homogametic species (males of *Oreochromis aureus* or *O. hornorum* with ZZ genotype, where Z is a sex chromosome) with females from a female-homogametic species (females of *O. niloticus* or *O. mossambicus* with XX genotype) yields predominantly male offspring (Mair et al. 1991). The advantage is that monosex culture is facilitated (fish need not be sorted by hand) and unwanted reproduction may be controlled. Unfortunately, the hybrid offspring are not always 100% male, probably because of the effect of sex-modifying genes that reside on the autosomes.

Sex-reversal has been used to produce monosex populations in various finfish and crustacean species (Dunham 2004; Mair et al. 2007). The work of Hunter et al. (1983) illustrates the use of sex-reversal to produce broods of completely female chinook salmon. Female chinook salmon are homogametic (homozygous for sex chromosomes, i.e., genotype XX where X is a sex chromosome), normal males are **heterogametic** (heterozygous for sex chromosomes, i.e., genotype XY). The sex of genetic females can be reversed by adding small amounts of male hormones to the feed of young fry. The sex-reversed males are functionally male but remain genetically female. When these functional males are mated to normal females the progeny are all XX, that is, genetically and functionally female. The monosex progeny produced in this manner have normal levels of heterozygosity, unlike the nearly homozygous progeny obtained using gynogenesis. Heterozygous progeny may be more useful in aquaculture than inbred, homozygous lines.

It is difficult to distinguish between normal and sex-reversed males when the sex-reversal process is begun with mixed sex populations. This problem may be circumvented by application of gynogenesis in the first generation (to give all female progeny), followed by sex-reversal (to give XX functional males). The sex-reversed males can be out-crossed to produce all female, heterozygous progeny.

CHAPTER THREE: GENETIC ISSUES IN FISHERIES MANAGEMENT

OVERVIEW

With information about genetic principles and techniques managers can address three broad categories of genetic issues in fisheries management:

- intentional and unintentional alterations of the genetic makeup of hatchery stocks
- inadvertent effects of management and exploitation on the genetics of wild stocks
- accidental or purposeful release of transgenic fish into natural waters and their possible effects on wild populations

THE GENETICS AND FITNESS OF HATCHERY STOCKS

Mating techniques, intentional and inadvertent selection, domestication, and inbreeding all affect the genetics and fitness of fish. In general, hatchery managers should try to maintain genetic variation in breeding populations while producing fish that are suitable for their intended use. Hatchery bred fish may be stocked in an aquacultural facility for food production, in a natural environment, or in some captive setting as broodstock. The reproductive fitness of fish destined for slaughter is not important; in fact, reproduction is often undesirable. In contrast, the fitness of fish used as broodstock or for stocking in natural environments is of paramount importance because the fitness of future generations depends upon their genetic characteristics. Principal hatchery concerns are: 1) that hatchery practices may detrimentally alter survival, yield or reproduction, and 2) small effective population sizes in hatcheries can lead to inbreeding and loss of genetic diversity.

FACTORS THAT AFFECT INBREEDING AND EFFECTIVE POPULATION SIZE

Principal factors affecting inbreeding and effective size of hatchery populations are:

- the number of breeding individuals in the population,
- the sex ratio,
- variation in the reproductive success of individual spawners, and
- effective population size during previous generations.

Unintentional inbreeding, which occurs in all populations of finite size, is an important concern in hatchery management. Inbreeding results in loss of genetic diversity (loss of alleles) and increased homozygosity, leading potentially to inbreeding depression. The rate of inbreeding in a population is inversely related to the effective size of the population; i.e., it is low in large populations and high in small populations (see Inbreeding Coefficients, page 30). Inbreeding is cumulative because it increases from one generation to the next.

Population size (numbers of individuals)

In an idealized population (infinitely large, random variation in reproductive success, non-overlapping generations, and balanced sex ratio) the effective size is simply the number of individuals in the population. In real populations the effective size is almost always less than the number of individuals in the population. The first requirement, therefore, for the reduction of inbreeding in hatcheries is the capability to hold large numbers of spawners. There is no guarantee that inbreeding will be minimized in large populations because the effective population size also depends on other factors like sex ratios and variation in reproductive success.

Sex ratio

Consider a population with N_m males and N_f females in which the number of males does not necessarily equal the number of females. The effective population size and the rate of inbreeding are given approximately by:

$$N_e = 4 N_m N_f / (N_m + N_f) \\ \Delta F = 1 / (2N_e) = 1 / (8N_m) + 1 / (8N_f)$$

Note that $N_e = N$ and $\Delta F = 1 / (2N)$ when the sex ratio is balanced ($N_m = N_f$). An unfortunate consequence of these relationships is that unbalanced sex ratios have large effects on N_e and ΔF . Consider two hatchery populations of 50 individuals. In the first population (with equal numbers of males and females) the effective population size and rate of inbreeding for the population are 50 and 1

percent, respectively; in the second population (with 40 females and 10 males) the effective size and rate of inbreeding for the population are 32 and 1.6%, respectively. The unbalanced sex ratio reduced the effective population size by 36 percent and increased the rate of inbreeding by 60%.

Variation in reproductive success

Non-random variation among individuals in reproductive success can be due to differences in fecundity, fertility, and longevity (fish that live longer have more opportunities to reproduce), variable conditions or disturbances on the spawning grounds, and accidents in the hatchery (e.g., fungus infections). The effective population size accounting for variation in family size (N_e) is given (approximately) by:

$$N_e = 4N / (V_k + 2)$$

In an ideal population with random variation in family size, $V_k = 2$, and therefore $N_e = N$. In real populations a few successful individuals may produce numerous offspring whereas many produce few or none. This increases V_k , which decreases the ratio N_e/N . Falconer and MacKay (1996) state that differences in reproductive success in real populations are “the most important cause of N_e being less than N ”

Temporal variability in effective population size

Inbreeding and loss of genetic diversity accumulate with each new generation so that the degree to which a population is inbred depends on the effective population size during each of the previous generations. It is important to consider how temporary reductions in the effective population size (**population bottlenecks**) affect the loss of genetic diversity in the long-term. The mean effective population size of a population over a period of n generations can be calculated (approximately) as the harmonic mean of the effective population sizes during each of the generations:

$$1 / N_e = (1 / N_1 + 1 / N_2 + \dots + 1 / N_n) / n$$

The mean rate of inbreeding can be obtained from N_e in the usual way. It is important to realize that population bottlenecks (small values of N_i) have large effects on N_e , inbreeding, and the loss of genetic diversity. Consider a population with the following effective population sizes during ten consecutive generations: 100, 50, 10, 50, 100, 100, 100, 100, 100, 100. The mean effective size and mean rate of inbreeding are 47.6 and 1%, respectively. With no three-year population bottleneck, the effective size and mean rate of inbreeding would have been 100 and 0.5%, respectively. A population bottleneck that lasted only three

generations reduced the mean effective size by 54% and doubled the mean rate of inbreeding.

RECOMMENDATIONS FOR EFFECTIVE SIZE OF HATCHERY POPULATIONS

The effective size of hatchery populations should be as large as possible in order to minimize loss of genetic diversity. Recommended values for N_e reflect compromises involving the value of the genetic diversity in a population (which is very difficult to measure), the rate at which genetic diversity is lost and the costs of raising fish and managing a hatchery. Recommendations in the fisheries literature for the minimum effective population size of hatchery populations vary tremendously. Gharrett and Shirley (1985), for example, cite values ranging from 60 to 200. This variation results from uncertainty about the minimum amount of genetic variation required for populations to persist in unpredictable natural environments. Domestic animals seem to tolerate inbreeding at the rate of about one percent per generation (equivalent to $N_e = 50$) without showing inbreeding depression. The figure $N_e = 50$ is thus a lower bound on the acceptable values of N_e for populations used in aquaculture. Acceptable values for hatchery populations used in stocking programs are higher. Kincaid (1983), for example, suggests that the effective size of a breeding population should be at least 100 (equivalent to $\Delta F = 0.5\%$) and Allendorf and Ryman (1987) recommended a minimum of 200. In the conservation genetics literature, a minimum N_e of 500 is recommended for conserving long-term evolutionary potential (Franklin 1980) and even higher minimums of a few thousand have been suggested (Lande 1995). Considering this uncertainty, an N_e of 100 – 200 should be regarded as a minimum acceptable range for hatchery populations that are used for enhancement of wild stocks. For restoration of severely depleted populations, this number will not always be achievable. Waples and Do (1994), however, showed that problems associated with small populations can be reduced if restoration is successful and the population rapidly increases in size.

As we have seen, the effective size of a population is often less than the number of fish in the spawning population. How many fish are required to insure that the effective size of a population is at least some specified value? The answer is not simple and will depend on the sex ratio of the stock, the breeding technique used, the means by which spawners are selected, etc. When the sex ratio is balanced and all spawners enjoy equal reproductive success, fewer fish will be required to obtain a given value of effective population size.

In a hatchery it may be possible to maximize N_e by equalizing family size

($V_k = 0$) resulting in an N_e that is almost twice as large as N . Note that family size is measured as the number of offspring that reach maturity and reproduce. Equalizing family size, therefore, requires a means of tracking family membership until maturity (e.g., separate rearing, physical tags, or genetic markers). As a matter of practicality, some hatchery programs have equalized family size at an earlier life stage (e.g., eyed-eggs) to reduce the variance in family size attributable to fecundity and fertility differences (Dale Bast, USFWS-Iron River, WI, personal communication).

Other hatchery practices can be used to directly reduce inbreeding. One approach is to employ a line crossing scheme such as rotational line crossing (Kincaid 1977) that minimizes matings between related individuals. In special cases involving captive broodstock for restoration of critically small populations, geneticists have used molecular genetic markers to pedigree fish to avoid mating close relatives (W. Ardren, USFWS-Abernathy Fish Technology Center, personal communication).

MATING TECHNIQUES

The rate at which genetic diversity is lost in a hatchery program due to inbreeding and genetic drift depends partly on how the fish are mated. For a fixed number of breeders, effective population size is maximized (inbreeding and genetic drift are minimized) when there are equal numbers of males and females and all breeders contribute equal numbers of progeny to the next generation. Miller and Kapuscinski (2003) provide guidelines for mating schemes. A baseline for making crosses is by pairs of a single male and a single female. In some situations unequal numbers of each sex will be available and the potential for gamete inviability should be taken into account. If one sex is in excess, divide gametes from individuals of the less numerous sex to allow crosses with all of the excess sex. Sperm from a single male is preferred for each cross because the alternative of using pooled sperm may cause unequal contribution of gametes from some males due to differences in potency of their sperm (Withler 1988). An exception is when it is suspected that many males are infertile. In this case, pool sperm from overlapping pairs of males and use each pooled pair to fertilize eggs of one female. Pooling pairs of males will reduce the incidence of unsuccessful fertilization because at least one of the pair is likely to be fertile (Gharrett and Shirley 1985). The degree of sperm potency differences versus numbers of completely infertile males will determine whether or not overlapping males is advantageous.

Another scheme used to increase genotypic diversity and N_e is **factorial matings**. Factorial matings involve dividing gametes from several individuals

and making all possible crosses between males and females. Factorial matings increase diversity by creating more genotype combinations and may increase N_e by reducing extremes in reproductive success (C. Busack, Washington Department of Fisheries and Wildlife, personal communication).

Selection

Artificial selection is useful for improving broodstocks in captive aquaculture and is usually intentional and desirable. Conversely, artificial selection of hatchery fish destined for stocking in natural environments may be unintentional and raises concerns. Artificially selecting hatchery populations that supply a stocking program may improve hatchery performance at the expense of the fitness of fish released in natural environments.

Domestication selection is any change in the selection regime of a cultured population relative to that experienced by the natural population (Waples 1999). The end result is that the genetic composition of a hatchery population will likely differ from that of its wild source. Domestication may be desirable in fish that are used for aquaculture but it is almost certainly undesirable in fish destined for stocking in the wild. A premise of our application of genetic principles to hatchery management is that natural selection can best produce distributions of genetically determined traits and that any significant alterations of these distributions should reduce a population's fitness in the wild (Reisenbichler 1997).

Domestication selection can take one of several forms (Busack and Currens 1995, Campton 1995, Waples 1999). The most obvious is intentional selection on traits such as size or run timing. In addition to directly changing a selected trait, intentional selection may adversely affect other correlated traits, particularly those traits that are fitness related. Improperly designed selection programs also reduce effective population size and encourage inbreeding and loss of genetic diversity (Hynes et al 1981). **Inadvertent selection** is unintentional artificial selection in hatcheries resulting from two causes. The first cause is nonrandom collection of broodstock. It is different from intentional selection because there is no purposeful selection on a trait. For example, gametes may be collected as soon as a spawning period begins and end when sufficient numbers are taken. The parents have been unintentionally selected for early spawning time, a trait known to have a genetic component in some fish species (e.g., Gharrett and Smoker 1993). Inadvertent selection may occur if the average size, age, or spawning locations of the spawners used in hatcheries are different from the averages for the entire population. The second cause of inadvertent selection is the unintentional selection that occurs in the hatchery environment. For example, changes in agonistic behavior due to crowding,

rearing conditions, or feeding methods (e.g., Berejikian et al. 1996). The final form of domestication selection is the **relaxation of natural selection** in the hatchery environment. Fish that would have been selected against in the wild may survive in the hatchery and pass on their genes that are maladaptive in the natural environment.

Domestication selection can be reduced, although not entirely avoided, by collecting large, random samples of broodstock, minimizing hatchery mortalities, collecting broodstock from the wild, and minimizing the time fish are held before stocking. Ford (2002) showed, however, that even these efforts might not prevent loss of fitness in the wild. Importantly, he found that the effect of domestication on the fitness of hatchery fish in the wild is sensitive to the carrying capacity of the environment and the population growth rate it can support. These findings indicate the importance of restoring or maintaining good habitat before considering population supplementation by stocking.

IMPACTS ON THE GENETICS OF WILD STOCKS

STOCKING

It is possible that the fitness of stocked fish will be less than the fitness of the wild population residing at a particular location. This is especially true for stock transfers (e.g., Waples 1995) but may also be true when stocking hatchery reared fish derived from the local population (Fleming and Petersson 2001). Stocked fish could affect the fitness and long-term adaptability of the population. Stocked fish could directly affect the genetics of native populations through hybridization, resulting in loss of between population genetic diversity and **outbreeding depression**. Stocked fish also could impose indirect genetic effects by reducing or fragmenting populations. These indirect effects could be induced through increased harvest, introduced diseases, or range reductions due to displacement of native fish (Utter 1998).

RECOMMENDATIONS

Whenever possible, manage wild populations so that stocking is unnecessary. When stocking is necessary, try to stock fish that are well adapted to the local environment. The best way to improve the chances that stocked fish will have high fitness in a particular environment is to choose a source stock following three similarity criteria (Miller and Kapuscinski 2003):

- Similarity in genetic lineage, which for the case of hatchery fish, is best assured by using the wild population as the source for broodstock. If this is not practical, then broodstock should be obtained with similar life histories.
- Similarity in life history patterns partly reflects similarity in genetic makeup for these evolutionarily important traits (Ricker 1972) and increase the chances that life history patterns of stocked fish will be adaptive in the new environment.
- Similarity in ecology of the originating environment. A similar originating environment is indicative of similarity of evolutionary history and increases the chances that the source population will be adaptive in the environment targeted for stocking.

A number of other methods can be used to help insure that stocked fish have high fitness (Miller and Kapuscinski 2003). Broodstock should be obtained by sampling randomly from spawners in a wild population in order to avoid inadvertent selection for body size, spawning time, etc. Mating schemes and hatchery management should aim to maximize effective population size. The hatchery rearing period for broodstock and production stock should be minimized because consequences of hatchery culture (i.e., domestication, inadvertent selection and inbreeding) accumulate with time. Experiments are underway to determine if simulating natural conditions (e.g., substrate, cover, underwater feeding) in the hatchery can increase the fitness of stocked fish upon release (Maynard et al. 1995). Finally, fish should be stocked at a size, time, and place so that they are similar to wild fish and integrate with wild fish rather than displace them.

HARVEST MANAGEMENT

Harvest management affects the genetics of wild fish stocks in at least two ways. First, high exploitation rates reduce the effective size of a stock so that the rates of genetic drift and inbreeding are increased. Second, fishing methods that “select” individual fish for harvest on the basis of some characteristic (e.g., size selectivity in a gillnet fishery) amount to artificial selection programs that can cause genetic changes in the stock over time.

Effects of management practices and harvest on within-population variation in fish are poorly documented (Reisenbichler 1997). Loss of genetic variation is recognized in the conservation literature as a problem that could reduce population fitness (e.g., Nelson and Soulé 1987). Harvest and alteration of spawning habitats may directly reduce population size, thus reducing the effective population size (which is inversely related to the rate of loss of genetic variation). Harvest and habitat alteration may also alter demographic factors (sex ratio and variance in family size) that reduce the ratio of effective to census population size. Because growth rates can differ between the sexes, size regulations or angler selection may preferentially target one sex, thus skewing the sex

ratio. Disturbances on spawning grounds may destroy the offspring of entire families and increase the variance in reproductive success among adults in the population. Although many plausible scenarios can be described that would result in genetic losses, declines in the productivity of wild populations from inbreeding and loss of alleles due to genetic drift have not been documented in fish species (Nelson and Soulé 1987, Reisenbichler 1997). Reisenbichler (1997) suggests that this is due in part to the lack of relevant data and confounding effects of other factors of decline.

Unintentional selection

Many fisheries amount to artificial selection programs that act on fitness-related traits or traits genetically correlated with fitness. Fish taken by a fishery are seldom a random sample from the population because fishing techniques and gear select individuals with certain characteristics. Consequently, fish that live to spawn will be different, on average, than fish in the population before exploitation. If the differences are heritable, then the next generation will be genetically and phenotypically different from the previous generation. This process can occur in every generation until substantial changes have occurred in the population. It is possible that inadvertent artificial selection will produce a stock of fish with inferior commercial value or reduced fitness.

Inadvertent selection has been cited repeatedly in connection with size selective commercial fisheries (Law 2000). For example, many nets are designed to select individuals larger than some minimum size; consequently fish that survive to spawn are relatively small. Growth rate is a heritable characteristic and reduced growth rates have been observed in several stocks following exploitation (Law 2000). Conover and Munch (2002) exposed captive fish populations to simulated size selective fishing pressure and caused genetic changes in growth that ultimately reduced yields from the exploited populations. Traits genetically correlated with growth rate, such as average age at sexual maturity, may also change in response to selection by the fishery (Beacham 1983; Ricker 1981). Inadvertent selection is not limited to commercial fisheries. Favro et al. (1979) discusses the genetic effects of inadvertent selection due to size limits in a recreational trout stream fishery.

HABITAT ALTERATION

Changes in the environment can affect the genetics of wild populations in two familiar ways: 1) by depressing the effective size of the population, which causes a loss of genetic diversity; and 2) by natural selection for increased fitness in the new environment, which may decrease the value of the resource.

Depression of effective population size (leading to increased inbreeding, increased genetic drift, and loss of genetic diversity) is a certain consequence of environmental changes that reduce habitat size (e.g., obstruction of spawning streams), kill fish (e.g., pollution), or limit reproductive success (e.g., acid rain).

Population fitness may be reduced by a major change in habitat because characteristics that maximize fitness in the old environment may not maximize fitness in the new environment. Selection that increases fitness in the new environment is a natural and constructive response of the population to an environmental change. A number of related consequences, however, should be kept in mind. Fish adapted to the new environment (e.g., tolerant of polluted water) may not be desirable for human consumption. Productivity of the population may remain low even after the population adapts to the new environment. Many generations might elapse before the population adapts to the new environment because the response to natural selection may be slow (although examples of rapid evolution in fish populations are being found [Koskinen et al. 2002; Unwin et al. 2003]). The population may never adapt if the environment continues to change.

ENVIRONMENTAL EFFECTS OF TRANSGENIC FISH

The ongoing development of transgenic fish and shellfish raises the need to assess and manage environmental risks imposed by intentional introductions and unintended escapes of transgenic fish into natural waters. This should involve a case-by-case consideration of the production system (aquaculture or otherwise) in which the fish would be used and characteristics of the transgenic fish line and potentially affected ecosystems. Most transgenic fish have been developed for aquaculture and many aquaculture systems are extremely vulnerable to accidental releases into the natural environment (e.g., damage to ocean net pens, flooding of outdoor ponds). Each line of transgenic fish should be assessed for how transgenic escapees might affect wild fish and other organisms through gene flow and ecological interactions (ABRAC 1995; Kapuscinski 2005). It is a challenge to *prospectively* assess these effects before transgenic fish enter a natural ecosystem. Therefore, efforts are underway to develop and validate environmental risk assessment and management methodologies that integrate confined experiments on transgenic fish and data on potentially affected ecosystems (Kapuscinski et al. 2007).

GENE FLOW FROM TRANSGENIC INDIVIDUALS TO WILD RELATIVES

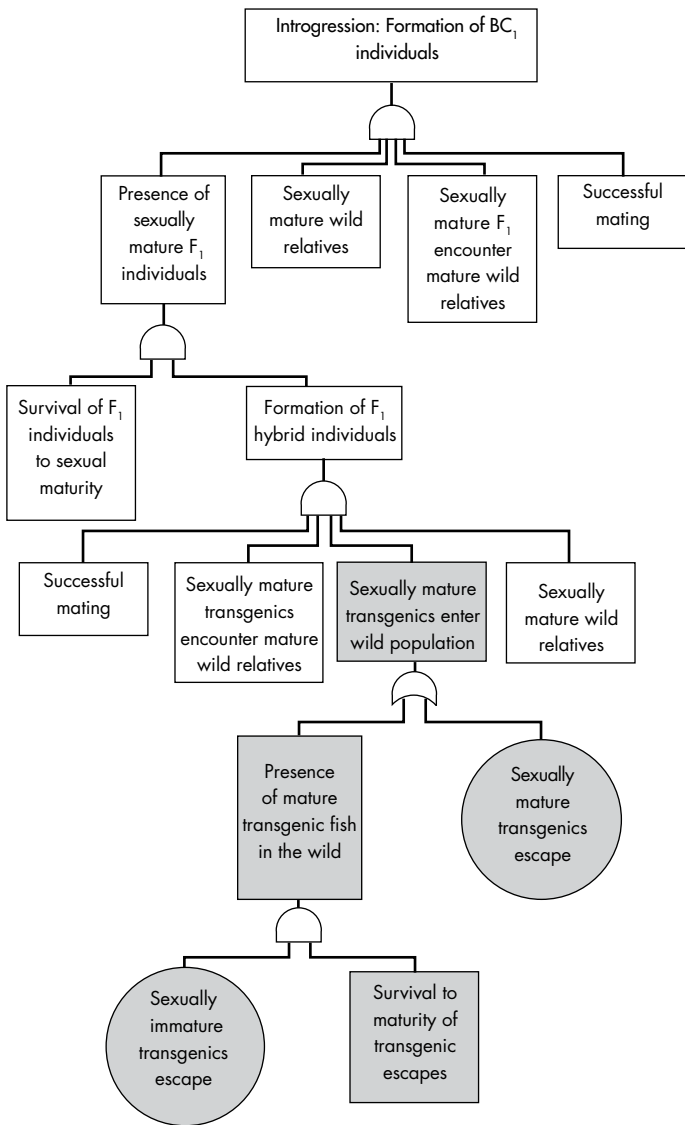
Gene flow from transgenic individuals to wild relatives is a major process through which transgenic fish may affect wild fish populations. The main concern is whether gene flow results in **introgression** (incorporation) of the transgenic genotype into the gene pool of wild relatives. Figure 15 indicates the chain of events that must occur to end up with introgression. Predicting the fate of transgenes requires data, obtained from well-confined experiments, on how the transgenic genotype affects the **net fitness** of the fish, as well as evidence of how the **genetic background** of the fish population and genotype-environment interactions might alter this net fitness (Kapuscinski et al. 2007-a). Also required are specific baseline data about the wild relatives, such as population genetic structure and spatial distribution of breeding adults.

Application of a 'net fitness' method, involving aquarium experiments and computer simulations, has suggested three potential scenarios of gene flow (Muir and Howard 1999, 2001, 2002). In a purging scenario, the net fitness of a transgenic fish is much lower than that of its wild relatives and natural selection purges any transgenes inherited by wild relatives. This is the most benign outcome but not completely risk free because purging is not instantaneous and may take a number of generations. If the affected wild population is already in decline, inheritance of maladaptive transgenes in some individuals could increase the loss of genetic variation and risk of extinction. In a spread scenario, introgression of transgenes could result in altered frequencies of native alleles, loss of genetic distinctiveness, and loss of genetic variation in the affected wild population. These genetic changes can undermine current adaptation of wild populations to their environment and their ability to adapt to future environmental change. In the worst case scenario, transgene spread under very specific conditions would trigger a population crash (Howard et al. 2004). Such predictions based on net fitness models need to be validated using more complete data about the transgenic fish line, wild populations, and particular ecosystem.

ECOLOGICAL EFFECTS

Transgenic fish may have ecological effects beyond their possible effects on the genetics of wild populations. Ecological effects are even possible when there is no gene flow and introgression of transgenes into wild populations. Consider, for example, a line of goldfish with antifreeze protein transgenes giving them increased cold tolerance (Wang et al. 1995). Large-scale aquaculture of these fish would raise the possibility that they could invade a broader range

Figure 15.








SYMBOL	MEANING OF SYMBOL
	TOP EVENT the primary undesired event of interest
	INTERMEDIATE EVENT caused by more primary level events described below
	BASIC INITIATING EVENT does not need to be developed further
	AND GATE logic gate where output occurs only if all inputs occur
	OR GATE logic gate where output occurs if any of the inputs occur

Figure 15. Fault tree of events leading to introgression of transgenes from escaping transgenic fish into the gene pool of wild relatives. (Modified from figure developed by K. R. Hayes.) The fault tree should be read from the bottom to the top. Grey backgrounds: events leading to entry of sexually mature transgenic fish into a breeding population of wild relatives. White backgrounds: post-entry events leading to introgression. F_1 : first-generation hybrids from transgenic and wild fish matings; BC_1 : first generation of backcrosses between F_1 hybrids and wild relatives. Formation of BC_n individuals would occur via the same events shown in the penultimate row but involving BC_1 , BC_2 , etc. instead of F_1 individuals. Additional lower-level events could contribute to events in rectangular boxes but they have not been shown for brevity. Reprinted with permission from CABI Publishing 2007.

than non-transgenic goldfish, already an established alien species in some inland waters, and through their prolific breeding and hardy nature, become a greater nuisance.

Adding a new element to an ecosystem can trigger the ecosystem to shift from an initial state to a new state of 'dynamic equilibrium'. This new state can have undesired changes in species composition (e.g., species extinctions and altered population abundance) and ecosystem functions (Parker et al. 1999). The main question is whether introduction of a line of transgenic fish can be a new disruptive element. An example of an undesirable disruption comes from food competition, experiments involving growth-enhanced transgenic coho salmon and non-transgenic counterparts (Devlin et al. 2004). When food was limited, a mixed population of transgenic and non-transgenic fish crashed whereas a purely non-transgenic population did not crash and its fish merely grew more slowly.

Devlin et al. (2007) have proposed an approach for assessing ecological effects. It involves four phases that build upon each other:

- (1) characterize the specific biotic and abiotic properties of the receiving ecosystem(s) that the transgenic fish might affect;
- (2) measure the intended and unintended changes in traits of the transgenic fish line;
- (3) determine the interactions anticipated between transgenic fish and ecosystem resources and services both used and provided by transgenic fish; and
- (4) estimate the scale and likelihood of ecological effects resulting from each transgenic fish-ecosystem interaction.

Each phase involves integration of information from several sources including experts and appropriate stakeholders, baseline data about potential receiving ecosystems and empirical data from well-confined experiments with the transgenic fish. In some cases, the assessment can also utilize specific field data from non-transgenic surrogate models. For example, field studies using salmonids with growth-hormone implants as surrogates for growth-enhanced transgenic fish found that these fish competed successfully with wild salmonids, counteracting the idea that growth enhancement might be a disadvantage in the wild (Johnsson and Bjornsson 2001). Even when applying this systematic four-phase approach, prospective assessment of ecological effects involves significant sources of uncertainty. One example is genotype-environment interactions that cause transgenic fish to behave differently in confined tests than in nature, thus reducing the value of applying the results to natural environments (Devlin et al. 2007).

RECOMMENDATIONS

Environmental risk assessment of transgenic fish and other aquatic species should be done through interdisciplinary scientific analysis and multi-stakeholder deliberation (Kapusinski et al. 2007). Transparent, equitable, and science-driven deliberation among the relevant stakeholders for each case, incorporating their knowledge and perspectives at key points in the process, ensures that the risk assessment leads to socially and scientifically credible conclusions (Nelson et al. 2007). All environmental risk assessments are subject to uncertainty. A scientifically defensible assessment, therefore, requires identifying and explicitly addressing the types and sources of uncertainty at relevant points in the risk assessment process (Hayes et al. 2007).

Risk management for a proposed use of a transgenic fish line aims to reduce identified risks to acceptable levels. It can include confinement measures and monitoring programs. Redundant confinement measures can focus on preventing escapes or reducing effects if escapes occur (Mair et al. 2007). Physical

barriers (e.g., lethal water temperatures or pH), mechanical barriers (e.g., screens), and geographical barriers (e.g., raising a marine species in an inland closed seawater system) can be used to prevent escapes. Biological barriers, such as induced triploidy which makes adults of some fish species functionally sterile (see Chapter 2), can be used to reduce gene flow and invasive species risks. But sterilization does not necessarily neutralize environmental risks. Escaped, sterile fish might still compete with wild fish for limited resources or engage in courtship and spawning behavior, disrupting breeding in wild populations (ABRAC 1995; NRC 2004). The only way to detect escapes and early signs of undesired ecological changes is through a well-designed monitoring program. Senanan et al. (2007) suggested six monitoring endpoints that are feasible to measure, occur over short time frames and allow early detection of ecological effects. For example, detecting transgenic fish at all life stages in a monitored area would indicate that transgenic individuals are reproducing well enough to interact extensively with other species and potentially alter fish community composition. Early detection of problems allows for remedial responses at the earliest point possible. Monitoring can also confirm a risk assessment's conclusion of environmental safety. A monitoring program, however, should not be used to circumvent the need to conduct a credible environmental risk assessment or make a well-informed regulatory decision.

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GLOSSARY

Additive Component (V_A) the portion of the total phenotypic variance in a population that is due to the additive effects of genes.

AFLP a multi-locus DNA marker technique in which DNA is digested with restriction enzymes and selectively amplified by PCR prior to gel electrophoresis.

Allele one of the alternative forms of the same gene; alleles for the same gene occur at the same locus.

Allelic Frequency the proportion of each allele in a population or sample, calculated as the number of times an allele occurs divided by $2N$, because each individual has two allele copies per locus.

Allozyme an enzyme produced by one allele at a locus; different allozymes are produced by different alleles.

Amino Acid a molecule that is one of the building blocks of proteins.

Anaphase the third stage in the division of a cell nucleus during mitosis or meiosis when the chromosomes migrate toward opposite ends of the cell.

Androgenesis production of offspring having all paternal inheritance (all chromosomes and genes obtained from the father).

Aneuploidy the condition in which cells have extra copies of one or more chromosomes.

Artificial Selection the process of choosing parents on the basis of a trait to affect a phenotypic and genetic change in the next generation.

Autosome any chromosome that is not a sex chromosome.

Base one of five molecules (guanosine, cytidine, thymidine, adenosine, and uracil) that are the building blocks of DNA and RNA.

Base Pair a pair of nucleotides containing bases (adenosine with thymidine or guanosine with cytidine) that are bonded together; chains of base pairs form the double helix of DNA.

Breeding Value the value of an individual as a breeder in an artificial selection program as judged from the mean phenotypic value of its progeny.

Captive Aquaculture raising aquatic organisms in captivity for the entire life cycle (e.g., aquaculture for human food, hobby aquaria, public aquaria, and research); does not include aquaculture in which organisms are deliberately released into the natural environment for part of the life cycle.

Chromosome a structure that contains DNA, carries genes and is found in the nuclei of cells.

Chromosome Duplication a mutation in which parts of a chromosome are duplicated.

Clone a group of genetically identical organisms descended from one common ancestor; genetically engineered replicas of a DNA sequence.

Cluster Analysis a statistical procedure that assigns similar units or samples to the same group or cluster.

Codominant the condition in which both alleles at a locus are expressed phenotypically to the same degree.

Codon a group of three adjacent nucleotides in DNA or RNA that code for a specific amino acid in a protein.

Combined Selection an artificial selection program that combines individual and family selection.

Combining Ability the value of a particular line in the production of hybrids (see general combining ability and specific combining ability).

Condensed Chromosome the state of chromosomes during division of the cell nucleus; condensed chromosomes have a characteristic shape and are visible under a light microscope.

Crossing Over the process in which arms of homologous chromosomes cross over and exchange DNA during production of eggs and sperm (meiosis).

Cytogenetics the study of genetics at the level of individual chromosomes and cells.

Cytokinesis the last stage of cell division in which the cytoplasm of a mother cell splits into two daughter cells.

Cytoplasm all living material inside a cell except the nucleus.

Cytoplasmic DNA DNA found in the cytoplasm of a cell (not in the nucleus) in connection with organelles (e.g., mitochondria); DNA not found in chromosomes within the nucleus.

Deleterious Allele an allele that has a deleterious effect on an organism.

Dendrogram a type of graph or chart that resembles a tree (with trunk and branches) and is used to illustrate similarity or relatedness among units or samples.

Deoxyribonucleic Acid (DNA) the chemical used to store genetic information in most organisms.

Diploid a cell or organism that has two complete sets of chromosomes.

Diploid Gynogenesis production of gynogenetic diploids; see gynogenesis.

Dispersive Process a process (either genetic drift or inbreeding) that causes random changes in allelic frequencies and loss of genetic diversity in populations over time.

DNA see deoxyribonucleic acid.

DNA Chip a chip spotted with any array of many DNA fragments. Used to simultaneously detect many genes and/or gene expression. Also called a microarray.

Domestication Selection any change in the selection regime of a cultured population relative to that experienced by the natural population.

Dominance the property of an allele that suppresses expression of other alleles at the same locus; a dominant allele is the only allele expressed phenotypically in a heterozygote.

Dominance Effect the effect of dominance at one or more loci on the phenotype of an individual or mean phenotype of a population.

Double Helix the shape of a DNA molecule; a chain of base pairs twisted in a spiral.

Effective Population Size (N_e) the size of an ideal population that would experience genetic drift and inbreeding at the same rate as the real population under consideration.

Electrophoresis a laboratory procedure for separating and observing proteins (usually enzymes) or DNA that can be used as genetic markers.

Environmental Effect the effect of the environment on the phenotype of an individual or mean phenotype of a population.

Enzyme a protein produced in living cells that speeds up a particular chemical reaction.

Epistasis interaction between genes at different loci such that one gene affects the phenotypic expression of the other.

Expected Response (R) the predicted response to one generation of artificial selection; calculated as the product of the selection differential (S) and the heritability (h^2).

Expressivity the intensity with which a gene is expressed phenotypically in different individuals.

Factorial Mating a mating technique used to increase genotypic diversity and effective population size in which all possible crosses are made between males and females.

Family Selection an artificial selection program in which superior families rather than superior individuals are chosen for breeding.

Female Factor a gene not found on a sex chromosome that promotes female characteristics.

Fertilization the fusion of an egg and sperm to initiate development of an embryo.

Fitness a measure of the reproductive success of an individual; the frequency distribution of reproductive success for a population of sexually mature individuals.

Fitness Related Traits quantitative traits that directly affect the fitness of an individual.

Fixation the loss from a population of all but one of the alleles at a locus due to inbreeding or genetic drift.

Fixation Index (FS_T) a measure of genetic divergence among populations.

Full-sibs individuals having both parents in common.

Gamete a mature egg or sperm cell.

Gametogenesis the formation of eggs and sperm (gametes).

Gene a segment of DNA that occupies a specific position (locus) on a chromosome, is heritable and has one or more specific effects upon the phenotype of an organism.

Gene Transfer the process of inserting genes into the DNA of a recipient cell by artificial means, which can confer novel traits not obtainable by artificial selection or hybridization. The inserted genes come from other organisms of the same or different species. Also called genetic modification or genetic engineering.

General Combining Ability a general measure of the value of a particular line in the production of hybrids; determined by crossing a line with a large number of other lines.

Genetic Background all genes of the organism other than the one(s) under consideration.

Genetic Correlation correlation between the phenotypic values for two traits (e.g., growth rate and age at maturity) due to genes that affect both traits.

Genetic Distance a statistical measure of the genetic similarity or difference between two populations.

Genetic Diversity all of the genetic variation in an individual, population, or species.

Genetic Drift random changes in allelic frequencies due to natural sampling errors that occur in each generation; the rate of genetic drift increases as effective population size decreases.

Genetic Engineering see gene transfer.

Genetic Mapping construction of an ordered listing of the genes or genetic markers occurring on the respective chromosomes of a species of interest.

Genetic Marker a phenotypic characteristic (e.g., allozyme, chromosome band, or pigmentation) that can be used to infer the genotype of an organism.

Genetic Modification see gene transfer.

Genomics the study of structure and function of the genetic material of an organism.

Genotype the set of alleles at one or more loci in an organism; the entire set of genes carried by an individual.

Genotype-Environment Interaction the effect of interaction between genes and the environment on the phenotype of an individual or mean phenotype of a population.

Genotypic Frequency the proportion of individuals in a population with a particular genotype.

Genotypic Value the mean phenotypic value of individuals in a population that have a particular genotype.

Gynogenesis the production of offspring having all maternal inheritance (all chromosomes and genes obtained from the mother).

Half-Sibs individuals having one parent in common and the other parent different.

Haploid a cell or organism with a single set of homologous chromosomes.

Haplotype a description of the alleles at two or more loci on the same chromosome; also refers to single-copy mtDNA patterns.

Hardy-Weinberg Equilibrium the relationship between allelic and

genotypic frequencies in an idealized population after a single generation of random mating.

Heritability (h^2) the fraction of the total phenotypic variance in a population that is due to the additive effects of genes; used to predict the response to artificial selection.

Hermaphrodite an organism that is both male and female at some time in its life.

Heterochromatin sections of condensed chromosomes that are readily observable when stained and examined under a light microscope; heterochromatin can be used to identify particular chromosomes or as a genetic marker.

Heterogametic the condition of having two different sex chromosomes.

Heterosis see hybrid vigor.

Heterozygote Advantage superior phenotypic value due to heterozygosity.

Heterozygote an organism or cell with two different alleles at a particular locus.

Homogametic the condition of having two copies of the same sex chromosome.

Homologous Chromosomes chromosomes that carry the same genes.

Homozygote an organism or cell with two copies of the same allele at a particular locus.

Hormone a chemical that controls and coordinates the condition of cells and tissues in organisms.

Hybrid Vigor increased phenotypic value of a hybrid strain relative to the parental strains used to produce the hybrids.

Hybridization interbreeding between different species, races, lines, or varieties.

Idealized Population an infinitely large population that has a balanced sex ratio, random mating, non-overlapping generations, no migration, no mutation, random variation in reproductive success and no artificial selection; a population to which the Hardy-Weinberg law applies exactly.

Inadvertent Selection unintentional artificial selection in hatcheries caused by nonrandom collection of broodstock and differences between the hatchery and natural environment.

Inbreeding the mating of related individuals.

Inbreeding Coefficient (F) a measure of the amount of inbreeding and genetic drift that a population has experienced; also called an F-statistic (see also Fixation Index).

Inbreeding Depression a reduction in fitness or vigor due to inbreeding and increased homozygosity.

Incomplete Dominance partial dominance by one allele so that both alleles at a locus are expressed phenotypically in a heterozygote but to different degrees.

Independent Assortment random and independent assortment of alleles or chromosomes during production of eggs and sperm.

Independent Culling a selection program designed to improve several traits simultaneously; individuals are selected for breeding only if they meet independent criteria for all of the traits under consideration.

Indirect Selection artificial selection applied to one character to improve some other, genetically correlated character.

Individual Selection selection of individuals, rather than family groups, for breeding in an artificial selection program.

Intensity of Selection a standardized measure of the intensity of selection in an artificial selection program; intensity is equal to the selection differential divided by the standard deviation of the trait in the population from which the breeders were obtained.

Interphase the stage of the cell cycle between cell divisions.

Introgression the incorporation of genes of one species (or genetically distinct population) into the gene pool of another by backcrossing of fertile hybrids with one or both parent species or population(s).

Inversion a mutation in which the linear sequence of genes in one segment of a chromosome is reversed.

Isozymes enzymes that promote the same chemical reaction but are the products of alleles at different loci.

Linked Genes alleles at two loci that tend to be inherited as a single unit because the loci are located near one another on the same chromosome; genes that do not assort independently.

Locus the location of a particular gene on a chromosome.

Male Factor a gene not found on a sex chromosome that promotes masculine characteristics.

Marker-Assisted Selection (MAS) the use of a genetic marker closely associated with a quantitative trait locus to choose parents with alleles linked to superior performance for the trait.

Mass Selection a form of artificial selection in which only individuals with phenotypic values greater or less than some threshold level are used for breeding.

Maternal Effects non-genetic influences of a mother on the phenotypes of her young.

Meiosis a sequence of cell divisions that lead to reduction in the number of chromosomes prior to the production of eggs and sperm.

Mendel's Principles 1) each gamete contains only one allele from every pair in the parent organism (the principle of segregation) and 2) alleles at different loci assort independently during gametogenesis (the principle of independent assortment).

Mendelian Trait a trait that is controlled by genes that segregate and assort independently during gametogenesis.

Meristic Trait a trait that displays discrete rather than continuous variation (e.g., number of ribs and number of scales along the lateral line).

Messenger RNA (mRNA) ribonucleic acid that is used to communicate genetic information obtained from DNA inside the cell nucleus to the sites of protein synthesis in the cytoplasm of the cell.

Metaphase the second stage in the division of a cell nucleus during mitosis or meiosis when condensed chromosomes line up midway between opposite ends of the cell.

Microarrays see DNA chip.

Microarray Expression Analysis detection of gene expression using a DNA chip spotted with DNA sequences from functional genes.

Microsatellite DNA also known as short tandem repeats (STRs), segments of nuclear DNA composed of 1-6 base-pair repetitive sequences (e.g., ACA-CACAC...) often used as genetic markers.

Mid-Parent Mean the average of the phenotypic value of both parents.

Migration the movement of individuals from one population to another.

Mitochondria organelles in the cytoplasm of cells that contain DNA and function in energy metabolism.

Mitochondrial DNA (mtDNA) DNA found in mitochondria.

Mitosis division of nuclei during cell divisions that do not lead to the production of gametes; chromosome number is not reduced during mitotic divisions.

Molecular Genetics the study of genetics at the level of molecules (e.g., structure of DNA, the genetic code, replication of DNA).

Monomorphic a locus that has just one allele in a population; a locus that is always homozygous for the same allele.

Monoploid an organism or cell having a single haploid set of chromosomes.

mRNA see messenger RNA.

mtDNA see mitochondrial DNA.

Multi-Locus nDNA Markers genetic markers for which genotypes at multiple loci are observed simultaneously. Often, it is not possible to infer allelic relationships among the bands so observed.

Multi-Locus DNA Fingerprinting a multi-locus DNA marker technique in which digested DNA is separated by gel electrophoresis and visualized after hybridization with probes containing specific DNA sequences.

Multiple Trait Selection artificial selection on the basis of two or more phenotypic traits.

Mutagen an environmental agent (e.g., radiation or chemicals) that is capable of inducing mutations.

Mutation a change in the DNA or chromosomes of a cell or organism.

Natural Selection the selection of successful breeders in natural environments on the basis of phenotypic traits related to fitness.

Net Fitness the degree to which an organism succeeds at passing on its genes to future generations. It is determined by the joint effect of six fitness traits spanning the entire life cycle of the organism: juvenile and adult viability, fecundity, fertility, mating success, and age at sexual maturity.

Non-Additive Component the portion of the total phenotypic variance for a trait in a population that is due to non-additive effects of genes (i.e., dominance effects and epistatic effects).

Non-Coding DNA sequence that does not code for or regulate production of gene products. The majority of DNA in higher organisms, including fish.

Nuclear DNA DNA in chromosomes within the nucleus of a cell.

Nucleotide a unit of the DNA molecule containing a phosphate, a sugar, and a base.

Nucleus an organelle that contains chromosomes in the cells of fish and other higher plants and animals.

One Gene - One Protein Concept a definition of a structural gene as the DNA that codes for a single protein.

Organelle a specialized part of a cell with particular functions.

Outbreeding Depression the phenomenon of reduction in fitness following intraspecific hybridization (matings between individuals from different populations), either in the immediate hybrids or delayed until the backcross or later generations.

Outcrossing matings between individuals from one line and entirely unrelated individuals.

Overdominance the condition that exists when heterozygotes have greater phenotypic value than homozygotes.

Penetrance the frequency with which a genotype is expressed phenotypically in different individuals.

Phenotype the detectable properties (i.e., one or more traits) of an individual that are produced by the genotype and the environment.

Phenotypic Value a measurement of some trait (e.g., weight, number of dorsal fin rays) obtained from an organism.

Plasmid a small DNA molecule usually found in bacteria that is capable of autonomous replication and is useful for transferring genes from one organism to another in the laboratory.

Pleiotropy the condition in which a single gene affects more than one phenotypic characteristic.

Polygenic traits that are determined by genes at many loci.

Polymerase Chain Reaction (PCR) a molecular genetic technique used to amplify the number of copies of a target DNA sequence.

Polymorphic a gene or qualitative trait that exists in two or more forms in a population.

Polyploid a cell or organism possessing three or more haploid sets of chromosomes.

Population a group of organisms that freely interbreed.

Population Bottleneck a temporary decline in population size that dramatically reduces mean effective population size over many generations; population bottlenecks increase the mean rates of inbreeding, genetic drift, and loss of genetic diversity in a population.

Population Fitness see fitness.

Progeny Selection the selection of breeders in an artificial selection program on the basis of the mean phenotypic value of their progeny.

Promoter regulatory DNA sequences that bind the enzyme RNA polymerase to initiate transcription of RNA, i.e., promote gene expression.

Prophase the first stage of mitotic or meiotic cell divisions during which chromosomes condense and become visible by light microscopy.

Protein a molecule composed of one or more chains of amino acids called polypeptides.

Qualitative Trait a phenotypic trait that is described qualitatively rather than by measurement (e.g., eye color).

Quantitative Trait a phenotypic trait that is described by a measurement (e.g., weight at maturity) and usually controlled by genes at many loci.

Quantitative Trait Loci (QTLs) genes determining expression of quantitative traits.

Randomly Amplified Polymorphic DNA (RAPD) a multilocus DNA marker technique that produces PCR products from genomic DNA template using one or a combination of short oligonucleotide primers (typically ca. 10 bp).

Recessive an allele, or trait that is expressed only in homozygotes.

Reciprocal Cross mating males of one strain to females of another and vice-versa.

Recognition Site a specific sequence of nucleotides in DNA that are recognized and cut by a restriction endonuclease.

Recombination exchange of alleles between homologous chromosomes due to crossing over during meiosis.

Regulator Gene a gene whose function is to control the rate at which other genes are transcribed.

Relaxation of Natural Selection a form of domestication selection whereby fish that would have been selected against in the wild survive in the hatchery and pass on genes that are maladaptive in the natural environment.

Replication duplication of a DNA molecule.

Response to Selection change in the mean phenotypic value of a population due to artificial selection.

Restriction Endonuclease an enzyme that cuts DNA at specific sequences of base pairs called recognition sites.

Restriction Fragment Length Polymorphisms (RFLPs) variation in the length of DNA fragments generated within a species when treating longer DNA segments with a restriction enzyme. The variants may be due to differences in DNA sequence at the recognition site where cleavage occurs (i.e., the enzyme does or does not cut) or to variations in length of the cleaved segment (often due to presence of varying numbers of tandem repeats).

RNA ribonucleic acid; present in several forms in a cell and involved in the production of proteins.

Rotational Line Crossing a breeding program involving three or more lines that minimizes inbreeding and loss of genetic diversity.

Secondary Character the trait used to select breeders in an indirect selection program.

Segregation the separation of homologous chromosomes or alleles during the production of gametes.

Selection the natural or artificial process by which breeders are chosen from a population on the basis of fitness or phenotypic value.

Selection Differential (S) the difference between the mean phenotypic value of selected breeders and the mean phenotypic value of the population.

Selectively Neutral the hypothesis that the genotype at a locus does not affect the fitness of an organism.

Semidominance the condition of an allele that is incompletely dominant; see incomplete dominance.

Sex Chromosome a chromosome that is involved in sex determination.

Sex Reversal the process of switching sex.

Sib Selection the selection of breeders in an artificial selection program based on the phenotypic value of their siblings.

Single-Locus nDNA Markers genetic markers for which single loci are genotyped. Both alleles can be scored so that homozygotes and heterozygotes can be distinguished.

Single Nucleotide Polymorphisms (SNPs) genetic markers based on single nucleotide changes in DNA sequence (e.g. AACTGC v. AAGTGC)

Somatic Cell a cell in the body of an organism that is not a gamete.

Specific Combining Ability the value of the hybrids obtained when two specific lines are crossed.

Statistical Power the ability of a statistical test to discriminate between alternate statistical hypotheses.

Stock a population of organisms that, sharing a common environment and participating in a common gene pool, is sufficiently discrete to be considered a self-perpetuating, manageable system (Larkin 1970).

Structural Gene a gene that codes for a protein.

Superior Sex Gene a gene that is a primary determinant of sex and is found on a sex chromosome.

Systematic Processes processes that change allelic frequencies of a population in some predictable, nonrandom fashion (i.e., mutation, migration, and selection).

Tandem Selection an artificial selection program designed to improve several traits; breeders are selected on the basis of one trait in the first generation, another trait in the second generation and so on.

Telophase the fourth and final stage in the division of a cell nucleus during mitosis or meiosis when the chromosomes group together at opposite ends of a cell just before the nucleus divides.

Tetraploid a cell or organism with four haploid sets of chromosomes.

Tetrasomic an otherwise diploid organism or cell that has four copies of one or more chromosomes or genes.

Trait any detectable, phenotypic property of an organism.

Transcription the process of forming messenger RNA from DNA.

Transfer Vector a segment of DNA that facilitates insertion of new genes into the genome of a recipient cell.

Transgenic Organism an organism created by gene transfer. Also called genetically modified organism (GMO), genetically engineered organism (GEO), or living modified organism (LMO).

Translation the process of protein synthesis in ribosomes using mRNA as a template.

Triplet Code a sequence of three nucleotides that code for one amino acid in a protein.

Tripliod a cell or organism having three haploid sets of chromosomes.

Trisomic an otherwise diploid cell or organism that has an extra copy of a chromosome or gene.

Within Family Selection selection of the best individuals within a family for breeding.

